

RESTORING THE ECOLOGICAL QUALITY OF RIPARIAN ECOSYSTEMS – A MULTI-LEVEL APPROACH

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**THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN
FORESTRY ENGINEERING AND NATURAL RESOURCES**

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Contemporary Attica may accurately be described as a mere relic of the original country. There has been a constant movement of soil away from the high ground and what remains is like the skeleton of a body emaciated by disease. All the rich soil has melted away, leaving a country of skin and bone. Originally the mountains of Attica were heavily forested. Fine trees produced timber suitable for roofing the largest buildings; the roofs hewn from this timber are still in existence. The country produced boundless feed for cattle, there are some mountains which had trees not so very long ago, that now have nothing but bee pastures. The annual rainfall was not lost as it is now through being allowed to run over the denuded surface to the sea, it was absorbed by the ground and stored... the drainage from the high ground was collected in this way and discharged into the hollows as springs and rivers with abundant flow and a wide territorial distribution. Shrines remain at the sources of dried up water sources as witness to this.

The Dialogues of Plato, Critias III (Thirgood, 1981)

[Thirgood, J.V. 1981. *Man and the Mediterranean Forest: A History of Resource Depletion*. Academic Press. London.]

ABSTRACT

Many European rivers and floodplains have been subjected to long periods of anthropogenic degradation. Activities like land drainage, construction of dams and weirs, channelization, water abstraction and pollution, resulted, among others, in the loss of floodplains and wetlands, high sediment runoff, biodiversity losses, lowering of the river and water table levels and increase in peak flows. Thus, this thesis focuses on a multi-level top to bottom approach to freshwater ecosystem restoration, addressing the legislation restoration drivers, as well as the restoration at the basin and river section levels. The main conclusions are: a) to improve freshwater restoration success in Europe it is highly recommended to create more ecosystem restoration soft law and reinforcement mechanisms related with governance, quality, stakeholders, publicity and research; b) there is a joint effect of climate change and land use on river water quality, meaning that proposed environmental conservation measures may be too conservative to have a significant effect in river nitrogen concentration, particularly in a climate change context; c) local population awareness and participation are as essential for habitat restoration success as grazing herbivores exclusion, river pollutant load, water table levels and tree installation techniques; d) the sampling of a river section to assess the influence of the liquid effluent from an acid bisulfite pulp mill on river water quality did not reveal particularly high levels of pollution directly related to the mill, in spite of relevant levels of total phosphorous and dissolved lignin; and e) cork and Tasmanian blue gum bark are capable of enhancing biological denitrification in laboratory batch tests. The implementation of ecologically effective restoration should be flexible to adjust to changing climate and societal priorities, retaining simultaneously the capacity to integrate information from new technologies into site assessment and restoration planning.

Keywords

Freshwater restoration; riparian ecosystems; Mediterranean; nitrates; denitrification.

RESUMO

Muitos rios e zonas aluviais da Europa têm sido historicamente sujeitos a degradação de origem humana. A drenagem de zonas húmidas, construção de barragens e diques, canalização, rega e poluição, entre outras, resultaram na destruição daquelas zonas húmidas, acréscimo da sedimentação, redução da biodiversidade e do nível do lençol freático, e aumento dos caudais de ponta de cheia. Assim, esta tese aborda o restauro dos ecossistemas fluviais numa perspetiva multi-escala: a legislação que fomenta o restauro, e o restauro ao nível da bacia e do troço fluvial. As principais conclusões foram: a) para melhorar o sucesso do restauro fluvial na Europa recomenda-se a criação de legislação adicional dirigida à governança, qualidade, *stakeholders*, publicidade e investigação; b) as alterações climáticas e do uso do solo têm efeitos conjuntos na qualidade da água fluvial, pelo que algumas medidas de conservação ambiental podem ser demasiado conservadoras para terem efeitos relevantes na dinâmica fluvial do azoto; c) a consciencialização e participação da população local no restauro fluvial é tão importante para o sucesso das intervenções como a exclusão do pastoreio e a carga de nutrientes do rio, o nível da toalha freática ou as técnicas de plantação; d) a avaliação da influência na qualidade da água fluvial do efluente líquido duma fábrica de pasta de papel (processo bissulfito ácido) não revelou níveis particularmente elevados de poluição diretamente imputáveis à fábrica, apesar dos valores relevantes de fósforo total e de lenhina dissolvida; e) a cortiça e a casca de eucalipto potenciaram a desnitrificação biológica em testes *batch* de laboratório. A implementação de ações de restauro ecologicamente eficazes deve possuir flexibilidade suficiente para se ajustar às alterações climáticas e das prioridades da sociedade, mantendo a capacidade de integrar no planeamento e avaliação do restauro a informação proveniente de novas tecnologias.

PALAVRAS-CHAVE

Restauro fluvial; ecossistemas ripícolas; Mediterrâneo; nitratos; desnitrificação.

RESUMO ALARGADO

A vegetação envolvente dos rios forma galerias ribeirinhas que funcionam como área de transição entre os sistemas terrestre e aquático. Normalmente possui uma riqueza de espécies, complexidade estrutural e produtividade de biomassa superiores às áreas envolventes, tendo um papel relevante na regularização e valorização do habitat aquático. O reconhecimento da influência dos processos que ocorrem nestas zonas na mitigação dos efeitos da poluição difusa originou um interesse crescente na utilização de zonas ripícolas tampão ao longo dos corredores fluviais. Porém, muitos rios e zonas aluviais da Europa têm sido historicamente sujeitos a degradação de origem humana. Devido ao seu carácter dinâmico as zonas ripícolas são especialmente vulneráveis a estes impactos. A drenagem de zonas húmidas, construção de barragens e diques, canalização, rega e poluição, entre outras ações antrópicas, resultaram na destruição daquelas zonas húmidas, com acréscimo da sedimentação, redução da biodiversidade e do nível do lençol freático, e aumento dos caudais de ponta de cheia. Esta situação deu origem a uma sensibilidade crescente para a necessidade de termos sistemas fluviais mais saudáveis, capazes de disponibilizar serviços de ecossistema e de sustentar níveis razoáveis de diversidade biológica à escala da paisagem. Deste modo, o restauro fluvial surgiu como uma ferramenta poderosa para deter e reverter a degradação dos sistemas fluviais. O restauro fluvial possui um âmbito ecológico claro, estando os seus objetivos frequentemente relacionados com o restauro de habitats e/ou da ictiofauna, ou com a recuperação ecológica e do ecossistema. Neste contexto, o restauro das comunidades ripícolas é fundamental para uma recuperação bem-sucedida dos processos fluviais naturais.

Assim, esta tese procura contribuir para o restauro dos ecossistemas fluviais Mediterrânicos através de uma abordagem multi-escala: a legislação que fomenta o restauro, e o restauro ao nível da bacia e do troço fluvial. Deste modo, procurou-se atingir os seguintes objetivos: a) analisar de que forma os mecanismos legislativos podem melhorar os padrões de restauro na Europa; b) avaliar por modelação SWAT os impactos dos efeitos combinados das alterações climáticas e da gestão do uso do solo na dinâmica fluvial do azoto numa bacia hidrográfica de cariz agrícola, com irrigação e problemas de

captação de água; c) avaliar os resultados do restauro de uma zona húmida; d) avaliar os resultados do restauro de troços fluviais degradados num rio Mediterrânico intermitente e regulado; e) avaliar os impactos na qualidade da água fluvial de um grande rio Ibérico do efluente líquido, particularmente nutrientes, duma fábrica de pasta de papel (processo bissulfito ácido); f) estudar a capacidade de remoção de nitratos de vários substratos de desnitrificação através de ensaios *batch* de laboratório.

As principais conclusões são apresentadas a seguir:

- a. Ainda são expectáveis ações significativas de restauro fluvial ao abrigo da atual legislação. No entanto, a experiência recente no que se refere à implementação da Diretiva Quadro da Água (DQA) e das Diretivas Habitats e Aves, demonstra que objetivos ambiciosos são difíceis de atingir. Assim, os resultados dos últimos 18 anos de implementação da DQA revelam que em 2015 menos de metade das massas de água dos países da União Europeia cumpriam o objetivo do bom estado ecológico. Deste modo, para melhorar o sucesso do restauro fluvial na Europa, recomenda-se a criação de legislação adicional dirigida à governança, qualidade, *stakeholders*, publicidade e investigação.
- b. Os cenários modelados indicam que a qualidade da água da bacia hidrográfica do Rio Sorraia se irá degradar ao longo do tempo. O aumento da concentração de nitratos na água parece estar relacionado com o uso do solo e com as práticas agrícolas, observando-se maiores concentrações nos cenários onde existe expansão da área agrícola e um aumento da fertilização. Adicionalmente, as alterações climáticas podem vir a originar uma forte redução do caudal médio anual do Rio Sorraia, com a consequente redução da capacidade de diluição do rio e aumento da concentração de nutrientes. Assim, as alterações climáticas e do uso do solo apresentam um efeito conjunto na qualidade da água fluvial, pelo que algumas medidas de conservação ambiental podem ser demasiado conservadoras para terem efeitos relevantes na dinâmica fluvial do azoto. Estes resultados realçam a importância de implementar soluções de gestão adaptativa que considerem alterações do clima e do uso do solo em paralelo.

- c. O restauro da galeria ripícola recorrendo a plantas produzidas em viveiro através de métodos florestais clássicos, juntamente com a utilização de técnicas de engenharia natural, foi bem-sucedido. Atualmente a zona alvo de restauro apresenta uma comunidade vegetal mais complexa, com abundante regeneração natural, e com disponibilidade de habitat de alimentação, reprodução e refúgio para aves aquáticas. O restauro desta área piloto proporcionou informação importante sobre as necessidades e problemas relacionados com este tipo de intervenção em zonas húmidas mediterrânicas, especialmente os associados à sobrevivência das plantas. No entanto, o pastoreio por parte de gado bovino e outras pressões de origem antropogénica antrópica podem colocar em perigo os resultados obtidos até este momento. Deste modo, a consciencialização e participação da população local no restauro é tão importante para o sucesso das intervenções como a exclusão do pastoreio, o nível do lençol freático ou as técnicas de plantação. Assim, o restauro ripícola é um processo de longo prazo, que necessita de monitorização contínua, de modo a implementar correções atempadamente.
- d. Observou-se um aumento do grau de cobertura de vegetação ripícola nas áreas restauradas e a estabilidade dos taludes também melhorou, especialmente nos Troços identificados como I e M. No entanto, o controlo da invasora exótica *Arundo donax* não foi tão bem-sucedido, verificando-se um aumento gradual (ainda que lento) do número de manchas desta espécie na área restaurada. Adicionalmente, apesar da qualidade e heterogeneidade do habitat para a ictiofauna ter melhorado, tal ainda não se refletiu num aumento das populações de *Squalius aradensis* e *Iberochondrostoma almakai*. Verificou-se igualmente que o restauro das áreas ripícolas em zonas Mediterrânicas através da utilização de técnicas de engenharia natural necessita de uma gestão muito cuidadosa nos primeiros anos após intervenção, nomeadamente no que se refere ao stress hídrico das plantas e ao controlo de exóticas invasoras. Outros fatores antropogénicos, como o pastoreio e a poluição orgânica do meio aquático, representam uma ameaça para o sucesso deste tipo de projetos de restauro.

- e. O tipo de amostragem realizado, não sistemático e limitado no tempo, não permitiu estabelecer um perfil da evolução espaço-temporal dos parâmetros avaliados, embora tenha possibilitado a caracterização da situação à data das amostragens. Assim, para obter conclusões mais abrangentes e detalhadas são necessárias mais datas de amostragem e uma maior cobertura espacial. A avaliação na qualidade da água fluvial não revelou níveis particularmente elevados de poluição diretamente imputáveis à fábrica, apesar dos valores relevantes de fósforo total e de lenhina dissolvida. No entanto, os níveis de fósforo a montante do emissário da fábrica atingem valores com alguma relevância, o que indica que o rio sofre os efeitos da poluição orgânica antes do troço amostrado. Ainda assim, a presença de níveis de fósforo total, lenhina dissolvida, pH, azoto total e zinco dissolvido com alguma relevância a jusante do emissário da fábrica aconselha a que se instale uma estação de monitorização integrada na rede de monitorização da Bacia Hidrográfica do Rio Tejo.
- f. Os substratos testados revelaram ter a capacidade de estimular a desnitrificação biológica, ainda que com diferentes graus de eficácia. Os substratos que apresentaram os melhores resultados foram a cortiça e a casca de eucalipto, com vantagem deste último, em especial nas taxas de remoção de nitratos. As cascas de acácia e de pinhão foram consideradas desadequadas como fonte de carbono para a desnitrificação devido a taxas excessivas de redução de nitrato para amónio (em ambos) e a taxas de remoção de nitratos reduzidas (no caso da acácia). Tanto quanto foi possível aferir na bibliografia, esta foi a primeira vez que se testou o potencial das cascas de eucalipto, acácia e pinhão como fonte de carbono para potenciar a desnitrificação biológica. Este ensaio *batch* permitiu seleccionar o substrato mais adequado para testes mais detalhados.

A implementação de ações de restauro ecologicamente eficazes deve possuir flexibilidade suficiente para se ajustar às alterações climáticas e das prioridades da sociedade, mantendo a capacidade de integrar no planeamento e avaliação do restauro a informação proveniente de novas tecnologias.

As linhas de investigação futuras devem incidir na melhoria da validação e calibração do modelo SWAT para a Bacia Hidrográfica do Rio Sorraia, através da obtenção de dados reais de caudal e de nutrientes mais fiáveis e abrangentes. Igualmente, importa melhorar o nível de conhecimento acerca do controlo e erradicação de plantas invasoras em ambiente fluvial, uso de metodologias mais holísticas (*i.e.*, que tenham em consideração o ecossistema fluvial como um todo) para o cálculo de caudais ecológicos, e desenvolver novas formas de abordagem para a cooperação e envolvimento dos proprietários de terrenos no restauro fluvial. Adicionalmente, a capacidade da casca de eucalipto para remover nitratos da água deverá ser testada através de ensaios de coluna e num ensaio de campo recorrendo a uma estação lisimétrica.

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SECTION I

INTRODUCTION

CHAPTER 1

Introduction

1. INTRODUCTION

1.1 River systems

Rivers are lotic four-dimensional systems that are characterized by a high spatio-temporal heterogeneity (Ward, 1989). These four dimensions characterize the longitudinal, lateral, vertical and temporal interactions that take place in the river systems. The longitudinal dimension integrates the upstream-downstream continuity of the river and deals with biotic and abiotic changes. The lateral dimension is where the exchanges of matter and energy between the river and floodplain take place. These interactions are fundamental for river productivity and biotic diversity (Junk *et al.*, 1989). The vertical dimension integrates the interaction between the channel and groundwater flow and includes the hyporheic zone. The last dimension incorporates a temporal hierarchy on the three spatial dimensions. For instance, the visualization of changes in three-dimensional connectivity over time is a valuable tool that helps to characterize anthropogenic impacts and ensuing responses to river restoration (Kondolf *et al.*, 2006). These four connectivity dimensions regulate the processes and patterns of river ecosystems at multiple scales (e.g. Stanford & Ward, 1988; Naiman *et al.*, 2005; González del Tánago & García de Jalón, 2006; Kawanishi *et al.*, 2013; Holt *et al.*, 2015; Gurnell *et al.*, 2016; Hug Peter *et al.*, 2017). Nevertheless, although river connectivity is normally considered a positive ecological attribute, the variability in spatial and temporal connectivity is important for sustaining the highest diversity of ecological structure and function (Kondolf *et al.*, 2006).

Furthermore, rivers are hierarchical systems that can be break down into different levels of organization (Werner, 1999). Their hierarchical classification allows for a systematic view of the spatial and temporal variation among and within river systems (Frissell *et al.*, 1986). That hierarchy is spatially nested, which means that the system of a higher level shapes the environment of the lower level subsystems. Thus, each river level has a number of variables that control the actions and capacities of the system within a spatio-temporal frame (Frissell *et al.*, 1986) (Figure 1). In permanent rivers that hierarchy translates into a longitudinal continuum of species and functional feeding groups based on stream size/order (Vannote *et al.*, 1980). Moreover, nutrient spiraling, i.e. the cycling of

nutrients while they are transported downstream (Webster & Patten, 1979), takes place along the same river continuum. This phenomenon is influenced by stream order (Ensign & Doyle, 2006) and by invertebrate consumers (Newbold *et al.*, 1982).

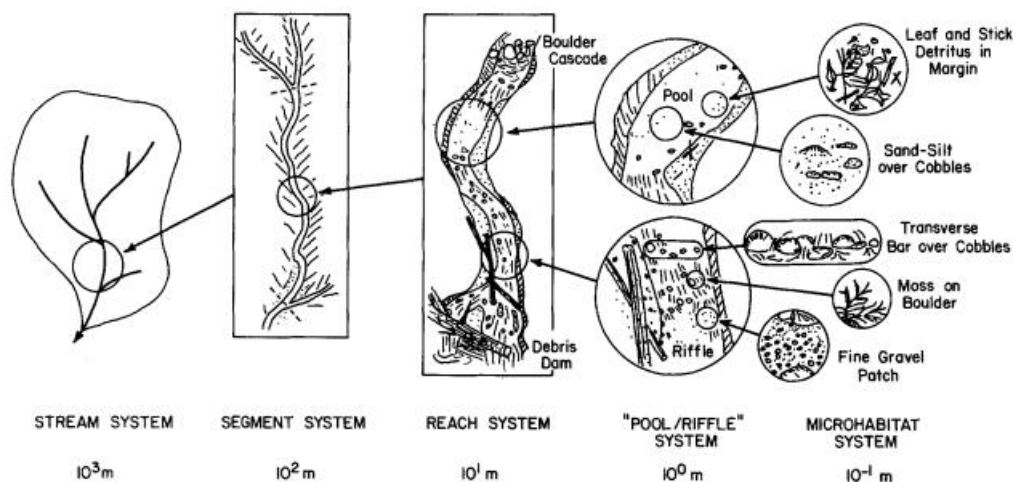


Figure 1. Geomorphological hierarchical organization of a river system and its subsystems, with indication of the approximated linear spatial scale between them (reprinted from Frissell *et al.*, 1986).

However, this view of rivers as continuous, longitudinal gradients in physical conditions is not consensual. Consequently, the Riverine Ecosystem Synthesis theory considers rivers as downstream clusters of large hydrogeomorphic patches created by catchment geomorphology and flow attributes (Thorp *et al.*, 2006). The patches spread longitudinally, laterally and vertically, with different time-scales (sub-seasonal to geological time periods) (Thorp *et al.*, 2008) (Figure 2).

Riparian vegetation (i.e. the assemblage of plant communities characteristic of riverbanks) is an important and dynamic element in the longitudinal and lateral river dimensions (FISRWG, 1998; González del Tánago & García de Jalón, 2006). The riparian zones are transition areas between the terrestrial and the aquatic systems (Gregory *et al.*, 1991). They usually display higher species richness, structural complexity and biomass productivity than the surrounding areas (Hunter Jr., 1990; Lewis *et al.*, 2009; Santos, 2010; Young-Mathews *et al.*, 2010). Its width is usually related with the geomorphological conditions of the

channel and with stream order, varying from a narrow strip at the headwaters and in lower order streams, to a wide area in the slow river sections of the main rivers (González del Tánago & García de Jalón, 2001, 2006).

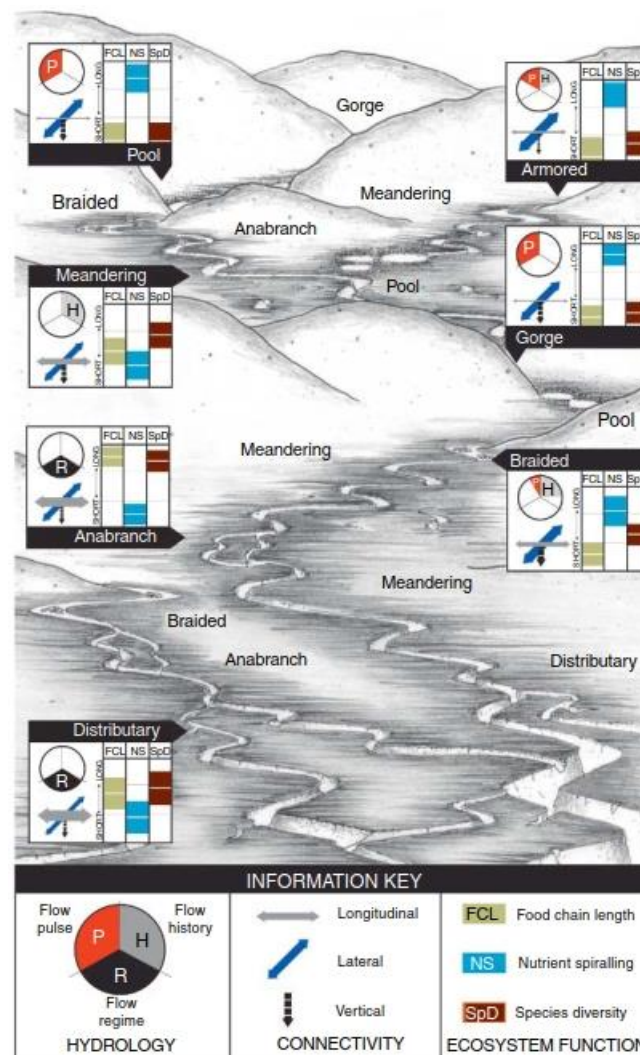


Figure 2. The River Ecosystem Synthesis (Thorp et al., 2006), with several functional process zones (FPZ) that may occur in a theoretical riverine landscape. Each FPZ results from the combination of different hydrogeomorphologic processes (reprinted from Thorp et al., 2008).

Moreover, the structure and heterogeneity of the riparian vegetation is mainly controlled by river flow, but also by longitudinal zonation and riverbank topography (Rood et al., 2003a; González del Tánago & García de Jalón, 2006; Rodríguez-González et al., 2010; Angiolini et al., 2011; Booth & Loheide, 2012; Magdaleno et al., 2014; Rivaes et al., 2014; Marques, 2016). Thus, this type of vegetation presents specific morphologic, physiologic and reproductive strategies to be able to thrive in the dynamic riverbank environment. Adaptative strategies

include the ability to withstand waterlogging (Hunter Jr., 1990; Blom & Voesenek, 1996; Hager & Schume, 2001), through aerenchyma and adventitious roots (Smirnoff & Crawford, 1983; Calhoun, 1999), seed release connected with the natural flow regime (Mahoney & Rood, 1998; Stella *et al.*, 2006), different water use strategies (Singer *et al.*, 2013), fast growth (Blanco Castro *et al.*, 2005) and good vegetative propagation capability (Rood *et al.*, 1994, 2003b).

The importance of riparian zones is far greater than the minor proportion of land area that they cover (Gregory *et al.*, 1991). They interact with the aquatic environment, acting as flux regulators and supplying matter and energy (Gregory *et al.*, 1991; Mitsch & Gosselink, 2015). Thus, riparian zones have an important role in the regulation and improvement of the aquatic habitat (*e.g.* Gregory *et al.*, 1991; Naiman *et al.*, 1993, 2005; Naiman & Décamps, 1997; Tabacchi *et al.*, 2000; Pusey & Arthington, 2003; Broadmeadow & Nisbet, 2004; Dosskey *et al.*, 2010; Van Looy *et al.*, 2013; Rood *et al.*, 2015). Accordingly, a healthy and mature riparian gallery regulates water temperature through overshadowing (Schiemer & Zalewski, 1991; Bowler *et al.*, 2012; Ryan *et al.*, 2013; Kalny *et al.*, 2017), influences fish assemblages (Schiemer & Zalewski, 1991; Growns *et al.*, 1998; Pires *et al.*, 2010; dos Santos *et al.*, 2015) and macroinvertebrate communities (Rios & Bailey, 2006; Shilla & Shilla, 2012). It also has an indirect effect on food webs (Nakano *et al.*, 1999; Baxter *et al.*, 2005; Wootton, 2012), influences stream bank stability (Simon & Collison, 2002; Easson & Yarbrough, 2002; Hubble *et al.*, 2010; Rood *et al.*, 2015) and aquatic habitat mainly by supplying large woody debris (Fausch & Northcote, 1992; Piégay & Maridet, 1994; Fetherston *et al.*, 1995; Gurnell *et al.*, 2005). Riparian areas are also important wildlife habitats (Doyle, 1990; Matos *et al.*, 2009; Gomes *et al.*, 2017), also functioning as corridors between different habitats (Machtans *et al.*, 1996; Hilty & Merenlender, 2004). Moreover, riparian vegetation enhances sediment retention (Steiger *et al.*, 2003; Noe & Hupp, 2009) and prevents pollutants and nutrients from entering the channels through direct runoff or subsurface flow (Lowrance *et al.*, 1984, 1997; Osborne & Kovacic, 1993; Fennessy & Cronk, 1997; Dosskey *et al.*, 2010). The recognition of the importance of riparian zone processes on water quality led to a growing interest in the use of riparian buffer zones along river corridors to mitigate the effects of non-point source pollution

(Hill, 1996). However, the spatial and temporal heterogeneity of these areas affects the rate of nitrate removal in the riparian zone because the major pathway for nitrate movement is through subsurface flow (Hill, 1996). Thus, the removal capacity of riparian zones is controlled by the water residence time and degree of contact between soil and groundwater (Gold *et al.*, 1998; Ocampo *et al.*, 2006; Noe *et al.*, 2013), but also by plant uptake and denitrification (Groffman *et al.*, 1992, 1996; Aguiar Jr. *et al.*, 2015). The relative influence of these factors depends on soil characteristics (Groffman *et al.*, 1992; Flite III *et al.*, 2001; Sabater *et al.*, 2003) and nitrogen input to the riparian zone (Hanson *et al.*, 1994). Consequently, nitrogen containing molecules applied to the landscape can interact with many different biological components, sometimes in close proximity or separated by great distances in time and space (Schmidt & Clark, 2012).

The importance of the riparian ecosystems goes beyond its ecological value. Accordingly, they also provide environmental services that are directly valued by human societies (Postel & Carpenter, 1997; Loomis *et al.*, 2000). Thus, riparian vegetation protects and improves water quality (Dosskey *et al.*, 2010; Gundersen *et al.*, 2010) and decreases flow peaks, reducing the flood risk (Dixon *et al.*, 2016). It also has the capability to sequester large amounts of atmospheric carbon (Rheinhardt *et al.*, 2012), although they also are potential sources of other greenhouse gases, like methane and nitrous oxide (Jones & Mulholland, 1998; Groffman *et al.*, 2000). Riparian areas also have an intrinsic aesthetic value (Brown & Daniel, 1991; Burmil *et al.*, 1999; Décamps, 2001; Pflüger *et al.*, 2010), and provide food and recreation, like game fishing (Holmes *et al.*, 2004) and bird watching (Villamagna *et al.*, 2014) zones.

1.2 Threats to river systems

The human footprint in the earth system has reached dangerous levels (Vitousek *et al.*, 1997b; Steffen *et al.*, 2015), and many ecosystems are threatened or strongly degraded (e.g. Bryant *et al.*, 1997; Bogardi *et al.*, 2012). Freshwater ecosystems are some of the most endangered in the world, being increasingly impaired by multiple stressors (Dudgeon *et al.*, 2006). Accordingly, biodiversity reduction is higher in freshwater than in most terrestrial ecosystems (Sala *et al.*, 2000). The threats to these ecosystems can be grouped under five

interconnected categories (Dudgeon *et al.*, 2006): overexploitation (e.g. Bromley *et al.*, 2001; Anthony & Downing, 2001; Alemayehu *et al.*, 2007), water pollution (e.g. Chandra *et al.*, 2006; Gascho Landis *et al.*, 2013; Eerkes-Medrano *et al.*, 2015), flow modification (e.g. Maingi & Marsh, 2002; FitzHugh & Vogel, 2011; Gao *et al.*, 2013), destruction or degradation of habitat (e.g. Sweeney *et al.*, 2004; Österling *et al.*, 2010; Bjelke *et al.*, 2016) and invasion by exotic species (e.g. Aguiar *et al.*, 2001; Cruz & Rebelo, 2007; Cushman & Gaffney, 2010) (Figure 3).

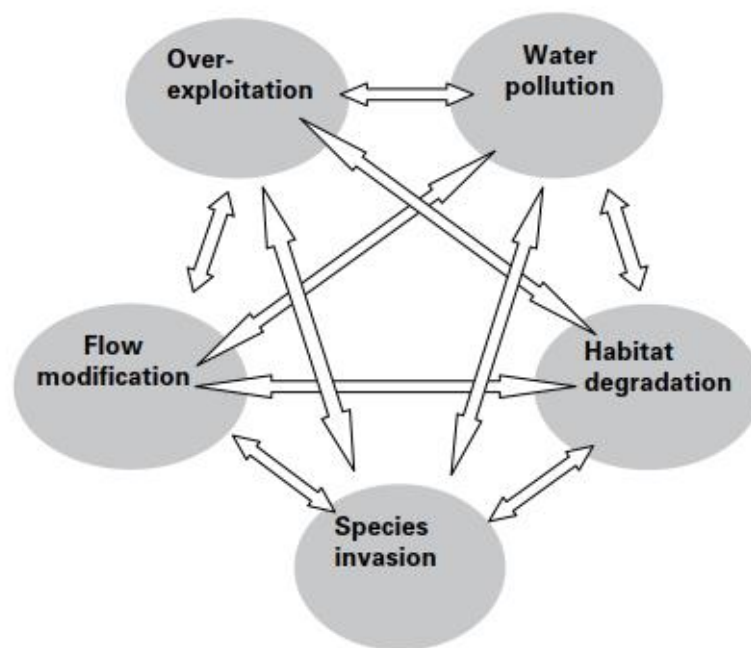


Figure 3. Major threat categories on freshwater ecosystems. The arrows represent established or potential impacts on biodiversity. Environmental scales taking place at the global scale are superimposed upon these categories (reprinted from Dudgeon *et al.*, 2006).

Water pollution from industrial sources and agricultural non-point sources still remains one of the main threats for river systems worldwide (e.g. Stokál *et al.*, 2016; Wright *et al.*, 2017; Ltifi *et al.*, 2017). For example, in spite of the improvement in wood pulp industry liquid emissions in the western countries over time (Suhr *et al.*, 2015), solids and organic matter are still discharged in large quantities to the watercourses (Hubbe *et al.*, 2016). Additionally, there are also difficulties within the industry towards achieving a meaningful reduction in the load of low biodegradable organic substances (Suhr *et al.*, 2015). Regarding agricultural non-point sources, the increase in the use of fertilizers and pesticides associated with the expansion and industrialization of agriculture resulted in

additional problems of surface and groundwater degradation (Donoso *et al.*, 1999; Zalidis *et al.*, 2002; Lawniczak *et al.*, 2016; Hundey *et al.*, 2016). Non-point source pollutants, like nitrogen, are transported by rainwater and melting snow overland and through the soil, ultimately finding their way into groundwater and aquatic ecosystems (Ongley, 1996). They can have severe ecological impacts on freshwater bodies, like acidification, eutrophication, hypoxia and N₂O emissions (Ongley, 1996; Vitousek *et al.*, 1997a; Howarth *et al.*, 2000; Rabalais, 2002; Camargo & Alonso, 2006; Phoenix *et al.*, 2006). The growth in nutrient discharge into aquatic ecosystems in recent years resulted in an increase of eutrophication problems in watercourses (Vitousek *et al.*, 1997a; Galloway & Cowling, 2002; Jørgensen *et al.*, 2013).

Because of their dynamic character, riparian areas are particularly vulnerable to anthropogenic impacts (Brinson & Verhoeven, 1999). Hydrologic disturbances, like the increased depth to ground water (Stromberg *et al.*, 1996; Chen *et al.*, 2006; Dott *et al.*, 2016), or stable flow regimes (Franklin *et al.*, 2008; Tonkin *et al.*, 2018; Bejarano *et al.*, 2018), may influence riparian species distribution and composition. Thus, the risk of exotic plant establishment and/or invasion increases (Catford *et al.*, 2011; Greet *et al.*, 2015), animal species richness may decrease (e.g. Matos *et al.*, 2009; Merritt & Bateman, 2012), recruitment sites for young riparian flora may be absent (Polzin & Rood, 2000), except for the river channel, where riparian vegetation may colonize and develop towards maturity (Picco *et al.*, 2017). However, the latter may increase flood risk due to the higher flow resistance of the mature, less-flexible, vegetation (Darby, 1999).

In spite of the importance of freshwater ecosystems, new threats are still emerging, like climate change impacts on freshwater physical environment (Knouft & Ficklin, 2017), the worldwide increase in hydropower projects due to international agreements about the reduction of greenhouse emissions (Hermoso, 2017), the water demand for hydraulic fracturing (Entrekin *et al.*, 2018) or the presence of microplastics and endocrine disruptors in river water and food webs (Eerkes-Medrano *et al.*, 2015; Ruhí *et al.*, 2016). Thus, riparian ecosystems may be highly vulnerable to climate change impacts (Capon *et al.*, 2013). Meteorological changes will significantly affect European river flow regimes, mainly through more pronounced low flow periods in the Mediterranean region

(Schneider *et al.*, 2013). Contrarily, the rise in the winter heavy rain events may increase the risk of flooding (IPCC, 2008). These expected modifications of the river flow regime will possibly be augmented by future climatic change interactions with anthropogenic pressures, such as increased water abstraction for human needs (Alcamo *et al.*, 2007; Murray *et al.*, 2012). Moreover, pluvial flow regimes with deep seasonal gaps between flooding and drought extremes, like the ones in southern European rivers, are expected to experience more conspicuous riparian vegetation changes (Rivaes *et al.*, 2014). Thus, younger individuals, which are more dependent on flow levels for survival, are expected to be the most affected by climate change (Rivaes *et al.*, 2013, 2014).

1.3 River restoration

As previously established, many European rivers and floodplains have been subjected to anthropogenic induced degradation for long periods (Brookes, 1988, 1996; Petts, 1994; Nienhuis & Leuven, 2001; Downs & Gregory, 2004; Mant *et al.*, 2012). Activities like land drainage, the construction of dams and weirs, channelization, water abstraction and pollution (Mant *et al.*, 2012; Mitsch & Gosselink, 2015), culminated in the loss of floodplains and wetlands, high sediment runoff, biodiversity losses, over widening and deepening of river channels, lowering of the river and water table levels and increase in peak flows (Mant *et al.*, 2012). These negative impacts gave rise to a growing awareness regarding the need for healthier river systems, able to provide ecosystem services and to sustain satisfactory levels of biological and ecological diversity at the landscape scale (Piégay *et al.*, 2008; Mant *et al.*, 2012). Moreover, that increase in societal environmental awareness provided the political background for the introduction of an assortment of legislation that created the conditions for river restoration to grow (Downs & Gregory, 2004; Wharton & Gilvear, 2007; Lemons & Victor, 2008). Accordingly, restoration emerged as a powerful tool to stop and reverse the degradation of river systems (Ormerod, 2004; Wheaton *et al.*, 2008). Nowadays, ecosystem and natural capital restoration is viewed as an important part of the move towards a green economy (United Nations, 2011; Smith *et al.*, 2014). Also, the compensation for ecological damage or biodiversity offsetting is one of the main policy approaches that seeks to achieve a no net loss of biodiversity when economic development leads to environmental

degradation (Lapeyre *et al.*, 2015; Calvet *et al.*, 2015). Accordingly, freshwater ecosystem restoration is a high priority at the International agenda due to the danger of these ecosystems not being able to secure the provision of freshwater for human consumption (United Nations, 2016; IPBES, 2018a). Thus, numerous International conventions and treaties mention restoration practices at global scale and the need for cooperation between States to effectively achieve Sustainable Development Goals. Accordingly, the European legislation currently has a high set of laws that drive member states to develop restoration practices, like the Water Framework Directive (WFD) (European Commission, 2000), the Habitats Directive (European Commission, 1992), the Birds Directive (European Commission, 2010), the Floods Directive (European Commission, 2007), or the Nitrates Directive (European Commission, 1991).

In spite of the above, there is still no official definition of ecological restoration, although there is scientific consensus over several definitions (Telesetsky, 2013). Thus, ecological restoration is not defined in national legislations or in international law (Telesetsky, 2013; Palmer & Ruhl, 2015; Richardson, 2016). That lack of common legal and technical definitions still leaves room for discussion among sectors and for different approaches which may be harmful to freshwater ecosystems, since in some cases the implementation of compensation for ecological damage mechanisms do not generate sufficient positive effects (Schoukens, 2017a; IPBES, 2018b). Nevertheless, an EU Commission working paper defined restoration as "... actively assisting the recovery of an ecosystem that has been degraded, damaged or destroyed, although natural regeneration may suffice in cases of low degradation. The objective should be the return of an ecosystem more or less equal to its original community structure, natural species composition and ecosystem functions to ensure in the long term a continued provision of services, although in cases of extreme degradation, the focus on specific services may be justified" (European Commission, 2011). However, one of the most consensual definition is the one from the Society for Ecological Restoration (SER), which states that ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (SER, 2004). Accordingly, ecological restoration is one of several types of intervention that tries to modify the biota and

physical conditions of a site (SER, 2004). While restoration is a technique to enhance and promote habitats and populations, conservation focuses exclusively on slowing down or stopping degradation or on maintaining the remnants of the original population or ecosystem (Young, 2000; Hilderbrand *et al.*, 2005). Thus, ecological restoration differs from other forms of ecosystem repair because it aims to assist in the recovery of a natural or semi-natural ecosystem instead of imposing a new direction or form upon it (McDonald *et al.*, 2016). Consequently, restoration seeks to guide an ecosystem on a recovery trajectory following a temporary loss (Young, 2000; McDonald *et al.*, 2016). It encompasses both passive measures, like restrictions seeking to remove disturbances or limiting human pressures, and active measures, aiming to shift an impacted ecosystem towards its recover (Schoukens, 2017b). However, full ecological restoration is often difficult to achieve because the nature of the original ecosystem may be unknown or impossible to accomplish due to historical events or complex evolution trajectories (Hughes *et al.*, 2005; Lamb, 2009; Dufour & Piégay, 2009; Bouleau & Pont, 2015; Jacobs *et al.*, 2015). Therefore, the practice of ecological restoration currently evolves around six main concepts (McDonald *et al.*, 2016):

- The existence of a pre-degradation reference system that provides information regarding the target of the restoration project. However, some authors argue that this approach should be replaced by an objective based strategy that considers the limitations of developing sustainable landscapes and the growing importance of accounting for human services of the target ecosystem (Dufour & Piégay, 2009);
- Identification of the target ecosystem key attributes before establishing longer term goals and shorter-term objectives;
- The work of recovering the ecosystem is carried out by the biota. Restoration actions aim to assist those natural recovery processes, supplementing the impaired natural recover potential. Those actions include the removal of the pressures affecting the target ecosystem;
- Full recovery, when possible, may take a very long time to occur. Thus, the implementation of a continuous improvement strategy, with a long-

term goal of full recovery, is paramount for the enduring success of the restoration effort;

- The use of all relevant knowledge, i.e., a multidisciplinary approach;
- The active engagement of the stakeholders from the start of the project.

So, river restoration has a clear ecological focus, and its objectives are frequently related with habitat restoration, fisheries improvement or ecological and ecosystem recovery (Smith *et al.*, 2014). Thus, restoration priorities are related with the type of problem being addressed, and differ between the different European countries (Smith *et al.*, 2014). The main priority may be water quality, fisheries restoration, improving in-stream flows, or floodplain restoration (Mant *et al.*, 2012). Also, the restoration of the riparian communities is paramount for the successful recovery of the natural river processes (Palmer *et al.*, 2014). Nevertheless, despite the ecosystem context, ecological river restoration is also a social undertaking (Kates *et al.*, 2001). Social perceptions and expectations regarding ecosystem performance help to decide if restoration is an attainable management option, so stakeholder participation is fundamental (Wohl *et al.*, 2005). At an early stage, European river restoration approaches focused on individual river sections (Clarke *et al.*, 2003; Gregory & Downs, 2008). Also, these initial projects were frequently implemented at locations that had a single, willingly to cooperate, landowner (Mant *et al.*, 2016). Therefore, many of those early restoration approaches were fragmented and site-specific eco-engineering projects, that did not take into account the dominant hydrological and geomorphological processes (Brierley & Fryirs, 2009; Mant *et al.*, 2016). However, European best practice nowadays focus on river restoration on the long term, catchment-scale context, as indicated by the WFD (European Commission, 2000; Clarke *et al.*, 2003; Gregory & Downs, 2008; Brierley & Fryirs, 2009). Thus, the WFD and other Directives require that the Member States implement integrated river basin management plans, which must include restoration measures to improve or prevent further deterioration of the ecological status of river systems. Still, in order to improve ecological restoration success, and achieve the WFD good ecological status objectives, there is a need to coordinate

land use and rural development policies with water resources and river management (González del Tánago *et al.*, 2012).

1.4 Objectives

The thesis aims to contribute to freshwater ecosystem restoration in the Mediterranean region, through a multi-level top to bottom approach: the legislation restoration drivers, the basin level and the river section level.

Accordingly, the specific objectives of the thesis are the following:

- a. To analyze how restoration standards in Europe can be improved, through soft law and reinforcement mechanisms recommendations;
- b. To assess the impacts that the combined effects of climate change and management practices may have on nitrate concentrations in the water of an agricultural river basin with crop irrigation and water abstraction problems;
- c. To assess the results of a wetland restoration;
- d. To assess the results of habitat restoration in selected river sections of a regulated intermittent Mediterranean river;
- e. To assess the impacts of the liquid effluent, notably nutrients, of an acid bisulfite pulp mill on the river water of a major Iberian river;
- f. To study the nitrate removal capability of several alternative denitrification substrates in laboratory batch tests.

1.5 Thesis structure

The thesis comprises five sections, with 8 chapters. Thus, Section I – Introduction, frames the thesis aim by presenting the theoretical background for a better comprehension of the subjects presented in the ensuing sections. Additionally, the objectives and thesis structure are also detailed in this section. The following three sections – Sections II, III and IV – refer to the studies developed to fulfil the objectives of the thesis. Lastly, Section V refers to the general conclusions of the research presented in the previous Sections, summarizing the more relevant findings of this thesis. In more detail:

Section II – Legislation restoration drivers. This section addresses specific objective a.

- Chapter 2 – Review of River Restoration Policies and Recommendations for their Improvement in Europe and China.

Section III – Restoration at basin level. This section addresses specific objective b.

- Chapter 3 - Restoration at Basin Level: The Influence of Future Land Use and Climate Scenarios on River Nitrates Levels.

Section IV – Restoration at river section level. This section addresses specific objectives c, d, e and f.

- Chapter 4 – Environmental Restoration of a Degraded Wetland.
- Chapter 5 – Riverbank Restoration in a Temporary Mediterranean River.
- Chapter 6 – Point Sources of Pollution and Restoration: Influence of the CAIMA Paper Mill on the Water Quality of the Tagus River.
- Chapter 7 – Alternative Organic Substrates for Nitrate Removal from Water.

Section V – General Conclusions.

- Chapter 8 – Conclusions.

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SECTION II

LEGISLATION RESTORATION DRIVERS

CHAPTER 2

**Review of river restoration policies and
recommendations for their
improvement in Europe and China**

1. INTRODUCTION

Our planet resources are limited (Foley, 2017), and many ecosystems are threatened and profoundly degraded (e.g. Bryant *et al.*, 1997; Bogardi *et al.*, 2012). As a society we will need to make major transitions in energy, food, mobility and urban systems, which will require deep changes in major institutions practices, technologies, policies and lifestyles (UNEP/UNECE, 2016). Therefore, there is an urgent need for new governance alliances involving national and subnational levels of government, business and citizens (UNEP/UNECE, 2016). Hence, different policy instruments are needed to promote these transitions (IPBES, 2016), each having a specific policy mix for biodiversity conservation and restoration (Ring & Schröter-Schlaack, 2015).

Both government and governance refer to intentional behavior, to goal oriented activities to systems of rule (Rosenau, 1992). However, the latter is a more encompassing phenomenon that embraces governmental institutions, but it also includes informal, non-governmental mechanisms and multiple actors (Rosenau, 1992). Governance processes occur at various spatial (local to international) and temporal scales, and affect different societal, economic and public sectors (Lange *et al.*, 2013). Hierarchical governance (centralized) models have mainly central governmental bodies with top-down command-control mechanisms of interactions (Meuleman, 2008), where decentralized governmental actors at lower levels decide autonomously within top-down determined boundaries. Nevertheless, self-governance and private governance models also play a role in these centralized and decentralized governance models as they can contribute to bring bottom up approaches that may reinforce and enlarge impacts of political decisions.

The European Union (EU) is often regarded as a sui generis organization regarding governance model with strong elements of legal interactions (Tömmel, 2011) since Member States have voluntarily and democratically transferred competences to the EU (European Commission, 2016). Even though EU combines hierarchical governance with decentralized models incorporating also public-private partnerships, it has been experimenting, with success, soft law enforcement mechanisms such as guidelines and standards that may develop

into binding treaties or being recognized as customary law (Ahmed & Mustofa, 2016).

China is seen by the international community as a very hierarchical top-down centralized governance model (Mol & Carter, 2006), and water governance is no exception (Wang, 2017). There is a strong hierarchy from State Council and its Ministries with very well-defined command-control mechanisms (Mol & Carter, 2006; Huang, 2008; Perry & Heilmann, 2011). Though this high hierarchical top-down process, Chinese culture and organization also allows public participation and is willing to test and scale up new approaches that prove to be efficient (Economy, 2006). A good example is the River Chief system, also known as River Leader or River Captain system, which was first tested in 2007 in the city of Wuxi (Jiangsu Province) and has since been adopted as a national policy (Dai, 2015; Chien & Hong, 2018; Qiu, 2018). Furthermore, recent changes increased the independence of provincial environmental protection departments from local governments (Zhang *et al.*, 2017a).

Freshwater ecosystem restoration is a high priority at the International agenda due to the threat of insufficient ability of these ecosystems to secure the provision of freshwater for human consumption (United Nations, 2016; IPBES, 2018a). A myriad of International conventions and treaties mentions restoration practices at global scale and the need for cooperation between States to effectively achieve Sustainable Development Goals. European and Chinese legislation currently have a high set of laws that drive member states and provinces to develop restoration practices (e.g. the EU Water Framework Directive or the China Water Pollution and Control Action Plan). However, a lack of common legal and technical definitions still leaves room for discussion among sectors and for different approaches which have proved to harm the restoration of freshwater ecosystems since in some cases the implementation of compensation mechanisms do not generate sufficient positive effects (Schoukens, 2017a; IPBES, 2018b).

Most rivers and floodplains in Europe have been degraded for long periods (Brookes, 1988, 1996; Petts, 1994; Nienhuis & Leuven, 2001; Downs & Gregory, 2004; Mant *et al.*, 2012). They have suffered from the influence of different anthropogenic activities, like land drainage, reservoirs and dams, weirs,

channelization, water abstraction and water pollution (Mant *et al.*, 2012; Mitsch & Gosselink, 2015). These degradation drivers resulted in the loss of floodplains and wetlands, high sediment runoff, biodiversity losses, over widening and deepening of river channels, lowering of the river and water table levels and increase in peak flows (Mant *et al.*, 2012). The negative impacts of these historical activities created a growing awareness in the scientific and political communities towards the need to have healthy riverine environments, able to provide ecosystem services to the human society and to sustain adequate levels of biological and ecological diversity at the landscape scale (Piégay *et al.*, 2008; Mant *et al.*, 2012). Thus, restoration arose as an efficient way to stop and reverse the degradation of river systems (Ormerod, 2004; Wheaton *et al.*, 2008). Therefore, according to Smith *et al.* (2014) the main technical drivers of river restoration are related with habitat restoration, fisheries improvement and ecological and ecosystem recovery. Hence, there is a clear ecological focus in the way in which river restoration is outlined (Smith *et al.*, 2014).

The restoration of ecosystems and natural capital is now viewed as an important part of the move toward a green economy (United Nations, 2011; Smith *et al.*, 2014). Additionally, nowadays the compensation for ecological damage or biodiversity offsetting is one of the main policy approaches that seeks to achieve a no net loss of biodiversity when economic development leads to environmental degradation (Lapeyre *et al.*, 2015; Calvet *et al.*, 2015).

Starting in the 1970s, the increase in societal environmental awareness provided the political background for the introduction of an assortment of legislation that created the conditions for river restoration to grow (Downs & Gregory, 2004; Wharton & Gilvear, 2007; Lemons & Victor, 2008). In the European Union binding legal instruments take the form of Regulations and of Directives, which create specific legal obligations to Member States. Regulations are focused on harmonizing legislation in a certain field to promote the integration of the Member State and proper function of the internal market (Jans & Vedder, 2012). Directives are legally binding in terms of results to be achieved but leave Member States with the autonomy on the form and method to apply (Article 4.3 of the Treaty on European Union) (European Commission, 2016), though frequently producing non-binding documents (e.g. guidelines) to advise Member States on the best

way to achieve intended results. Summarising, national environmental legislation is driven by Directives at the European level, which are then transposed into national laws (Smith *et al.*, 2014).

For a long time, China's economic development made great achievements (Ravallion & Chen, 2007; Qiang *et al.*, 2011; World Bank, 2018). However, the neglect of environmental protection and overexploitation of natural resources resulted in tremendous issues, including serious environmental pollution, ecological degradation and biodiversity losses (Johnson *et al.*, 1997; Economy, 2007; Fu, 2008; Ma *et al.*, 2013). For example, population growth, economic development and technical shifts are some of the major factors related to freshwater scarcity in China (Varis & Vakkilainen, 2001; Chen *et al.*, 2005; Hubacek & Sun, 2005; Jenerette *et al.*, 2006; Cai, 2008). In recent years, the government increased the focus on environmental problems (Zhang & Wen, 2008). At the 2012 18th National Congress of the Communist Party of China (CPC) "Ecological Civilization" was included in the Constitution of the CPC (He *et al.*, 2013; Wang *et al.*, 2014; Marinelli, 2018). Later, at the 2018 13th National People's Congress of the People's Republic of China it was also included in the Chinese Constitution (in the Preamble and in Chapter III The Structure of the State, Section 3 The State Council, Article 89) (Liangyu, 2018; Wei, 2018). Therefore, the revision of the Constitution shows the ambition and determination of China on its ecological development in a new era (You & Liu, 2013).

Ecological restoration is one of the most significant approaches adopted in China for ecological development, because a traditional pollution control approach could hardly meet the requirements of mitigating accumulated environmental issues, and ecological restoration becomes an increasingly significant tool to rebuild degraded ecosystems and improve environmental services (Baker & Eckerberg, 2013). Therefore, in order to achieve the concepts of "Sustainable Development" and "Ecological Civilization", a series of policies and legislations have been established in China to facilitate ecological restoration practices, where a variety of policy instruments are employed.

Laws and regulations are the primary approaches to protect the environment and direct ecological restoration in China (Ma *et al.*, 2013). The new Environmental Protection Law came into action in 2015, and is considered "the strictest

Environmental Law ever” (Zhang *et al.*, 2015). Besides, under the guidance of the “ecological civilization” concept, several regulations involving ecological restoration have been established, such as the Law on Prevention and Control of Water Pollution, the Action Plan for Prevention and Control of Water Pollution, the Act on Construction of National Water Ecological Civilization City, among others. Mandatory regulations are also widely known as “Command-and-Control” approaches (Hahn & Stavins, 1992), which are straightforward and relatively uncomplicated for the authorities as well as the public to understand and execute. However, as criticisms suggest, “Command-and-Control” seldom provides incentives for actors to engage environmental protection or ecological restoration (Hahn & Stavins, 1992; Oh & Svendsen, 2015). Furthermore, it requires sound knowledge and resources to become established and implemented (Hodge, 1995). Flexible instruments are thus needed, and it is argued that economic instruments provide a better mechanism, since these provide economic motivations for stakeholders or broader actors to become involved in environmental activities (Hahn & Stavins, 1992; Oh & Svendsen, 2015). Economic instruments also try to address the indirect and longer-term effects of pollution and resource depletion. A single policy instrument is hard to be comprehensive, but different policies can be complementary (Connelly *et al.*, 2012). Therefore, in China in recent years, policy instruments have been employed increasingly more than regulatory instruments, such as economic and information-based instruments. Large amounts of funding are supplied for water pollution control and river restoration (e.g. Zhang *et al.*, 2013; Mi *et al.*, 2015; Xu *et al.*, 2016; Zhu *et al.*, 2016; Li *et al.*, 2017; Huang *et al.*, 2018). Payment of Ecological Services (PES) schemes have been considerably used in many ecological programmes (Pan *et al.*, 2017). More notably, “Ecological Civilization” is deeply rooted into the mainstream thinking of the whole society (Shikui Dong, 2017; Xin, 2018a) and the concept of “A Community of Shared Future (for All Mankind)” is firstly initiated as a global value for better international cooperation in a new era (Jun & Hongjin, 2017; Bijian Zheng, 2017), especially when facing global environmental challenges.

This review provides an analysis of river restoration policies in Europe and China, together with soft law and reinforcement mechanisms recommendations. Global,

European and Chinese policy drivers of restoration will be briefly summarised as well as main outcomes of these policy instruments. This work aims to contribute to the support of effective and successful freshwater ecosystem restoration on both regions.

2. METHODS

A survey of the available body of literature about policy drivers of freshwater restoration was made using google and google scholar web-sites. This literature survey was used to collect the most relevant data, through expert knowledge review of abstract or summary.

Systematic literature surveys regarding restoration in Europe and China were done using the following combinations of words: “freshwater restoration”; “river restoration” + “China” or “Europe”; “river restoration flood risk”; “river restoration flood risk management”; “floods directive river restoration”; “ecological restoration definitions”; “legal definition restoration”; “legal definition of ecological restoration”; “climate change riparian restoration”; “nitrate reduction river restoration”. Additionally, all relevant European Union Directives were collected, and the documents were searched using the words: “restoration” and “restore”.

Additionally, an online questionnaire (available at: <https://pt.surveymonkey.com/r/J99J3BS>) was made with the objective to collect information about river restoration projects in Europe and China. Thus, the questionnaire focused on the evaluation of past restoration projects that took place in both regions. The questionnaire was divided into five sections, arranged to i) identify the organizations and practitioners involved in restoration projects, ii) characterize the location, basic design and initial site characteristics of the project, iii) identify the strategy and measures implemented during the restoration project, iv) collect information on the amount of financial resources spent in the different phases of the projects, as well as about the financial supporters of the actions, and v) to evaluate the restoration project using the International Standards for the Practice of Ecological Restoration (McDonald *et al.*, 2016).

The critical analysis of the collected information was used to make policy recommendations based on the information gathered and most significant trends found in the literature.

3. DEFINITION OF RESTORATION

There is no official global definition of ecological restoration, although there is scientific consensus over several definitions (Telesetsky, 2013). Ecological restoration is mostly neither defined in national legislations nor in international law (Telesetsky, 2013; Palmer & Ruhl, 2015; Richardson, 2016). One of the most accepted definitions is the one from the Society of Ecological Restoration (SER), which states that ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (SER, 2004). Ecological restoration is one of several activities that seek to modify the biota and physical conditions of a site (SER, 2004). The main difference between ecological restoration and other forms of ecosystem repair is that it aspires to assist the recovery of a natural or semi-natural ecosystem instead of imposing a new direction or form upon it (McDonald *et al.*, 2016). Thus, restoration aims to place an ecosystem on a recovery trajectory after a temporary loss (Young, 2000; McDonald *et al.*, 2016). It encompasses both passive measures, like restrictions seeking to remove disturbances or limiting human pressures, and active measures, aiming to shift an impacted ecosystem towards its recovery (Schoukens, 2017b). However, full ecological restoration is often difficult because the nature of the original ecosystem may be unknown or impossible to achieve due to historical events or complex evolution trajectories (Hughes *et al.*, 2005; Lamb, 2009; Dufour & Piégay, 2009; Jacobs *et al.*, 2015). While restoration is a technique to enhance and promote habitats and populations, conservation focuses exclusively on slowing down or stopping degradation or on maintaining the remnants of the original population or ecosystem (Young, 2000; Hilderbrand *et al.*, 2005).

The Conference of the Parties (COP) of the Convention on Biological Diversity (CBD) Decision XI/16 of 2012 urged to develop clear terms and definitions of ecosystem rehabilitation and restoration and clarify desired outcomes of implementation of restoration activities (UNEP/CBD/COP, 2012; Cliquet *et al.*,

2015). In China, the term restoration is frequently used but the legal definition has still to be established. Nevertheless, restoration standards have been issued through Chinese Standard SL 709-2015, “Guidelines for Aquatic Ecological Protection and Restoration Planning”. An EU Commission working paper defined restoration as “The restoration of ecosystem and their services is understood as actively assisting the recovery of an ecosystem that has been degraded, damaged or destroyed, although natural regeneration may suffice in cases of low degradation. The objective should be the return of an ecosystem more or less equal to its original community structure, natural species composition and ecosystem functions to ensure in the long term a continued provision of services, although in cases of extreme degradation, the focus on specific services may be justified” (European Commission, 2011a).

Restoration priorities depend on the type of problem being addressed, and differ between the different European countries (Smith *et al.*, 2014). The main priority may be water quality (like in Luxembourg), fisheries restoration (like in Ireland), improving in-stream flows (like in southern Spain), or floodplain restoration (like in the Netherlands) (Mant *et al.*, 2012).

In Europe, for a time most river restoration approaches focused on individual river reaches (Clarke *et al.*, 2003; Gregory & Downs, 2008). These early projects were frequently implemented for practical reasons at locations that had a single, willingly to cooperate, landowner (Mant *et al.*, 2016). Therefore, many of those early restoration approaches were fragmented and site-specific eco-engineering projects, that did not take into account the dominant hydrological and geomorphological processes (Brierley & Fryirs, 2009; Mant *et al.*, 2016). However, European best practice nowadays focus on river restoration on the long term, catchment-scale context, as indicated by the Water Framework Directive (European Commission, 2000; Clarke *et al.*, 2003; Gregory & Downs, 2008; Brierley & Fryirs, 2009).

4. POLICY DRIVERS OF RESTORATION PRACTICES

According to the recent Assessment on land Degradation and Restoration (IPBES, 2018b), “the economic benefits of restoration actions to avoid, reduce

and reverse land degradation have been shown to exceed their costs in many places (established but incomplete), but their overall effectiveness is context dependent (well established)”. Restoration practices and actions generally produce positive results, but their effectiveness depends on the degree to which they address the nature, extent and severity of underlying drivers and processes of degradation, and the biophysical, social, economic and political settings in which they are implemented (IPBES, 2018b).

4.1 Global and Regional policy drivers of restoration

Existing multilateral environmental agreements provide a platform of unprecedented scope and ambition for action to avoid and reduce land degradation and promote restoration (IPBES, 2018a). The Convention on the Protection and Use of Transboundary Watercourses and International Lakes, the Convention on Biological Diversity, the Convention on Wetlands of International Importance (Ramsar Convention), the United Nations Framework Convention on Climate Change, the 2030 Agenda for Sustainable Development and its Sustainable Development Goals and other agreements all have provisions to avoid, reduce and reverse land degradation. However, greater commitment and effective cooperation in using and implementing these established mechanisms at the national and local levels are vital to enable these major international agreements to create a world with no net land degradation, no loss of biodiversity and improved human well-being (IPBES, 2018a). The following section summarizes key International treaties that link Europe and China in the challenge of promoting freshwater ecosystem restoration.

Convention on the Protection and Use of Transboundary Watercourses and International Lakes

The Convention on the Protection and Use of Transboundary Watercourses and International Lakes (Water Convention) (UNECE, 1992) was adopted in 1992 and entered into force in 1996 (UNECE, n.d.). The majority of the countries that share transboundary waters in the United Nations Economic Commission for Europe (UNECE) region are Parties to the Convention. In 2003, the Water Convention was amended to allow its extension to countries outside the UNECE region (UNECE, 2004). The amendment entered into force on 6 February 2013, turning

the Water Convention into a legal framework for transboundary water cooperation worldwide (UNECE, n.d.). Starting on the 1st March 2016, all United Nations Member States can accede to the Convention.

The Water Convention aims to improve the transboundary water cooperation and the measures for the ecologically-sound management and protection of transboundary surface waters and groundwaters (UNECE, n.d.). It promotes the implementation of integrated resources management, notably at basin level. Therefore, the Water Convention requires Parties to prevent, control and reduce transboundary impact. It also calls for the use of transboundary waters in a reasonable and equitable way, thus preventing potential water related conflicts, as well as their sustainable management (UNECE, 1992). Conservation and restoration of freshwater ecosystems is a specific obligation under this convention, which requires parties to take “all appropriate measures” to this end, including the establishment of water-quality objectives and criteria, and development of concerted action programs for the reduction of pollution. Parties bordering the same transboundary waters must cooperate by taking part in specific agreements and establishing joint bodies. The Water Convention has currently 42 signatories from Europe and Central Asia. Although China is not a signatory, there are a number of water cooperation agreements under this convention where China participates (Nikiforova, 2010). One of particular importance is the 2011 China-Kazakhstan water quality agreement (UNECE, 2012).

Convention for Biological Diversity

The Convention for Biological Diversity (CBD) was signed at the United Nations Conference on Environment and Development in Rio de Janeiro in June 1992 (Secretariat of the Convention on Biological Diversity, 2000). It came into force at the end of 1993 and is a legally binding commitment to conserve biological diversity, to sustainably use its components and to share equitably the benefits arising from the use of genetic resources (FAO, 1992).

The Convention on Biological Diversity identifies a common problem, sets overall goals and policies and general obligations, and organizes technical and financial cooperation. However, the responsibility for achieving its goals rests largely with

the countries themselves (Secretariat of the Convention on Biological Diversity, 2000). Thus, governments are required to develop national biodiversity strategies and action plans, and to integrate these into broader national plans for environment and development (Secretariat of the Convention on Biological Diversity, 2000).

In its Article 8 the CBD states that “Each Contracting Party shall, as far as possible and as appropriate: (f) Rehabilitate and restore degraded ecosystems and promote the recovery of threatened species, inter alia, through the development and implementation of plans or other management strategies, and (h) Prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species” (FAO, 1992).

The 2030 Agenda for Sustainable Development

The 2030 Agenda for Sustainable Development was adopted through the United Nations Resolution 70/1 in October 2015 (UN, 2015). It was built on the experience gathered with the Millennium Declaration and Millennium Development Goals, which expired in 2015 (DG DEVCO, n.d.). The 2030 Agenda takes an integrated and balanced approach to poverty eradication, good governance, the rule of law, peaceful societies, and to the economic, social and environmental dimensions of sustainable development (UN, 2015). At the basis of the 2030 Agenda are 17 Sustainable Development Goals (UN, 2015), which are implemented through a global partnership characterized by shared responsibility and mutual accountability (DG DEVCO, n.d.). Other important elements of the 2030 Agenda are the Means of Implementation and Follow-Up and Review, which help to ensure that it is implemented for all.

The 2030 Agenda goals that are directly related with river restoration are the following (UN, 2015):

- Goal 6. “Ensure availability and sustainable management of water and sanitation for all”, mainly through target 6.6:
 - Target 6.6. “By 2020, protect and restore water-related ecosystems, including mountains, forests, wetlands, rivers, aquifers and lakes.”

- Goal 15. “Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss”, mainly through targets 15.1, 15.2, 15.3 and 15.8:
 - Target 15.1 “By 2020, ensure the conservation, restoration and sustainable use of terrestrial and inland freshwater ecosystems and their services, in particular forests, wetlands, mountains and drylands, in line with obligations under international agreements.”
 - Target 15.2 “By 2020, promote the implementation of sustainable management of all types of forests, halt deforestation, restore degraded forests and substantially increase afforestation and reforestation globally.”
 - Target 15.3 “By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world.”
 - Target 15.8 “By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species.”

Strategic Plan for Biodiversity 2011–2020 and the Aichi Targets

The Strategic Plan for Biodiversity 2011-2020, and its Aichi Biodiversity Targets, is a global framework for action adopted at the tenth meeting of the Conference of the Parties to the Convention on Biological Diversity (CBD) held in Nagoya, in October 2010. The Strategic Plan is comprised of a shared vision, a mission, strategic goals and twenty targets, commonly known as the Aichi Targets. The five strategic goals are the following (CBD, n.d.):

- Strategic Goal A: Address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society;

- Strategic Goal B: Reduce the direct pressures on biodiversity and promote sustainable use;
- Strategic Goal C: Improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity;
- Strategic Goal D: Enhance the benefits to all from biodiversity and ecosystem services;
- Strategic Goal E: Enhance implementation through participatory planning, knowledge management and capacity building.

The Parties to the CBD were expected to translate this framework into revised National Biodiversity Strategies and Action Plans (NBSAPs). Up to March 2018, 97% of the Parties have developed NBSAPs (CBD, 2018). In recognition of the urgent need for action, the United Nations General Assembly has also declared 2011-2020 as the United Nations Decade on Biodiversity.

Convention on Conservation of Migratory Species of Wild Animals

The Convention on the Conservation of Migratory Species of Wild Animals, also known as CMS or the Bonn Convention, aims to conserve terrestrial, marine and avian migratory species throughout their range (UNEP/CMS, 1983). It entered into force on 1 November 1983 and is the only global convention specializing in the conservation of migratory species, their habitats and migration routes (CMS, n.d.).

The CMS provides strict protection for the endangered species in Appendix I and requires Range States to conclude multilateral agreements for the conservation of species in Appendix II (Lyster, 1989). According to article III, point 4a), parties shall “conserve and, where feasible and appropriate, restore those habitats of the species which are of importance in removing the species from danger of extinction” for Appendix I species. Therefore, the Convention encourages the Range States to conclude global or regional agreements, acting as a framework Convention, mentioning in article V, point 1, that “The object of each AGREEMENT shall be to restore the migratory species concerned to a favorable conservation status or to maintain it in such a status” (UNEP/CMS, 1983). The agreements can be adapted to the requirements of specific regions, and may be

formal, legally binding, treaties, or less formal instruments, like Memoranda of Understanding (CMS, n.d.; EEA, n.d.). This capability to develop models adapted to the conservation needs throughout the species migratory range is unique to the CMS (Bertouille, 2012).

Convention on International Trade of Endangered Species of Wild Flora and Fauna

The Convention on International Trade in Endangered Species of Wild Fauna and Flora, commonly known as CITES, was signed in Washington in 1973 and entered in force in 1975 (Wijnstekers, 2011). It is an international agreement between governments which aims to ensure that international trade in specimens of wild animals and plants does not threaten their survival (CITES, 1973). The Convention establishes a fundamental international legal framework for the prevention of trade in endangered species and for an effective regulation of trade in others (Wijnstekers, 2011). Restoration is only mentioned in article XI point 3c indicating that parties may “review the progress made towards the restoration and conservation of the species included in Appendices I, II and III”.

Convention on Wetlands of International Importance Especially as Waterfowl Habitat

The Convention on Wetlands of International Importance Especially as Waterfowl Habitat (Ramsar Convention) is an intergovernmental treaty that provides the foundation for national action and international cooperation for the conservation and wise use of wetlands and their resources (Taylor, 2002; Ramsar Convention Secretariat, 2014). It was adopted in 1971 and entered into force in 1975. As of 2014, almost 90% of United Nations member states have joined the Ramsar Convention “Contracting Parties” (Ramsar Convention Secretariat, 2014).

Under the three pillars of the Convention, the Contracting Parties commit to (Ramsar Convention Secretariat, 2014):

- “Work towards the wise use of all their wetlands through national plans, policies and legislation, management actions and public education”;
- “Designate suitable wetlands for the list of Wetlands of International Importance (the “Ramsar List”) and ensure their effective management”;

- “Cooperate internationally on transboundary wetlands, shared wetland systems, shared species, and development projects that may affect wetlands”.

The Convention defines the wise use of wetlands as “the maintenance of their ecological character, achieved through the implementation of ecosystem approaches, within the context of sustainable development”. Therefore, wise use may be considered as “the conservation and sustainable use of wetlands and all the services they provide, for the benefit of people and nature” (Ramsar Convention Secretariat, 2014). The strategies for the implementation of National wetland policies should address areas of national and international interest or priority, like wetland restoration (Ramsar Convention Secretariat, 2010). In fact, Resolution VII.6, adopted by the 7th Conference of the Contracting Parties, “encourages Contracting Parties to recognize the benefits of incorporating into National Wetland Policies appropriate measures to ensure that wetland restoration is given priority” (COP, 1999).

Along the years the Convention provisions have been clarified, amplified and developed, mainly through Conference of the Contracting Parties (COP) resolutions (Bowman, 1995, 2013). This has enhanced the potential of the Ramsar Convention to advance the cause of wetland conservation (Bowman, 2013).

The Ramsar Convention is one of the worldwide basis of the management, protection and restoration of wetlands (Verhoeven, 2014; Hettiarachchi *et al.*, 2015). It has promoted wetlands in the environmental agenda and supported the development of a broad institutional framework for wetland governance (Hettiarachchi *et al.*, 2015).

International Plant Protection Convention

The International Plant Protection Convention (IPPC) is a 1951 multilateral treaty deposited with the Food and Agriculture Organization of the United Nations (FAO). It aims to secure coordinated, effective action to avoid and to control the introduction and spread of pests of plants and plant products (IPPC, n.d.). The Convention scope goes beyond the protection of cultivated plants and encompasses the protection of natural flora and plant products. It also considers

both direct and indirect damage by pests, so it includes weeds (IPPC, n.d.; FAO, 1997). As of March 2017, the Convention has 183 parties (IPPC, n.d.). The work of the IPPC is directly correlated to several of the United Nations 2030 Sustainable Development Goals (SDGs) (IPPC, n.d.).

4.2 Policy drivers of restoration in Europe

The following paragraphs briefly present policy drivers of restoration in Europe (summarized in Table 1) and problems they raise on their application by member states. Policy instruments are used in combination (policy mix); however, many of them interact with each other leading to complementarity, redundancy, overlap, synergies, competition, conflict, sequential interaction and replacement problems (Santos *et al.*, 2015). Examples of these are the interactions between Water Framework Directive, Nature Directive and Floods Directives (DG Environment, 2011).

Table 1. Summary of policy drivers of restoration in Europe (excluding International Treaties and Conventions).

	Legal and regulatory instruments	Economic and financial instruments	Social and Information-based instruments	Right-based instruments and customary norms
Europe	<ul style="list-style-type: none"> - Water Framework Directive - Habitats Directive - Birds Directive - Floods Directive - Environmental Liability Directive - Nitrates Directive - Ground Water Directive - European Union 2020 Biodiversity Strategy 	<ul style="list-style-type: none"> - Rural Development Programs - Common Agricultural Policy - Resource Efficiency Programs - Interregional Cooperation Programs 	<ul style="list-style-type: none"> - European Union Adaptation to Climate Change 	<ul style="list-style-type: none"> - Environment for Europe - European Platform for Biodiversity Research Strategy

Water Framework Directive (WFD)

The Water Framework Directive (2000/60/EC) (European Commission, 2000) was born from the need for a more integrated approach to water policy. At the time the existing legislation was fragmented, in both objectives and means, so the WFD was implemented to resolve those problems. Nowadays the WFD is considered one of the most far-reaching and ambitious piece of European environmental legislation (Voulvoulis *et al.*, 2017). The Directive is implemented mainly through River Basin Management Plans (RBMP), on a six-year cycle. The WFD objectives were to be met by 2015, although the Member States can invoke exemptions or extensions up to 2027 (European Commission, 2012). Its key objective is to achieve good status throughout the European Union (EU) waterbodies. This comprises the objectives of good ecological and chemical status for surface waters and good quantitative and chemical status for groundwater (European Commission, 2012). More detailed objectives are to (European Commission, 2000):

- “Prevent further deterioration and protect and enhance the status of aquatic ecosystems and, with regard to their water needs, terrestrial ecosystems and wetlands directly depending on the aquatic ecosystems”;
- “Promote sustainable water use based on a long-term protection of available water resources”;
- “Enhance protection and improvement of the aquatic environment, *inter alia*, through specific measures for the progressive reduction of discharges, emissions and losses of priority substances and the cessation or phasing-out of discharges, emissions and losses of the priority hazardous substances”;
- “Ensure the progressive reduction of pollution of groundwater and prevent its further pollution”;
- “Contribute to mitigating the effects of floods and droughts”.

The WFD offers an integrated and coordinated approach to water management in Europe based on the concept of river basin planning (European Commission, 2000; Voulvoulis *et al.*, 2017). Thus, the WFD requires a comprehensive

knowledge of catchments and management measures that adjust human-nature interconnection with the goal to improve the system as a whole (Voulvoulis *et al.*, 2017). It adopts an ecological view in which human activities are a source of disturbance and ecological degradation (Kelly, 2013).

The WFD strongly influences conservation practices in the EU firstly because it covers all bodies of water, but it also requires the Member States to improve ecological status in degraded locations, as well as calling for the identification, monitoring and protection of networks of high ecological status sites (Wharton & Gilvear, 2007). It is a powerful driver for river restoration since it sets ecologically based objectives and considers ecological status as an aspect of the structure and functioning of aquatic systems, but also on the grounds that it recognizes the river basin as the cornerstone natural, geographical and hydrological unit (European Commission, 2000; Wharton & Gilvear, 2007).

Though its high impact as a restoration driver, the fact is that the WFD uses the term “restore” only twice: 1) in article 4 – Environmental objectives – point 1a)ii) – “Member States shall protect, enhance and restore all bodies of surface water...”; and 2) point 1b)ii) “Member States shall protect, enhance and restore all bodies of groundwater...”. The term “restoration” is only used in Part B of Annex VI, indicating “recreational and restoration measures” as supplementary measures Member States may choose to adopt as part of the programme of measures (article 11 – point 4). Oddly, there is no definition of “restore” or “restoration” in article 2, “Definitions”, which makes the achievement of the directive objectives difficult to frame legally, though all Member States are obliged to have their water bodies in good ecological status and take the necessary measures (that is, restoration measures - implicitly understood) to achieve this based on well framed technical instruments. Moreover, the way that in the WFD water bodies are assessed against a reference condition is questioned by several authors (e.g. Bouleau & Pont, 2015). Although the historical trajectories of systems help to understand the main processes to be restored (Bouleau & Pont, 2015), that information may be impossible to obtain due to historical events, or complex evolution trajectories (Hughes *et al.*, 2005; Lamb, 2009; Dufour & Piégay, 2009; Jacobs *et al.*, 2015). Therefore, in order to minimize this problem,

Bouleau & Pont (2015) suggest the use of adaptive management to handle restoration in the context of the WFD.

Habitats Directive

The Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (European Commission, 1992), commonly designated Habitats Directive, was adopted to meet the European Union (EU) obligations under the 1979 Council of Europe's Convention on the Conservation of European Wildlife and Natural Habitats, also known as the Bern Convention. Together with the Birds Directive (European Commission, 2010) it forms the mainstay of Europe's nature conservation policy. Habitats and species listed in both Directives as valuable or threatened are safeguarded against potentially damaging developments through the EU wide Natura 2000 ecological network of protected areas. Article 17 of the Directive requires EU Member States to report on the state of their protected areas every six years. The main objective of the Habitats Directive is to ensure biodiversity conservation. To achieve that goal Member States are required to make efforts to maintain or restore to favourable conservation status natural habitats and wild animal and plant species listed at the Directive Annexes (European Commission, 1992). Thus, this Directive acts as a driver for large scale ecological restoration. However, care should be taken not to assume that any damage to nature will be repairable (Schoukens, 2017a). There is a trend to increase the use of habitat restoration as an instrument to accommodate project development with no net loss of biodiversity, as required under the EU Birds and Habitats Directives (Schoukens, 2017a). However, the concept that the negative effects of economic developments are offset by restoration actions linked to infrastructure projects may not follow the precautionary foundations of the Habitats Directive (Schoukens & Cliquet, 2016).

Birds Directive

The Council Directive on the conservation of wild birds (79/409/EEC), amended in 2009 to become the Directive on the conservation of wild birds (2009/147/EC) (European Commission, 2010), commonly known as the Birds Directive, is the oldest piece of European Union (EU) legislation on the environment. It aims to protect the wild bird species that naturally occur in the EU. Thus, the Directive

places great emphasis on the preservation, maintenance and restoration of biotopes and habitats for endangered and migratory species (European Commission, 2010). Accordingly, Member States must establish a network of Special Protection Areas (SPAs) which encompasses the most suitable territories for these species (European Commission, 2010). Nowadays the SPAs are included in the Natura 2000 ecological network. The term restore is not mentioned in the directive and restoration is mentioned only once in the introduction of the Directive.

Conflicts between the Water Framework Directive and Nature Directives

The Habitats and Birds Directives are also known as Nature Directives. Their relationship with the WFD is strong and in some cases conflicting (DG Environment, 2011). According to the WFD Article 4.1, the WFD objectives may need to be complemented by other additional directive objectives and Article 4.2 mentions that “where more than one of the objectives... relates to a given body of water, the most stringent shall apply”. However, the authorities need to determine precisely which objective is the most stringent, since the WFD and Nature Directives are not defined in the same way (DG Environment, 2011). The ecological status/potential of the aquatic fauna and flora is assessed in the WFD in terms of species composition and abundance (in line with ecological restoration principles). In the Nature Directives the focus is on selected species and habitats of Community interest. Thus, the Nature Directives do not directly focus at all the species that occur in a given water body. The articulation between restoration objectives is even more confusing since in articles 1 of the Habitats Directive the term restoration is used but no definition is provided and in the Birds Directive the terms restoration and restore are not mentioned in the law itself, only in the introductory note.

In order to elucidate member States on how to articulate both directives a recommendation was made indicating that “in principle, restoration towards good ecological status prevails (WFD objectives), because the whole ecosystem is benefiting and not only specific species or habitats, in conflicting cases, objectives of Nature directives should be brought in line with the objectives of the WFD” (example of the Brandenburg re-connection of oxbows where the

reconnection to achieve good ecological status destroyed habitat type 3150) (DG Environment, 2011).

However, there are exceptions (based on concrete legal cases that were judge by the European court of law) (DG Environment, 2011):

- “When restoring a WFD water body to make it “more natural” would lead to the loss of protected habitats and species which have developed in an artificially modified or managed environment (e.g. cut off ox-bows or freshwater marshes in a reclaimed area protected by an artificial flood bank – Veluwerandmeren wetland case)”;
- “When a compensation requirement under HD Art 6.4 will lead to a water body type change (e.g. from a freshwater marsh to a tidal lagoon)”;
- “When managed realignment promoted by a shoreline management plan would lead to a change from an impounded (low turbidity freshwater) river to a saline, high turbidity transitional water body”.

In summary, the WFD and Nature Directives do not allow derogation from the requirements set under each of them. The impact of the use of an exemption under the WFD must take account of the possible impact on the objectives of Nature Directives and vice-versa; this implies coordination and consultation between different stakeholders (DG Environment, 2011).

Another important example about the different views in the Nature Directives and the WFD is the use of exemptions due to socio-economic reasons: Article 6.4 of Habitats Directive foresees compensatory measures in order to maintain the overall coherence of Natura 2000 when “overriding public interest exists”, whereas article 4.7 d) of the WFD requires demonstration that there is no other technically viable alternative providing the same benefits. The latter is a better environmental option and does not entail disproportionate costs, but no compensation measures are mentioned.

Floods Directive

In an eleven-year period, between 1998 and 2009, Europe suffered more than 213 major floods, including the summer 2002 floods in the Danube and Elbe

ivers (EEA, 2010). Severe floods in 2005 further highlighted the need for concerted action. In the same eleven-year timeframe, floods have caused 1126 deaths, the displacement of about half a million people and at least €52 billion in insured economic losses (EEA, 2011). These catastrophic events gave rise to the Directive 2007/60/EC on the assessment and management of flood risks (European Commission, 2007), commonly designated as Floods Directive. It aims to manage and decrease flood risks, in order to protect human health, the environment, cultural sites and economic activities. Member States were required to do a preliminary assessment by 2011 to identify the river basins and related coastal areas at risk of flooding. By 2013 those zones were required to have flood risk maps, and established flood risk management plans by 2015. The Directive applies to inland waters as well as all coastal waters across the whole territory of the EU.

There is an obligation to coordinate flood risk management plans and river basin management plans (from the Water Framework Directive), including the public participation procedures in the preparation of these plans. It is mandatory that all assessments, maps and plans are made available to the public.

Flood risk management in shared river basins must be coordinated between Member States (and third countries, if any), and measures that may increase the flood risk in neighboring countries should not be implemented. Member States shall take into consideration long term developments, including climate change, as well as sustainable land use practices in the flood risk management cycle addressed in this Directive. River restoration may contribute to flood risk management by supporting the natural capacity of river systems to retain water (Baptist *et al.*, 2004; Dixon *et al.*, 2016) and there are a number of water retention measures that have been catalogued to increased awareness of the use of these measures to deliver multipurpose benefits to achieve different Directives objectives (<http://nwrn.eu/>).

Environmental Liability Directive

The Directive 2004/35/CE of the European Parliament and of the Council on environmental liability with regard to the prevention and remedying of environmental damage (European Commission, 2004), commonly designated

Environmental Liability Directive, establishes a framework based on the polluter pays principle to prevent and remedy environmental damage. The Directive defines "environmental damage" as damage to protected species and natural habitats, damage to water and damage to soil (European Commission, 2004). If environmental damage occurs the competent authority in each Member State may undertake restorative measures and recover costs later if an operator that has caused the environmental damage fails to undertake adequate restorative measures (European Commission, 2004; Telesetsky, 2013).

Nitrates Directive

The Council Directive concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC) (European Commission, 1991b), commonly known as the Nitrates Directive, was adopted in 1991. It aims to protect water quality from nitrate pollution from agricultural sources and to promote the use of good farming practices. Member States are required to (European Commission, 1991b):

- Identify polluted or at risk of pollution freshwater and groundwater;
- Designate as "Nitrate Vulnerable Zones" (NVZs) areas of land which contribute to nitrate pollution and that drain into polluted or at risk of pollution waters (Member States may instead apply measures to the whole territory);
- Establish good agricultural practices codes;
- Establish action programmes to be implemented by farmers within NVZs on a compulsory basis;
- National monitoring and reporting every four years.

The Nitrates Directive has close links with other EU policies, like the Water Framework Directive, Groundwater Directive, Common Agricultural Policy or climate change. In 2008 the application of the Nitrates Directive resulted in a EU-27 average 16% decrease in nitrogen leaching emissions (Velthof *et al.*, 2014). However, the effectiveness of the Directive is hampered by each Member State interpretation of some vague and ill prepared guidelines (Smith *et al.*, 2007).

Thus, the Directive success in reducing nitrate losses may vary between Member States (Smith *et al.*, 2007).

River and wetland restoration can play a pivotal role in the lowering of the annual riverine nitrogen export, with reductions up to 20-25% annually (García-Linares *et al.*, 2003; Passy *et al.*, 2012). The protection of riparian vegetation in legislation of Member States is a characteristic nitrate protection measure that in some cases gives revenues to farmers that adopt best practices (See Rural Development Programs and Common Agricultural Policy below).

Groundwater Directive

The Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration (European Commission, 2006b), commonly designated Groundwater Directive, has been developed in response to the requirements of Article 17 of the Water Framework Directive. The Directive establishes a system which sets groundwater quality standards and establishes measures to prevent or limit inputs of pollutants into groundwater. It establishes quality criteria that consider local characteristics and allows for further improvements to be made based on monitoring data and new scientific knowledge. Member States should establish standards at the most appropriate level and consider local or regional conditions.

Other Directives

There are other Directives focused on water quality improvement with some relevance in the context of integrated river basin management:

- Directive 2006/7/EC of the European Parliament and of the Council concerning the management of bathing water quality and repealing Directive 76/160/EEC, commonly known as the Bathing Water Directive (European Commission, 2006a).
- Council Directive 98/83/EC on the quality of water intended for human consumption, commonly designated as the Drinking Water Directive (European Commission, 1998).

- Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC), commonly known as the Urban Wastewater Directive (European Commission, 1991a).

Re-enforcement mechanisms

European Union 2020 Biodiversity Strategy

The European Union (EU) Biodiversity Strategy was adopted in 2011. With it, the EU intends to end the loss of biodiversity and ecosystem services in the EU and help stop global biodiversity loss by 2020. It is the EU reaction to the commitments taken at the international Convention on Biological Diversity. The strategy is built around six targets (European Commission, 2011b):

- Target 1: Protect species and habitats. By 2020, the assessments of species and habitats protected by EU nature law show better conservation or a secure status for 100% more habitats and 50% more species;
- Target 2: Maintain and restore ecosystems. By 2020, ecosystems and their services are maintained and enhanced by establishing green infrastructure and restoring at least 15% of degraded ecosystems;
- Target 3: Achieve more sustainable agriculture and forestry. By 2020, the conservation of species and habitats depending on or affected by agriculture and forestry, and the provision of their ecosystem services show measurable improvements;
- Target 4: Make fishing more sustainable and seas healthier. By 2015, fishing is sustainable. By 2020, fish stocks are healthy and European seas healthier. Fishing has no significant adverse impacts on species and ecosystems;
- Target 5: Combat invasive alien species. By 2020, invasive alien species are identified, priority species controlled or eradicated, and pathways managed to prevent new invasive species from disrupting European biodiversity;
- Target 6. Help stop the loss of global biodiversity. By 2020, the EU has stepped up its contribution to avert global biodiversity loss.

To assess if the EU was on track to achieve the objective of halting biodiversity loss by 2020, the strategy was subjected to a mid-term review in 2015. Regarding the 2020 Headline Target, the review concluded that there was “no significant progress towards the target” (European Commission, 2015). Regarding the six targets, the mid-term review concluded that Targets 1,2, 4 and 6 showed “progress, but at insufficient rate”, Target 3 showed “no significant progress towards the target” and that Target 5 was “currently on track to implementation” (European Commission, 2015).

Rural Development Programs

The specific rules relating to the European Agricultural Fund for Rural Development (EAFRD) for rural development programming between 2014 and 2020 are set out in Regulation (EU) N° 1305/2013 (European Commission, 2013b). Member States and regions write their Rural Development Programs (RDP) taking into account the needs of their territories and addressing at least four of the following six common EU priorities (European Commission, 2013b):

- Fostering knowledge transfer and innovation in agriculture, forestry and rural areas;
- Enhancing the viability and competitiveness of all types of agriculture, and promoting innovative farm technologies and sustainable forest management;
- Promoting food chain organization, animal welfare and risk management in agriculture;
- Restoring, preserving and enhancing ecosystems related to agriculture and forestry;
- Promoting resource efficiency and supporting the shift toward a low-carbon and climate-resilient economy in the agriculture, food and forestry sectors;
- Promoting social inclusion, poverty reduction and economic development in rural areas.

These priorities are divided into different “focus areas”. Within their RDP, each Member State (or region, in cases where powers are delegated to regional level) set quantified targets against these focus areas. Several measures are set to achieve those targets, and funding is allocated to each measure. At least 30% of funding for each RDP must be dedicated to measures relevant for the environment and climate change and at least 5% to *Liaison Entre Actions de Développement de l'Économie Rurale* (LEADER) Program. The LEADER Program is designed to support rural businesses to create jobs and support the rural economy.

European Union Adaptation to Climate Change

In 2013 the European Commission adopted an European Union (EU) strategy on adaptation to climate change, which aims to make Europe more climate-resilient (European Commission, 2013a). It sets a framework and mechanisms for taking the EU's preparedness for current and future climate impacts to a new level. The EU Adaptation Strategy is based on eight actions and has three key objectives (European Commission, 2013a):

- Promoting action by Member States.
 - Action 1: Encourage all Member States to adopt comprehensive adaptation strategies;
 - Action 2: Provide LIFE funding to support capacity building and step up adaptation action in Europe. (2013-2020);
 - Action 3: Introduce adaptation in the Covenant of Mayors framework (2013/2014).
- Promoting better informed decision-making.
 - Action 4: Bridge the knowledge gap;
 - Action 5: Further develop Climate-ADAPT as the ‘one-stop shop’ for adaptation information in Europe.

- Promoting adaptation in key vulnerable sectors.
 - Action 6: Facilitate the climate-proofing of the Common Agricultural Policy (CAP), the Cohesion Policy and the Common Fisheries Policy (CFP);
 - Action 7: Ensuring more resilient infrastructure;
 - Action 8: Promote insurance and other financial products for resilient investment and business decisions.

The strategy promotes greater coordination and information-sharing between Member States (for instance in cross border river basins) and ensures that adaptation considerations are addressed in all relevant EU policies.

Riparian restoration can offset some of climate change impacts (Seavy *et al.*, 2009; Perry *et al.*, 2015; Justice *et al.*, 2017). In fact, riparian environments are naturally resilient, but they also provide linear habitat connectivity and thermal refugia for wildlife, in addition to providing a link between the aquatic and terrestrial ecosystems (Naiman & Décamps, 1997; Milly *et al.*, 2002; Seager *et al.*, 2007). Therefore, riparian ecosystems can contribute to ecological adaptation to climate change (Seavy *et al.*, 2009). However, restoration experts need to incorporate climate change into riparian restoration planning in order to improve the system long term success (Perry *et al.*, 2015).

Common Agricultural Policy

The Common Agricultural Policy (CAP) was created in 1962 and is one of the oldest policies of the European Union (EU). It underwent major reforms in 1992, 2003 and 2013 to adjust to changing socio-economic and environmental factors. The CAP helps shape the economic and social fabric of rural communities and simultaneously establishes requirements for animal health and welfare, environmental protection and food safety.

Since 2013 four main regulations govern the common agricultural policy:

- Direct payments linked to environmental-friendly practices (European Commission, 2013c): a series of rules for direct payments to active farmers. It includes a binding "greening" component. Thus, farmers who

use their land more sustainably and care for natural resources as part of their everyday work benefit financially;

- Market measures (European Commission, 2013d): key elements for a common organization of markets in agricultural products;
- Rural development (European Commission, 2013b): supports the competitiveness, sustainable management of natural resources and job creation in rural areas. It outlines diverse priorities, such as energy or water efficiency in agriculture;
- Horizontal issues (European Commission, 2013e): outlines the rules for CAP expenditure, the farm advisory system, control systems set up by EU countries and the cross-compliance system.

As mentioned above, farmers that use agricultural practices beneficial for the climate and the environment are entitled to payment. Some of the agricultural practices eligible for payment are related with the control of non-point source pollution and bird and wildlife conservation (European Commission, 2013c), and are thus related with river restoration.

4.3 Policy drivers of restoration in China

The following paragraphs briefly present policy drivers of restoration in China (summarized in Table 2), according to a chronological order of establishment.

Table 2. Summary of policy drivers of restoration in China (excluding International Treaties and Conventions).

	Legal and regulatory instruments	Economic and financial instruments	Social and information-based instruments	Right-based instruments and customary norms
China	<ul style="list-style-type: none"> - The Ecological Civilization Construction - The Direction of National Water Ecological Civilization Pilots - The New Environmental Protection Law - Act of Water Pollution prevention and control - Action Plan for Prevention and Control of Water Pollution - River Chief System and Lake Chief System 	<ul style="list-style-type: none"> - The administrative measures of special funds for water pollution prevention and control - Temporary administrative measures of funds for rivers, lakes and reservoirs - The State Council's suggestion on mechanisms for ecological compensation 	<ul style="list-style-type: none"> - Ecological Civilization 	<ul style="list-style-type: none"> - A Community of Shared Future

“Ecological Civilization”

Ecological civilization is a brand-new stage of human civilization that came after the industrial civilization (Wei *et al.*, 2011; Hu, 2018). Ecological civilization is both material and spiritual fruit of human society's development by both following and co-existing harmoniously with nature (Feng & Fang, 2014). “The Construction of Ecological Civilization” was first mentioned on the 17th National Congress of the Communist Party of China (CPC) in 2007 (Xin, 2018a). In the CPC's report, the idea was regarded as a new requirement to build a moderately prosperous society (Xiaokang Shehui) nationwide and was needed because of increasingly serious environmental issues, both domestically and globally. It was proposed that “an energy & resource-saving, and environmental-protecting society should be basically built, involving industrial structure, patterns of growth and patterns of consumption... the idea of Ecological Civilization should become firmly established in the whole society.” (Zhao, 2007). In 2012, the 18th National

Congress of the Communist Party of China brought “Ecological Civilization Construction” to a new level. There, the President of the People's Republic of China, Xi Jinping, announced the vigorous promotion of ecological civilization construction (Hu, 2018). As a result, the overall arrangements for China’s development - the economic construction, political construction, cultural construction, social construction, namely the “Four-in-one” (*si wei yi ti*), has been expanded to the “Five-in-one” (*wu wei yi ti*) by taking the ecological civilization construction in (Xin, 2018b). Consequently, a series of remarkable policies were established:

- Opinions of the CPC Central Committee and the State Council on Accelerating the Ecological Civilization Construction (CPC Central Committee & State Council, 2015);
- Integrated Reform Plan for Promoting Ecological Progress (State Council, 2015a);
- The 13th Five-Year-Plan (Chapter 10) (CPC Central Committee, 2016);
- Opinions on Defining and Protecting Ecological Redlines (CPC Central Committee & State Council, 2017).

According to these policies, institutional frameworks for promoting ecological progress are supposed to be built gradually. Several notable ideas and objectives are:

- “Green, circular, and low-carbon development”;
- “Giving high priority to conserving resources, protecting the environment, and letting nature restore itself”;
- “System for payment-based resource use and compensation for ecological conservation”;
- “Mechanism for trans-regional and cross-watershed compensation for ecological conservation”;
- “Ecological conservation performance assessment and accountability”;

- “System of lifelong accountability for ecological and environmental damage”.

The Ecological Civilization Construction provides a guiding notion on a macro-level to shape the development and governance in China (Yang, 2015). It is a policy that strongly influences the conservation practices in China, due to its meaning of principle guidance (Wei *et al.*, 2011). However, these concepts still require institutional construction and other policy instruments to put into effect. In 2018, the 13th National People's Congress (NPC) of the People's Republic of China has written “Ecological Civilization Construction” into the constitution (Wei, 2018). Moreover, the bureaucratic fragmentation on environmental governance will soon become history, because an act to reshuffle cabinet level ministries has also been passed at the meeting of the 13th NPC. Two new cabinet level ministries, Ministry of Ecological Environment and Ministry of Natural Resource, will jointly govern natural resources and strive for ecological progress (Ma & Liu, 2018). With regard to river restoration, this is a powerful driver. According to Jørgensen *et al.* (2014), “Policy language matters because scientific information will be incorporated into environmental policy only when stakeholders perceive the information as credible, salient and legitimate”. Therefore, it sets ecologically-based ideas and considers ecological status as a primary principle of all conservation and restoration practices, which has made profound groundworks for following policies and practices.

Three red lines of Most Stringent Water Resources Management (2012)

A resources management scheme known as the Most Stringent Water Resources Management System (MSWRMS) was proposed by Ministry of Water Resources on the foundation of China's basic water situation at the national conference on water conservancy held in 2009. In 2012, in accordance with No. 1 Document of central government and the 18th National Congress of the Communist Party of China, the MSWRMS and Three Red Lines were established as the critical guiding ideology of China's water conservancy for the next generation (State Council, 2012):

- A red line of control on water resources development and utilization. Until 2030, the total water use in China is planned to be controlled within 700 billion m³;
- A red line of control on water use efficiency. Until 2030, Irrigation water use efficiency coefficient is planned to be increased to over 0.6, and water consumption per industry GDP to be decreased to below 40 m³/10,000 CNY;
- A red line of on restricted pollutant discharge and water function river reach ratio. Until 2030, total pollutant discharge into river is planned to be controlled within the carrying capacity, and ratio of water function river reach that meet the standard to be increased to over 95% of all rivers.

National Water Ecological Civilization City Construction (2013)

The 18th National Congress of the Communist Party of China declared that ecological civilization construction is a far-reaching plan for people's wellbeing and the nation's future. As the source of life and development, water is an important part of ecological civilization. In 2013, the Ministry of Water Resources officially issued the *Speeding up the National Water Ecological Civilization Construction Pilot* (Zhang *et al.*, 2017b). In it 45 cities were first elected as water ecological civilization construction Pilot cities (Zhang *et al.*, 2017b), including Beijing, Shanghai and Wuxi. In 2014 the number of cities increased to 105, to explore different types of water ecological civilization construction modes and experiences (Zhang, 2017). Six principles are highlighted in the policy:

- Executing the strictest water resource management legislations;
- Improving water resource allocation;
- Improving water resource conservation;
- Enforcing water quality management;
- Boosting watershed ecological restoration;
- Highlighting ecological idea's in hydraulic engineering.

It indicated that the management of urban rivers should be based on ecological principles, using ecological approaches to implement ecological restoration of rivers as a sustainable way of governance.

Action Plan for Prevention and Control of Water Pollution (2015)

In 2014, the Chinese Prime Minister Li Keqiang announced the ‘war on pollution’ (Reuters, 2014). Since then, a series of influential policies have been released by the Chinese central government (Branigan, 2014). The Action Plan for Prevention and Control of Water Pollution is commonly called “Ten-point water plan” (*shui shi tiao*), following the terminology of “Ten-point air plan” (*da qi shi tiao*), i.e. Air Pollution Prevention and Control Action Plan. Established in 2015 by the State Council, the “Ten-point water plan” was regarded as the strongest action plan on water governance to date (Qiu, 2018). The core objective is to improve national water quality and aquatic ecological situation by 2020. Furthermore, aquatic environmental restoration is an outstanding concern in the “Ten-point water plan”. The requirements about river ecological restoration are (State Council, 2015b):

- To promote outstanding technologies of ecological restoration;
- To research and develop advanced technology on ecological restoration;
- To industrialize restoration technologies and instruments;
- To increase government funding;
- To explore and establish an integrated ecological system protection and restoration mechanism for land and sea as a whole;
- To establish pilots on aquatic environmental restoration;
- To carry out restoration practices in urban rivers, wetlands and marine ecosystems.

The Action Plan is one of the most comprehensive regulations on water governance in China in recent years (Han *et al.*, 2016). This plan has sharply overturned the previous approaches to mitigate the water crisis in China, which primarily focused on large-scale hydraulic or chemical engineering solutions for providing clean water (e.g. Liu & Yang, 2012). It uses a more holistic approach

(Qiu, 2018), focusing into the overall ecosystem and water cycle, including groundwater, surface water and marine water, and their interactions. The monitoring and compliance responsibilities have also been clearly appointed to specific departments and persons (Han *et al.*, 2016; Qiu, 2018).

Ecological protection red line (2015)

An ecological protection red line was explicitly put forward for the first time at the Third Plenary Session of the 18th Central Committee of the Communist Party of China (PB of the CPC Central Committee, 2013; Wang *et al.*, 2015). The ecological red line is the ecological baseline area needed to provide ecosystem services to ensure and maintain ecological, living environment, and biological safety (Bai *et al.*, 2016). In 2015, the ecological red line concept was taken into the Environmental Protection Law of the People's Republic of China (State Council, 2015c). It includes a red line system of key ecological functional areas, ecological sensitive areas, and ecological weak areas. The overall aim of the ecological red line concept is to protect the integrity of important ecosystems, being similar to the natural protected areas of other regions of the world (e.g. United States) (Bai *et al.*, 2016). In 2017, the Chinese Ministry of Environmental Protection published a technical guideline for the establishment of Ecological Protection Red Lines (Speed *et al.*, 2016).

Environmental Protection Law of the People's Republic of China (2015)

The Environmental Protection Law of the People's Republic of China is a national law that aims to protect and improve the environment, prevent and control pollution and other public hazards, safeguard public health, promote ecological civilization improvement and facilitate sustainable economic and social development (State Council, 2015c). The current, amended, law was adopted at the 8th Meeting of the Standing Committee of the Twelfth National People's Congress of the People's Republic of China, and came into force in 2015 (State Council, 2015c). The original 47-article 1989 Environmental Protection Law has been expanded to 70 articles. Some basic environmental protection rules, including those on environmental planning, standards, and monitoring, have been updated in the new amended version. The Environmental Protection Law

provides basic principles and regimes, while the Water Pollution Prevention and Control Law provides specific rules on water pollution prevention and control.

The Environmental Protection Law addresses the need for restoration in several articles (State Council, 2015c):

- In Chapter III - Protection and Improvement of the Environment, Article 30, it states that ecological protection and restoration programs shall be developed and implemented;
- In Chapter IV - Prevention and Control of Pollution and Other Public Hazards, Article 47, it states that the government shall conduct proper risk control, emergency preparation, emergency response and post-emergency restoration for environmental accidents;
- In Chapter VI - Legal Liability, Article 61, it states that if a construction project starts without a submitted or approved Environmental Impact Assessment report the government shall order the work to stop and impose a monetary penalty. The government may also require the restoration of the construction location.

China's National Plan on Implementation of the 2030 Agenda for Sustainable Development (2016)

The National Plan on Implementation of the 2030 Agenda for Sustainable Development was developed to guide and promote the Chinese implementation efforts of the 2030 Agenda (MEE, 2016). The Plan analyzes challenges and opportunities in implementing the 2030 agenda, outlines guidelines, general principles and approaches, as well as specific plans for the implementation of the 17 Sustainable Development Goals and 169 targets.

Law for Prevention and Control of Water Pollution (2018)

The revised Water Pollution Prevention and Control Law came into effect on January of 2018 (Jiaqi, 2018). This law is an empirical summary of the effective practices on preventing and controlling water pollution and protecting the water environment in China. It is a legal system that guarantees to promote the

prevention and control of water pollution, to solve outstanding water environmental problems, and to facilitate ecological river restoration.

The law stipulates that, in accordance with the ecologically functional requirements of each river basin, local governments at or above the county level shall organize the protection and restoration of rivers, lakes and wetlands (Jiaqi, 2018). Constructed wetlands, water conservation forests, vegetation buffer zones along rivers and lakes, and other ecological management projects shall be constructed with respect to local conditions (State Council, 2017). The black and dirty water bodies shall be cleaned, and the carrying capacity of environmental resources along the river basins shall be improved (State Council, 2017).

The Chinese Minister of Environmental Protection, Li Ganjie, expressed in a national environmental meeting that this newly revised law standardizes and legalizes the higher requirements of construction of an ecological civilization and the new measures proposed by the “Ten-point water plan”, *i.e.* Action Plan for Prevention and Control of Water Pollution.

River Chief System (2017) & Lake Chief System (2018)

River Chief System (RCS) and Lake Chief System (LCS) are significant institutional innovations in China under its unique political system, in which members from local Communist Party of China (CPC) Committees are assigned as river or lake leaders to execute and coordinate the governance of appointed river basin or lakes (Chien & Hong, 2018). Their future career advancement is determined by the achievement of specific milestones for improving and maintaining river governance, which guarantees that they are politically motivated to mobilize the resources at their disposal to achieve the assigned goals (Dai, 2015; Chien & Hong, 2018). Initially created in Wuxi City, Jiangsu Province, in 2007, the success of the River Chief System resulted in its adoption as a nationwide policy by the central government (Chien & Hong, 2018; Qiu, 2018). Thus, after almost a decade after its introduction, the CPC Central Committee and the State Council jointly released the *Opinion on Comprehensively Promoting the River Chief System* (State Council, 2016), which triggered the start of the nationwide implementation for RCS (Guan, 2016). Following the RCS, the similar administrative system LCS was established for lake management. It was

announced that the LCS will be implemented in the whole country before the end of 2018 (Pan, 2018).

The River Chief System is a four-tier unified management system that is established at the provincial, municipal, county and township levels (State Council, 2016; Shaofeng, 2017). Each province has its own general river chief, that must be a top element of the Government or of the Central Committee of the Communist Party of China (State Council, 2016). Likewise, a river chief is set up for the main rivers and lakes within the administrative region of each province (autonomous regions and municipalities directly under the central government), which is to be acted by a provincial principal (State Council, 2016). In the lower tier, a river chief is set up for each level and section of each river and each lake in the respective city, county and township, which is to be acted by a principal of the same level (State Council, 2016). A relevant river chief system office is to be established for river directors at the county level and above, in accordance with the specific local situations (State Council, 2016). A River Chief has the responsibility to manage its river, including water pollution prevention and control, water resource management, aquatic environment management, river restoration, and law enforcement (Guan, 2016).

The River Chief System, as an institutional innovation, significantly improves administrative efficiency of river governance in China through establishing clear responsibilities and tasks to carry out legislation and other policies. Furthermore, fragmentation in governance still is an outstanding issue of water governance in China, not only because of the trans-jurisdiction conflicts, but also due to complicated overlaps of multiple functional departments, such as the hydraulic department, the urban construction department, the land department, or the environmental department, among others (Qiu, 2018). This problem is now mitigated by the RCS, since there is a river basin management plan and a River Chief. The River Chief acts as the immediate leader of the different governmental sectors, creating a unified management and control system (Dai, 2015).

Notwithstanding its qualities and success, the RCS has some shortcomings (Dai, 2015; Chien & Hong, 2018). It is not implemented in major rivers that run across several provinces and neither in small rivers (with less than 5 km in length) and lakes (with area lower than 10 km²) (Chien & Hong, 2018). Therefore this policy

is only suitable for regional rivers (Chien & Hong, 2018). Additionally, although the river chief policy seems to be quite effective in the management of pollution problems and in short term actions, it is less successful when dealing with broader social and agricultural problems (Chien & Hong, 2018). Perhaps the most significant shortcoming is the fact that the environmental benefits from good management tend to appear after a certain period of time, which may lead to the unfair assessment of river chiefs (Dai, 2015).

In order to illustrate this keystone policy, the case study of the City of Wuxi is briefly described below (Zhou, 2008; Zhang, 2010; Dai, 2015):

The City of Wuxi was the pioneer on the application of the River Chief System. It is one of the most industrialized cities in eastern China, and its water quality was a major problem. In 2007 a devastating blue algae bloom stroke the Taihu Lake, and Wuxi was badly impacted by the disaster. Therefore, a series of measures were taken by the local government to mitigate water issues and improve water quality. The Wuxi Central Committee of the Communist Party of China and Wuxi government decided to assign the management of its 64 rivers to party heads and government officials of all levels. They had to sign a responsibility agreement, linking their career path to the performance of river governance. Besides, a special guarantee deposit account has been set by the Wuxi Government for its RCS. Annually each river chief must deposit a certain amount into that account. The fund is exclusively used to reward or penalize the management success of each river chief. An improvement on river water quality makes the river chief eligible to a refund of the double amount of their deposit. When the river quality status remains the same the river chief is entitled to have its money returned, but if the river quality status decreases its deposit is confiscated. This instrument has achieved an extraordinary success, with big improvements in water quality. In 2008 74.4% of the rivers reached the established standards, a 50% increase over the previous year when the system had not yet been adopted.

5. QUESTIONNAIRE RESULTS

The questionnaire was sent to 72 experts and the reply rate was 39%, which meant that only 28 experts accessed the online survey. Furthermore, only 46%

of the experts that accessed the questionnaire where able to complete it. The global average completion time was 10 minutes, but this value was strongly influenced by the low completion rate. The average completion time for the experts that did fill the entire questionnaire was 30 minutes.

All the Chinese experts that answered the questionnaire work for a University or a Research Institution (Figure 4). European experts came from different working backgrounds, being that the majority (47.8%) work for a University or a Research Institution and 21.7% work in the private sector (Figure 4).

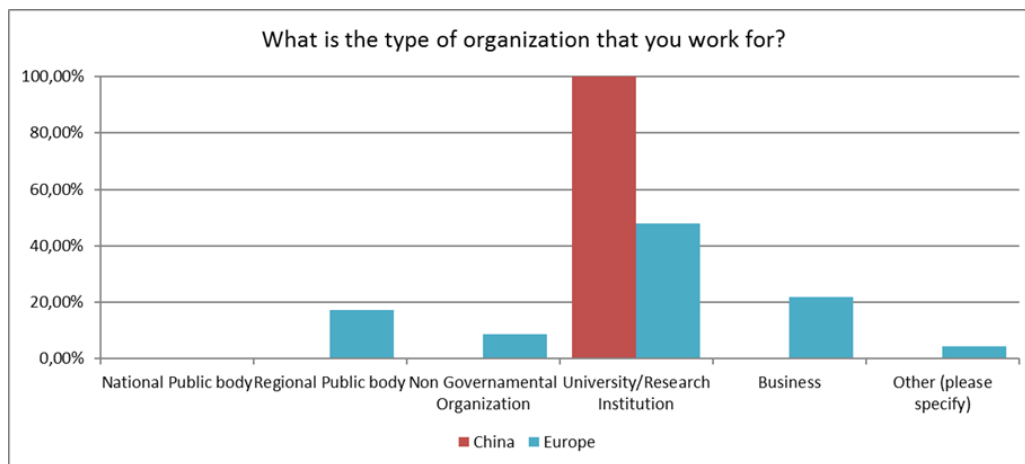


Figure 4. Professional affiliation of the experts in China (n=5) and Europe (n=23).

The experts that accessed the questionnaire came from twelve different countries (Figure 5). The majority came from Portugal (8) and China (5), although only two Portuguese experts completed the questionnaire.

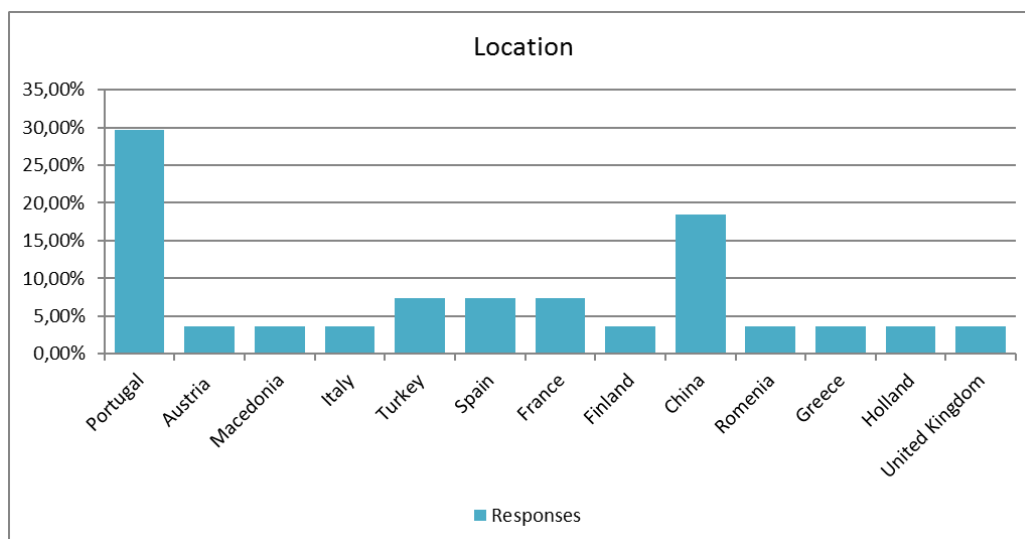


Figure 5. Country origin of the experts that accessed the questionnaire.

The global average experience on freshwater restoration of the experts was 16.8 years. Chinese experts had a 13 years average freshwater restoration experience and the European ones 17.8 years.

Regarding the types of ecosystem subject to restoration, European restoration projects focused mainly on wetland restoration (50%), as opposed to Chinese ones, which targeted mainly lotic systems (60%) (Figure 6).

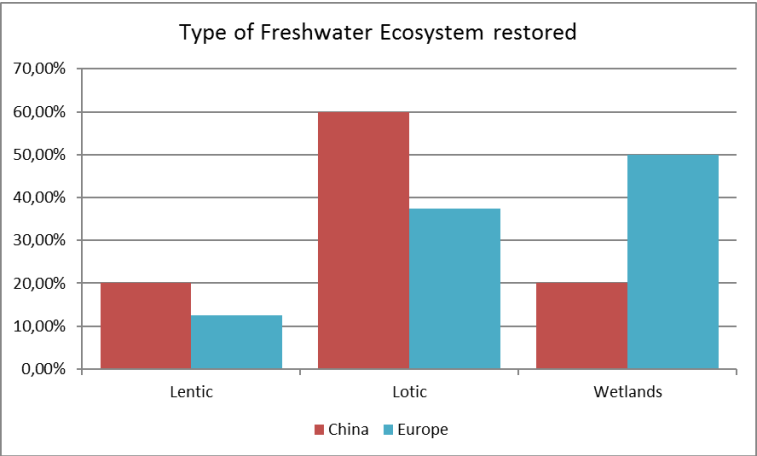


Figure 6. Type of ecosystem restored in China (n=5) and Europe (n=8).

The average implementation period of the restoration projects surveyed is longer in Europe (48 months) than in China (12 or 24 months) (Figure 7). The design phase of the projects reviewed typically takes 6 months in Europe and 12 months in China (Figure 8).

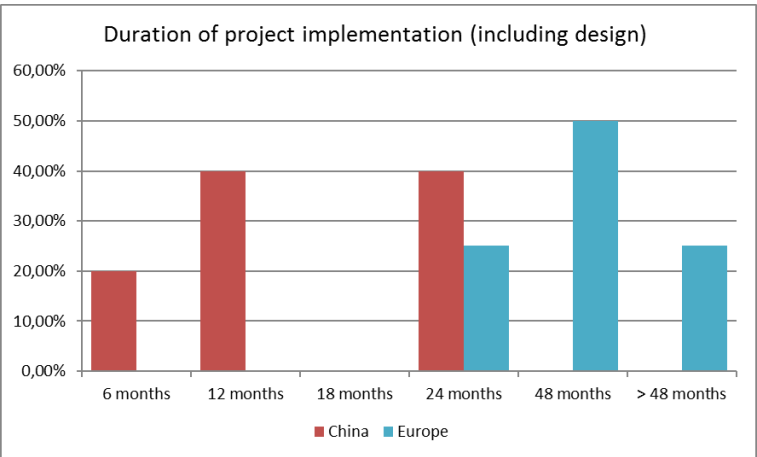


Figure 7. Duration of the surveyed projects implementation in China (n=5) and Europe (n=8).

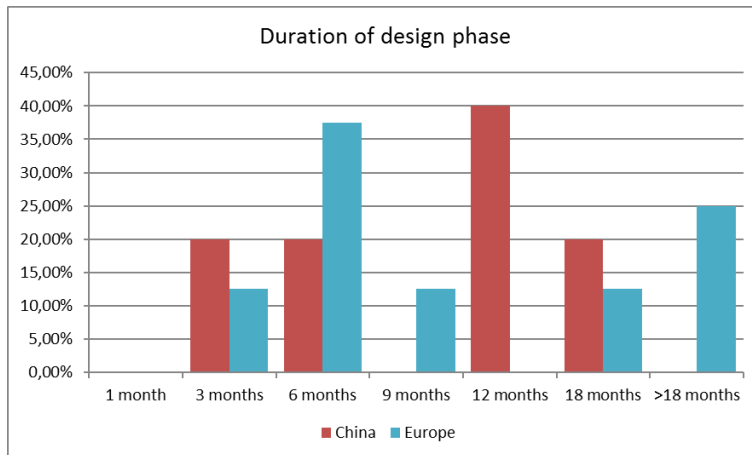


Figure 8. Duration of the surveyed projects design phase in China (n=5) and Europe (n=8).

The stakeholder community participated in the implemented projects from an early stage more in Europe (75.0%) than in China (40.0%) (Figure 9). There was no stakeholder participation in 20% of the surveyed Chinese restoration projects.

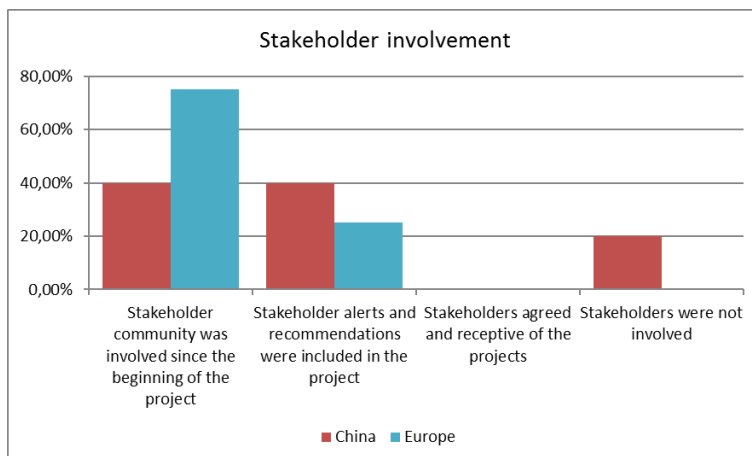


Figure 9. Stakeholder participation in the surveyed restoration projects in China (n=5) and Europe (n=8).

The main driver of degradation to be restored in the European projects was the over-utilization of water resources (21.0%) and in China was water pollution (29.4%) (Figure 10).

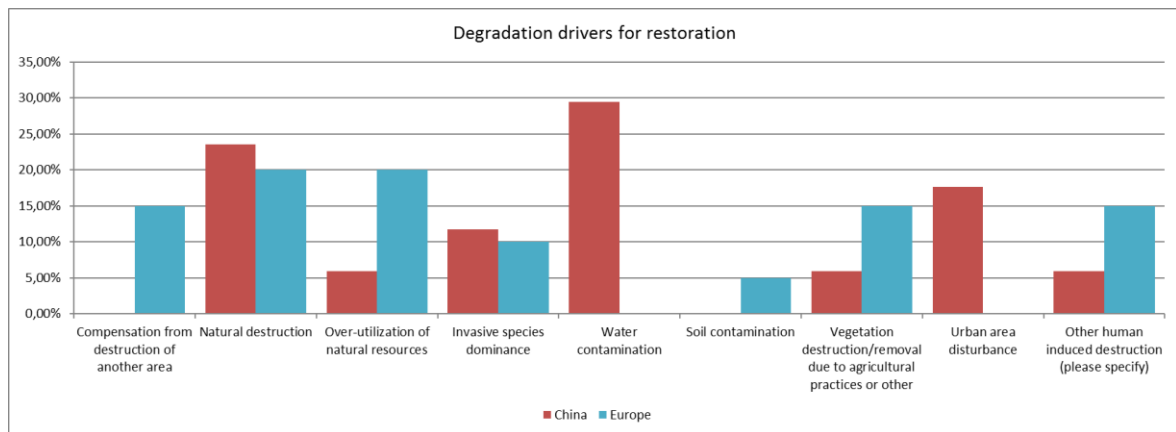


Figure 10. Degradation drivers for restoration in China and Europe in China (n=5) and Europe (n=8).

The main restoration measure applied in the European projects is hydro-morphology restoration (29.2%), as opposed to threats removal in China (30.8%) (Figure 11).

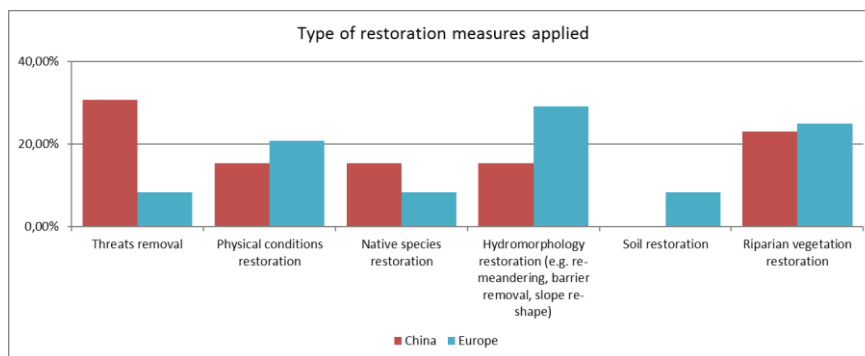


Figure 11. Measures applied in restoration projects in China(n=5) and Europe (n=8).

The majority of the Chinese restoration projects (75%) did not use soil bioengineering techniques to achieve project objectives. Regarding the European projects, 37.5% resorted to these type of techniques (Figure 12).

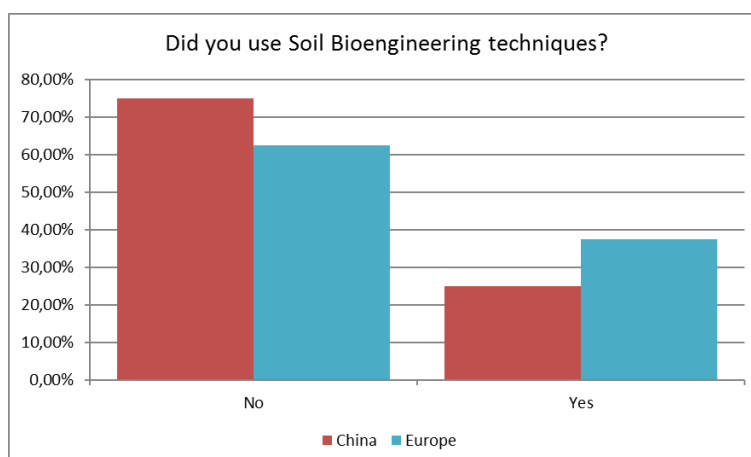


Figure 12. Use of soil bioengineering techniques in Chinese (n=4) and European (n=8) restoration projects.

The main soil bioengineering technique employed in European restoration projects was the vegetated log cribwall (16.7%). The only technique used in the Chinese restoration projects was reed structures for bank stabilization (Figure 13).

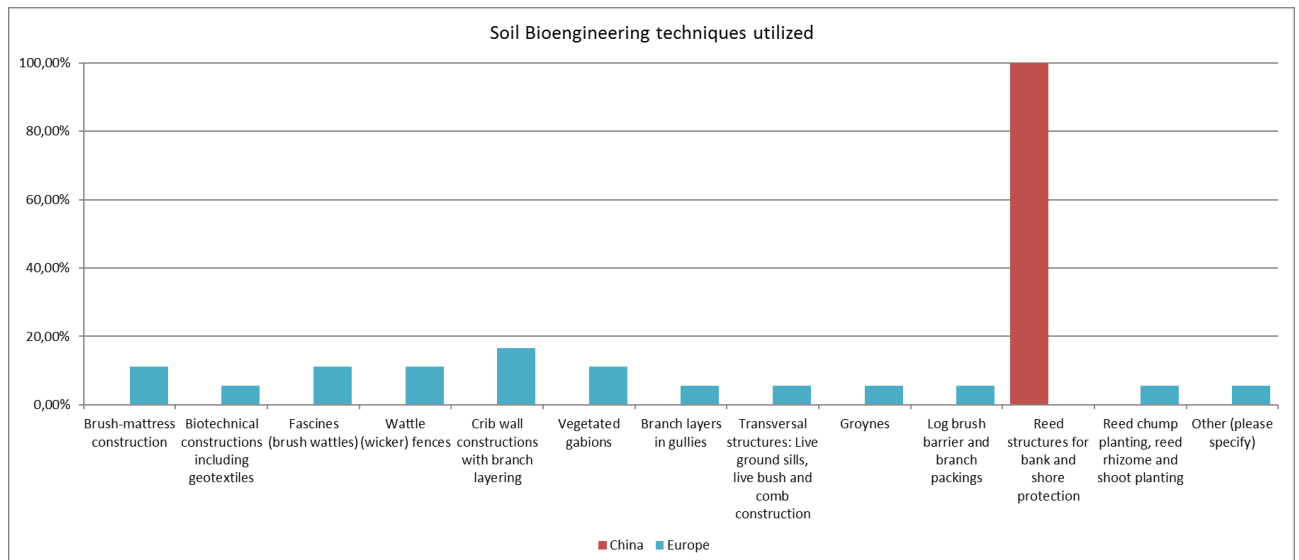


Figure 13. Soil bioengineering techniques utilized in Chinese (n=1) and European (n=3) restoration projects.

The budget of the majority of the surveyed European restoration projects is in the 1 000 000 to 5 000 000 Euros range (28.6%) or in the 100 000 to 250 000 Euros range (28.6%) (Figures 14 and 15). The surveyed Chinese restoration projects have varied budgets, ranging from very small-scale projects (5 000 to 10 000 Euros), to very large-scale ones (budget higher than 5 000 000 Euros) (Figures 14 and 15).

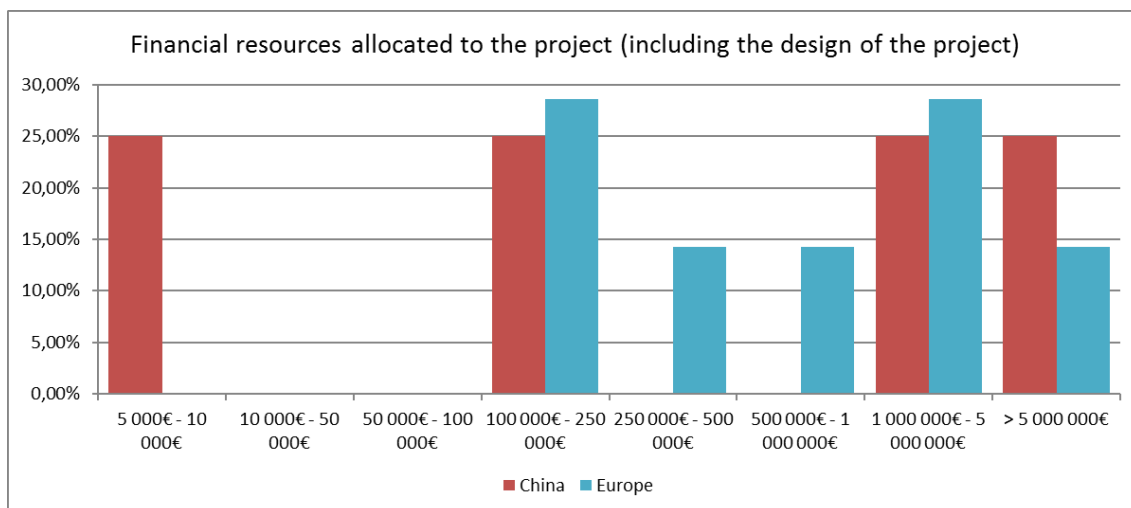


Figure 14. Budget allocation for the surveyed Chinese (n=4) and European (n=7) restoration projects.

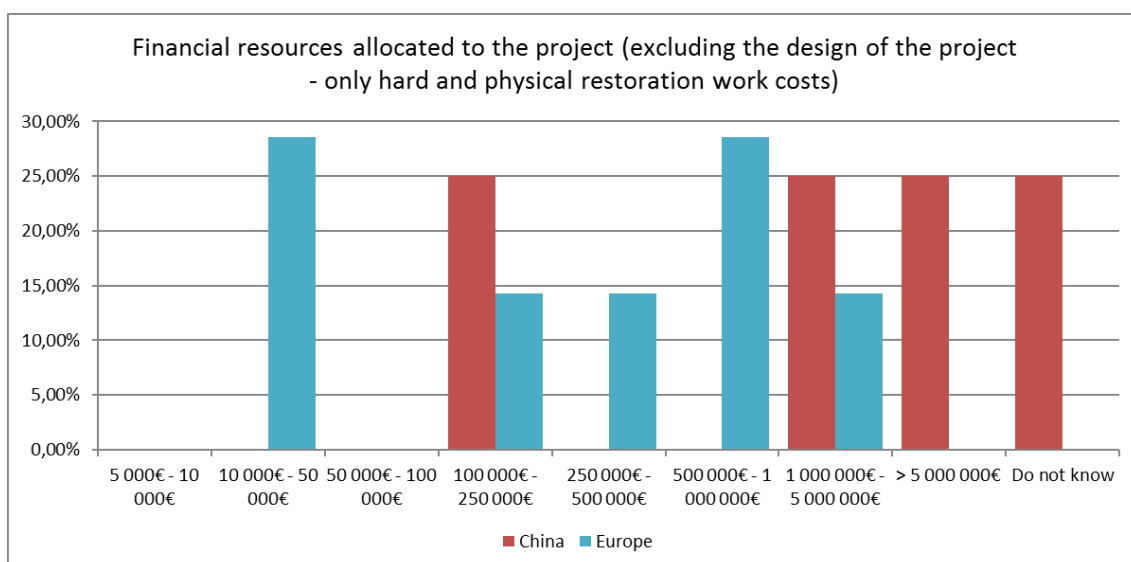


Figure 15. Budget allocation for physical restoration works in the surveyed Chinese (n=4) and European (n=7) restoration projects.

In Europe it may take up to 5 years after identifying the degradation problem to implement a restoration project, whereas in China half the projects were implemented one year after the identification of the degradation problem (Figure 16).

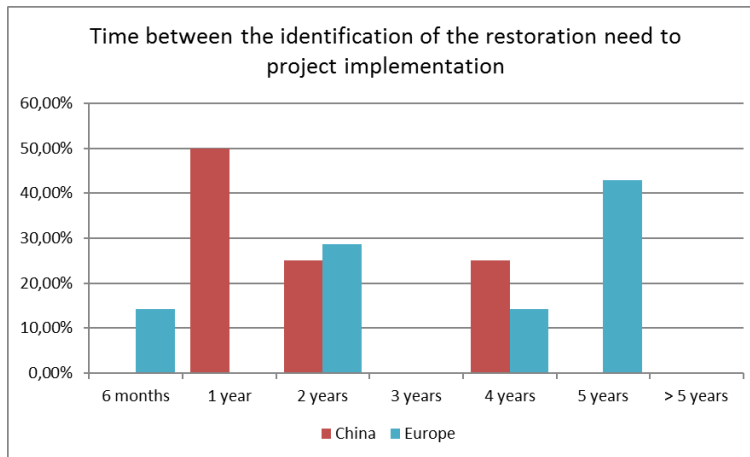


Figure 16. Time between the identification of the degradation problem to restoration project implementation in China (n=4) and Europe (n=7).

Regarding the mitigation of threats, 42.9% of the European projects initiated the process of mitigation and management of threats. Additionally, 28.6% of projects mitigated or managed all threats to low extent (Figure 17). Regarding the Chinese projects, 66.7% of them succeed to mitigate or manage all threats to intermediate extent (Figure 17).

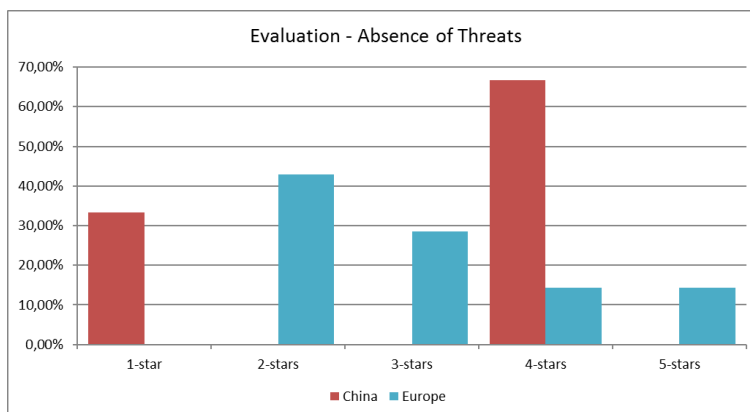


Figure 17. Success in threat mitigation in Chinese (n=3) and European (n=7) restoration projects. Please see McDonald et al. (2016) for a generic 1-5 stars recovery scale.

Regarding the improvement of physical conditions, 42.9% of the European projects succeed in stabilizing the substrate within natural range. A similar percentage of projects managed to put the chemical and physical properties of substrate on track to stabilization (Figure 18). Regarding the surveyed Chinese projects, 33.3% managed to obtain substrate conditions suitable for ongoing growth and recruitment of characteristic biota. A similar percentage of Chinese projects only managed to remediate gross physical and chemical problems (Figure 18).

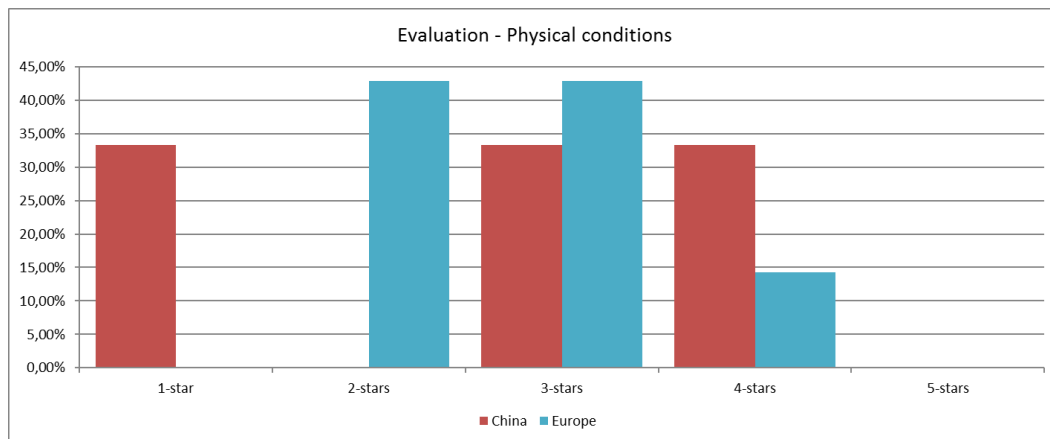


Figure 18. Success in the improvement of physical conditions in Chinese ($n=3$) and European ($n=7$) restoration projects. Please see McDonald et al. (2016) for a generic 1-5 stars recovery scale.

Regarding the evolution of species composition, 57.1% of the European projects managed to establish a substantial subset of key native species over the area, together with a very low onsite threat from undesirable species. As regards to the Chinese projects, 66.7% managed to secure the genetic diversity of stock and establish a small subset of characteristic native species (Figure 19).

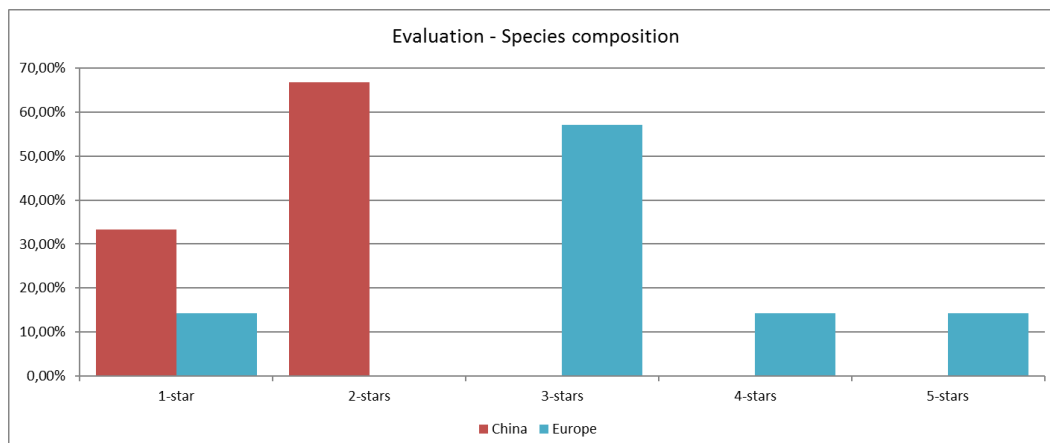


Figure 19. Success in the improvement of species composition in Chinese ($n=3$) and European ($n=7$) restoration projects. Please see McDonald et al. (2016) for a generic 1-5 stars recovery scale.

Concerning the structural diversity, 33.3% of the European projects accomplished the goal of having more strata present, together with some spatial patterning and trophic complexity relative to the reference site (Figure 20). A similar percentage of projects managed to achieve increased levels of trophic complexity and spatial pattern, in the presence of all vegetation strata. On the

other hand, 66.7% of the Chinese projects presented lower results, only managing to have one or fewer strata present, with no spatial patterning or trophic complexity relative to reference ecosystem (Figure 20).

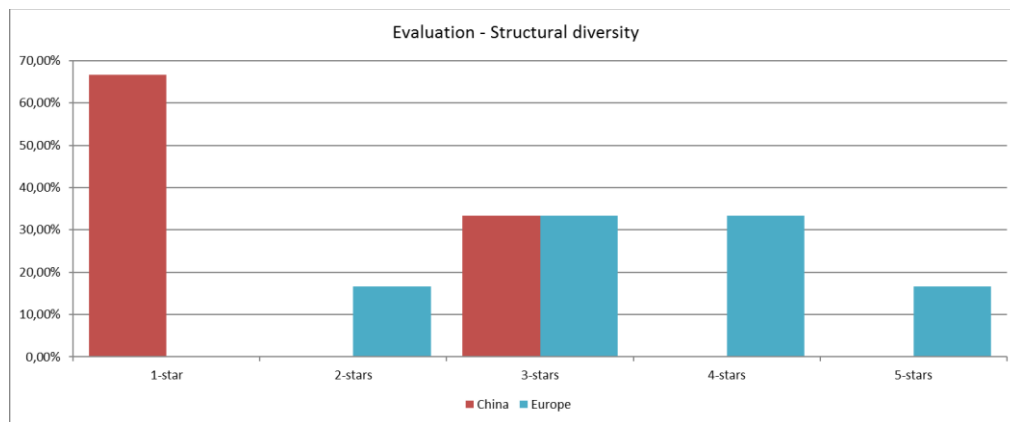


Figure 20. Success in the improvement of structural diversity in Chinese (n=3) and European (n=6) restoration projects. Please see McDonald et al. (2016) for a generic 1-5 stars recovery scale.

Regarding the ecosystem functionality, 42.9% of the European projects managed to improve substrates and hydrology to an extent able to provide a wide range of functions, including nutrient cycling and provision of habitats/resources for other species (Figure 21). The Chinese projects achieve a lower result so far, with 66.7% only managing to improve substrates and hydrology up to a foundational stage, but capable of future development functions similar to reference (Figure 21).

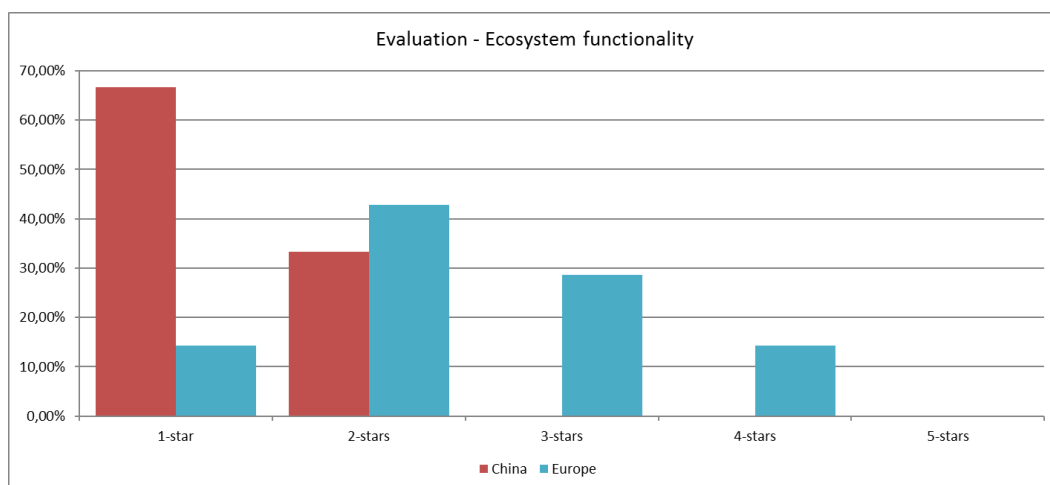


Figure 21. Success in the improvement of ecosystem functionality in Chinese (n=3) and European (n=7) restoration projects. Please see McDonald et al. (2016) for a generic 1-5 stars recovery scale.

Regarding the external exchanges, 28.6% of the European projects managed to improve connectivity up to the point of evident exchanges between the site and the external environment (Figure 22). Concerning the Chinese projects, 66.7% only managed to achieve potential exchanges with surrounding landscape or aquatic environment (Figure 22).

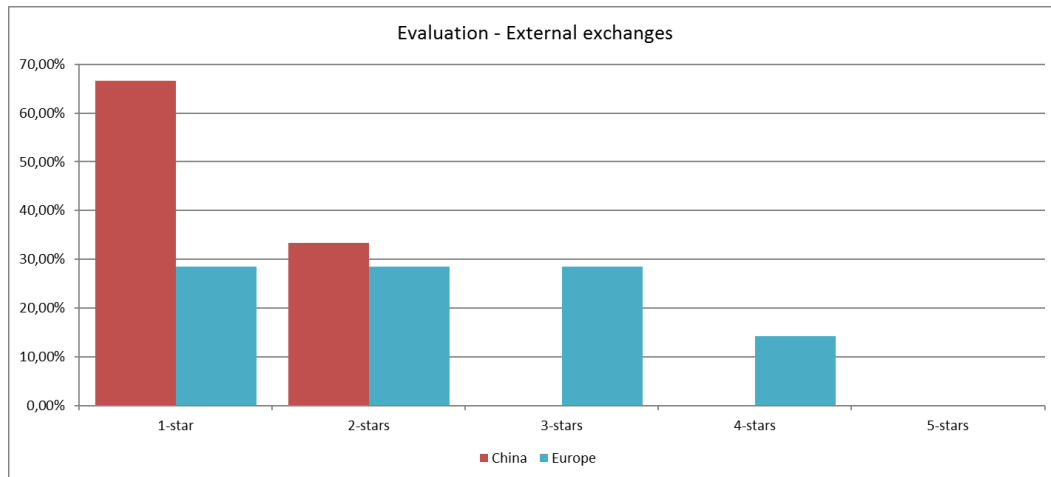


Figure 22. Success in the improvement of external exchanges in Chinese ($n=3$) and European ($n=7$) restoration projects. Please see McDonald *et al.* (2016) for a generic 1-5 stars recovery scale.

Figure 23 summaries the evaluation of the surveyed Chinese and European restoration projects. On average, the Chinese projects so far reached a two-stars level: “threats from adjacent areas starting to be managed or mitigated. Site has a small subset of characteristic native species and low threat from undesirable species onsite. Improved connectivity arranged with adjacent property holders” (McDonald *et al.*, 2016). The European projects averaged a three-stars level: “adjacent threats being managed or mitigated and very low threat from undesirable species onsite. A moderate subset of characteristic native species are established and some evidence of ecosystem functionality commencing. Improved connectivity in evidence” (McDonald *et al.*, 2016).

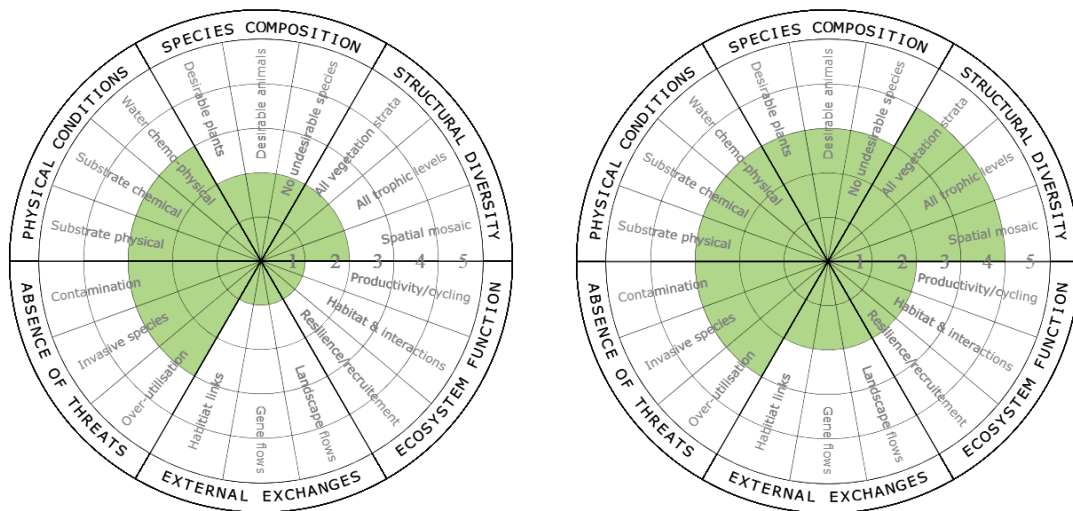


Figure 23. Recovery wheel with the average recovery levels for each ecosystem attribute for the surveyed Chinese restoration projects (left; $n=3$) and European restoration projects (right; $n=7$) (adapted from McDonald et al., 2016).

Analysis of the on-line questionnaire and policy mixes in Europe and China

Notwithstanding the limited number of completed replies of the on-line questionnaire, it is possible to identify possible restoration implications of the different policy mixes used in China and Europe. The diverse array of legislative pieces and obligations is set for different types of environmental problems, which is probably also a reflection of the maturation status of implementation of the several legislative pieces. As illustrated in Figure 10, the main degradation driver for restoration in Europe was the over-utilization of water resources (21.0%) and in China it was water pollution (29.4%). Another interesting aspect is depicted on Figure 11, with hydro-morphology restoration (28.6%) as the main restoration measure applied in the European projects, as opposed to threats removal in China (30.8%). This is probably due to the different implementation drivers in Europe and China, since the Water Framework Directive calls for the need on hydro-morphologic restoration, and in China all the main restoration drivers (the Three red lines of Most Stringent Water Resources Management, Action Plan for Prevention and Control of Water Pollution and the Law for Prevention and Control of Water Pollution) call for pollution control and removal. This implication is also illustrated on Figures 17 to 22, where the differences between the classification of projects in China and Europe are clearly seen, especially on Figures 17 and 18, where Chinese projects are mainly rated as 4 stars, which is clearly in line

with current legislative pieces and standards. Other difference to Europe is that in the latter the Water Framework Directive imposes stricter implementation of restoration standards, which includes some ecosystem functions. However, this does not guarantee that results are achieved (see Figure 23 for overall classification of projects between Europe and China). Regarding financial resources and time from problem identification to resource allocation and project implementation, in Europe project implementation is a lengthy process (5 year, see Figure 16), whereas in China the time from project design to implementation is smaller (1 year, see Figure 16). That is probably related with the urgent need for pollution control, as was the case of past European environmental legislation, because water pollution control is the first step for environmental problems resolution.

6. POLICY RECOMMENDATIONS

Considering the policy context and the most significant trends found in the literature, we recommend several soft law and reinforcement mechanisms, divided into Governance (Table 3), Quality (Table 4), Stakeholder (Table 5), Publicity (Table 6) and Research measures (Table 7).

Table 3. Recommended Governance measures.

Description	Geographic area of implementation
Development of legislation that initiates, supports and guarantees ecological restoration	Europe and China
Development of official restoration standards that take in consideration International Standards for the practices of Ecological Restoration	Europe and China
Development of guidelines to assist policy makers on funding restoration projects in rural and urban contexts	Europe and China
Development of guidelines for reference sites	China using past European experience

Create official definitions of “ecological restoration” and “restore” in the national and international law context	Europe and China in cooperation
Development of monitoring system and database for resources and ecosystem	China using past European experience
Development of institution or mechanism for assessing status and tendency of ecosystem and restoration projects	China using past European experience

Table 4. Recommended Quality measures.

Description	Geographic area of implementation
Ensure ecosystem resilience over time, <i>i.e.</i> , ensure that ecological restoration focusses on the protection and restoration of natural ecosystem's structure, function, composition and dynamics within the constraints imposed by medium to long-term changes	Europe and China
Ensure that restoration projects protect native flora and avoid genetic pollution	Europe and China
Introduction and definition of provenance regions for vegetative material	Mostly China, but also Europe
Discourage the use of concrete/cement on riverbanks; stimulate the use of natural materials instead of concrete; develop a code of good practice for the use of concrete in river banks.	China and Europe
Develop guidelines for protection and development of riparian vegetation and for biodiversity protection and enhancement	Mostly China in cooperation with Europe

Table 5. Recommended Stakeholder measures.

Description	Geographic area of implementation
Take advantage of synergistic partnerships; develop collaborative learning in local communities; perform/stimulate stakeholder-mapping to understand relationships, possible sources of conflict and organize the restoration interventions in the most suitable way for all involved parties	Europe and China
Develop working group/platform that involves designers, developers of projects, practitioners, and academia so that guidelines are used Nation-wide	Europe and China in cooperation
Implementation of Stakeholder involvement practices so that regional adaptation is taken in consideration as well as local community needs	China using accumulated experience from Europe

Table 6. Recommended Publicity measures.

Description	Geographic area of implementation
Raise public awareness on the importance of improved standards	China and Europe
Give awards to reinforce good restoration standards; River Prize model	China and Europe
Promote citizen science for data collection and monitoring of restoration projects	China and Europe

Table 7. Recommended Research measures.

Description	Geographic area of implementation
Development of typologies for river classification	China in cooperation with Europe
Foster creativity, innovation and knowledge sharing to ensure best science and practices	China in cooperation with Europe
Measures and technologies for freshwater restoration	China in cooperation with Europe

Several forms of sustainability standards such as certification schemes, voluntary corporate initiatives, public-private partnerships have become an institutionalized approach to sustainable management (Visseren-Hamakers *et al.*, 2012), and may be used by institutions as reinforcement and soft law mechanisms that will certainly make their contribution to freshwater ecosystem restoration.

7. CONCLUSIONS

Water bodies in the EU are the most degraded and fragmented ones in the world. A significant amount of restoration is still expected to take place under existing legislation (European Commission, 2011b), though the oldest piece of nature restoration legislation exists already for 39 years (the 1979 Birds Directive). Recent European experience regarding the implementation of the WFD and Nature Directives shows how difficult it can be to achieve ambitious goals. Results from the implementation of the WFD in these past eighteen years indicate that by 2015 slightly less than half of the Member States water bodies complied or were expected to comply with the good ecological status target (EEA, 2012). A recent review (Cliquet *et al.*, 2015) of restoration practices in Europe indicated the need for:

- The EU Commission to work out further guidelines and not leave choices entirely to Member States, in order to prevent “easy choices” (e.g. restoring nature only in protected areas or restoring nature towards a lower standard).

- Criteria for defining restoration priorities and evaluation of restoration.
- More specific guidelines on restoration.

China is facing unprecedented serious environmental pollution, ecological degradation and biodiversity losses (Ma *et al.*, 2013). A new set of environmental governance structures and recent legislations in China (e.g. State Council, 2012, 2015c, 2017) make the future of restoration practice a challenge since enormous amounts of funds have been and are still to be spent to achieve proposed water quality standards and to restore ecosystems (e.g. Zhang *et al.*, 2013; Mi *et al.*, 2015; Xu *et al.*, 2016; Zhu *et al.*, 2016; Li *et al.*, 2017; Huang *et al.*, 2018). River and Lake chiefs across China will be responsible for achieving pollution control and restoration of freshwater ecosystems. Capacity building and cooperation with other regions of the globe that may help shorten the learning process to implement the most effective restoration practices has been put into place. Thus, the China Europe Water Platform (CEWP) was launched at the 6th World Water Forum in Marseille, France (CEWP, 2012). The CEWP is a reliable mechanism for cooperation and joint sharing of knowledge that can also reinforce the achievement of International Treaties and Conventions such as the CBD, Ramsar Convention and Sustainable Development Goals, as well as promoting internal catalytic processes that may overcome current failures of the European legal framework.

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SECTION III

RESTORATION AT BASIN LEVEL

CHAPTER 3

**Restoration at basin level: the
influence of future land use and
climate scenarios on river nitrates
levels**

1. INTRODUCTION

Agriculture is considered the main origin of non-point source pollution (Ongley, 1996; Haag & Kaupenjohann, 2001; Lam *et al.*, 2010). The expansion and industrialization of agriculture resulted in surface and groundwater degradation due to the increase in the use of fertilizers and pesticides (Donoso *et al.*, 1999; Zalidis *et al.*, 2002; Lawniczak *et al.*, 2016; Hundey *et al.*, 2016). Furthermore, the negative impact of agricultural practices may compromise vital ecosystem services (Segurado *et al.*, 2018). The runoff from precipitation inputs pollutants from human activities, like agriculture, into surface and groundwater (Ongley, 1996), promoting water quality degradation. In Portugal, roughly 80% of the total water consumption is for agricultural uses (EEA, 2012), and the demand for water for irrigation is increasing. Future pressures on water resources are predicted to increase, and climate change scenarios bring greater uncertainty to water resources availability (Arnell, 1999; Vörösmarty *et al.*, 2000; Middelkoop *et al.*, 2001; Milly *et al.*, 2005; Cosgrove & Loucks, 2015). In fact, the majority of the Global and Regional Circulation Models that simulate the Earth's climate system predict an increase in mean annual temperature and a decrease in mean annual rainfall in the Mediterranean regions (IPCC, 2013). Moreover, precipitation is expected to be concentrated into shorter periods, with longer and harsher droughts (IPCC, 2013). Land use may also affect the provision of hydrologic services (Foley *et al.*, 2005; Brauman *et al.*, 2007). For instance, forested river basins usually have less available surface water than grass-dominated basins (Andréassian, 2004), but also lower nitrate concentrations (Cameron *et al.*, 2013). Thus, to improve land management options it is important to evaluate how different land use scenarios will affect the supply of hydrological services (Kepner *et al.*, 2012).

To address non-point source pollution, the European Union developed agricultural policies and environmental regulations that aim to improve the ecological status of surface and groundwater. Accordingly, the Nitrates Directive (91/676/EEC) aims to protect water quality from nitrate pollution from agricultural sources and to promote the use of good farming practices (European Commission, 1991). It has close links with other EU policies, like the Water Framework Directive, Groundwater Directive (2006/118/EC), Common

Agricultural Policy or adaptation to climate change. In 2008 the application of the Nitrates Directive resulted in a EU-27 average 16% decrease in nitrogen leaching emissions (Velthof *et al.*, 2014). However, the Directive success in reducing nitrate losses may vary between Member States (Smith *et al.*, 2007). Another related piece of legislation is the Water Framework Directive (WFD) (2000/60/EC), which aims to achieve good ecological and chemical status for surface waters and good quantitative and chemical status for groundwater (European Commission, 2000). For the WFD to be successful, an effective reduction of Nitrates in surface and groundwater is needed.

The SWAT (Soil and Water Assessment Tool) model has been commonly used to predict nutrient budgets at the catchment scale (Saleh *et al.*, 2000; Santhi *et al.*, 2001; Borah & Bera, 2003; Saleh & Du, 2004; Stewart *et al.*, 2006; Gassman *et al.*, 2007; Rode *et al.*, 2008; Ferrant *et al.*, 2011; Boithias *et al.*, 2014; Cerro *et al.*, 2014; Molina-Navarro *et al.*, 2018). Moreover, it is an effective tool to evaluate alternative land uses, best management practices and other causes of pollution through the simulation of hypothetical scenarios (Gassman *et al.*, 2007; Ullrich & Volk, 2009). Therefore, we applied the SWAT model to the Sorraia River basin to assess the impacts that the combined effects of climate change and management practices may have on its water quality. The main objectives of this study were to simulate the nitrate loads in a Mediterranean type agricultural river basin with water abstraction problems, and to predict nitrate behavior in the basin using three different storylines which combine alternative tendencies in the evolution of society and ecosystems with climate change scenarios.

2. METHODS

2.1 Study Area

The Sorraia basin has an area of 7730 Km² and a length of 155km (Figure 24). It flows towards the Tagus river estuary and it is the Tagus tributary with the largest basin area. The climate of the region is dry sub-humid, with hot and dry summers and mild and wet winters. According to the data of 14 local weather stations (1981-2011), mean annual temperature is 15.0°C and mean annual rainfall is 600 mm, with an average monthly precipitation of 50 mm (APA, 2017). Dominant soil

types in the basin are Cambisols, Luvisols, and Regosols, with Fluvisols also present in the downstream irrigated areas (IUSS Working Group WRB, 2015). About one half of the Sorraia watershed is covered by cork-oak forest, while the other half includes the biggest irrigated area in Portugal (approximately 15 500 ha). In detail, the land cover in the Sorraia watershed is distributed as follows (EEA, 2016): 34% broadleaf forest, 28% range-grasses, 20% agricultural crops, 9% pine forest, 5% orchards, 2% pasture, 1% urban and industrial, 1% others. There are two major reservoirs in the watershed, Maranhão (1957) and Montargil (1958), built during the implementation of the Sorraia Valley Irrigation Plan. The main pressures in the basin are hydro-morphological changes, diffuse pollution, municipal discharges, flow regulation and water abstraction (APA, 2012). Thus, the basin is under significant anthropogenic influence, with significant water abstraction for irrigation and nutrient enrichment problems (Cordovil *et al.*, 2018; Segurado *et al.*, 2018). These pressures are expected to increase in the future.

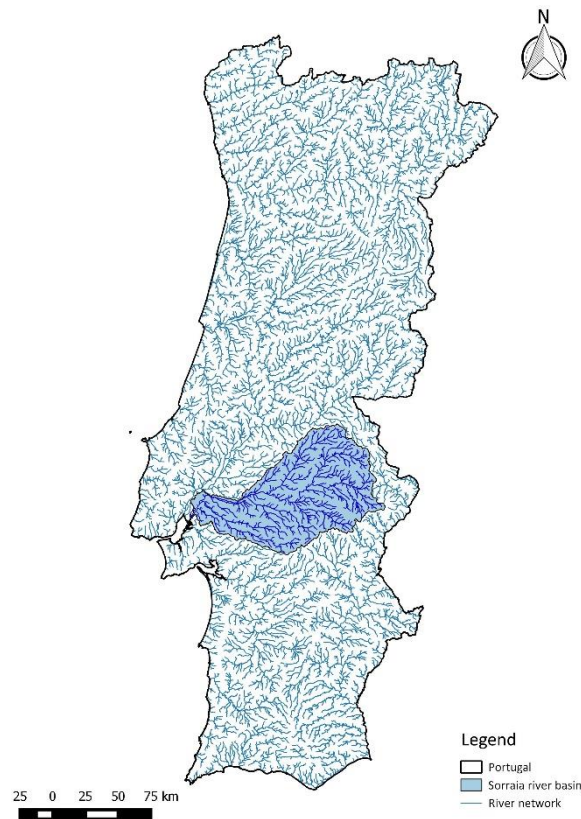


Figure 24. Location of the Sorraia watershed.

2.2 The Soil and Water Assessment Tool (SWAT) model

The software used for hydraulic and nitrogen modelling was the Soil and Water Assessment Tool (SWAT) model (Neitsch *et al.*, 2011). SWAT is a continuous time hydrological model designed to predict the impact of land management on water, sediment and non-point source pollution at basin scale (Gassman *et al.*, 2007). It has widespread use in the simulation of watershed level processes (*e.g.* Durão *et al.*, 2012; Amaral *et al.*, 2013; Liu *et al.*, 2015; Lee *et al.*, 2016; Segurado *et al.*, 2018). The SWAT model uses the basic principles of the hydrologic cycle to simulate the behavior of a watershed (Neitsch *et al.*, 2011; Mutenyo *et al.*, 2013). The hydrology of the model is based on the water balance equation which includes runoff, precipitation, evaporation, infiltration and lateral flow in the soil profile. Major model components include weather, hydrology, soil temperature, plant growth, nutrients, pesticides, and land management (Gassman *et al.*, 2007). SWAT divides the watershed into homogeneous areas, the Hydraulic Response Units (HRUs). Each HRU is based on unique combinations of soil, land-use, and slope characteristics (Neitsch *et al.*, 2011).

SWAT is able to model the nitrogen cycle in the soil profile and in the shallow aquifer (Neitsch *et al.*, 2011). Accordingly, the nitrogen is represented by five different pools, which encompass mineral and organic forms. The mineral nitrogen is divided into ammonia (NH_4^+) and nitrate (NO_3^-) pools. The organic nitrogen is divided into fresh organic N, stable N and active N. Fresh organic N is associated with crop residues and microbial biomass, while the active and stable organic N pools are associated with the soil humus. Nitrate leaching algorithms in SWAT take into account nitrate loss in surface runoff and lateral flow (Neitsch *et al.*, 2011). SWAT is also able to model the groundwater nitrate loads over time.

The SWAT model was applied to the Sorraia basin using the ArcSWAT interface, which is an extension for ArcGIS (©ESRI, Redlands, CA).

2.3 Input data

The SWAT model requires detailed information on the climate, soils, and land use for the study watershed. Table 8 gives an overview of the input data and Figure 25 shows the physical characteristics of the Sorraia basin.

Table 8. List of input data.

Data type	Source	Data description	Year
DEM	United States Geological Survey (USGS)	Shuttle Radar Topography Mission (30 meters spatial resolution)	2000
Soils	Serviço de Reconhecimento e de Ordenamento Agrário (SROA)	Soil physical properties, 1:25 000	1965
Land Use	Copernicus Programme	GSE Land M2.1 Regional Land Cover	2012
Climate	Sistema Nacional de Informação de Recursos Hídricos (SNIRH)	Precipitation, temperature, relative humidity and wind speed	1980-2012

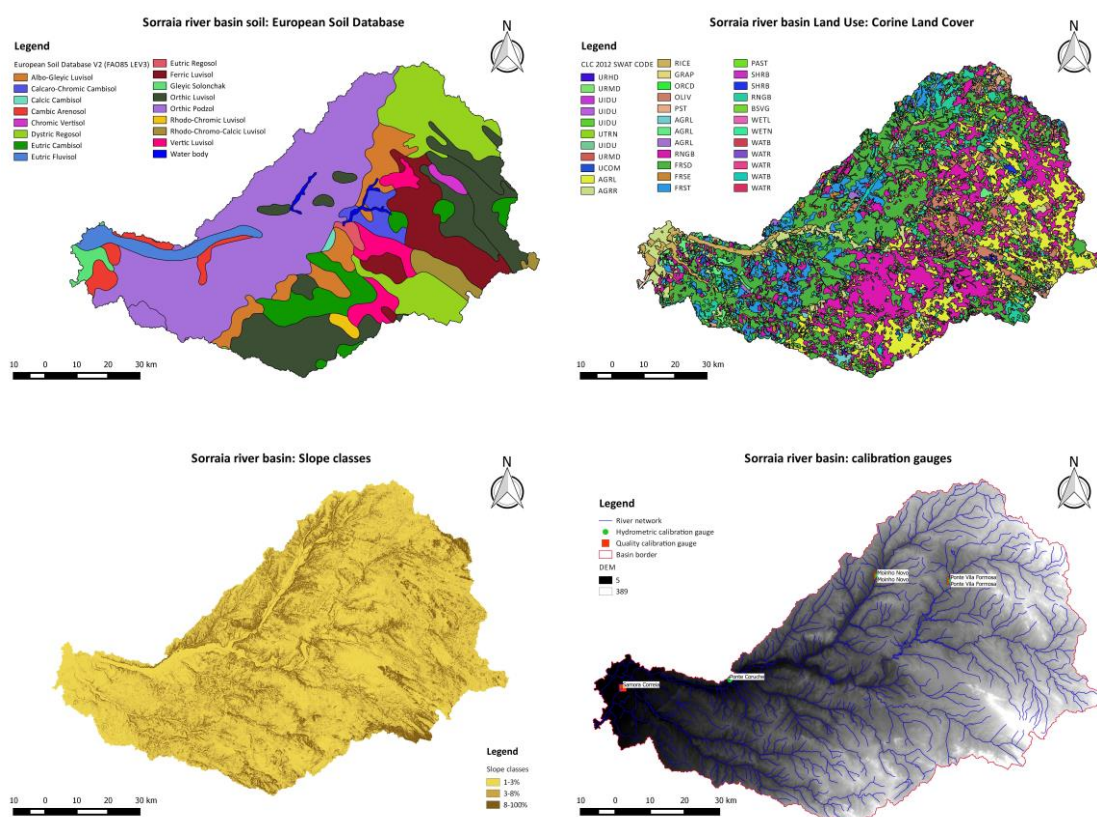


Figure 25. Physical characteristics of the Sorraia watershed. (a) soil type, (b) land use, (c) slope classes, and (d) spatial location of hydrometric and water quality model calibration gauges.

2.4 Calibration and validation

The model calibration was done manually by adjusting parameter values within an allowable range, following the technical guidelines of the SWAT model. Nitrate fluxes are strongly related with water fluxes, so the parameters that control water balance were calibrated first. Parameters related to water flow were modified to minimize deviations between model outputs and measured flow data. Thus, following the analysis of the hydrograms, several parameters that affected flow peaks and baseflow were selected for calibration (Table 9). The calibrated parameters were validated by comparing results of simulations with an independent measurement set. Model calibration was performed for the 1996-2005 timeframe, and validation comprised the 2006-2015 period.

Table 9. List of calibrated parameters.

Parameter	Description	Default	Calibrated Value
CN2	SCS runoff curve number for moisture condition II.	25 to 92	80 to 92
ALPHA_BF	Base flow alpha factor (1/days).	0.048	1
GW_Delay	Groundwater delay time (days)	31	3
SOL_AWC	Available water capacity of the soil layer (mm H ₂ O/mm soil).	0.11 - 0.14	- 40%
SOL_ZMX	Maximum rooting depth of soil profile. (mm).	-	500
SOL_Z1	Depth from soil surface to bottom of first layer (mm).	300 to 800	800 slope_cd 0-3
			500 3-8
			300 8-9999
SOL_Z2	Depth from soil surface to bottom of second layer (mm).	300 to 800	1000 slope_cd 0-3
			800 3-8
			500 8-9999

The discharge data from the monitoring stations of Moinho Novo (Lat. 39.228°; Long. -8.029°) and Ponte Vila Formosa (Lat. 39.216°; Long. -7.784°) (APA, 2017) (Figure 25d) was used for calibrating and validating the model. Removing the influence of hydraulic structures ensured that the model was more precise in the simulation of natural flows. The following statistical performance measures were considered: coefficient of determination (R^2), the root mean square error (RMSE),

the Nash–Sutcliffe model efficiency coefficient (NSE) (Nash & Sutcliffe, 1970), and the Model Bias (Bias). The comparison between model outputs and observations of the nitrogen data was done visually, due to limitations on the available measured data. Accordingly, this comparison focused on the magnitude of the simulated and observed values.

2.5 Storylines

The storylines established for this study were developed within the Project MARS – Managing Aquatic Ecosystems and Water Resources Under Multiple Stress (Hering *et al.*, 2015). The IPSL-CM5A-LR climate model (Dufresne *et al.*, 2013) was adapted for the storylines. The storylines were created using Shared Socioeconomic Pathways (SSP's) (O'Neill *et al.*, 2014) and Representative Concentration Pathways (RCP's) for greenhouse gas emission (Moss *et al.*, 2010). The former are reference scenarios that describe reasonable alternative trends in the evolution of society and ecosystems over a century timescale in the absence of climate change or climate policies (O'Neill *et al.*, 2014). The RCP's considered for storyline definition were RCP 4.5, which considers that greenhouse gas emission will peak around 2040, then decline up to 2080, followed by stabilization until the end of the century, and RCP 8.5, which assumes the greenhouse gas emissions will increase throughout the 21st century (Moss *et al.*, 2010; Vuuren *et al.*, 2011). Therefore, the following storylines were used in this study:

- “Techno World” (STL1): Represents a rapid global economic growth, enabling technological development but with high energy demands and no real drive to specifically enhance or ignore natural ecosystem health. This world is based on a combination of SSP 5 and climate scenario RCP 8.5.
- “Consensus world” (STL2): Represents a world where current policies continue after 2020, economy growing at the same pace as now, with awareness for environment preservation. This world is based on a combination of SSP 2 and climate scenario RCP 4.5.
- “Survival of the fittest” (STL3): Represents a fragmented world driven by countries own interests, with fast economic growth in NW Europe but

decrease in other regions, with minimal or no investment and effort in environmental protection, conservation and restoration. This world is based on a combination of SSP 3 and climate scenario RCP 8.5.

Tables 10 and 11 details the differences between the storylines.

The climate models were dynamically downscaled for the period 1996-2099 at a 20 km resolution. The period 1996-2016 was selected as a reference for the baseline simulation (present), and for bias correction (temperature and precipitation) of climate outputs.

The management practices associated with each storyline are detailed in Table 12. Fertilizer and irrigation inputs were related with the degree of agriculture increase and environmental protection consciousness considered in each storyline. The simulations were run in two different time periods, 2030 (10-year average from 2025 to 2034) and 2060 (10-year average from 2055 to 2064).

Table 10. Element change according to Storyline 1, 2 and 3 (Ferreira et al., 2016).

Attribute	Element	Storyline 1		Storyline 2		Storyline 3	
		2030	2060	2030	2060	2030	2060
Environment and Ecosystems	<i>Desertification</i>	- 20% natural forest areas and shrubland	- 25% natural forest areas and shrubland	- 10% natural forest areas and shrubland	- 15% natural forest areas and shrubland	- 30% natural forest areas and shrubland	- 35% natural forest areas and shrubland
Land use change	<i>Non-native plantations</i>	+ 10% in eucalyptus	+ 15% in eucalyptus	+ 10% in eucalyptus	+ 15% in eucalyptus	+ 30% in eucalyptus	+ 35% in eucalyptus
	<i>Urbanization</i>	+ 5% urban areas	+ 10% urban areas	No change	No change	+ 15% urban areas	+ 20% urban areas
	<i>deforestation</i>	- 20% forest areas	- 25% forest areas	- 10% forest areas	- 15% forest areas	- 30% forest areas	- 35% forest areas
Agriculture	<i>Nutrient load</i>	+ 10% of fertilizers due to biofuel crops	+ 15% of fertilizers due to biofuel crops	- 10% of fertilizers	- 15% of fertilizers	+ 30% of fertilizers	+ 35% of fertilizers
	<i>Efficient use of resources</i>	- 30% of water for irrigation	- 35% of water for irrigation	- 20% of water for irrigation	- 20% of water for irrigation	+ 30% of water for irrigation	+ 30% of water for irrigation
	<i>Agricultural areas for crops</i>	+ 5% agricultural areas for crops	+ 10% agricultural areas for crops	No change	No change	+ 15% agricultural areas for crops	+ 20% agricultural areas for crops
	<i>Efficient irrigation</i>	+ 30% of efficiency	+ 35% of efficiency	+ 20% of efficiency	+ 25% of efficiency	- 30% of efficiency	- 35% of efficiency
	<i>Industrialization</i>	+ 15% industry areas	+ 20% industry areas	No increase of industry areas	No increase of industry areas	+ 10% industry areas	+ 10% industry areas
	<i>Use of fertilizers</i>	+ 10% fertilizers	+ 15% fertilizers	- 10% fertilizers	- 15% fertilizers	+ 30% fertilizers	+ 35% fertilizers
	<i>Water pollution</i>	5% events of faecal coliforms	5% events of faecal coliforms	+ 10% of events in faecal coliforms	+ 10% of events in faecal coliforms	+ 30% in events of faecal coliforms	+ 30% in events of faecal coliforms
	<i>Local agriculture</i>	Biofuel crops	Biofuel crops	No change	No change	+ 30% agriculture	+ 35% agriculture
	<i>Environmental flow needs covered</i>	10% flow retained for environmental needs	15% flow retained for environmental needs	35% flow retained for environmental needs	40% flow retained for environmental needs	No flow retention for environmental needs	No flow retention for environmental needs
Water levels	<i>Natural flood retention</i>	Hydropower will increase	Hydropower will increase	Environmental policies persist past 2020; climate change force dams and weirs to be built	Environmental policies persist past 2020; climate change force dams and weirs to be built	Hydropower will increase	Hydropower will increase
	<i>Increase water reservoirs and weirs</i>	+ 20%	+ 25%	+ 10%	+ 15%	+ 30%	+ 35%
	<i>Overexploitation of water resources</i>	+ 20%	+ 25%	+ 10%	+ 15%	+ 30%	+ 35%
	<i>Water use efficiency</i>	+ 30%	+ 35%	+ 10%	+ 15%	- 30%	- 35%
	<i>Riparian restoration</i>	No change	No change	+ 10% in riparian width	+ 10% in riparian width	- 30% in riparian width	- 30% in riparian width

Table 11. Evolution of the land use area (km²) for each of the storylines (Ferreira et al., 2016).

Land Use	Area (km ²)						
	Baseline	STL1_2030	STL1_2060	STL2_2030	STL2_2060	STL3_2030	STL3_2060
Agriculture	1606.70	1687.50	1765.60	1606.70	1606.70	2088.30	2175.40
Forest	3458.20	2763.60	2589.00	3114.10	2828.30	2417.70	2214.90
Industrial	2.03	2.40	2.60	2.03	2.03	2.19	2.38
Water bodies	18.90	18.90	18.90	18.90	18.90	18.90	18.90
Eucalyptus	-	0.10	0.15	0.10	0.15	0.32	0.32
Urban	-	0.07	0.10	-	-	0.11	0.28
Others	2485.10	3098.30	3194.50	2829.10	3114.90	3043.40	3158.80

Table 12. Input values used for simulating the storylines with SWAT.

Storyline	Timeline	Management Practices	Variation (%)	Present	Scenario
STL1	2030	Fertilization (kg/ha)	10+	492	541
	2060		15+		566
	2030	Irrigation (mm)	10-	430	387
	2060		15-		366
STL2	2030	Fertilization (kg/ha)	10-	492	443
	2060		15-		418
	2030	Irrigation (mm)	20-	430	344
	2060		25-		323
STL3	2030	Fertilization (kg/ha)	30+	492	640
	2060		35+		664
	2030	Irrigation (mm)	30+	430	559
	2060		35+		581

3. RESULTS

3.1 Model calibration and validation

The calibration presented a good relation between model and measured data at the Moinho Novo gauging station, particularly with monthly data (Table 13). The statistical indicators for the validation period were similar, meaning that the model was well calibrated. The calibration results for the Ponte Vila Formosa gauging station were slightly worse than at Moinho Novo, particularly regarding the model efficiency parameter, which was lower than zero, both with monthly and daily outputs.

Due to data limitations, the default parameters of the SWAT model were used for the nitrogen simulation. That fact prevented a more detailed calibration. Thus, the comparison between model outputs and measured values focused on observed peaks and in the order of magnitude of values, which allowed to conclude that the model results were realistic and adjusted. Nevertheless, it was possible to obtain calibration statistical indicator for total N in the Moinho Novo gauging station: $R^2 = 0.59$; Bias = 0.22; NSE = -0.98.

Table 13. Daily and monthly flow ($m^3 s^{-1}$) statistics analyses at the Moinho Novo and Ponte Vila Formosa gauging stations.

	Moinho Novo				Ponte Vila Formosa			
	Calibration		Validation		Calibration		Validation	
	Daily	Monthly	Daily	Monthly	Daily	Monthly	Daily	Monthly
Observed Average	6.05	6.71	7.57	7.07	3.17	3.31	5.68	5.61
Modelled Average	6.95	7.04	6.5	5.81	6.09	6.27	5.22	5.19
Bias	0.9	0.33	-1.07	-1.27	2.93	2.97	-0.46	-0.42
RMSE	13.1	6.00	16.6	7.51	12.61	6.04	15.21	5.93
R²	0.41	0.71	0.41	0.68	0.31	0.58	0.24	0.54
NSE	0.22	0.71	0.39	0.67	-3.05	-1.26	0.11	0.4

3.2 Land use and nitrogen evolution

The average annual flow decreased from the current baseline of $56.0 \pm 13.8 m^3 s^{-1}$ to $18.7 \pm 3.6 m^3 s^{-1}$ and $9.1 \pm 5.4 m^3 s^{-1}$ in the 2030 and 2060 timeframes, respectively. The modeled average annual flow in the 2030 timeframe was lower in STL2, with $18.3 \pm 6.3 m^3 s^{-1}$, and similar in STL1 and STL3, with $18.7 \pm 6.5 m^3 s^{-1}$ on both storylines (Figure 25). Regarding the 2060 timeframe, STL1 presented the lowest average annual flow, with $8.3 \pm 2.9 m^3 s^{-1}$, and STL3 the highest, with $9.6 \pm 3.5 m^3 s^{-1}$ (Figure 25). The average annual flow in STL2 for the same timeframe was $9.5 \pm 3.5 m^3 s^{-1}$. Also, the modeled average discharge shows an increase of the dry no flow period from the current two months (July and August) to four months (May to September) (Figure 25).

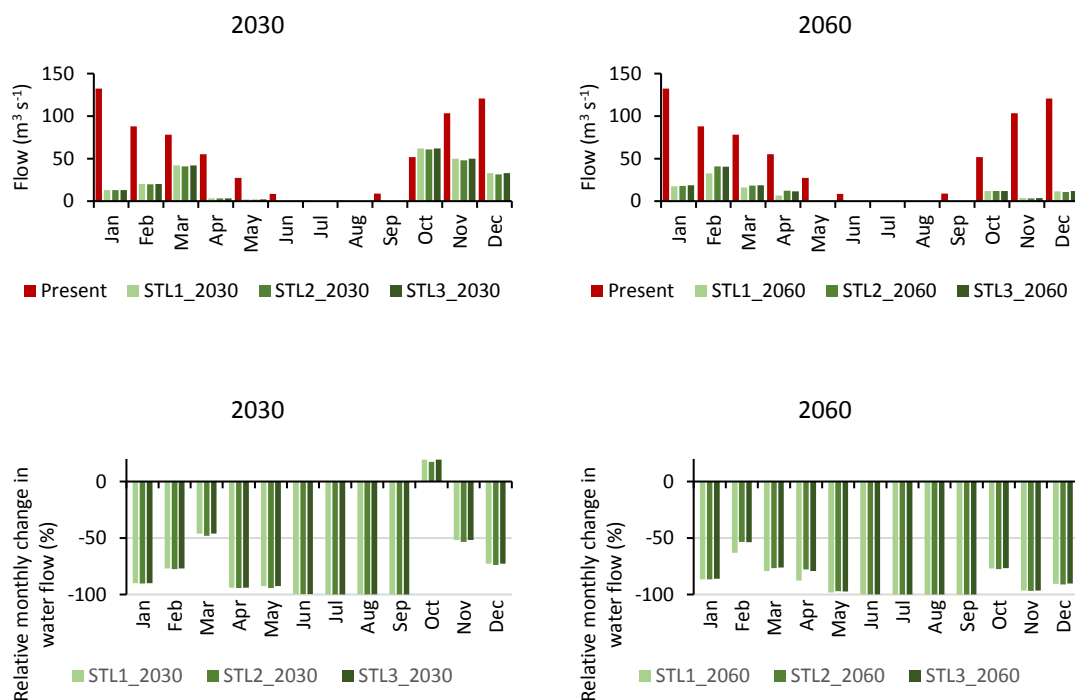


Figure 25. Average monthly flow ($m^3 s^{-1}$) for the current baseline situation and for each of the modelled storylines (top). Relative monthly change in water flow ($m^3 s^{-1}$) from baseline for each of the modelled storylines (bottom).

There was an increase in Total N concentrations in all storylines, particularly in STL 3 (Figure 26). Thus, the predicted Total N concentrations increased from the current baseline annual average of $0.67 \pm 0.12 \text{ mg N L}^{-1}$ to $1.05 \pm 0.13 \text{ mg N L}^{-1}$ in the 2030 timeframe, and to $1.35 \pm 0.11 \text{ mg N L}^{-1}$ in the 2060 timeframe. The increase is more evident between October and December (Figure 26). STL2 presented the lowest annual Total N average, with $0.90 \pm 0.19 \text{ mg N L}^{-1}$ in the 2030 timeframe, and $1.20 \pm 0.17 \text{ mg N L}^{-1}$ in the 2060 timeframe (Figure 27). An annual average of $1.05 \pm 0.24 \text{ mg N L}^{-1}$ and $1.18 \pm 0.25 \text{ mg N L}^{-1}$ was observed respectively for STL1 and STL3 in the 2030 timeframe, and of $1.35 \pm 0.18 \text{ mg N L}^{-1}$ and $1.49 \pm 0.21 \text{ mg N L}^{-1}$ in the 2060 timeframe (Figure 27).

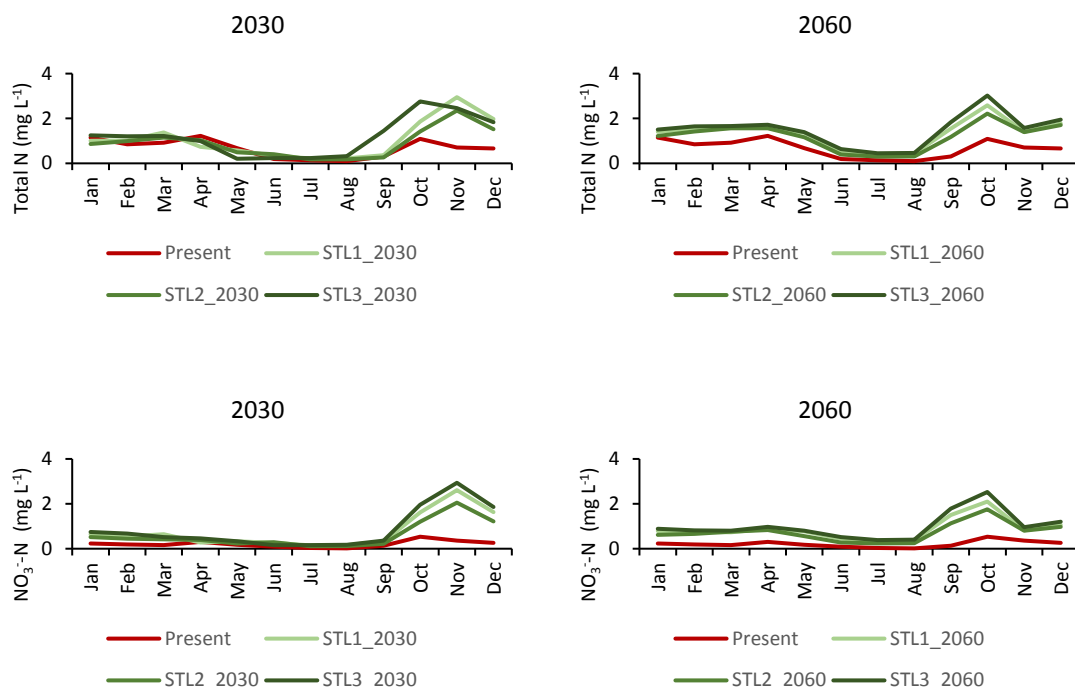


Figure 26. Average monthly Total N (mg L^{-1}) (top) and Nitrate (mg L^{-1}) (bottom) for the current baseline situation and for each of the modelled storylines.

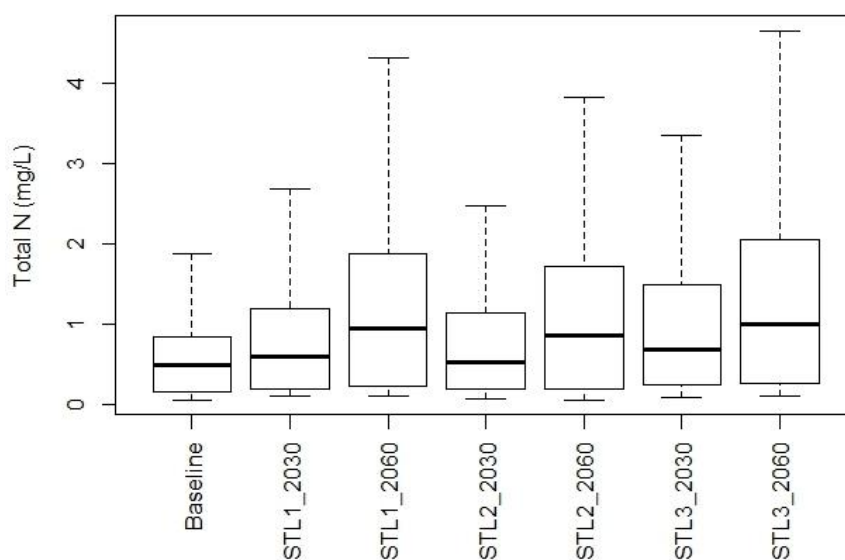


Figure 27. Boxplot (median and interquartile range) of the average monthly Total N concentration (mg L^{-1}) along the simulation period for the current baseline situation ($n=236$) and for each of the modelled storylines ($n=132$).

The evolution of nitrate concentrations is similar to the one for Total N, with an increase from the baseline annual average of $0.21 \pm 0.04 \text{ mg NO}_3^- \text{N L}^{-1}$ to

0.74±0.12 mg NO₃⁻-N L⁻¹ in the 2030 timeframe, and 0.87±0.09 mg NO₃⁻-N L⁻¹ in the 2060 timeframe. The increase is particularly evident between October and December (Figure 26). Again, the lowest annual average concentration was observed in STL2, with 0.60±0.17 and 0.74±0.12 mg NO₃⁻-N L⁻¹ respectively in the 2030 and 2060 timeframe (Figure 28). In the 2030 timeframe, the annual average concentration was 0.76±0.22 mg NO₃⁻-N L⁻¹ in STL1 and 0.86±0.26 mg NO₃⁻-N L⁻¹ in STL3. The trend was similar in the 2060 timeframe, with higher annual average concentrations in STL3 (1.01±0.18 mg NO₃⁻-N L⁻¹) than in STL1 (0.88±0.14 mg NO₃⁻-N L⁻¹) (Figure 28).

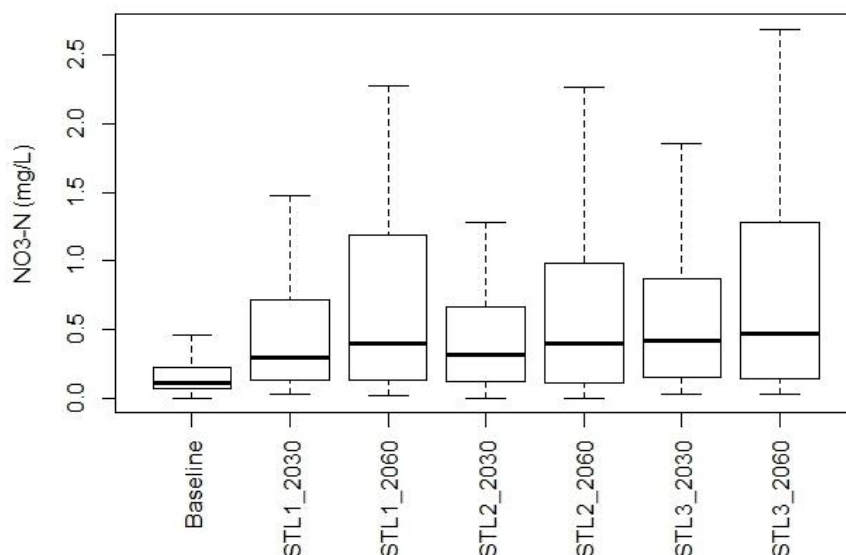


Figure 28. Boxplot (median and interquartile range) of the average monthly (mg NO₃⁻-N L⁻¹) along the simulation period for the current baseline situation (n=236) and for each of the modelled storylines (n=132).

4. DISCUSSION

The simulated scenarios showed a strong decrease of the average annual flow, with a 67% and 84% decrease, respectively in the 2030 and 2060 timeframe. Our results show that the average streamflow is lower when the decrease of forest area is smaller, which is in accordance with the literature (Brown *et al.*, 2005; Farley *et al.*, 2005). The forest area increase is associated with more evapotranspiration and thus less flow and more concentration of elements in the

water. However, in the 2060 timeframe the average annual flow in STL1 was lower than in STL2, despite the larger forest area in the former. This is probably related to the fact that STL1 uses the RCP 8.5 climate scenario, which is less conservative than the RCP 4.5 used in STL2. Other authors reported similar trends of flow reduction due to climate change in Mediterranean river catchments (Quintana Seguí *et al.*, 2010; Molina-Navarro *et al.*, 2014; von Gunten *et al.*, 2015; Pascual *et al.*, 2015; Serpa *et al.*, 2015; Coppens *et al.*, 2016; Pekel *et al.*, 2016; Shrestha *et al.*, 2017; Bucak *et al.*, 2018). This was expected because the IPSL model predicts a significant decrease in precipitation, particularly evident in the Mediterranean region (Erol & Randhir, 2012). The predicted reduction in precipitation is the consequence of a positive trend in the North Atlantic Oscillation (NAO) caused by climate change (Coppola *et al.*, 2005; Giorgi & Lionello, 2008; Hoerling *et al.*, 2011; Gillett *et al.*, 2013; Delworth *et al.*, 2016).

Although precipitation has a major impact on the streamflow of the Sorraia river, other factors, like the presence of irrigation agriculture and the predicted increase of evapotranspiration rates, also influence streamflow (von Gunten *et al.*, 2015). Moreover, the river flow in the dry periods may be higher than in natural conditions when irrigation is present (Kendy & Bredehoeft, 2006; von Gunten *et al.*, 2015). Thus, base flow rates of river basins which have a strong presence of irrigated agriculture are more influenced by climate change (Vano *et al.*, 2010; Ferguson & Maxwell, 2012). With the predicted reduction in the average annual flow, the base flow of the Sorraia river may change rapidly. That may have a strong impact in the river ecology, because fast changes in flow rate are difficult to overcome by ecological communities (Bradford & Heinonen, 2008; Sandel *et al.*, 2011; Cid *et al.*, 2017), or on water quality, because the lower dilution will increase nutrient and pollutant concentration (Whitehead *et al.*, 2009; Blasco *et al.*, 2015).

Total N concentrations increased by 57% and 101% in the 2030 and 2060 timeframe, respectively. These results indicate a future degradation of the Sorraia river water quality. Total N concentrations were higher when the agricultural area increased, and the forest area decreased. Similar trends were reported in the literature, with the proportion of agricultural land in a catchment being positively related with the nitrogen concentration in river water (Hayakawa *et al.*, 2006;

Kaushal *et al.*, 2011; Yevenes & Mannaerts, 2011; Lawniczak *et al.*, 2016; Carvalho-Santos *et al.*, 2016). Moreover, the nitrate leaching potential from forest soils is usually lower than in agricultural soils (Cameron *et al.*, 2013). Total N and non-point source organic pollution are known freshwater fish stressors (Branco *et al.*, 2016; Segurado *et al.*, 2018). The lower annual streamflow may explain part of the increase in Total N concentration, particularly in STL2, due to the lower dilution capability (Whitehead *et al.*, 2009; Blasco *et al.*, 2015). The higher increase in Total N concentration in STL1 and STL3 is also explained by the above-mentioned land use changes, with less forested and more agricultural areas. Other factor that helps to explain these results is the increase in fertilizer application on both storylines, when compared with the more ecological approach of STL2. The simulations revealed that nitrate was the most abundant N form found in the river. This is related with the type of fertilizers used by the basin farmers, but also with nitrate's high solubility and leachability, particularly in periods of precipitation (Cameron *et al.*, 2013). Accordingly, the movement of nitrate out of the terrestrial plant root zone depends on the soil hydraulic properties, the amount of irrigation and/or precipitation, the quantity applied, the N chemical form in the fertilizer and the time of the application (Cameira *et al.*, 2003). The nutrient concentration increased in all storylines between October and December, has a result of the harvesting of the corn crops in the irrigated areas. The high temperature and the low soil moisture after harvesting enhance the mineralization of crop residues, which produces ammonium. It is then oxidized to nitrate at a rate that increases with higher moisture content in the soil, typical of late fall conditions, and leaches to the river (Whitehead *et al.*, 2006; Cameron *et al.*, 2013). The increase in nitrates, together with streamflow reduction and the predicted temperature rise, may promote river eutrophication (Whitehead *et al.*, 2009). The lower flow rates increase the residence time of the water, which enhances the settling rate of sediments. Thus, turbidity is lower and light penetration is improved, increasing the algae growth potential (Whitehead *et al.*, 2009), with the subsequent severe impacts on freshwater fish populations (Pusey & Arthington, 2003).

Despite the conservation actions proposed in STL2, like the increase of the riparian buffer width, the decrease in the amount of fertilizers applied in the basin,

or the stabilization at 2012 levels of the urban and industrial areas, the Total N results were very similar between storylines. This may be connected with a possible high nitrogen content of the overland flow component, because surface removal of nitrogen is partly related to riparian buffer width (Mayer *et al.*, 2007). Thus, the simulated increase of the riparian buffer width may not be enough to offset the predicted increase of nitrogen in the basin. Also, the annual fertilizer quantity may be exceeding the crops needs, even with the simulated decrease of the fertilizer amount, which would increase nitrate leaching. The results indicate that much more effort must be developed to achieve the Water Framework Directive goals, considering the regression of nutrient levels from diffuse sources.

The combined effects of climate and land use change thus result in lower streamflow and higher nitrogen pollution in the Sorraia river basin. Accordingly, future river management plans for this basin should focus on limiting the input of nutrient loads to the river system (Segurado *et al.*, 2018). Also, the implementation of improved irrigation systems may increase the water use efficiency (Fader *et al.*, 2016). However, further adaptative measures, like the implementation of cover crops (Flower *et al.*, 2012; Ward *et al.*, 2012), no-till practices (Soane *et al.*, 2012), or the introduction of drought tolerant crops (Jacobsen *et al.*, 2012) are needed to help to mitigate the effects of climate and land use change on freshwater ecosystems.

5. CONCLUSIONS

The Sorraia river water quality is expected to deteriorate in the modeled timeframe, with Total N concentrations likely to increase up to 101% by 2060. There is a joint effect of climate change and land use on the river water quality, in spite of management activities. Land use and agricultural practices seem to explain part of the Total N increase, with higher concentrations of nutrient pollution particularly in scenarios where there is agricultural expansion and an increase in fertilization. However, climate change will also result in a strong reduction of annual mean streamflow in the Sorraia river, a decrease of the river's dilution capability, and an increase of nutrient concentration. Extreme agricultural practices (like the ones simulated in STL3), can aggravate the negative impacts of climate change in the ecological quality of rivers. These results highlight the

importance of implementing adaptative management solutions that contemplate both climate and land use changes.

In this work, three different storylines, each with its own environmental measures scenario, were proposed to assess the impacts that the combined effects of climate change and management practices may have on the water quality of the Sorraia river basin. One of the scenarios (STL2) simulates fewer pressures in the basin and includes environmental conservation measures, like increasing the riparian buffer width. However, the simulation results do not show a relevant improvement of the river nitrogen concentrations when compared with the other storylines. Thus, the proposed environmental conservation measures may be too conservative to have a significant effect in the river nitrogen concentration, particularly in a climate change context. Therefore, there is a need for further research on river basin management and its effects on river water quality in a climate change context, in order to improve the ecological quality of our river systems and fulfill the Water Framework Directive obligations.

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SECTION IV

RESTORATION AT RIVER SECTION LEVEL

CHAPTER 4

Environmental restoration of a degraded wetland

1. INTRODUCTION

Wetlands are characterized by the presence of water within the rooting zone during the growing season, which affects soil processes and plant growth (Calhoun, 1999). They develop in badly drained areas, in topographic lows, in locations where the water table is high, or where there is significant flooding from rivers, lakes, or ocean tides (Calhoun, 1999). Those occurring along river margins function as elongated transition zones between the terrestrial and aquatic environments (Calhoun, 1999). They are influenced by river dynamics, being subjected to periodic flooding, erosion and sedimentation (Lewin, 1992).

Wetlands are some of the most productive and economically valuable ecosystems in the world (Costanza *et al.*, 1997), in particular those related to watercourse margins. Their characteristic plant communities contribute to stabilize water supplies and to mitigate the erosive damage that otherwise could result from the seasonal alternation of flood and drought periods (Millennium Ecosystem Assessment, 2005; Mitsch & Gosselink, 2015). Simultaneously, they also provide the recharge of groundwater aquifers, the protection of shorelines and the removal of pollutants from water (Millennium Ecosystem Assessment, 2005; Mitsch & Gosselink, 2015).

The composition of plant communities associated with those riparian galleries is strongly influenced by the soil water regime (Booth & Loheide, 2012; Rivaes *et al.*, 2014). Seasonal and inter-annual hydrologic fluctuations may be challenging even for the more tolerant species (Rodríguez-González *et al.*, 2010). Thus, riparian vegetation has specific morphologic, physiologic and reproductive strategies. Some species are able to withstand temporary or permanent waterlogging, making the riparian environment a unique biotope. (Hunter Jr., 1990; Hager & Schume, 2001). Other species adapted to riverbank morphology changes by fast growth and strong vegetative propagation capability (Blanco Castro *et al.*, 2005). Adaptation to soil hypoxic conditions include spongy tissue that forms spaces or air channels (aerenchyma) in the stems and roots of some plants, which allows exchange of gases between the leaves and the root (Calhoun, 1999). In addition, to withstand hypoxic conditions and shifting soil, some species like poplars (*Populus* spp.), willows (*Salix* spp.) and alder (*Alnus*

spp.) developed adventitious roots with some mechanical flexibility (Cortes & Ferreira, 1998; Calhoun, 1999).

Therefore, these areas have different plant and animal communities than the surrounding ones, usually including higher species richness, structural complexity and biomass productivity than the neighborhood zones (Hunter Jr., 1990). However, riparian areas may become a difficult environment for plant and animal establishment (Manci, 1989; Cortes & Ferreira, 1998) and, in the Iberian Peninsula, plant communities are frequently dominated by pioneer species with fast growth and easy propagation (González del Tánago & Garcia de Jalón, 2001). The dynamic character of these areas makes them particularly vulnerable to changes caused by human activity (Brinson & Verhoeven, 1999). Several authors (e.g. Lewin, 1992; Cortes & Ferreira, 1998; Brinson & Verhoeven, 1999; Gasith & Resh, 1999; Tkach, 2001; Aguiar & Ferreira, 2005; Salinas & Casas, 2007; Mitsch & Gosselink, 2015) have reported negative effects of anthropogenic impacts on river plant communities, namely hydrological disturbance caused by lowering of the water table. Additionally, the expansion of both urban and agricultural areas has resulted in the degradation and disappearance of riparian galleries, which have been replaced by other types of plant cover, since the ready availability and access to water provide strong incentives for economic development (Larsen, 1994; Gasith & Resh, 1999; Duarte *et al.*, 2002; Angradi *et al.*, 2004). Land drainage, the indiscriminate clearing of trees, and river impounding have been cited as some of the factors giving rise to the degradation of river systems and wetlands (Lewin, 1992; Klimo, 2001; Machar, 2001; Mitsch & Gosselink, 2015). These degraded areas sometimes become sought for the removal of substrates, mainly sand and/or gravel for the construction industry (e.g. Brookes, 1996; Picco *et al.*, 2012). This type of activity affects not only the already degraded margins but also the channel stability and wetland integrity (Brookes, 1996).

Restoration aims to return an ecosystem to a more natural state after human disturbance (Frelich & Puettmann, 1999). However, full ecological restoration is often difficult because the nature of the original ecosystem may be unknown or impossible to achieve due to historical events, or complex evolution trajectories (Hughes *et al.*, 2005; Lamb, 2009; Dufour & Piégay, 2009; Jacobs *et al.*, 2015).

In order to be sustainable in the long term, a restoration project needs clear and properly defined objectives (Jacobs *et al.*, 2015). Nowadays there are widespread efforts to restore forests (Jacobs *et al.*, 2015) and degraded wetlands (Moreno-Mateos *et al.*, 2012; Mitsch & Gosselink, 2015). Wortley *et al.* (2013), surveyed 301 ecological restoration scientific articles and concluded that only 9% addressed restoration in riparian zones, with plantation being the most common method used to restore the ecological condition. In this context, revegetation is essential for the recovery of ecosystem functions (Aust *et al.*, 1990). Nevertheless, the degree of recovery of ecosystem functioning and structure from these efforts is frequently lower than in reference sites (Benayas *et al.*, 2009; Moreno-Mateos *et al.*, 2012). In fact, success with tree survival and growth is not necessarily equal to forest ecosystem restoration, being a necessary but not always a sufficient condition (Avera *et al.*, 2015). In addition, although there is extensive experience in the restoration of herbaceous marshes, the base knowledge regarding forested wetlands restoration is more limited (Mitsch & Gosselink, 2015). Forests need more time to recover, which turns restoration outcome more uncertain (Jones & Schmitz, 2009; Mitsch & Gosselink, 2015; Jacobs *et al.*, 2015).

Moreover, climate change is transforming our environment and creating new challenges to ecological restoration. Due to its characteristics, riparian environments can contribute to ecological adaptation to climate change (Seavy *et al.*, 2009). Management practices to improve ecosystem resilience to climate change include enhancing connectivity, promoting redundancy and buffers, realigning significantly disrupted conditions, anticipating surprises and threshold effects and reducing landscape synchrony (Millar *et al.*, 2007).

A wetland restoration project should allow natural succession processes to proceed (Mitsch & Gosselink, 2015). Therefore, one possible strategy is to establish several native plant species and allow for species selection through natural processes (Mitsch & Gosselink, 2015). However, to have an ecosystem with a high adaptive capacity, the combination of species incorporated into restoration efforts must be stress resistant and competitive in the longer term (Jacobs *et al.*, 2015). Additionally, the utilization of high quality seedlings is paramount for plantation success (Villar-Salvador *et al.*, 2009).

Most of the above considerations are particularly well adapted to the historical journey of most Portuguese wetlands, especially those related to large river systems. That is the case for the study reported here. In the distant past, the Paul da Goucha area would have been a permanent freshwater body with partially immersed emergent vegetation during the growing season. The vegetation would probably have been dominated by mixed stands of willows (*Salix* spp.) (Mendes *et al.*, 2008). Despite its ecological significance, the area is located within the lowlands of the river Tagus basin, has a prevailing dense human occupation, and has been influenced by human activities, especially those related with wood extraction, agriculture and animal husbandry.

In the early 20th century agricultural pressure led to the drainage of the area and clearing of the forest. The water level was kept low by river regulation to allow the cultivation and irrigation of traditional crops such as maize and rice. Because of this regulation, the area silted up due to natural factors (sediment transport during periods of rainfall and periodic flooding) and human impacts (removal of gravel, upstream). The sedimentation, the confined space and the successive flooding events, together with population movement to the cities, led to the abandonment of agricultural activities in the early 1970's; as a result, this area rapidly underwent a transition/succession to a wetland area, as it was in the past. In its present state, the drainage basin of the small river in whose mouth the wetland is located has undergone considerable sedimentation, which impedes water flow.

Intensive quarrying of gravel in part of the area began in the 1980's. Although this land use exploitation ended in the beginning of the 21st century, it produced significant changes in the vegetation cover and created small artificial lakes. In the latest decades the area was also used for the disposal of garbage and debris (e.g. bricks, concrete, asphalt, car batteries, refrigerators or pesticide containers), both in lakebed and banks.

A conventional restoration approach in such intensively transformed floodplain area was unrealistic, but local-scale restoration of some ecosystem functions (Capon & Pettit, 2018) through the recovery of riparian plant communities, was considered an achievable and priority target. Therefore, it was decided to restore one of the affected areas to mitigate the environmental impact of this land use. The objective was to improve selected ecosystem functions (biodiversity, aquatic

habitat, light and temperature control, bank stabilization), through the triggering/improvement of the natural riparian vegetation colonization in a small pilot area in the northern part of the Paul da Goucha wetland, to serve as restoration guidance. We hypothesized that planting riparian forest species seedlings produced through classical forest nursery methods and established following good forestry practices might have enough quality to guaranty restoration success.

2. METHODS

2.1 Study Area

The Paul da Goucha wetland is located in an alluvial depression within the Tagus river basin in south-central Portugal (Figure 29). It is part of the Vale de Atela watershed (23 km long, 92 km² basin area), which is a small left bank Tagus river tributary. With almost 100 ha, it is one of the largest willow woodlands in Portugal (Mendes *et al.*, 2008). It is also one of the rare wetland woods of significant size still occurring in the South of the Iberian Peninsula (Mendes *et al.*, 2008).

Tree cover is dominated by rusty sallow (*Salix atrocinerea* Brot.), but *Salix salviifolia* Brot., white willow (*Salix alba* L. (Ser.) subsp. *vitellina*), alder buckthorn (*Frangula alnus* Mill.), narrow-leaved ash (*Fraxinus angustifolia* Vahl.) and common alder (*Alnus glutinosa* (L.) Gaertn.) are also present (Rodríguez-González *et al.*, 2008). The exotic invader parrot's feather (*Myriophyllum aquaticum* (Velloso) Verdc.) is also abundant. The Paul da Goucha is a priority habitat, within the aim of the Habitats Directive (92/43/EEC), namely due to the occurrence of willow and alder wet woodlands (habitat code 91E0), willow formations on intermittent watercourses (92B0) and transition mires and quaking bogs (7140).

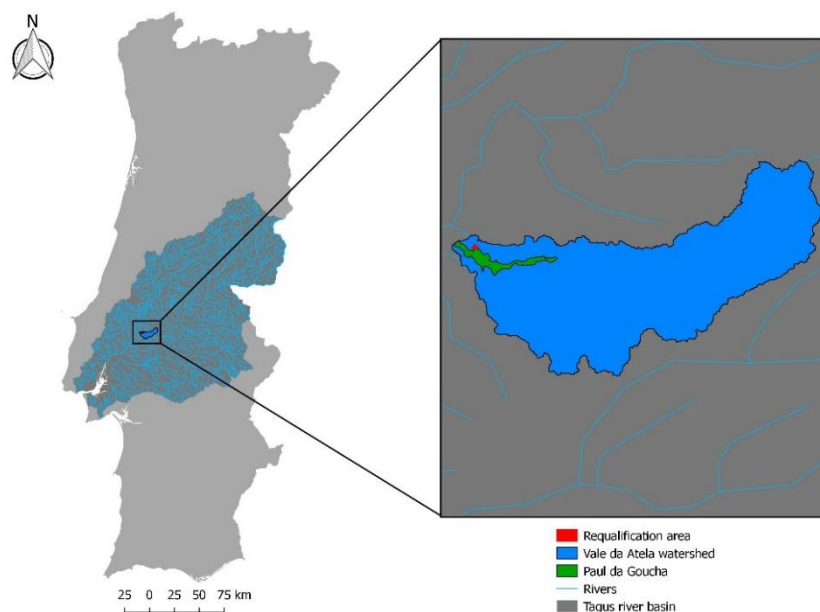


Figure 29. Location of the Paul da Goucha and the restoration area.

Regarding wildlife, there are 11 recorded fish species, 13 species of amphibians, 17 species of reptiles, 167 bird species and 27 mammal species (Mendes *et al.*, 2008). Twenty-five of the recorded bird species are listed in Annex 1 of the Habitats Directive, 8 of which are protected because they are listed as endangered by the Vertebrate Red Data Book for Portugal (Cabral *et al.*, 2006); 82 species are known to nest in the area. One of the 27 mammal species is classified as critically endangered and another as vulnerable (Cabral *et al.*, 2006).

The climate of the site is of the Mediterranean type, characterized by hot and dry summers and mild and wet winters. According to the weather survey station of Santarém (39° 15' lat. N, 08° 54' long. W, 54 m a.s.l., 1971-2000), mean annual rainfall is 695.5 mm, with 5% of it occurring between June and August and the mean annual temperature is 16.0°C, ranging from a monthly mean of 9.6°C in January to 22.7°C in August (IPMA, 2017). According to the Rivas-Martínez bioclimatic classification system (Rivas-Martínez *et al.*, 2011), this area is classified as Mediterranean Pluviseasonal Continental, with a Lower inframediterranean thermotype and an upper dry ombrotype (Figure 30).

According to the World Reference Base soil classification system (IUSS Working Group WRB, 2015), soils are mostly Haplic Arenosols (ESBD v2.0, 2004), with low organic matter content. The Paul da Goucha is an area of sedimentary

formation from the Plistocenic and Holocenic eras, with peat deposits up to 8 meters deep (CONSMAGA, 2002).

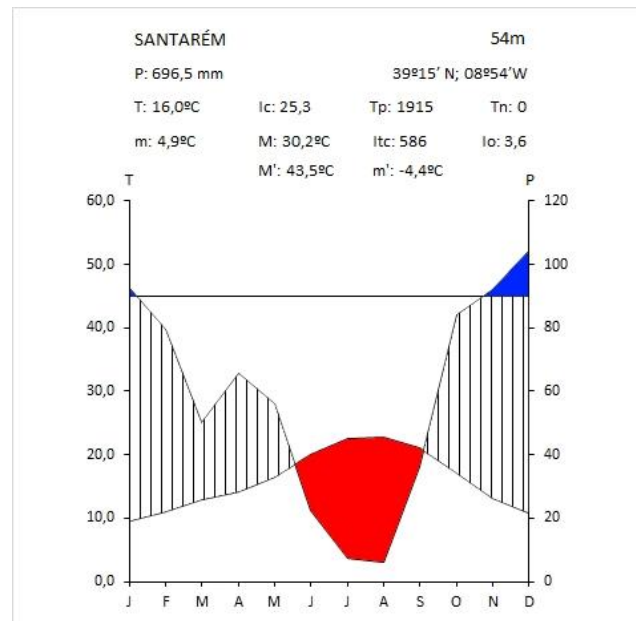


Figure 30. Bioclimograph from Santarém weather station (1971-2000) according to (Rivas-Martínez et al., 2011). Blue color represents the wet period (precipitation higher than 90 mm), red color represents the dry period (precipitation lower than twice the average monthly temperature) and vertical lines represents precipitation higher than twice the average monthly temperature. Graphics are represented in a Cartesian coordinate system with a double scale, adjusted to $P \text{ mm} = 2T^{\circ}\text{C}$. Y-axis shows the monthly temperature and precipitation averages, and the x-axis shows the months of the year.

2.2 Site Preparation

The restoration work started with the mechanical and manual removal of all the garbage and debris present on and around a small artificial lake within the northern part of the site. It was followed by the removal of the exotic invader giant reed (*Arundo donax* L.) including removal of the rhizomes as thoroughly as possible. The slope of the artificial lake banks was smoothed using heavy machinery, as well as the margins of a small island present inside the lake. This allowed for a bigger plantation area, with an easier access to the water table for the installed plants. The lakebed slope was reduced to 14%, using heavy machinery. A layer of clean topsoil at least 15 cm thick was spread over the plantation areas with a bulldozer.

2.3 Plant Production

The plants were produced in the Instituto Superior de Agronomia forest nursery. Floristic surveys were carried out within the Tagus river basin, in areas ecologically similar to the study site, to identify suitable plant propagule collection areas. Seeds and cuttings were collected in those areas, in trees free from diseases, vigorous, of known identity, in a minimum of 10 and distant from each other at least 25 meters (to avoid a narrow genetic base). Depending of the species characteristics, the plants were produced either by seed or by vegetative propagation from shoot cuttings (Table 1). The nursery techniques used for the riparian species propagation followed the ones in Prada & Arizpe (2008). A combination of peat and vermiculite (1:1) was used as propagation medium, in plastic containers with 300 cm³ of volume per cell. After 5 months the plants were transferred to 2.5 liters forest pots, filled with a peat and vermiculite medium (2:1).

2.4 Plantation

Plantation took place in February 2008. A total of 575 plants, from 12 different species, were established in an area of 11000 m² (Table 14). To facilitate the plantation, the area was divided into several plots, each one with its specific species mix. There were two types of plots, riparian high density and dry low density (Figure 31), each one with a specific species mix and tree density (2460 trees/hectare in the former, 280 trees/hectare in the latter). The riparian species were planted in areas with high water table and the less flood tolerant strawberry tree (*Arbutus unedo* L.), umbrella pine (*Pinus pinea* L.), cork oak (*Quercus suber* L.) and tamarisk (*Tamarix africana* Poir.) were planted in dryer locations. All the plant pots were color coded to avoid plantation errors, each color combination corresponding to a specific species. In addition, each plant location was marked using a wood stake with the same color-coded combination. The planting holes were opened using a mechanical mini backhoe and the plantation was done manually.

Table 14. Number of plants per species, propagation method and plant age at installation time.

SPECIES	NUMBER PLANTS	OF PROPAGATION METHOD	AGE (YEARS)
<i>Alnus glutinosa</i> (L.) Gaertn.	51	Seed	2-2.5
<i>Arbutus unedo</i> L.	36	Vegetative	2-2.5
<i>Celtis australis</i> L.	43	Seed	2-2.5
<i>Frangula alnus</i> Mill.	23	Vegetative	2-2.5
<i>Fraxinus angustifolia</i> Vahl.	71	Seed	2-2.5
<i>Pinus pinea</i> L.	37	Seed	1-1.5
<i>Populus nigra</i> L.	80	Vegetative	2-2.5
<i>Quercus suber</i> L.	22	Seed	1-1.5
<i>Salix atrocinerea</i> Brot.	72	Vegetative	2-2.5
<i>Salix salviifolia</i> Brot.	64	Vegetative	2-2.5
<i>Salix alba</i> L. (Ser.) subsp. <i>vitellina</i>	51	Vegetative	2-2.5
<i>Tamarix africana</i> Poir.	25	Vegetative	2-2.5



Figure 31. General schematics of the riparian high-density plots (light green) and the dry low-density plots (dark green). Light blue represents water surfaces.

2.5 Soil Bioengineering

The east bank of the lake had scarce natural vegetation but was not included in the plantation effort. Thus, to improve bank stabilization and bird habitat, in April

2008 soil bioengineering techniques were employed on that location. Bioengineering comprises a series of techniques that use live vegetation as an engineering material, alone or in combination with inert structures, for environmental remediation (Sangalli, 2008). The techniques used in the restoration were the following: two wattle fences (Figure 32), two live fascines (Figure 33), one live brush mattress (Figure 34), a cribwall (Figure 35), two coconut fiber planted rolls (Figure 36) and rhizome planting (20 units) (Figure 37). Technique descriptions are available in Zeh (2007) and Sangalli (2008). The live vegetation was collected on the undisturbed sections of the Paul da Goucha. All the poles and live cuttings were of *Salix atrocinerea* Brot. Planted fiber roles and rhizome planting employed locally sourced yellow flag (*Iris pseudacorus* L.) rhizomes.

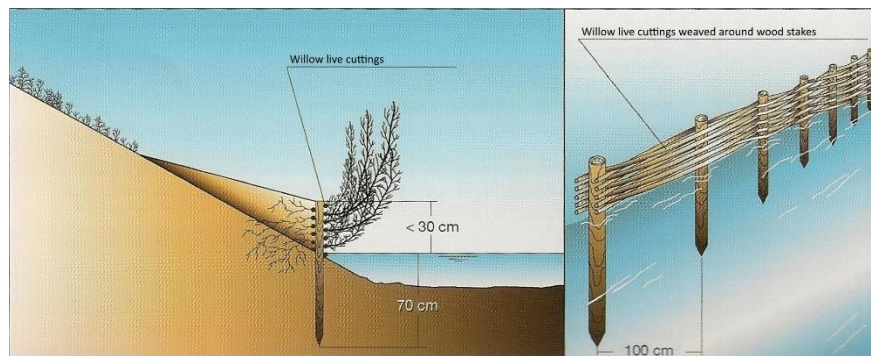


Figure 32. Wattle fences (adapted from Basora & Gutiérrez, 2008).

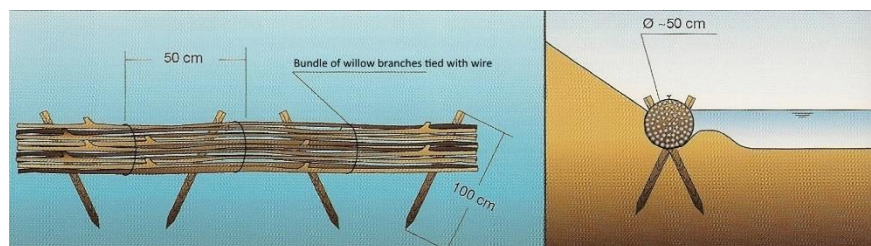


Figure 33. Live fascines (adapted from Basora & Gutiérrez, 2008).

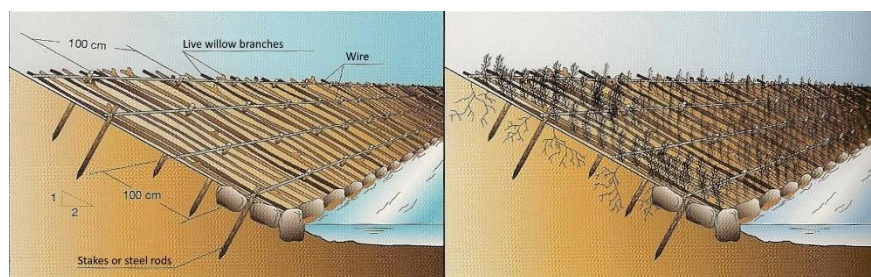


Figure 34. Live brush mattress (adapted from Basora & Gutiérrez, 2008).

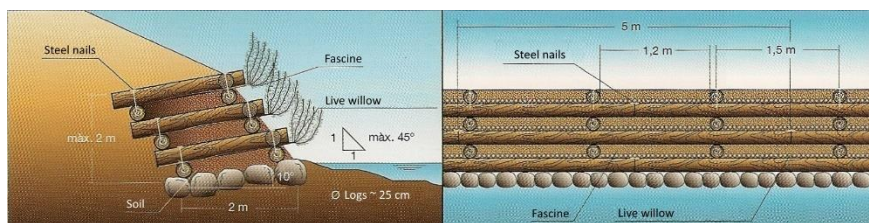


Figure 35. Cribwall (adapted from Basora & Gutiérrez, 2008).

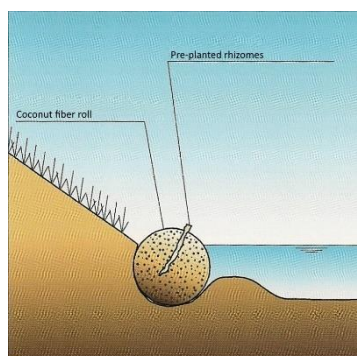


Figure 36. Coconut fiber planted rolls (adapted from Basora & Gutiérrez, 2008).

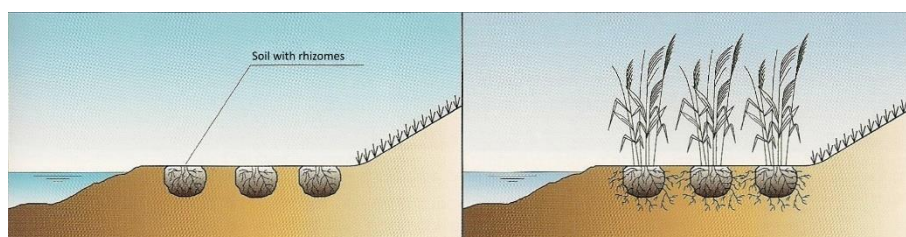


Figure 37. Rhizome planting (adapted from Basora & Gutiérrez, 2008).

2.6 Monitoring

Monitoring took place in October 2008 (6 months after planting), August 2014 (ca. 6 years after planting) and October 2017 (9 years after planting), always done by the same team. Evaluated parameters were plant survival and diameter at breast height (DBH), the latter only in 2014 and 2017. DBH was measured with a Mantax Blue caliper (©Haglöf). Stolen plants were recorded but counted as dead. Soil bioengineering interventions were evaluated through expert judgement.

2.7 Data analysis

Data analysis was made using IBM SPSS Statistics 23 (Release 23.0.0, 2015; IBM®, SPSS®) statistical software.

Data was analyzed using non-parametric tests, because normality assumptions were not met (according to the Kolmogorov-Smirnoff test for normality). A Kruskal-Wallis test was performed to ascertain if DBH distribution was the same between the different species. In order to compare DBH differences between species pairs, a Games-Howell multiple comparisons test using mean ranks was performed (Ruxton & Beauchamp, 2008; Maroco, 2010). A Mann-Whitney test was performed to assess for statistically significant differences in global average DBH between 2014 and 2017.

3. RESULTS

3.1 Plant survival

Average plant survival, globally and by species, for the three monitoring periods is shown in Figure 38. Global average survival after 6 months was 67.8%. In this period, the best survival results were achieved by *T. africana* (100%), *C. australis* (95.4%) and *Q. suber* (81.8%). The lowest survival rates were those from *A. unedo* (52.8%) and *P. nigra* (53.8%), although these lower results were influenced by the number of plants remaining after theft events. These two species were the most attractive for robbers, with respectively 16.7% and 22.5% of the installed plants being stolen. Other appealing species for robbers were *S. alba* subsp. *vitellina* (8.3% theft) and *A. glutinosa* (7.8% theft).

Global average survival after 6 years was 60.7%. The best survival results were achieved by *S. alba* subsp. *vitellina* (101.4%) and *S. atrocinerea* (100.0%), followed by *F. angustifolia* (74.7%). The lowest survival rates were those from *T. africana* (4.0%) and *F. alnus* (4.4%). Most of the highest values (including those above 100%) observed 6 and 9 years after planting account for natural regeneration from the seed bank and adjacent reproductive tree sources.

Global average survival after 9 years was 69.7%. The best survival results are the ones from *P. pinea* (148.7%), *Q. suber* (118.2%), *S. atrocinerea* (109.4%) and *F. angustifolia* (81.7%). The lowest survival rates were those from *T. africana* (0.0%), *F. alnus* (0.0%) and *A. unedo* (27.8%).

Figure 39 illustrates the vegetation induced landscape change along the years.

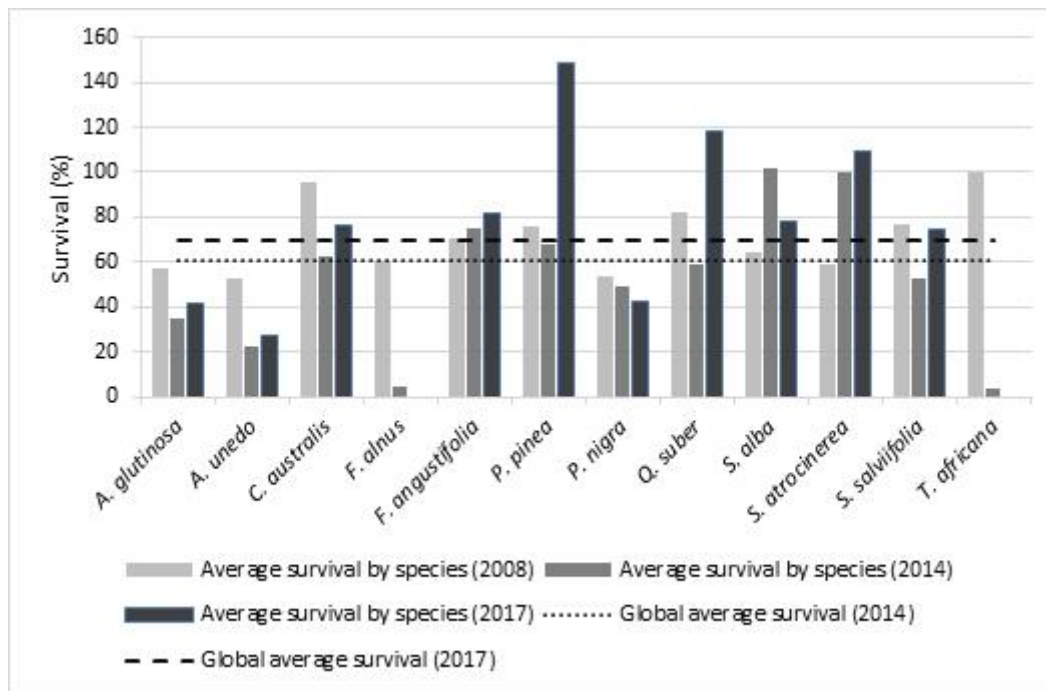


Figure 38. Average plant survival, globally and by species, after 6 months, 6 years and 9 years.

3.2 Plant DBH

Global average DBH was significantly different between the two monitoring periods ($U=33703.5$ $p \leq 0.05$) (Figure 40). There were significant differences between the average DBH of the installed plant species after 6 years [$H(6)=106.155$, $p=0.000$, $n=263$]. The average DBH of *P. nigra* (17.5 ± 1.6 cm, $n=38$) and *S. alba* subsp. *vitellina* (12.2 ± 0.9 cm, $n=61$) was significantly higher than in the other species, except for *A. glutinosa* (10.0 ± 1.2 cm, $n=18$) (Figure 41). The average DBH of *P. pinea* (3.6 ± 0.4 cm, $n=23$) and *F. angustifolia* (4.3 ± 0.5 cm, $n=46$) was significantly lower than in the other species, except for *S. salviifolia* (7.3 ± 1.6 cm, $n=17$) (Figure 41). *C. australis* (2.1 ± 0.6 cm, $n=5$) was not subjected to statistical analysis due to the very low number of individuals with a measurable DBH. Dominant DBH class was in the 5-7.5 cm class, with 60 cases, with 86% of the measured DBH's fitting into the 0-15 cm class (Figure 42).



Figure 39. Vegetation growth during the analyzed period. From top to bottom: February 2008; October 2008; September 2011; August 2014; October 2017.

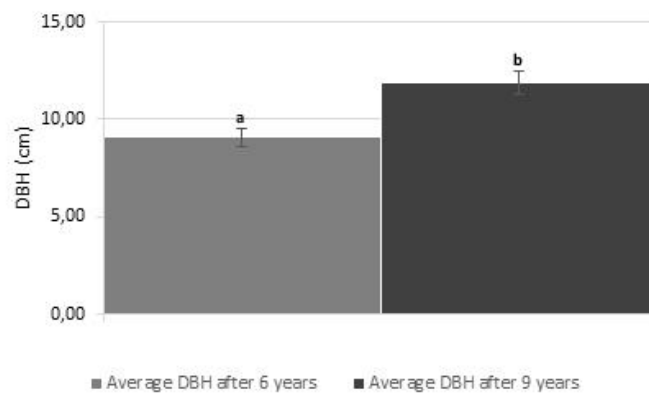


Figure 40. Global average DBH 6 and 9 years after plantation. The vertical bars are standard errors ($n=268$, $n=301$, respectively after 6 and 9 years). Different letters indicate significant DBH pairwise differences between monitoring season after Mann-Whitney U-test ($p<0,05$).

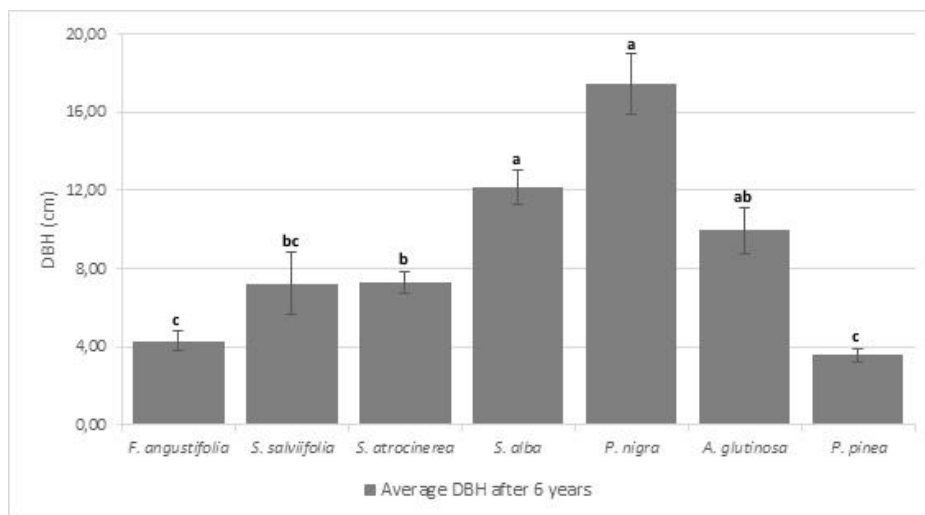


Figure 41. Average DBH by species 6 years after plantation. The vertical bars are standard error ($n=46$, $n=17$, $n=60$, $n=61$, $n=38$, $n=18$, $n=23$, respectively, from left to right). Different letters indicate significant DBH pairwise differences between species after Games-Howell's test ($p<0.05$). *C. australis* was excluded from the statistical analysis due to the very low number of individuals with a measurable DBH.

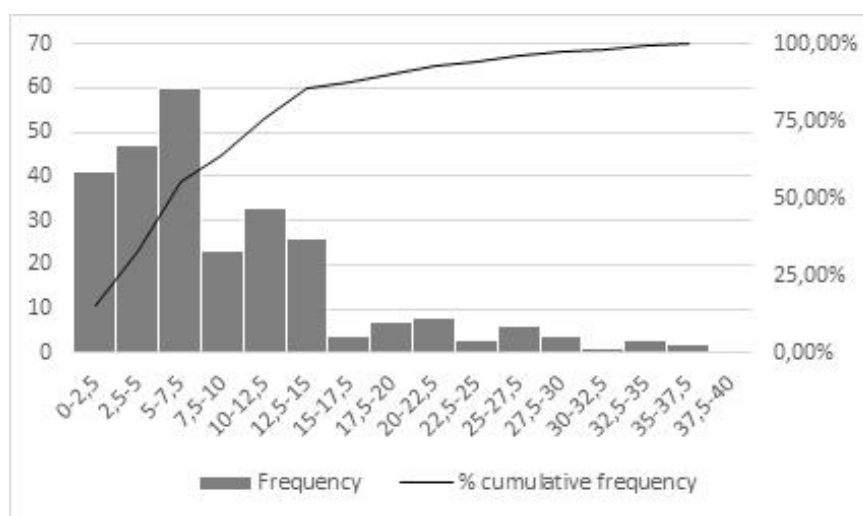


Figure 42. Frequency histogram with DBH class distribution (cm) 6 years after plantation.

There were also significant differences between the average DBH of the installed plant species after 9 years ($H(7)=126.302$, $p=0.000$, $n=297$). The average DBH of *P. nigra* (27.2 ± 2.0 cm, $n=34$) was significantly higher than those in the other species, except for *A. glutinosa* (16.1 ± 1.7 cm, $n=20$) (Figure 43). The average DBH of *C. australis* (1.7 ± 0.6 cm, $n=20$) was significantly lower than in the other species (Figure 43). *A. unedo* (0.9 ± 0.2 cm, $n=4$) was not subjected to statistical analysis due to the very low number of individuals with measurable DBH. Dominant DBH classes was in the 0-2.5 cm class, with 43 cases, and in the 5-

7.5 cm class, with 42 cases, with 90% of the measured DBH's fitting into the 0-22.5 cm class (Figure 44).

Exotic plant invaders present at the restoration area 9 years after planting are *Myriophyllum aquaticum* (Velloso) Verdc., *Arundo donax* L., silver wattle [*Acacia dealbata* Link (n=2)] and black locust (*Robinia pseudoacacia* L.) (average DBH 4.5 ± 1.3 cm, n=18). Also present is the native species *Rubus ulmifolius* Schott, which likewise displays invasive behavior.

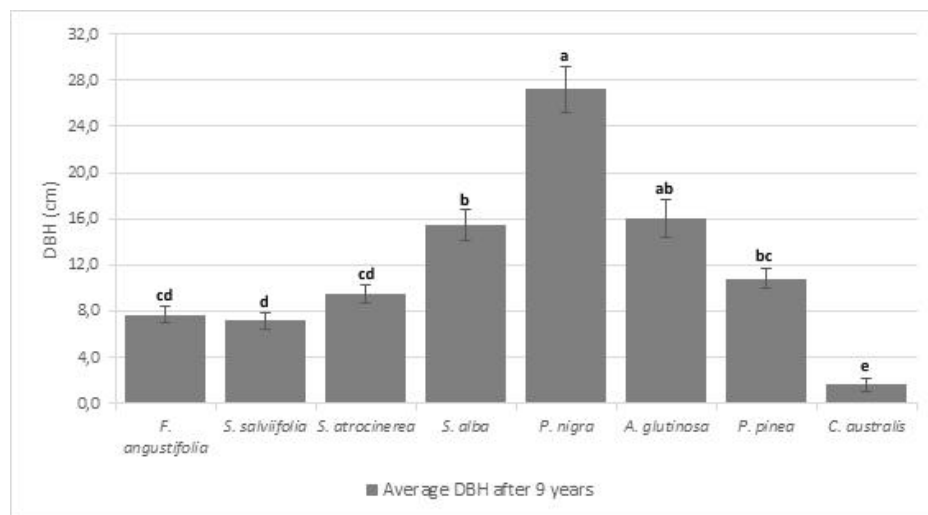


Figure 43. Average DBH by species 9 years after plantation. The vertical bars are standard error (n=54, n=29, n=64, n=52, n=33, n=20, n=24, n=20, respectively, from left to right). Different letters indicate significant DBH pairwise differences between species after Games-Howell's test ($p < 0.05$). *A. unedo* was excluded from the statistical analysis due to the very low number of individuals with a measurable DBH.

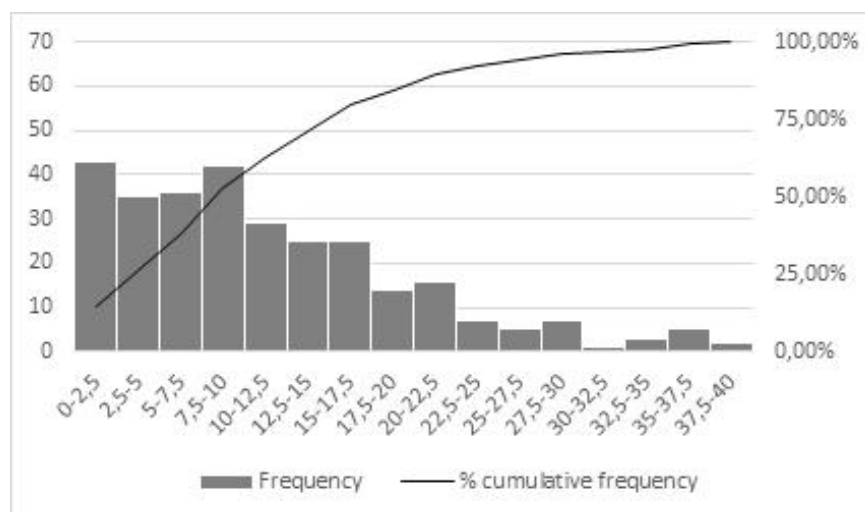


Figure 44. Frequency histogram with DBH class distribution 9 years after plantation.

3.3 Soil bioengineering

After 6 years, the wattle fences and the live fascines were successful, presenting multiple sprouts. The *I. pseudacorus* plants in the coconut fiber rolls and those installed from rhizomes fragments presented good vegetative vigor. The live brush mattress was destroyed by human intervention and must be considered as unsuccessful. Nevertheless, it still had some sprouts. The cribwall only had one vigorous sprout. After 9 years, both the wattle fences and the live fascines were fully successful, with the former having a higher number of sprouts than the latter. The planted coconut fiber rolls and the rhizomes had a 100% survival rate. All those techniques are indistinguishable from the surrounding vegetation, providing a significant contribution for margin stabilization and wildlife habitat. The cribwall structure, although only exhibiting a single adult *S. atrocinerea* tree, is contributing for the stabilization of an access road embankment.

4. DISCUSSION

Global average plant survival was positively influenced by the natural regeneration process. Therefore, the average plant survival was good, especially due to the high natural regeneration of *Salix* spp. and *F. angustifolia*. There was also high natural regeneration of *Q. suber* (from seed and eventual root sprouts) and *P. pinea* (from seed sources). That result was expected, due to the surrounding vegetation composition (mixed stands of cork oak and stone pine). Gravity, small rodents and birds (Olrik *et al.*, 2012) disperse oak seeds. It was established that bird dispersal of *Q. suber* acorns can go as far as 500 meters from the parent tree (Pons & Pausas, 2007). Also, *Q. suber* has the capacity to sprout from the lignotuber, a swollen underground root structure with dormant buds (Verdaguer *et al.*, 2001). *Pinus pinea* seeds are dispersed under or nearby the parent tree, usually no more than two crow radii for the average tree (van Wilgen & Siegfried, 1986; Manso *et al.*, 2012). Plant survival was particularly high in spots installed in locations with a higher water table. This should be expected, as water is the main regulation factor in forested wetlands (Calhoun, 1999). Nevertheless, the restoration area is located at the edge of the Paul da Goucha, at a slightly higher quota than the adjacent wetland. Thus, it is less prone to periodic high flows that cause channel movement and sediment deposition.

Therefore, some of the conditions necessary for the natural regeneration of some plant species are less frequent (Hughes, 2003; Hughes *et al.*, 2005).

The fact that *F. alnus* individuals were planted mostly in waterlogged locations may be the reason for the high mortality of this species. That is because although they require water during the growing season, they do not tolerate long periods of inundation (Evette *et al.*, 2009; Fiedler & Landis, 2012). Other possible cause was the rapid development of helophytes, like the native common reed (*Phragmites australis* (Cav.) Trin ex.Steud.), that muffle some of the installed plants. *Tamarix africana* failure was also probably related to the location chosen for this species, in an exposed sandy and very dry hill slope.

After 9 years, plant cover in the riparian high-density plots is higher than 90%. Plant cover in the dry low-density plots is sparse, partly intentionally, owing to the low planting density, but also due to the recent introduction of cattle into the restoration area. Initial plant density in these plots was quite low, averaging 280 plants/hectare, much less than the recommended 2000-5000 plants/hectare (Mitsch & Gosselink, 2015). Cattle grazing, together with mechanical understory cleaning, destroyed the majority of the smaller natural regeneration and destroyed or damaged some trees. Future restoration efforts should therefore consider cattle exclusion. Also, future *Salix* spp. and *P. nigra* cutting collection for nursery plant production should consider the sex of the donor plants, to have a proper mixture of male and female plants in the community (Landis *et al.*, 2003), improving the conditions for natural regeneration from seed.

Although *P. nigra* and *S. alba* subsp. *vitellina* had high average DBH, plant numbers did not increase between the 2014 and 2017 monitoring seasons. Concerning the former, the decrease in plant numbers may be related to the lowering of the water table, because riparian cottonwoods are dependent on shallow alluvial groundwater (Rood *et al.*, 2003). Regarding the latter, one possible explanation may be the fact that some *Salix* spp. individuals were cut down to facilitate cattle access to water.

Global DBH class distribution after 9 years is more balanced than 3 years before, with a gradual frequency reduction from the lower class to the higher class, *i.e.*, a DBH distribution close to that typical of a unevenaged, multicohort stand with

natural regeneration (Smith *et al.*, 1997). This may be considered a very favorable indicator of the restoration success, taking into account the objectives that were defined for this experiment.

Less favorably, several alien invaders were present and spreading in the restoration area. The smoothing of the banks of the lake favored the expansion of *M. aquaticum*, already occurring in several places of Paúl da Goucha. This species has a preference for habitats with low flow velocity and low water depth (Ochs *et al.*, 2018). The removal of *A. donax* rhizomes does not seem to have been enough by itself to avoid further colonization by this species. It is an environment tolerant invader (Quinn & Holt, 2008), that disperses mainly by vegetative propagation, and its clones can spread for hundreds of meters along streams (Mariani *et al.*, 2010). It is widespread in Portugal, developing dense stands in disturbed river corridors, particularly in coastal calcareous areas (Aguiar & Ferreira, 2005; Aguiar *et al.*, 2007). Frequently its control requires chemical methods, notably with glyphosate (Spencer *et al.*, 2008), although its use may have specific detrimental effects on keystone macroinvertebrate species (Puértolas *et al.*, 2010). After 9 years, this invasive species occupies an area of about 1600 m² within the study site. The relatively large number of *R. pseudoacacia* present at the site is intriguing, one possible cause being the contamination of the topsoil with seeds from this species. *Robinia pseudoacacia* is a problematic riparian invader in Europe (Vítková *et al.*, 2017), and may cause plant richness loss and shifts in species composition (Benesperi *et al.*, 2012). It is likely that the restoration activities facilitated alien plant invasions (Catford & Jansson, 2014). That problem can be attenuated with permanent monitoring and support of the requalified area (Lapin *et al.*, 2016).

Six months after the end of the restoration works there was a fast colonization of the aquatic surfaces by aquatic birds, like *Ardea cinerea* (Linnaeus, 1758), *Anas platyrhynchos* (Linnaeus, 1758), *Gallinula chloropus* (Linnaeus, 1758) and *Charadrius dubius* (Scopoli, 1786) (Mendes *et al.*, 2008).

The cribwall relative failure was probably due to being constructed on a dry location, to help stabilize a road embankment. This should be expected, as survival of willow cuttings is influenced, among others, by elevation relative to water table (Pezeshki *et al.*, 2007). Additionally, the top of the cribwall should

have been finished with a layer of soil up to the road quota, followed by the installation of rooted plants. That final step was skipped in this experimental restoration, due to logistic difficulties. Nevertheless, the bioengineering techniques developed for Northern and Central European countries, need to be adapted to withstand the Mediterranean environmental conditions. The seasonal dryness of the Mediterranean climate, where most of the annual rainfall is concentrated in the winter months, compromises the survival of the tree cuttings, especially at the early stages after installation. Due to these harsh environmental conditions, it would be interesting to test the use of rooted plants (rather than live stakes) on some bioengineering techniques.

5. CONCLUSIONS

The project objectives were achieved as the area submitted to intervention has now a more complex plant community structure, with abundant natural regeneration and the presence of multiple feeding, breeding and shelter habitats for waterfowl. The pilot area restoration provided a good insight into the restoration needs and problems, notably concerning plant survival. It is also clear that riparian restoration is a long-term process and that it needs continuous monitoring to guide adaptive corrections. Tree survival and growth were satisfactory, although it is unclear if this restoration effort restored all of the ecological functions associated with the native wetland ecosystem (Avera *et al.*, 2015). Moreover, cattle grazing, and other types of human disturbance may endanger what was achieved so far. Thus, local population awareness and participation are as essential as water table levels and tree installation techniques for wetland restoration success.

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CHAPTER 5

Riverbank restoration in a temporary Mediterranean river

1. INTRODUCTION

Riverbanks are tridimensional spaces adjacent to the river that function as connectors between the aquatic and terrestrial systems (Dix *et al.*, 1997). They are subjected to periodic flooding and significant sedimentation and erosion processes. Its width may vary from a narrow strip at the headwaters and along the less important river sections, to a wide area on the slow river sections of the main rivers (González del Tánago & García de Jalón, 2001). Regardless of its width, usually they have different communities of plants and animals when compared to neighborhood zones outside the influence of the river hydrological regime, usually including higher species richness, structural complexity and biomass productivity than the surrounding areas (Hunter Jr., 1990; Cortes, 2004).

The assemblage of plant communities occurring along riverbanks is called riparian vegetation. These riparian galleries are a primordial component of the riverside environment. Their structure and heterogeneity is mainly controlled by the watercourses hydrological regime, as mentioned above, but also by longitudinal zonation and riverbank topography (González del Tánago & García de Jalón, 2006; Rodríguez-González *et al.*, 2010; Angiolini *et al.*, 2011; Booth & Loheide, 2012; Magdaleno *et al.*, 2014; Rivaes *et al.*, 2014; Marques, 2016). Thus, riparian vegetation evolved with specific morphologic, physiologic and reproductive strategies, such as the adaptation to the seasonal alternation of flooding and drought. As a result of this adaptive flexibility, some species are able to withstand temporary or permanent waterlogging (Hunter Jr., 1990; Hager & Schume, 2001), and others adapted to riverbank morphology changes by fast growth and strong vegetative propagation capability (Blanco Castro *et al.*, 2005). One of the adaptations to soil hypoxic conditions consists in the presence of aerenchyma, a spongy tissue that forms spaces or air channels in the stems and roots of some plant species (Calhoun, 1999); this tissue allows the exchange of gases between the leaves and the root system. On the other hand, species like poplars (*Populus* spp.), willows (*Salix* spp.) and alder (*Alnus* spp.) developed adventitious roots with some mechanical flexibility to withstand hypoxic conditions and shifting soil (Cortes & Ferreira, 1998; Calhoun, 1999).

Riparian woody species are important to supply matter and energy, as well as to regulate fluxes in aquatic and riparian ecosystems (Mitsch & Gosselink, 2015). Riparian vegetation influences water temperature (Schiemer & Zalewski, 1991; Bowler *et al.*, 2012; Kalny *et al.*, 2017) and prevents pollutants and nutrients from entering the channels through direct runoff or subsurface flow (Lowrance *et al.*, 1984, 1997; Schiemer & Zalewski, 1991; Osborne & Kovacic, 1993; Dosskey *et al.*, 2010). Also, in Mediterranean streams, pools well shaded by the riparian vegetation may have more diverse and abundant native fish populations (Pires *et al.*, 2010). In addition, riparian forests are a food source for aquatic organisms (Gregory *et al.*, 1991; Barnes *et al.*, 1998; González del Tánago & Garcia de Jalón, 2001). They also influence many geomorphological processes, mainly by reducing riverbank erosion, enhancing sediment retention, creating habitats and feeding the river channel with woody debris that contribute to river habitat structuring (Gregory *et al.*, 1991; Piégay & Maridet, 1994).

Due to its dynamic character, these areas are especially vulnerable to changes caused by human activity (Brinson & Verhoeven, 1999). Thus, river plant communities are very susceptible to anthropogenic impacts like hydrological disturbance caused by the lowering of the water table (e.g. Lewin, 1992; Cortes & Ferreira, 1998; Brinson & Verhoeven, 1999; Gasith & Resh, 1999; Tkach, 2001; Aguiar & Ferreira, 2005; Salinas & Casas, 2007; Mitsch & Gosselink, 2015). Additionally, riparian galleries may be severely affected by the expansion of urban and/or agricultural areas along riverbanks, since the ready availability and access to water provide strong incentives for economic development (Larsen, 1994; Gasith & Resh, 1999; Duarte *et al.*, 2002; Angradi *et al.*, 2004). Some other factors giving rise to the degradation of river systems and wetlands are land drainage, tree clearing, river channelization and river impounding (Lewin, 1992; Klimo, 2001; Machar, 2001; Aguiar *et al.*, 2001; Mant *et al.*, 2012; Mitsch & Gosselink, 2015).

Restoration aims to return an ecosystem to a more natural state after human disturbance (Frelich & Puettmann, 1999). However, full ecological restoration is often difficult because the nature of the original ecosystem may be unknown or impossible to achieve due to historical events or complex evolution trajectories (Hughes *et al.*, 2005; Lamb, 2009; Dufour & Piégay, 2009; Jacobs *et al.*, 2015).

Wortley *et al.* (2013), who surveyed 301 ecological restoration scientific articles, reports that only 9% addressed riparian restoration, with plantation being the most common method used to restore the ecological condition. In this context, revegetation is essential for the recovery of ecosystem functions (Aust *et al.*, 1990), and the utilization of high quality seedlings is paramount for plantation success (Villar-Salvador *et al.*, 2009).

Due to their characteristics, riparian environments can contribute to ecological adaptation to climate change (Seavy *et al.*, 2009), which is transforming our environment and creating new challenges to ecological restoration. However, meteorological changes will significantly affect European river flow regimes, mainly through more pronounced low flow periods in the Mediterranean region (Schneider *et al.*, 2013). In contrast, increased heavy rain events in winter may increase the risk of flooding (IPCC, 2008). These potential modifications in river flow regimes will likely be amplified by future climate change interactions with anthropogenic pressures, such as increased water withdrawals to satisfy human needs (Alcamo *et al.*, 2007; Murray *et al.*, 2012). Pluvial flow regimes with deep seasonal gaps between flooding and drought extremes, like the ones in southern European rivers, are those likely to experience more pronounced riparian vegetation changes (Rivaes *et al.*, 2014). Moreover, younger and more water-dependent individuals are expected to be the most affected by climate change (Rivaes *et al.*, 2013, 2014).

Located in a sensitive Natura 2000 protected area, the construction of the Odelouca river dam (Algarve region, Portugal) was subject to diverse compensation measures. Therefore, a detailed appraisal of the entire catchment was carried out and rehabilitation guidelines were defined (Fernandes *et al.*, 2007; Cortes *et al.*, 2015). Within this context, it was decided to undertake the environmental restoration of selected river segments downstream from the dam, which were also impacted by intensive permanent agricultural crops (Cortes *et al.*, 2015). The well-preserved middle course riparian communities, to be cleared and submerged by the dam, were used as reference sites for the rehabilitation of the selected degraded river sections. Fully restoring natural riparian forest in such intensively transformed river segments was unrealistic, but local-scale recovery of riparian plant communities has been considered an achievable target. The

objective was to trigger/improve natural riparian vegetation colonization, increase riverbank stability, control invasive species (mainly giant reed, *Arundo donax* L.), and improve river channel habitat for endemic freshwater fish populations. We hypothesized that the implementation of classical soil bioengineering techniques, combined with the plantation of riparian forest species seedlings produced through forest nursery methods, might have enough quality to achieve the proposed objectives.

2. METHODS

2.1 Study Area

The Odelouca River (83 km long, 511.4 km² basin area) is the largest tributary of the Arade River, which is situated in the Algarve region, in the south of Portugal (Figure 45). The river raises in the Caldeirão Mountain, at 509 meters altitude, and flows through a relatively narrow valley (NEMUS, 2006). Floodplain width ranges from 20 to 200 meters, and drainage basin average slope is 26% (NEMUS, 2006). It is an intermittent Mediterranean type fluvial system, with limited water availability and a hydrological regime characterized by a strong climatic induced seasonality (Ferreira & Aguiar, 2006).

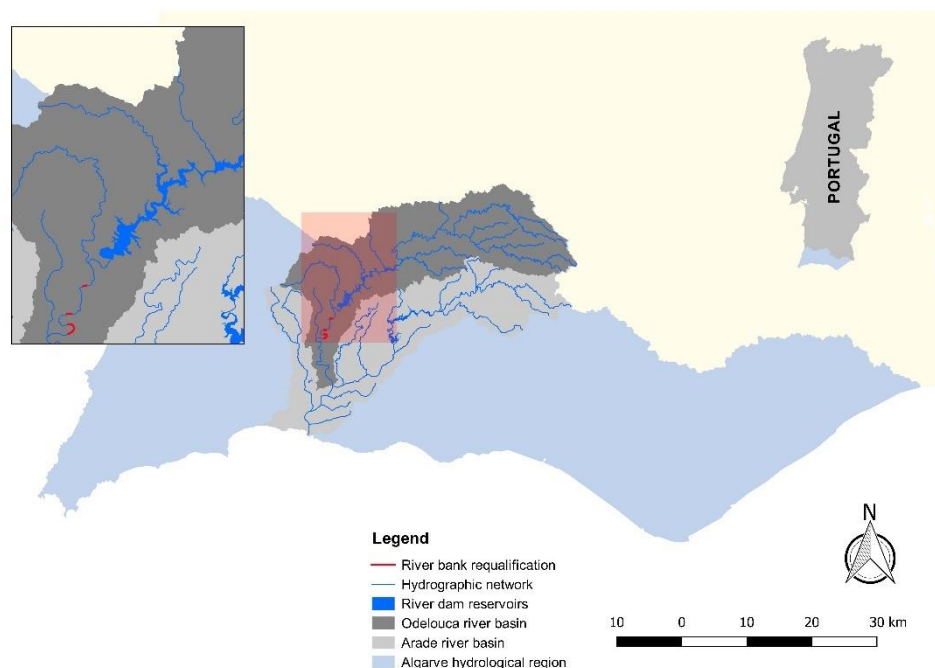


Figure 45. Location of the Odelouca river basin with the requalified river sections.

The Odelouca dam main purpose is to provide drinking water for the Algarve region. It started operating in May 2012 (AdA, 2016). The Odelouca reservoir's water surface elevation level ranges from 72 meters (minimum level requirement for operating the dam) to 102 meters (maximum storage capacity); its storage capacity is of 157 hm³ (NEMUS, 2006; AdA, 2016).

The climate of the site is of the Mediterranean type, characterized by hot and dry summers and mild and wet winters. According to the weather survey station of Faro (37° 01' lat. N, 07° 59' long. W, 8 m a.s.l., 1971-2000), mean annual rainfall is 509.1 mm, with 2.4% of it occurring between June and August and the mean annual temperature is 17.4°C, ranging from a monthly mean of 11.7°C in January to 23.7°C in August (IPMA, 2017). According to the Rivas-Martínez bioclimatic classification system (Rivas-Martínez *et al.*, 2011), this area is classified as Mediterranean pluviseasonal continental, with a lower inframediterranean thermotype and a lower dry ombrotype (Figure 46).

The lithology of the Odelouca basin is composed essentially of sedimentary and metamorphic formations, mainly shales and greywacke (NEMUS, 2006). According to the European Soil Database (ESBD v2.0, 2004; IUSS Working Group WRB, 2015), soils in the basin are mostly Eutric Regosols (82% of the area), with the presence of some Haplic Luvisols (13% of the area).

There are three Natura 2000 Network Sites of Community Importance (SCIs) that cover large portions of the Odelouca river basin, namely SCI Caldeirão (PTCON0057), SCI Monchique (PTCON0037) and SCI Arade/Odelouca (PTCON0052) (Figure 47). Two critically endangered endemic fish species are present in the Odelouca basin (Santos & Ferreira, 2008), namely the Iberian Chub [*Squalius aradensis* (Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998)] and the Iberian nase [*Iberochondrostoma almakai* (Coelho, Mesquita & Collares-Pereira, 2005)].

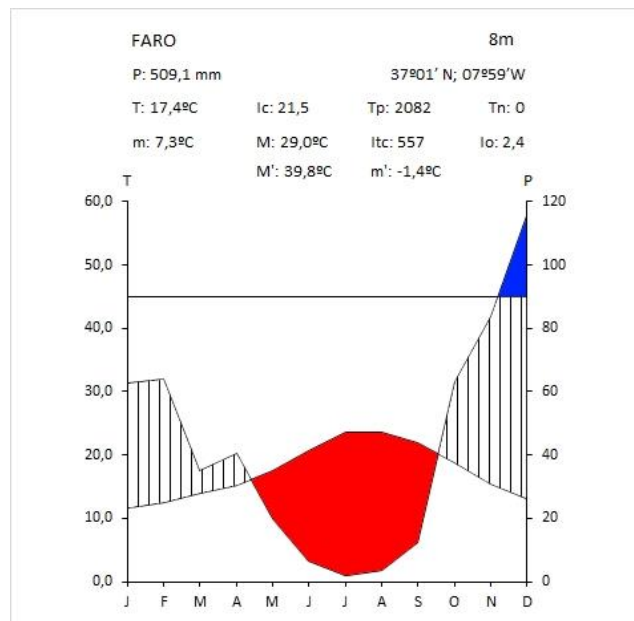


Figure 46. Bioclimograph from Faro weather station (1971-2000) according to (Rivas-Martínez et al., 2011). Blue color represents the wet period (precipitation higher than 90 mm), red color represents the dry period (precipitation lower than twice the average monthly temperature) and vertical lines represents precipitation higher than twice the average monthly temperature. Graphics are represented in a Cartesian coordinate system with a double scale, adjusted to $P \text{ mm} = 2T^{\circ}\text{C}$. Y-axis shows the monthly temperature and precipitation averages, and the x-axis shows the months of the year.

Riparian tree cover in the Odelouca river basin is dominated by *Salix salviifolia* Brot., common alder (*Alnus glutinosa* (L.) Gaertn.), oleander (*Nerium oleander* L.), and narrow-leaved ash (*Fraxinus angustifolia* Vahl.) (Hughes et al., 2009). Other common riparian species in the basin are rusty sallow (*Salix atrocinerea* Brot.), tamarisk (*Tamarix africana* Poir.), alder buckthorn (*Frangula alnus* Mill.), common hawthorn (*Crataegus monogyna* Jacq.), and Spanish heath (*Erica lusitanica* Rudolphi) (Hughes et al., 2009). The exotic invader giant reed (*Arundo donax* L.) is also abundant. Upland contiguous forests are dominated by cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L. subsp. [Desf.] Samp. *ballota*), with the presence of Tasmanian blue gum (*Eucalyptus globulus* Labill.) and maritime pine (*Pinus pinaster* Aiton) commercial plantations (Hughes et al., 2009; Rivaes et al., 2013). Cork oak woodland downstream from the dam was replaced by agriculture (mainly citrus groves) and by domesticated ruminants grazing (cattle, goats and sheep) (Hughes et al., 2009).

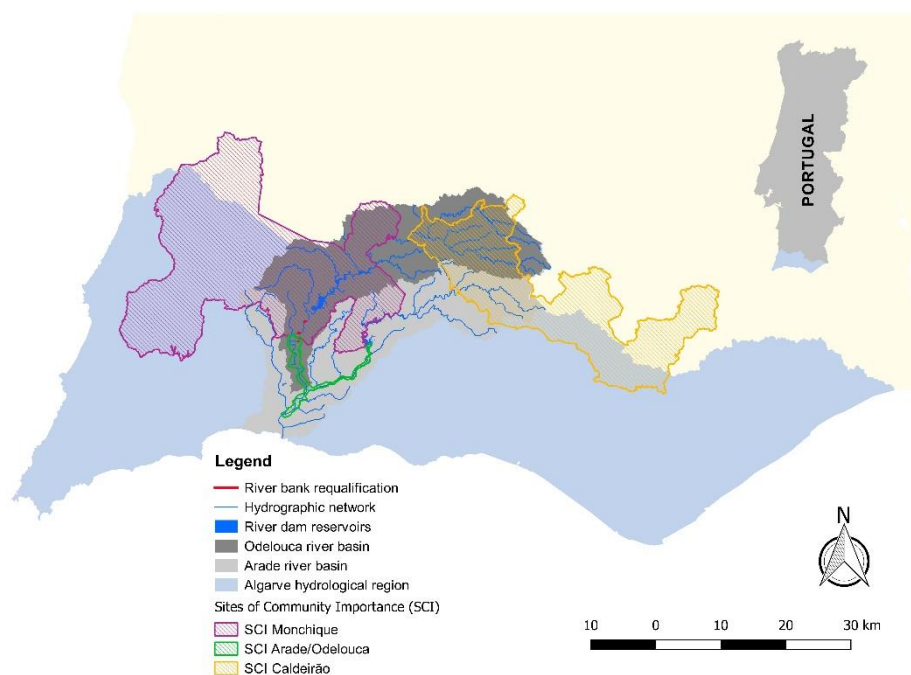


Figure 47. Natura 2000 Network Sites of Community Importance (SCIs) in the Odelouca river basin (APA, 2017).

2.2 Plant Production

The plants were produced in the Instituto Superior de Agronomia forest nursery. Floristic surveys were carried out in the Odelouca river basin, but also within the Algarve region, in areas ecologically similar to the study site, to identify suitable plant propagule collection areas. Seeds and cuttings were collected in those areas, in trees free from diseases, vigorous, of known identity, in a minimum of 10 and distant from each other at least 25 meters (to avoid a narrow genetic base). Depending of the species characteristics, the plants were produced either by seed or by vegetative propagation from shoot cuttings (Table 15). The nursery techniques used for the riparian species propagation followed the ones in (Prada & Arizpe, 2008). A combination of peat and vermiculite (1:1) was used as propagation medium, in plastic containers with 300 cm³ of volume per cell. After 5 months the plants were transferred to 2.5 liters forest pots, filled with a peat and vermiculite medium (2:1).

Table 15. Plant species, propagation method and plant age at installation time.

SPECIES	PROPAGATION METHOD	AGE (YEARS)
<i>Frangula alnus</i> Mill.	Vegetative	2-2.5
<i>Fraxinus angustifolia</i> Vahl.	Seed	2-2.5
<i>Nerium oleander</i> L.	Seed and Vegetative	2-2.5
<i>Salix salviifolia</i> Brot.	Vegetative	2-2.5
<i>Tamarix africana</i> Poir.	Vegetative	2-2.5

2.3 Riverbank restoration

The restoration area is located downstream of the Odelouca dam and consists of six different river sections (Figure 48). The riverbank restoration was done using classical bioengineering techniques modified to use mostly rooted plants instead of live stakes. The majority of the techniques were used to rehabilitate highly eroded and degraded riverbanks due to human disturbance (Table 16).

Table 16. Restoration sections characteristics.

River section	Length (m)	Bank	Characterization (AdA, 2011)
F	115	right	Unstable riverbank; giant reed invasion
G	85	right	Some giant reed; no riparian vegetation
H	12	n/a	n/a
I	240	left	Unstable steep riverbank; giant reed invasion
K	205	right	Strong erosion; coarse sediments; no riparian vegetation
M	155	right	Strong erosion; vertical slope

The restoration work took place in the Spring of 2012, following a project made by Professor Rui Cortes from the Universidade de Trás-os-Montes e Alto Douro (UTAD), and was carried out as follows (AdA, 2011):

Section F - *A. donax* removal, followed by the application of two overlapped organic geotextile mattresses (Figure 49) with 20 cm of topsoil between them. The toe of the bank was protected through a planted riprap foundation (Figure

49), that also functions as geotextile support; plantation of *S. salviifolia* (383 un.), *F. angustifolia* (153 un.), *N. oleander* (77 un.) and *T. africana* (51 un.).

Section G – Like section F; plantation of *S. salviifolia* (283 un.) and *F. angustifolia* (113 un.).

Section H - Construction of two islands to augment fish habitat heterogeneity. Each island was constructed with a foundation layer of large boulders, followed by an upper layer of two boulders, with a geotextile mattress between layers; gaps between boulders where filled with topsoil and gravel; plantation of *S. salviifolia* (16 un.) and *T. africana* (14 un.).

Section I - *A. donax* removal, followed by the reshaping of the bank with a geotextile mattress and topsoil, to reduce the slope. Construction of a vegetated log cribwall (Figure 49) over a planted riprap foundation; plantation of *S. salviifolia* (689 un.), *F. angustifolia* (65 un.), *N. oleander* (203 un.) and *T. africana* (130 un.).

Section K - The toe of the bank was protected through riprap (using stones obtained by the removal of material from the embankment). Installation of an organic mattress, followed by plantation of *F. angustifolia* (50 un.), *N. oleander* (340 un.), *T. africana* (400 un.) and *F. alnus* (30 un.); application of topsoil in each planting hole.

Section M - Construction of two overlapping rows of vegetated hard gabions (Figure 49), with rooted plants in the toe and between rows. Upper part of the embankment covered with topsoil and planted with riparian vegetation; plantation of *S. salviifolia* (312 un.), *F. angustifolia* (105 un.) and *N. oleander* (105 un.).

2.4 Monitoring

Monitoring took place in May 2017 (5 years after planting). Evaluated parameters were plant survival, stem basal diameter (SBD), and diameter at breast height (DBH). In sections H and I it was impossible to take SBD measurements due to stand density and strong undergrowth. SBD and DBH where measured with a Mantax Blue caliper (©Haglöf). Soil bioengineering interventions were evaluated through expert judgement.

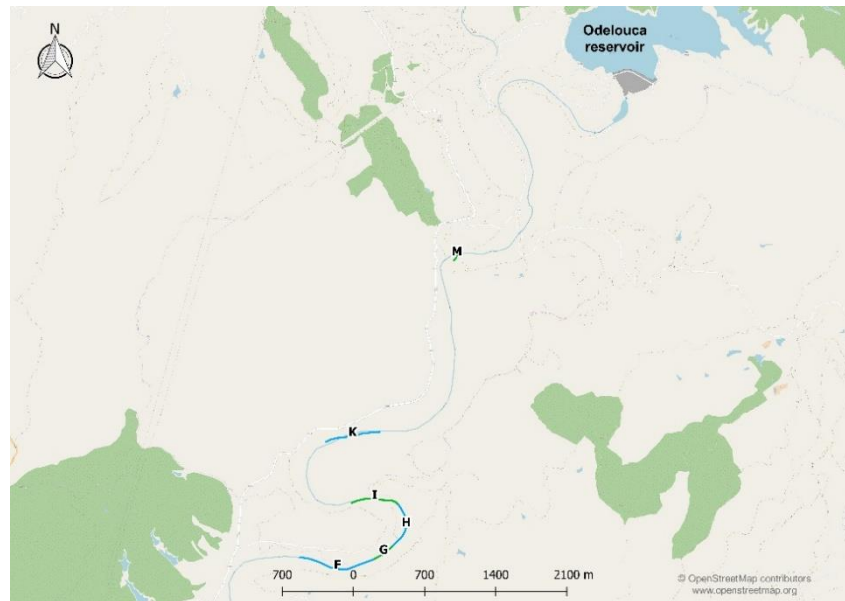


Figure 48. Spatial location of the restoration river sections. Section F is the furthest away from the dam and section M is the nearest.

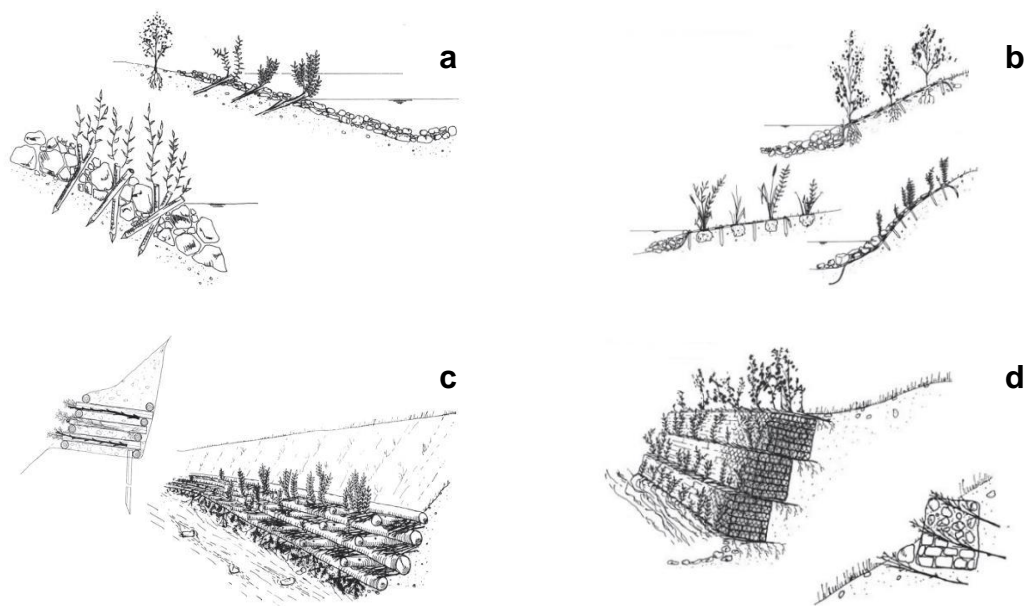


Figure 49. Soil bioengineering techniques. Planted riprap (a); Vegetated mattress (b); Vegetated log cribwall (c); Vegetated hard gabions (d). Adapted from Zeh (2007).

2.5 Data analysis

Data analysis was made using the integrated development environment RStudio (version 1.0.136) (RStudio Team, 2016) and R (version 3.3.2) (R Core Team, 2016) statistical software. Data was analyzed using non-parametric tests,

because the normality of residuals and homoscedasticity assumptions were not met. The distribution of the residuals was assessed using the D'Agostino Normality Test (D'Agostino *et al.*, 1990) through the fBasics R Package (version 3011.87) (Rmetrics Core Team *et al.*, 2014) and visually, through histograms and normal Q-Q plots. Homoscedasticity was assessed using the Brown-Forsythe Test (Brown & Forsythe, 1974) through the lawstat R Package (version 3.1) (Gastwirth *et al.*, 2017) and visually through residuals vs. fitted values plots. A Kruskal-Wallis test was performed to ascertain if DBH and SBD distribution were the same between the different species and between the different restoration sections. In order to compare DBH and SBD differences between species pairs and between section pairs, a Dunn multiple comparisons test using rank sums was performed (Dunn, 1964; Zar, 2010). This was done using the FSA R Package (version 0.8.17) (Ogle, 2017).

3. RESULTS

3.1 Plant survival

Global average plant survival was 46.2%. Survival results were very similar between species (Figure 50), except for *F. alnus*, which had a 0% survival rate. The best survival results were achieved by *T. africana*, with 51.6%, followed by *S. salviifolia* with 47.2%. *Fraxinus angustifolia* and *N. oleander* had very similar survival results, 43.6% and 43.0% respectively. Regarding plant survival in each requalified river section, the best results were achieved in section I, with 90.4%, followed by section H, with 43.3% (Figure 51). Section G had the lowest survival results, with 11.6%.

3.2 Plant SBD and DBH

There were significant differences between the average DBH of the installed plant species [$H(3)=50.601$, $p=0.000$, $n=266$]. The average DBH of *S. salviifolia* (3.4 ± 0.1 cm, $n=129$) was significantly higher than in the other species, except for *T. africana* (2.9 ± 0.3 cm, $n=36$) (Figure 52). The average DBH of *N. oleander* (1.6 ± 0.1 cm, $n=26$) was significantly lower than in the other species, except for *F. angustifolia* (2.8 ± 0.3 cm, $n=75$) (Figure 52).

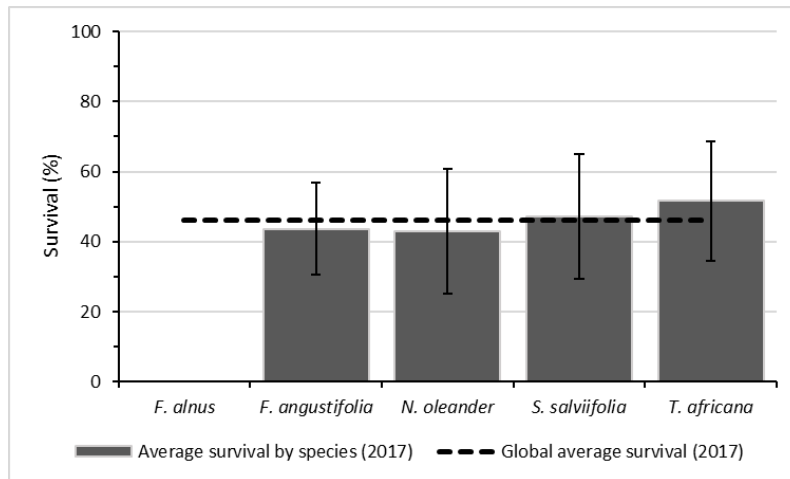


Figure 50. Average plant survival, globally and by species.

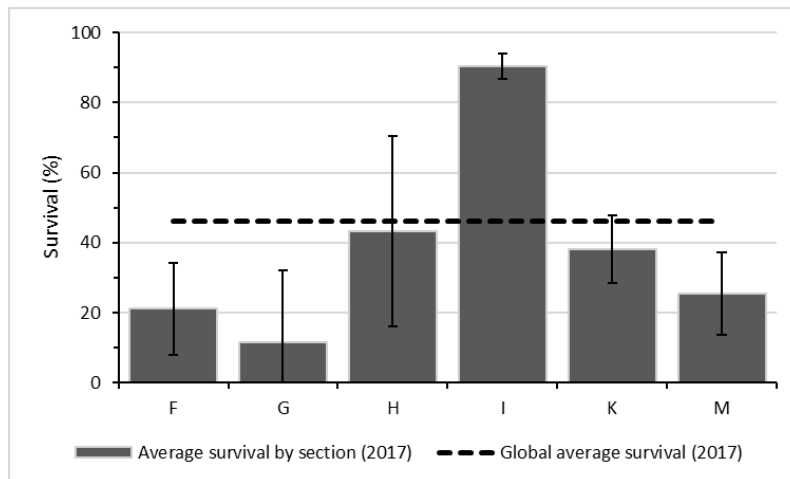


Figure 51. Average plant survival globally and by section.

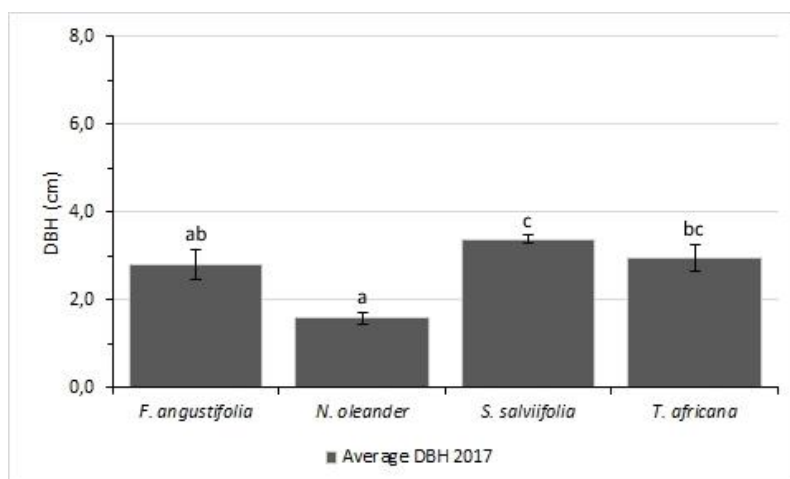


Figure 52. Average DBH by species. The vertical bars are standard error ($n=75$, $n=26$, $n=129$, $n=36$, respectively, from left to right). Different letters indicate significant DBH pairwise differences between species after Dunn's test ($p < 0.05$).

There were significant differences between the average SBD of the installed plant species [$H(3)=60.016$, $p=0.000$, $n=190$]. There were no significant differences between the average SBD of *S. salviifolia* ($5.7\pm0.8\text{cm}$, $n=8$), *N. oleander* ($5.0\pm0.3\text{cm}$, $n=41$) and *T. africana* ($3.6\pm0.3\text{cm}$, $n=50$) (Figure 53). The average SBD of *F. angustifolia* ($2.3\pm0.2\text{cm}$, $n=91$) was significantly lower than in the other species (Figure 53).

There were significant DBH differences between the requalified river sections [$H(5)=80.216$, $p=0.000$, $n=266$]. The average DBH in sections H ($4.5\pm0.4\text{cm}$, $n=13$) and M ($3.5\pm0.2\text{cm}$, $n=52$) was significantly higher than in the other sections (Figure 54). The average DBH in sections G ($0.9\pm0.1\text{cm}$, $n=21$) and K ($1.0\pm0.2\text{cm}$, $n=7$) was significantly lower than in the other sections (Figure 54).

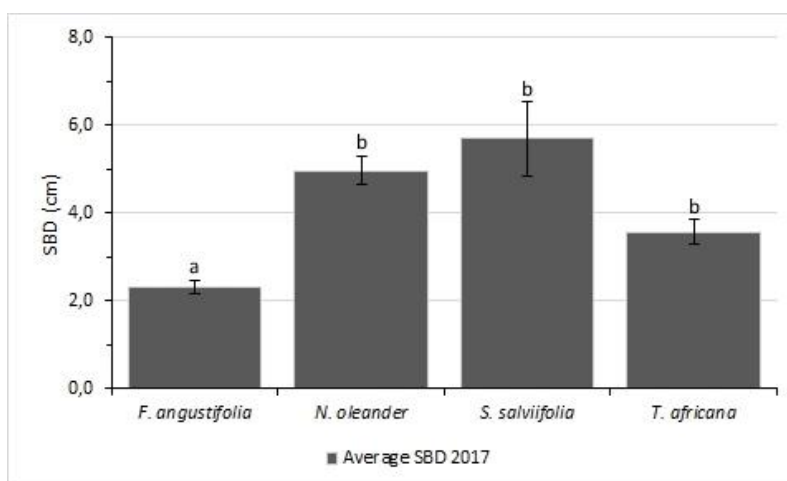


Figure 53. Average SBD by species. The vertical bars are standard error ($n=91$, $n=41$, $n=8$, $n=50$, respectively, from left to right). Different letters indicate significant DBH pairwise differences between species after Dunn's test ($p<0.05$).

There were significant SBD differences between the requalified river sections [$H(3)=34.011$, $p=0.000$, $n=190$]. The average SBD in section K ($4.1\pm0.2\text{cm}$, $n=85$) was significantly higher than in the other sections (Figure 55). The average DBH in section G ($1.9\pm0.1\text{cm}$, $n=24$) was significantly lower than in the other sections, except for section M ($2.0\pm0.2\text{cm}$, $n=10$) (Figure 55).

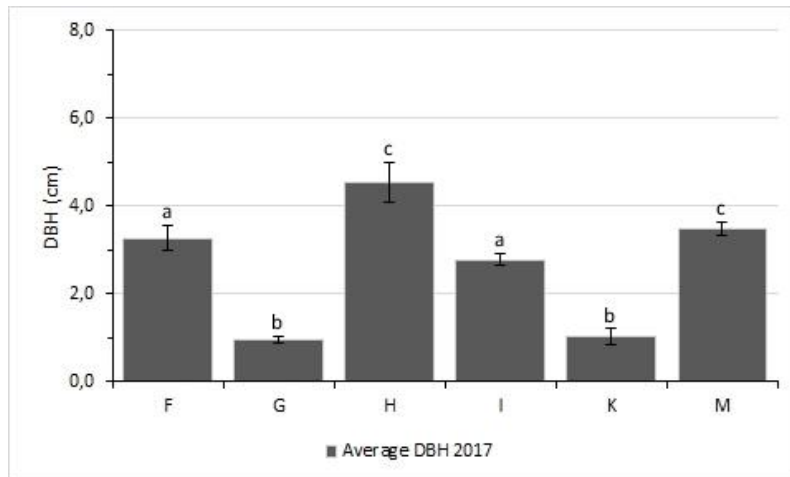


Figure 54. Average DBH by river section. The vertical bars are standard error ($n=89$, $n=21$, $n=13$, $n=84$, $n=7$, $n=52$, respectively, from left to right). Different letters indicate significant DBH pairwise differences between species after Dunn's test ($p<0.05$).

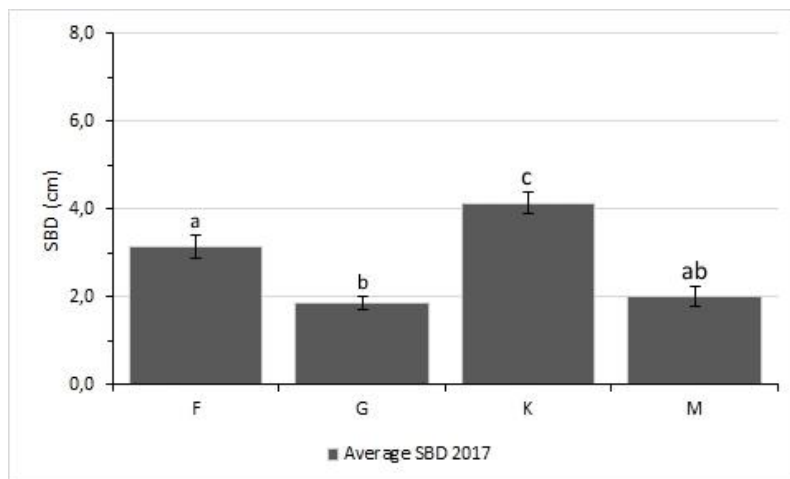


Figure 55. Average SBD by river section. The vertical bars are standard error ($n=71$, $n=24$, $n=85$, $n=10$, respectively, from left to right). Different letters indicate significant DBH pairwise differences between species after Dunn's test ($p<0.05$).

3.3 Soil bioengineering

Section F – River bank stabilization is being provided mainly by the geotextile mattress and spontaneous herbaceous vegetation, particularly in the higher sections of the embankment. There were signs of grazing in the surviving installed vegetation, mainly in *F. angustifolia*. It was observed strong sprouting of *A. donax*, with more than 100 clusters identified.

Section G - River bank stabilization is being provided mainly by the geotextile mattress and spontaneous herbaceous vegetation. Signs of grazing were

observed in the planted *F. angustifolia*, as well as sprouting of *A. donax*, with 10 identified clusters.

Section H – The islands are providing fish habitat, mainly to young of the year, although mean water level on the section is very low. Once more, there were signs of cattle grazing.

Section I – Riverbank is stabilized, and fully integrated with the surrounding natural area. There is a small area of *A. donax* sprouting.

Section K – The river bank is not fully stabilized due to the mortality and slow growth of the planted vegetation. Signs of frequent cattle grazing were also observed in this section. The topsoil was washed away.

Section M – Erosion was contained, the bank is fully stabilized. Gabions were covered by the planted vegetation. Vegetation in the upper part of the embankment was destroyed by mechanical intervention (*circa* 2015) and by grazing.

4. DISCUSSION

Plant survival and growth was conditioned by dry winters (2011/12, 2014/15 and 2016/17) and dry to very dry springs (2012, 2014, 2015 and 2017) (IPMA, 2012, 2013, 2014, 2015, 2016). This phenomenon put the planted species under considerable hydric stress and presented a threat to their survival.

Global plant survival rates were as expected for riparian forest restoration (e.g. Sweeney *et al.*, 2002; Keeton, 2008), although in our case heavily influenced by the above average survival results of Section I. Plant survival was highest in the lower areas of the riverbank. This should be expected, as species have different preferences along riverbank gradients, indicating their differential ability to cope with water stress (Magdaleno *et al.*, 2014). Additionally, water is the main regulation factor in forested wetlands (Calhoun, 1999). The very dry conditions, and the consequent water deficit, may be the reason for the high mortality of *F. alnus*. This species requires moist soils and weak summer drought in order to survive (Evette *et al.*, 2012; Castroviejo & Pizarro, 2015). Climate change, with

the reduction of spring rainfalls has adverse effects on *F. alnus* seed production and may help to explain its decline at its southwestern range limit (Hampe, 2005).

The Mediterranean climate is characterized by the striking annual (Rivas-Martínez *et al.*, 2011) and inter-annual variation in precipitation levels as a result of the North Atlantic Oscillation (NAO), which drives large variations in the river flow regime of the southern Iberia rivers (Trigo *et al.*, 2004). Thus, large scale climatic patterns should be taken into consideration when defining rehabilitation interventions (Hughes *et al.*, 2008).

The *F. angustifolia* average DBH values were positively influenced by the individuals in Section F that were in the lower zone of the bank, nearest to the river channel. Tree position relative to active channel is the main factor controlling *F. angustifolia* growth in the riverine environment (Marques, 2016). Survival and average DBH values of *S. salviifolia* and particularly *F. angustifolia* were negatively affected by grazing in the requalified sections. There was ample evidence of branch, twig and leave foddering. Livestock damaged the main shoots of many individuals, with new shoots sprouting from the remaining stem. *Fraxinus angustifolia* and *S. salviifolia* leaves are palatable to livestock, and are traditionally used as fodder in southern Europe (Fabião, 1996; Moore *et al.*, 2003; FRAXIGEN, 2005; Pereira *et al.*, 2008; Caudullo & Durrant, 2016). Grazing damage seemed to be more intense in Sections K, F and M than in the others. Livestock exclusion is paramount for the success of soil bioengineering based riparian restoration (e.g. Anstead *et al.*, 2012). This type of management action may also help to improve river water quality (Wilcock *et al.*, 2009, 2013).

The success of riverbank protection structures depends on the restoration area conditions (Buchanan *et al.*, 2012), the type of materials used (Evette *et al.*, 2009, 2012), and the implementation of a proper monitoring and maintenance program (Eubanks & Meadows, 2002; Zeh, 2007; Kondolf *et al.*, 2011). The post-intervention analysis of the soil bioengineering structures used in this restoration showed that the technical solutions employed were adequate. Regarding the visual impact of the techniques that employ large quantities of inert material in its construction, such as the vegetated log cribwall and the vegetated hard gabions, the former appears to be more similar to spontaneous patterns than the latter. This is similar to the results obtained by Cavaillé *et al.* (2015). However, some

problems related with anthropogenic pressure are evident. Besides the already mentioned livestock grazing, there was also a non-authorized mechanical intervention on a portion of the upper part of the Section M embankment. Apparently, the damage was related with the creation of a new access to the river for cattle use.

According to Cortes *et al.* (2015), three years after the restoration actions, the river sections presented highly degraded fish communities, dominated by very tolerant species, before and after restoration. The same author reported that the numbers of alien species varied considerably between river sections and years, but generally represented more than 25% of the total species composition. Also, the requalified sections have a low proportion of native invertivores cyprinids and native lithophilics (Cortes *et al.*, 2015). Although fish habitat has improved, the low native fish recover is probably related with pressures from organic non-point discharges, namely pig farm sewage, downstream from the Odelouca dam. *Squalius aradensis* and *I. almaiai* are strongly affected by this type of threat (Robalo *et al.*, 2009; Sousa-Santos *et al.*, 2009). In fact, large-scale disturbances may limit the capacity of river fishes to respond to restoration projects that take place in a relatively small area (Fausch *et al.*, 2002; Pretty *et al.*, 2003; McClurg *et al.*, 2007).

Future *Salix salviifolia* cutting collection for nursery plant production should consider the sex of the donor plants, to have a proper mixture of male and female plants in the community (Landis *et al.*, 2003), thus improving the conditions for natural regeneration from seed.

The removal of *A. donax* rhizomes does not seem to have been enough by itself to avoid further colonization by this species. The use of heavy machinery may have inadvertently helped to spread the species. The majority of new recruitments of *A. donax* grow from rhizomes fragments and land managers should avoid the use of heavy machinery to eradicate this species (Boland, 2008). *Arundo donax* is an environment tolerant invader (Quinn & Holt, 2008), that disperses mainly by vegetative propagation, and its clones can spread for hundreds of meters along streams (Mariani *et al.*, 2010). It is widespread in Portugal, developing dense stands in disturbed river corridors, particularly in coastal calcareous areas (Aguiar & Ferreira, 2005; Aguiar *et al.*, 2007). Its control

usually requires the use of chemical methods, notably glyphosate (Spencer *et al.*, 2008), although this herbicide may have specific detrimental effects on keystone macroinvertebrate species (Puértolas *et al.*, 2010).

The implementation of the restoration program was initially met with suspicion and resistance by landowners. The general perception was that the risk of flooding would increase with the restoration and that the planted riparian vegetation was of no commercial value. Thus, they expressed their preference for olive tree (*Olea europaea* L.) orchards, or similar cultures. After 5 years some of them still feel that the restoration was a useless intrusion on their land, and that the planted riparian vegetation does not serve any useful function. An effective approach to requalify and maintain riparian galleries must respect the concerns of landowners regarding flooding, economy or landscape, but also needs to emphasize the fundamental role of riparian forests in the ecosystem (Dutcher *et al.*, 2004; Chambers *et al.*, 2017). Thus, local stakeholder participation should be improved in future restoration efforts.

5. CONCLUSIONS

Although the elapsed time is still short for definitive conclusions, project objectives were partially fulfilled. Natural riparian vegetation cover has improved in the requalified areas and riverbank stability was enhanced, particularly in Sectors I and M. The control of the exotic invader *A. donax* was less successful, with a slow but steady increase of the number of patches of this species. Also, although native fish habitat heterogeneity and quality has improved, it was not followed by an increase in *S. aradensis* and *I. almakai* populations, probably because the populations in the area are too impoverished to respond to such short period and limited area of restoration. Riverbank restoration in Mediterranean areas using soil bioengineering techniques needs careful management in the early years, particularly regarding plant water stress, more so in the view of future climatic changes. This study showed the likely need to irrigate and control invasive weeds in the years following restoration. Anthropogenic factors, like livestock grazing and organic pollution are other major threats to the success of this type of restoration project. The implementation of an ecologically effective restoration should have enough flexibility to adjust to changing climate

and societal priorities, retaining simultaneously the capacity to integrate information from new technologies into site assessment and restoration planning (Kondolf *et al.*, 2011).

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CHAPTER 6

**Point sources of pollution and
restoration: influence of the CAIMA
paper mill on the water quality of the
Tagus River**

1. INTRODUCTION

Worldwide pulp production in 2015 was 178.8 million tons, 24.5% of which produced in Europe (CEPI, 2017). In the same time frame, world paper production was 407.6 million tons, 26.1% of which produced in Europe (CEPI, 2017). In 2016, Portugal was the third biggest paper pulp producer among the members of the Confederation of European Paper Industries (CEPI), representing 7.3% of CEPI members pulp production (CEPI, 2017).

Pulp making involves four basic steps, that can be carried out by several different methods (Ali & Sreekrishnan, 2001):

- Debarking - removes the bark and converts the wood fibers into smaller pieces, the woodchips;
- Pulping - turns the woodchips into pulp. It also removes most of the lignin and hemicellulose from the raw material;
- Bleaching - bleaches the brown pulp to achieve the final product desired color;
- Washing - removes the bleaching agents from the pulp.

Regarding the pulping step, commercial pulps can be grouped into chemical (35 to 65% pulp yield), semi-chemical (70 to 85% pulp yield), chemo-mechanical (85 to 95% pulp yield), and mechanical types (93 to 97% pulp yield) (Sjöström, 1993; Pokhrel & Viraraghavan, 2004). Chemical pulping is a process in which the lignin is dissolved in digesters, resulting in the release of the wood fibers (Sjöström, 1993). Although it has a lower pulp yield, chemical pulping produces a higher quality pulp. Chemical pulps are produced through the kraft (alkaline medium) or sulfite process (acid medium) (Pokhrel & Viraraghavan, 2004). In the kraft process the woodchips are cooked in a sodium hydroxide and sodium sulfide solution, and in the sulfite process they are cooked in a mixture of sulfurous acid and bisulfide ions (Pokhrel & Viraraghavan, 2004).

Dissolving pulp is a type of chemical pulp that possesses a content of α -cellulose higher than 90% (Sixta, 2006a). Nowadays it is being produced in large quantities worldwide and has many applications, such as regenerated cellulose (e.g.

viscose), cellulose esters and ethers, and other cellulose based products (Sixta *et al.*, 2013; Miao *et al.*, 2014). Dissolving pulp production and consumption has been growing, and this trend is expected to remain for the next decades (Sixta *et al.*, 2013). There are two main dissolving pulp production processes, steam pre-hydrolysis kraft (PHK) and acid sulfite (Sixta, 2006a). The sulfite process produces pulp with an α -cellulose content of 90–92% (up to 96% using special alkaline purification treatments), whereas the PHK process usually produces pulp with an α -cellulose content of 94–96% (up to 96% using special alkaline purification treatments) (Sixta, 2006a). Both methods need additional purification stages when compared with conventional pulp production (Sixta, 2006b). The dominant process to produce dissolving pulps is the acid sulfite, which accounted for 60-63% of the total worldwide production in 2003 (22-25% originated from PHK process and 12-16% was produced from cotton linters) (Sixta, 2006a). However, efforts have been made to develop other forms of production of dissolved pulp (Sixta *et al.*, 2013). Thus, technical advances that occurred at the cooking level of the kraft process resulted in the development of the Visbatch® and VisCBC processes (Sixta, 2006b; Sixta *et al.*, 2013). These new dissolving pulp technologies combine the advantages of displacement technologies and steam pre-hydrolysis (Sixta, 2006b; Sixta *et al.*, 2013). These processes are less detrimental to the environment and have shorter cover to cover times, low energy needs, as well as producing a very homogeneous high quality end product (Sixta, 2006b).

The pulp and paper industry is the sixth largest polluter worldwide, discharging liquid, gaseous and solid waste into the environment (Ali & Sreekrishnan, 2001). However, the main pollution impact of this sector is on watercourses, as the production process produces large volumes of liquid effluent (Hewitt *et al.*, 2006). These effluents have a strong organic matter load, because the pulp produced corresponds to only 40-45% of the weight of the wood used (Ali & Sreekrishnan, 2001). Table 17 details the typical wastewater pollutants from pulp mills.

Table 17. Potential water pollutants from pulp processes (Smook, 1992; EPA, 2002; Ince *et al.*, 2011; Lopes, 2012).

Pulp production steps	Pollutants released
Debarking	Solids; Biochemical Oxygen Demand (BOD); Color
Pulping	BOD; Volatile Organic Compounds (VOC's); Adsorbable Organic Halides (AOX); Chemical Oxygen Demand (COD); Resins; Fatty acids; Dissolved lignin
Bleaching	COD; AOX; VOC's
Washing	Solids; BOD; High pH; COD; Color

The discharging of this wastewater without treatment would cause negative ecological effects on the watercourses, like depletion of dissolved oxygen, toxic effects on fish and other aquatic organisms, and changes in the water color, turbidity, temperature and solid content (Van Der Kraak *et al.*, 1992; Tremblay & Kraak, 1999; Mattsson *et al.*, 2001; Chandra *et al.*, 2006; Ferreira *et al.*, 2009; Hubbe *et al.*, 2016). Thus, it is necessary to remove or reduce the concentration of this pollutants before the effluent is discharged into the environment. Typically, this takes place in two different steps, the primary and secondary treatments. In the former, the suspended solids are removed using gravity methods, like clarifiers or sedimentation basins (Süss, 2006; Hubbe *et al.*, 2016). Floating methods, like dissolved air flotation units can be an alternative to clarifiers (Hubbe *et al.*, 2016). This process is cost effective for the treatment of large water flows with high solid content, and is capable of removing up to 98% of the suspended solids (Hubbe *et al.*, 2016). In the secondary treatment, the toxic substances are removed through sorption and sedimentation processes, together with biologic treatment, like activated sludge, in which microorganisms decompose the biodegradable material (Süss, 2006; Hewitt *et al.*, 2006; Hubbe *et al.*, 2016). This process significantly improves the quality of the effluent by reducing the biochemical oxygen demand and reducing the levels of toxic organic compounds (Kovacs & Voss, 1992; Schnell *et al.*, 1997; Kostamo & Kukkonen, 2003).

The recognition of the potential environmental impact of the adsorbable organic halogens (AOX) from the pulp bleaching step resulted in the implementation of

extensive processes changes by the industry in the last thirty years (Süss, 2006; Suhr *et al.*, 2015). These compounds may present high toxicity to fish and humans and are the result of the reaction between the remaining lignin and the chlorine used in the bleaching process (Süss, 2006; Savant *et al.*, 2006). Thus, the environmental authorities of several countries have imposed severe restrictions on the discharge of AOX in the environment (Suhr *et al.*, 2015). Accordingly, the industry started to use chlorine dioxide instead of molecular chlorine (Chlorine Free) and/or molecular oxygen, hydrogen peroxide or peracetic acid (Totally Chlorine Free) in the bleaching process in order to reduce AOX levels (Süss, 2006; Suhr *et al.*, 2015).

In the western countries pollutant emissions from the pulp industry have improved dramatically over time (Suhr *et al.*, 2015). Nowadays solids and organic matter are the main pollutants discharged to the watercourses (Hubbe *et al.*, 2016). In a modern kraft pulp mill less than 3.5% of the AOX compounds formed during the bleaching process are discharged in the final effluent (Freire *et al.*, 2003). Totally chlorine free mills do not discharge chlorinated organics (they are not formed in bleaching) (Suhr *et al.*, 2015). However, reducing the load of poorly biodegradable organic substances, including some chemical additives such as chelating agents (EDTA), nutrients (nitrogen and phosphorus) and suspended solids, remains a challenge for the pulp industry (Suhr *et al.*, 2015).

In this study we aim to evaluate the effects of the pulp mill CAIMA – Indústria de Celulose S.A. liquid effluent on the water quality of the Tagus river. The pulp is produced through the acid bisulfite process, using magnesium as the cationic base (Ferreira, 2016). The pulp bleaching step does not resort to the use of chlorine (TCF pulp - Totally Chlorine Free), being carried out through alkaline extraction, oxygen and hydrogen peroxide delignification stages (Ferreira, 2016). We hypothesized that the evaluation of the concentration of selected pollutants upstream and downstream of the CAIMA sewage outfall would provide enough data to achieve the proposed objective.

2. METHODS

2.1 The CAIMA – Indústria de Celulose S.A. pulp mill

The pulp mill CAIMA - Indústria de Celulose S.A. started operating in 1962 and is located in the Municipality of Constância, Tagus river basin, Portugal. The mill has the capability to produce pulp for paper production or dissolved pulp for the textile industry. It uses eucalyptus (*Eucalyptus globulus* Labill.) as raw material. Nowadays the production is directed to the chemical and textile industry (dissolved pulp), with an installed production capacity of 125000 Air Dried ton (ADt)/year (342 ADt/day in a 365 days/year working regime).

The CAIMA pulp mill is an IPPC (Integrated Pollution Prevention and Control) installation. The Environmental License (LA N.º 606/01/2016) was issued in the 21st of April of 2016 and it is valid up to the 21st of April 2021.

The CAIMA pulp mill has three separate sewage networks (APA, 2016):

1. Industrial wastewater with wood fibers from the production process. These wastewaters undergo primary treatment to recover the fibers.
2. Industrial wastewater without fibers. The condensates are subjected to anaerobic treatment and the remaining wastewater goes through aerobic treatment.
3. Domestic wastewater (blackwater and greywater).

The effluents are routed through the mill's wastewater treatment plant (WWTP), which has a 719 m³/hour treatment capacity. The mill's WWTP also receives the Constância Municipality wastewater. The latter is mixed with the mill's domestic wastewater and represents an average 5% of the WWTP flow and an estimated pollutant load of 1% of the effluent that enters the WWTP (APA, 2016). The urban runoff (rainwater) flows through an independent network directly into the river.

The CAIMA WWTP carries out the primary and secondary treatment of the liquid effluents, which are later discharged through an emissary into the Tagus River (Figure 56).

The CAIMA pulp mill Water Use Permit – Wastewater Rejection (L000668.2016.RH5) establishes a maximum discharge rate of 20000 m³/day and maximum monthly volume of raw effluent of 520833.3(3) m³ (APA, 2016). The effluent discharge conditions are indicated in Table 18.

Table 18. CAIMA effluent discharge conditions according to the Water Use Permit – Wastewater Rejection (L000668.2016.RH5) (APA, 2016). ADt – air dried ton; ELV – Emission Limit Value.

Parameter	ELV	Monitoring obligations
pH (Sørensen scale)	6 a 9	
Biochemical Oxygen Demand (Kg/ADt)	5	
Chemical Oxygen Demand (Kg/ADt)	45	Quarterly, in the left bank of the river; 100 m downstream and 30 m upstream of the WWTP outlet
Total Suspended Solids (Kg/ADt)	3	
Total Nitrogen (Kg/ADt)	0,4	
Total Phosphorous (Kg/ADt)	0,16	

2.2 Sampling

Sampling took place in the river Tagus during the 18th of July and the 7th of August of 2017. Samples were obtained at the same locations on both dates. The sampled river section was divided into five cross river transects. Two transects were located upstream of the EH1 wastewater outlet and three downstream from the same location (Figure 56). The two upstream transects represent the state of the river before it receives the CAIMA wastewater (control transects). Each transept is composed of three sampling points, equidistant from each other, totaling fifteen sampling points per sampling date (Figure 56; Table 19).

The circulation between sampling points was done with a semi-rigid boat (Figure 57), and the points coordinates were determined using an GPS with sub-metric accuracy (Ashtech MobileMapper 100). The water samples were collected at a depth of one meter with a Van Dorn Sampler (Van Dorn, 1956), transferred to high-density polyethylene (HDPE) bottles and transported in a cooled storage box. The following physical and environmental parameters were taken for each sampling point: air temperature (°C), sample temperature (°C), dissolved oxygen

(mg/L), pH, conductivity ($\mu\text{S}/\text{cm}$) and depth (m) (Figure 2). The biocide sodium azide (0.02% solution) was added to the samples collected for total solids content, soluble lignin, phenolic compounds and cellulose quantification (2.5 ml NaN_3/L per sample).

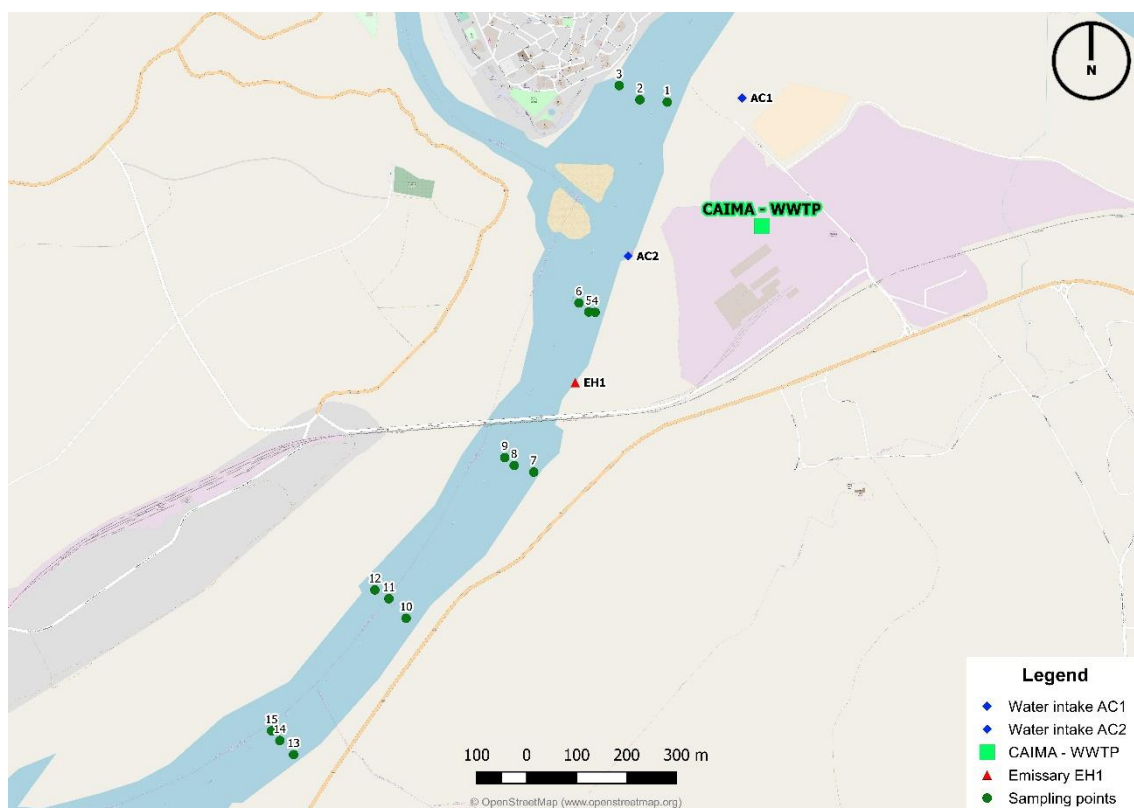


Figure 56. Location of the sampling points to monitor the impact of the discharge of the liquid effluents from the CAIMA pulp mill on the Tagus River.

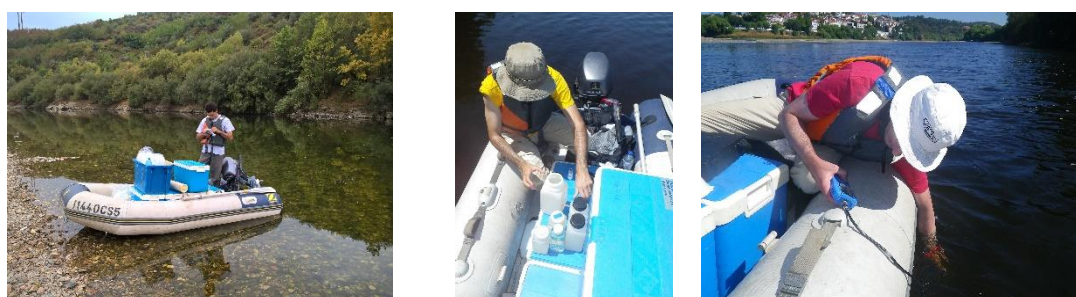


Figure 57. Sample collection.

Table 19. Geographical coordinates of the sampling points (Hayford-Gauss, Datum Lisbon).

Sampling point	Y (m)	X (m)
CAIMA 1	182513	278481
CAIMA 2	182459	278485
CAIMA 3	182417	278514
CAIMA 4	182368	278062
CAIMA 5	182355	278063
CAIMA 6	182336	278081
CAIMA 7	182244	277744
CAIMA 8	182205	277757
CAIMA 9	182187	277773
CAIMA 10	181989	277453
CAIMA 11	181954	277492
CAIMA 12	181926	277510
CAIMA 13	181763	277182
CAIMA 14	181735	277210
CAIMA 15	181719	277229

2.3 Laboratory analysis

The parameters to be analyzed were chosen by the Portuguese Environmental Agency (APA), which considered the ones relevant in the context of liquid effluents from the pulp mill industry. These parameters were analyzed in the Environmental Reference Laboratory (LRA) of the APA. The Forest Research Centre (CEF) selected four parameters related with woody material, that were analyzed in the Forest Technologies Laboratory (LTF) of the Instituto Superior de Agronomia (ISA). The APA parameters were analyzed according to the LRA internal methodologies (Table 20).

Table 20. Parameters selected by the APA and analytical methods employed.

Parameter	Technique / Method (APA, 2017a)
Total nitrogen (mg/L N)	Segmented Continuous Flow (SCF)
Biochemical Oxygen Demand (mg/L O ₂)	Electrochemical
Total water hardness (mg/L CaCO ₃)	Calculation
Total phosphorous (mg/L P)	SCF; Molecular absorption spectroscopy
Nitrate (mg/L NO ₃)	SCF; Ion chromatography
Total suspended solids (mg/L)	Gravimetry
Dissolved arsenic (µg/L As)	Inductively coupled plasma mass spectrometry (ICP-MS)
Dissolved cadmium (µg/L Cd)	
Dissolved lead (µg/L Pb)	
Dissolved copper (µg/L Cu)	
Dissolved chromium (µg/L Cr)	
Dissolved nickel (µg/L Ni)	
Dissolved zinc (µg/L Zn)	
Dissolved Organic Carbon (mg C/L)	Combustion with infra-red radiation detection
Chloroform (µg/L)	Solid-liquid micro-extraction and detection / quantification by Gas Chromatography Coupled to Mass Spectrometry (GC-MS)
Ethylbenzene (µg/L)	
Toluene (µg/L)	
Xylenes (ortho, meta and para isomers) (µg/L)	

The parameters analyzed by the LFT (ISA) and analytical methods were the following:

- Total solids content

Total solids content (suspended and dissolved solids) was obtained through the evaporation of 250 mL of a homogeneous water sample up to constant weight at 105 °C. The sample was placed in a dry, pre-weighted capsule. The capsule weight increase equals to the total solids content. Results were given in g L⁻¹.

- Soluble lignin

The soluble lignin in the water samples was obtained through the Tappi T um 250 method (TAPPI UM 250, 1991). Absorbance was read directly in the water samples using a spectrophotometer at 205 nm (Soluble lignin = Absorbance at 205 nm / molar absorptivity (110 L g^{-1})). Results were given in mg L^{-1} .

- Total phenol content

The quantification of the total phenolic compounds present in the samples was performed using the Folin-Ciocalteu colorimetric assay (Folin & Ciocalteu, 1927; Singleton *et al.*, 1999). Four milliliters of Folin-Ciocalteu reagent 1/10 (v/v) and 4 ml of Na_2CO_3 were added to 100 μl of each sample. The absorbance was read on a spectrophotometer at 765 nm. The results were given in mg L^{-1} of gallic acid equivalent (GAE).

- Tannin content

The tannin content was obtained through reaction with vanillin (Abdalla *et al.*, 2014). Two and a half milliliters of vanillin solution (10 g L^{-1} in methanol) and 2.5 ml of H_2SO_4 solution 25% (v/v) in methanol were added to 1 mL of sample. The absorbance was read on a spectrophotometer at 500 nm. The results were given in mg L^{-1} catechin equivalents (CE).

- Cellulose content

The cellulose content was determined by quantifying the glucose content after total hydrolysis of the residue with a 72% (v/v) H_2SO_4 solution. The glucose content was determined by the phenol-sulfuric colorimetric method (DuBois *et al.*, 1956). One milliliter of a 5% phenol solution was added to 1 mL of the hydrolyzed sample. The optical density of each mixture was read in a spectrophotometer at 490 nm. The results were expressed as mg L^{-1} glucose equivalent.

2.4 Additional data

In order to study the historical evolution of some water quality parameters, additional data was downloaded from the National Water Resources Information

System (SNIRH) (APA, 2017b). Thus, the water quality data from the Almourol (17G/02), Albufeira de Belver Estação 1 – superfície (17J/03S) and Albufeira de Belver (17J02) monitoring stations was obtained. These monitoring stations are located upstream (Albufeira de Belver) and downstream (Almourol) from the sampling locations. The data periods available for each monitoring station are shown in Table 21.

Table 21. Available data time-frame for the selected monitoring stations in the SNIRH database (APA, 2017b).

Monitoring station	START	END
Almourol	15 th of October 1985	5 th of December 2016
Albufeira de Belver Estação 1 - superfície	17 th of January 2012	1 st of August 2017
Albufeira de Belver	15 th of October 1985	9 th of February 2017

2.5 Data analysis

Data analysis was made using the integrated development environment RStudio (version 1.1.383) (RStudio Team, 2017) and R (version 3.4.3) (R Core Team, 2017) statistical software. Data was analyzed using non-parametric tests, because the normality of residuals and homoscedasticity assumptions were not met. The distribution of the residuals was assessed using the D'Agostino Normality Test (D'Agostino *et al.*, 1990) through the fBasics R Package (version 3042.89) (Rmetrics Core Team *et al.*, 2017) and visually, through histograms and normal Q-Q plots. Homoscedasticity was assessed using the Brown-Forsythe Test (Brown & Forsythe, 1974) through the lawstat R Package (version 3.2) (Gastwirth *et al.*, 2017) and visually through residuals vs. fitted values plots. A Wilcoxon signed-rank test for paired data was performed using the Stats R Package (version 3.4.3) (R Core Team, 2017) to assess for statistically significant differences between the July and August sampling seasons for each parameter.

3. RESULTS

3.1 Monitored parameters

The summary of the analysis of the sampled physicochemical parameters is presented in Table 22.

The levels of biochemical oxygen demand, dissolved lead, dissolved copper, dissolved chromium, chloroform, ethylbenzene, toluene and xylenes were below the limit of quantification at all sampling points and at both sampling dates. In addition, total phenols and condensed tannins were not detected in any of the water samples collected in both sampling dates.

There were significant average pH differences between both sampling dates ($V=83.5$, $p=0.0084$, $n=30$). The average pH in July (7.86 ± 0.052) was significantly higher than in August (7.61 ± 0.112) (Figure 58). The pH levels were relatively constant along the river in the July samples, although with a slight downstream increase tendency (0.3% increase between transept 1 and 5), more noticeable on the right margin. In August there was also a downstream pH increase, stronger than in the previous month (12.7% increase between transept 1 and 5), also with higher values on the right margin of the river (Figure 58).

The average total nitrogen in July (1.00 ± 0.023 mg L⁻¹) was also significantly higher ($V=120.0$, $p=0.0007$, $n=30$) than in August (0.82 ± 0.019 mg L⁻¹) (Figure 59). In both sampling dates there was a slight downstream reduction trend. The highest values came from the samples of the left margin (Figure 59).

Table 22. Mean results of the parameters sampled in Constância, Rio Tejo, in July and August of 2017. LoQ – Limit of quantification.

Parameter	18/07/2017		08/08/2017	
	Mean	Standard error	Mean	Standard error
pH	7.86	0.052	7.61	0.112
Sample temp. (°C)	25.9	0.150	23.8	0.380
Total N (mg/L)	1.00	0.023	0.82	0.019
BOD (mg/L O ₂)	<3.0 (LoQ)	---	<3.0 (LoQ)	---
Total water hardness (mg/L CaCO ₃)	138.0	2.225	145.3	2.906
Total P (mg/L P)	0.128	0.004	0.195	0.008
NO ₃ (mg/L)	1.68	0.113	1.69	0.017
Total Suspended Solids (mg/L)	1.55	0.183	1.64	0.203
Dissolved arsenic (µg/L)	3.45	0.070	4.20	0.201
Dissolved cadmium (µg/L)	<0.05 (LoQ)	---	0.029	0.003
Dissolved lead (µg/L)	<1.0 (LoQ)	---	<1.0 (LoQ)	---
Dissolved copper (µg/L)	<5.0 (LoQ)	---	<5.0 (LoQ)	---
Dissolved chromium (µg/L)	<1.0 (LoQ)	---	<1.0 (LoQ)	---
Dissolved nickel (µg/L)	2.03	0.381	3.01	1.014
Dissolved zinc (µg/L)	3.6	0.618	4.3	0.715
Dissolved Organic Carbon (mg C/L)	5.15	0.289	7.52	0.467
Chloroform (µg/L)	<1.0 (LoQ)	---	<1.0 (LoQ)	---
Ethylbenzene (µg/L)	<1.0 (LoQ)	---	<1.0 (LoQ)	---
Toluene (µg/L)	<1.0 (LoQ)	---	<1.0 (LoQ)	---
Xylenes (ortho meta para isomers) (µg/L)	<1.0 (LoQ)	---	<1.0 (LoQ)	---
Total solids content (mg/L)	300.13	6.14	325.73	9.37
Soluble lignin (mg/L)	4.06	0.32	5.76	0.37
Total phenol content (mg/L gallic acid equivalent)	< LoQ	---	< LoQ	---
Tannin content (mg/L catechin equivalents)	< LoQ	---	< LoQ	---
Cellulose content (µ glucose/L)	12.16	0.46	14.10	0.85

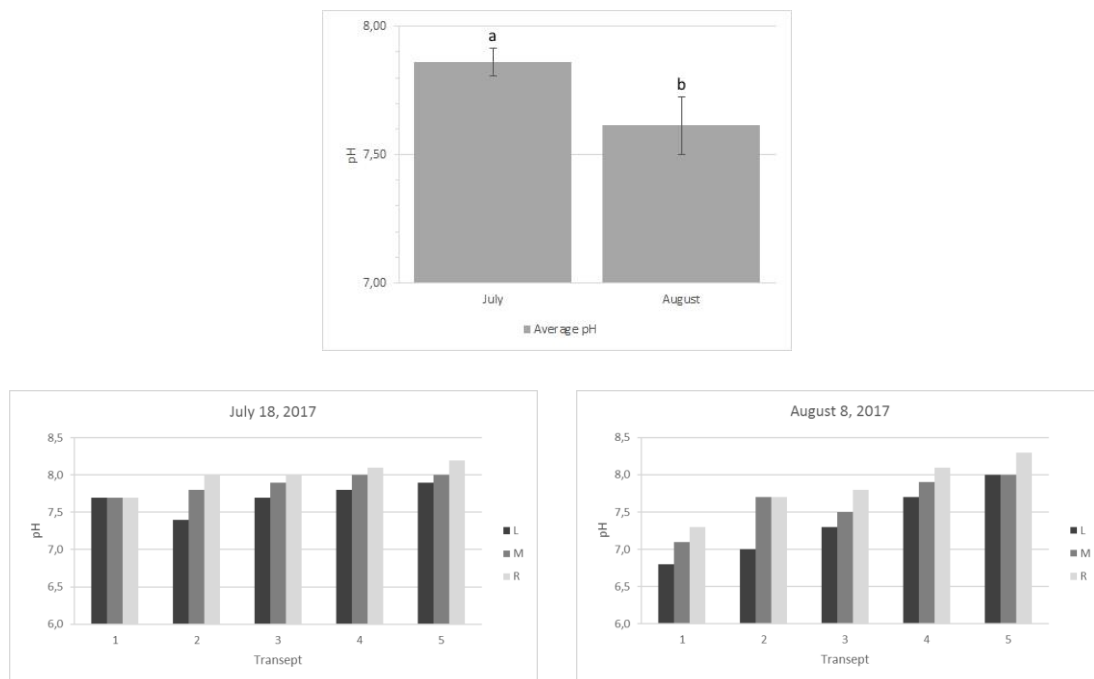


Figure 58. Top: Average pH levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: pH levels along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

There were significant average total phosphorus differences between both sampling dates ($V=120.0$, $p=0.0007$, $n=30$). The average total phosphorus in July ($0.128 \pm 0.004 \text{ mg L}^{-1}$) was significantly lower than in August ($0.195 \pm 0.008 \text{ mg L}^{-1}$) (Figure 60). In both sampling dates there was a trend for phosphorous increase on the left bank of the transects downstream from the CAIMA emissary.

There were no significant average nitrate differences between both sampling dates ($V=62.0$, $p=0.932$, $n=30$) (Figure 61). However, in the July transect 2, there were nitrate spikes on the middle of the river and on the right margin. Nitrate levels in August were homogeneous among all transects, with a slight downward trend downstream (Figure 61).

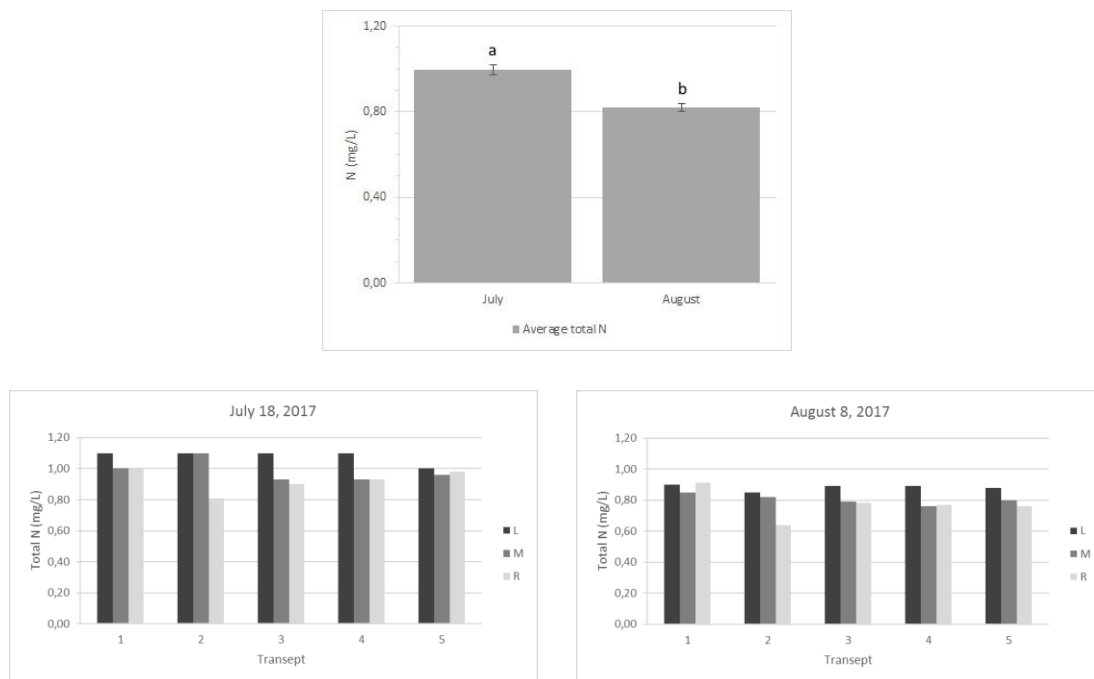


Figure 59. Top: Total nitrogen levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: total nitrogen levels (mg L^{-1}) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

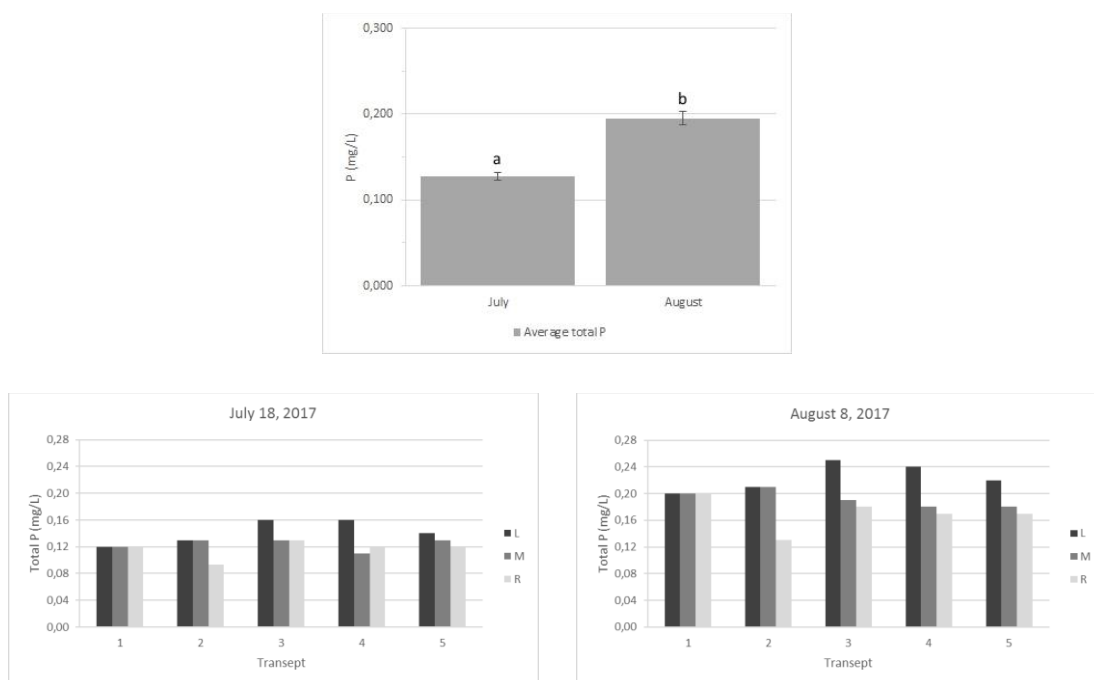


Figure 60. Top: Total phosphorous levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: total phosphorous levels (mg L^{-1}) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

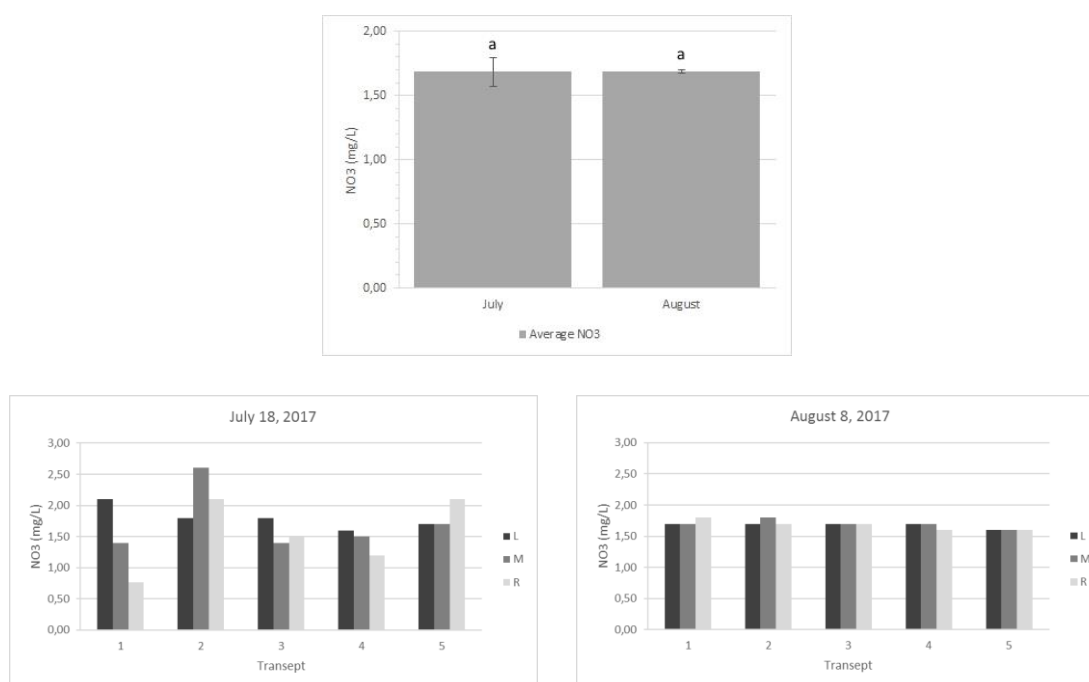


Figure 61. Top: Nitrate levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: Nitrate levels (mg L^{-1}) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

Differences in total suspended solids between both sampling dates were not statistically significant ($V=20.0$, $p=0.8121$, $n=30$) (Figure 62). However, there was an increase in the levels of this parameter in transept 3 at both sampling dates, especially in July (a 50% increase between transept 2 and transept 3, followed by a 56% reduction between transept 3 and 4). Sampling results were below the limit of quantification (LoQ) at more than 50% of the sampling points (Figure 62).

There were significant average dissolved arsenic differences between both sampling dates ($V=0.0$, $p=0.0007$, $n=30$). The average arsenic in July ($3.45 \pm 0.070 \mu\text{g/L}$) was significantly lower than in August ($4.20 \pm 0.201 \mu\text{g L}^{-1}$) (Figure 63). Additionally, in July this parameter remained relatively constant among the 5 transects, while in August it presented higher levels in transects 1 and 2, with special emphasis on the left bank of the river. There was also a tendency for arsenic dilution towards downstream at both sampling dates (Figure 63).

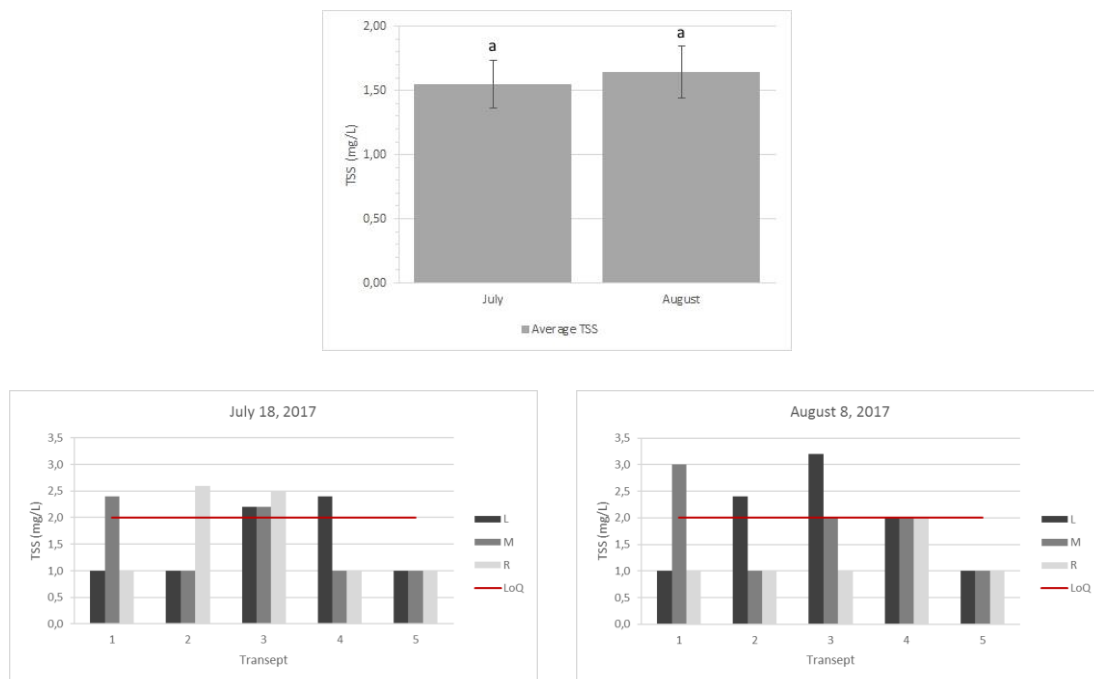


Figure 62. Top: total suspended solids levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: total suspended solids levels (mg L^{-1}) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

Contrary to dissolved arsenic, there were no significant average nickel differences between both sampling dates ($V=67.0$, $p=0.3788$, $n=30$) (Figure 64). The levels of this parameter were uniform in the July transects, except for a peak on the right margin on transept 4. In the August sampling the behavior of this parameter was similar, except for two peaks on the left margin on transept 1 and on the right margin on transept 3 (Figure 64).

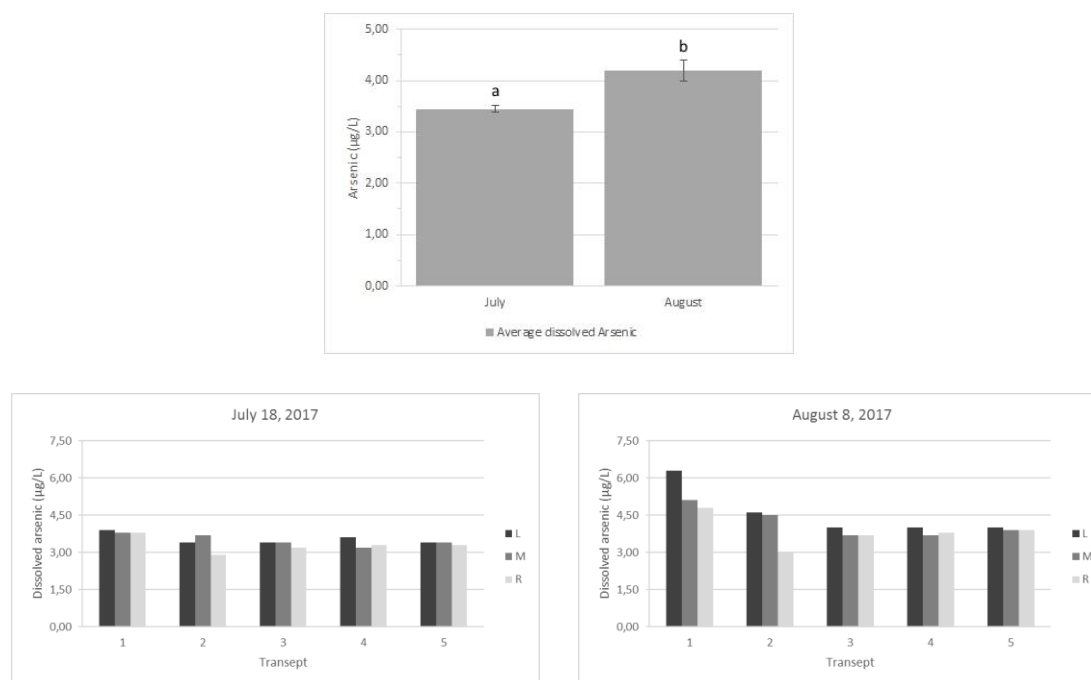


Figure 63. Top: dissolved arsenic levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: dissolved arsenic levels ($\mu\text{g L}^{-1}$) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

Differences in average dissolved organic carbon between the two sampling dates were statistically significant ($V=5.0$, $p=0.0006$, $n=30$). The average dissolved organic carbon in July ($5.15 \pm 0.289 \text{ mg L}^{-1}$) was significantly lower than in August ($7.52 \pm 0.467 \text{ mg L}^{-1}$) (Figure 65). In the July sampling, this parameter seems to have been influenced by the CAIMA emissary (transects 3, 4 and 5), with samples from the left margin showing higher values than the others. However, in the August sampling, this influence is no longer evident, with very small differences between transects 2 and 3 (Figure 65).

There were no significant average zinc differences between both the July and August samplings ($V=14.0$, $p=0.6236$, $n=30$) (Figure 66). Dissolved zinc levels in July did not show a clear relationship with the CAIMA emissary, with only three measurable samples (*i.e.* above the limit of quantification - LoQ): left margin on transept 1, middle of the channel on transept 2 and left margin on transept 5. In August there were five samples above the limit of quantification, with emphasis on the left margin and middle channel on transects 3 and 4. In the latter sampling

date there seems to be some kind of relation between the observed levels and the CAIMA emissary (Figure 66).

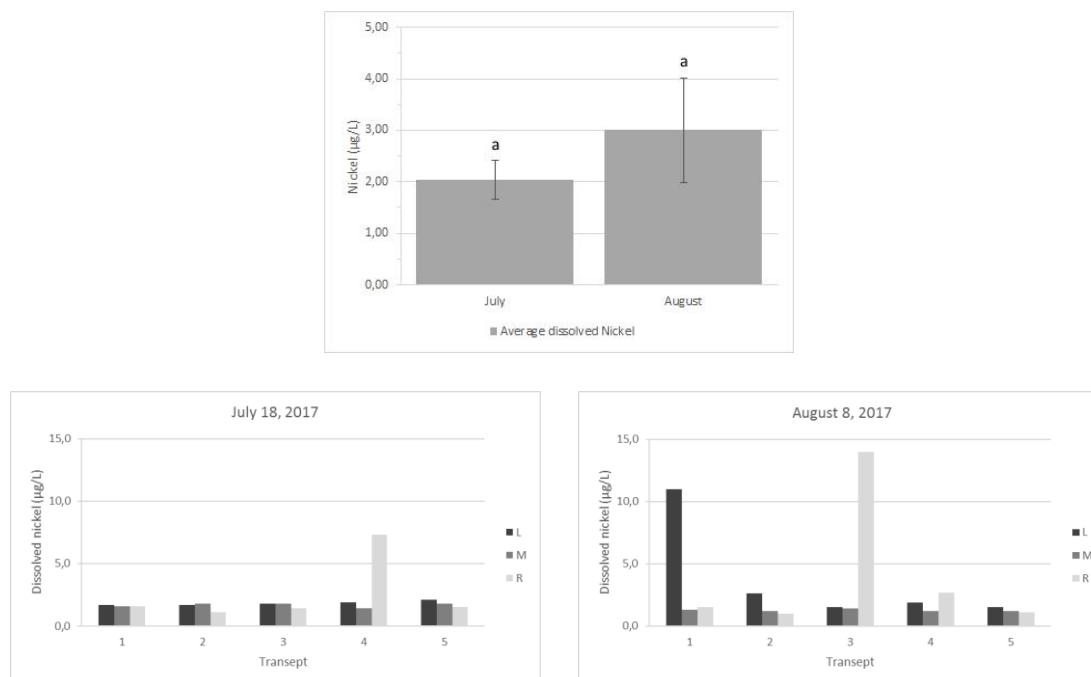


Figure 64. Top: dissolved nickel levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: dissolved nickel levels ($\mu\text{g L}^{-1}$) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

There were significant average total solids content differences between both sampling dates ($V=4.5$, $p=0.0018$, $n=30$). The average total solids content in July ($300.13 \pm 6.14 \text{ mg L}^{-1}$) was significantly lower than in August ($325.73 \pm 9.37 \text{ mg L}^{-1}$) (Figure 67). There were no differences between the samples collected in the transects upstream of the CAIMA emissary (control transects 1 and 2) and the samples collected in the downstream transects (transects 3, 4 and 5). However, there was a slight decrease in total solids levels from the left margin to the right margin (Figure 67).

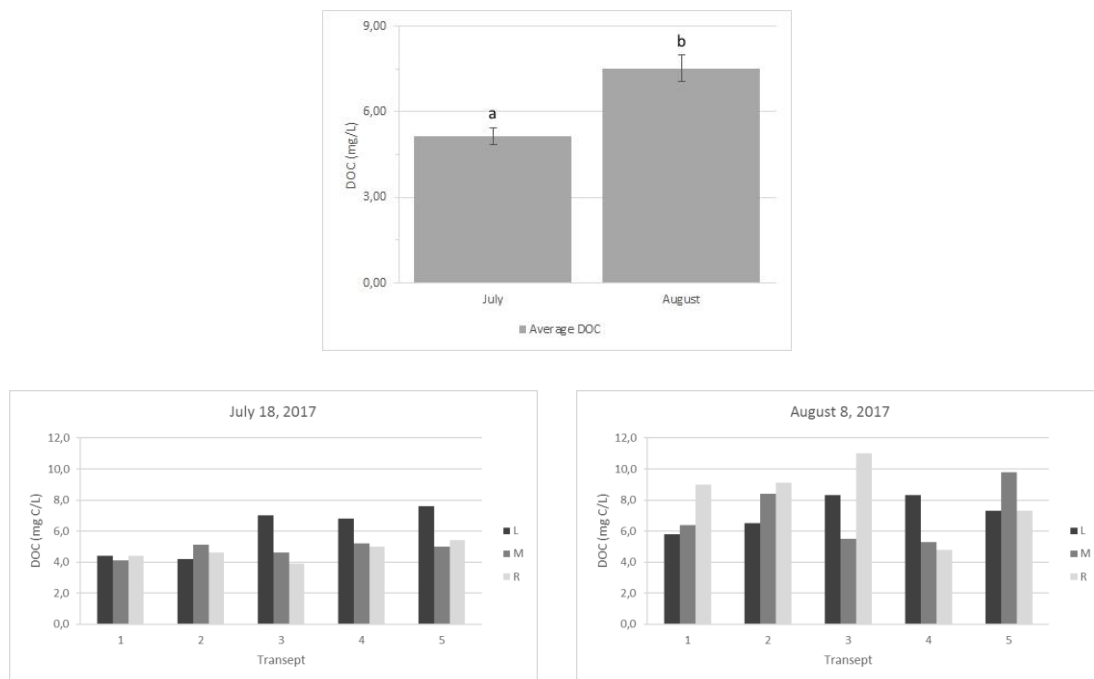


Figure 65. Top: dissolved organic carbon levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: dissolved organic carbon levels (mg C L⁻¹) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

There were no significant average cellulose differences between both sampling dates ($V=26.0$, $p=0.0554$, $n=30$) (Figure 68). There was no clear variation pattern within the transects (left bank and right bank) as well as along the sampled river section. In the July sampling, the glucose levels of the upstream transects (transects 1 and 2) were similar to those of the downstream transects (transects 3, 4 and 5). However, in the August sampling there was an increase in the glucose content in samples from the left margin, downstream of the CAIMA emissary (transects 4 and 5) (Figure 68).

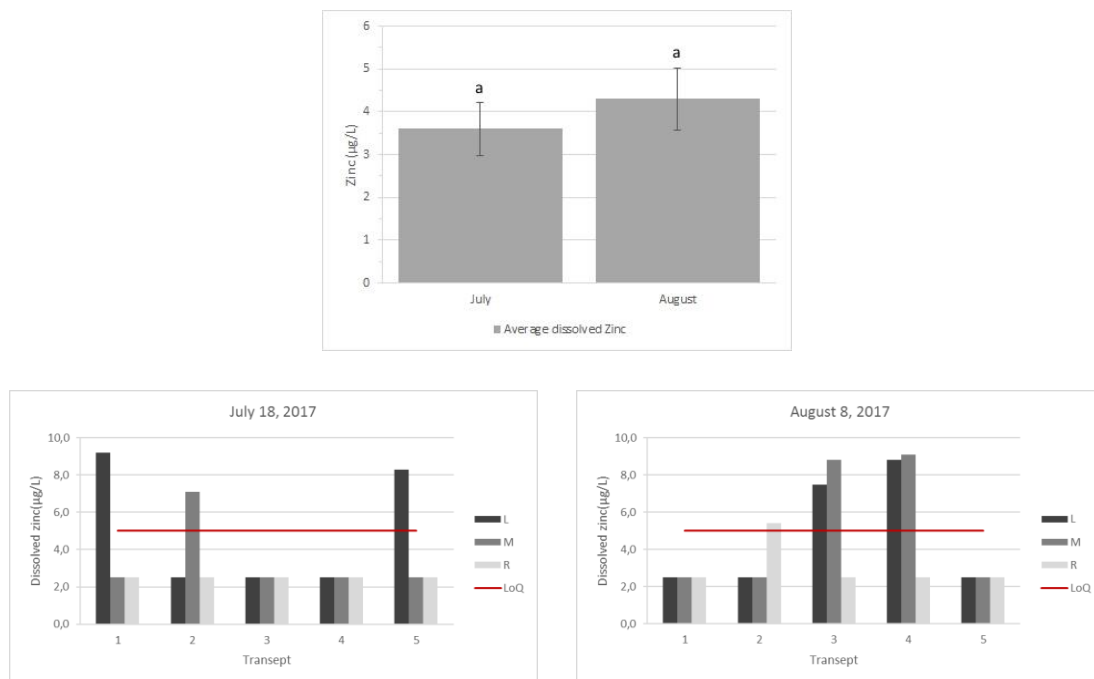


Figure 66. Top: dissolved zinc levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: dissolved zinc levels ($\mu\text{g L}^{-1}$) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

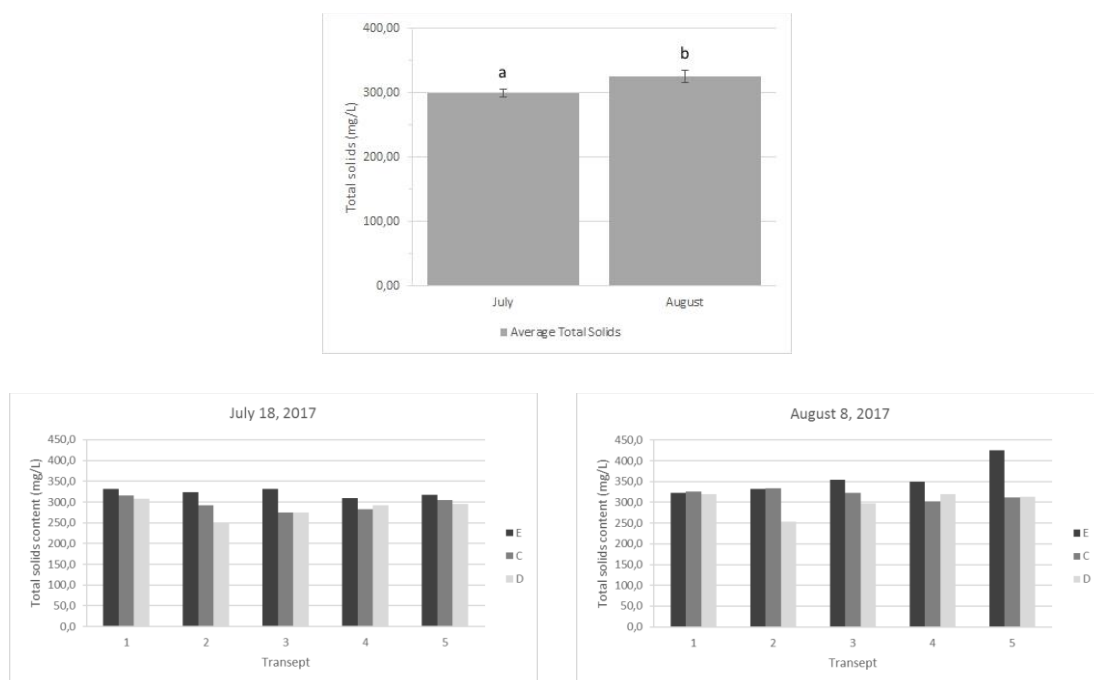


Figure 67. Top: total solids levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: total solids levels (mg L^{-1}) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

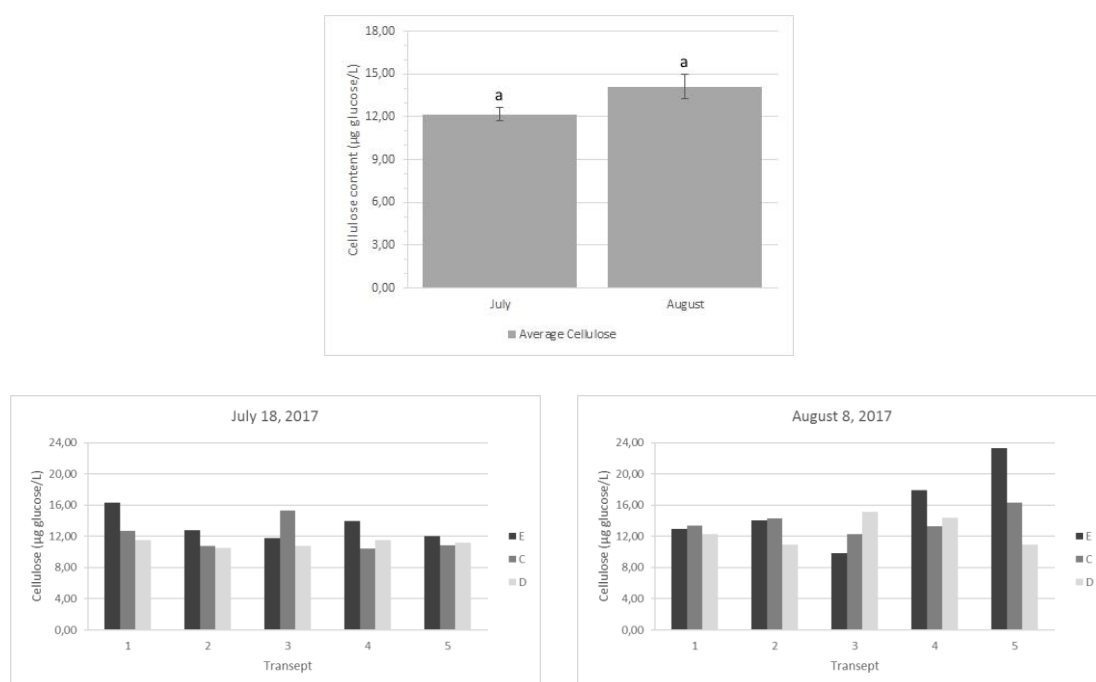


Figure 68. Top: cellulose levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: cellulose levels ($\mu\text{g glucose L}^{-1}$) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

Average soluble lignin levels differed significantly between both sampling dates ($V=6.0$, $p=0.0008$, $n=30$). The July value ($4.06 \pm 0.32 \text{ mg L}^{-1}$) was significantly lower than that determined in August ($5.76 \pm 0.37 \text{ mg L}^{-1}$) (Figure 69). The soluble lignin levels in the samples collected in July were relatively constant along the river, with higher levels from the samples on the left margin. In August there was an increase in soluble lignin levels in the transects downstream from the CAIMA emissary, with higher levels in the samples from the left margin of transects 3, 4 and 5 (Figure 69).

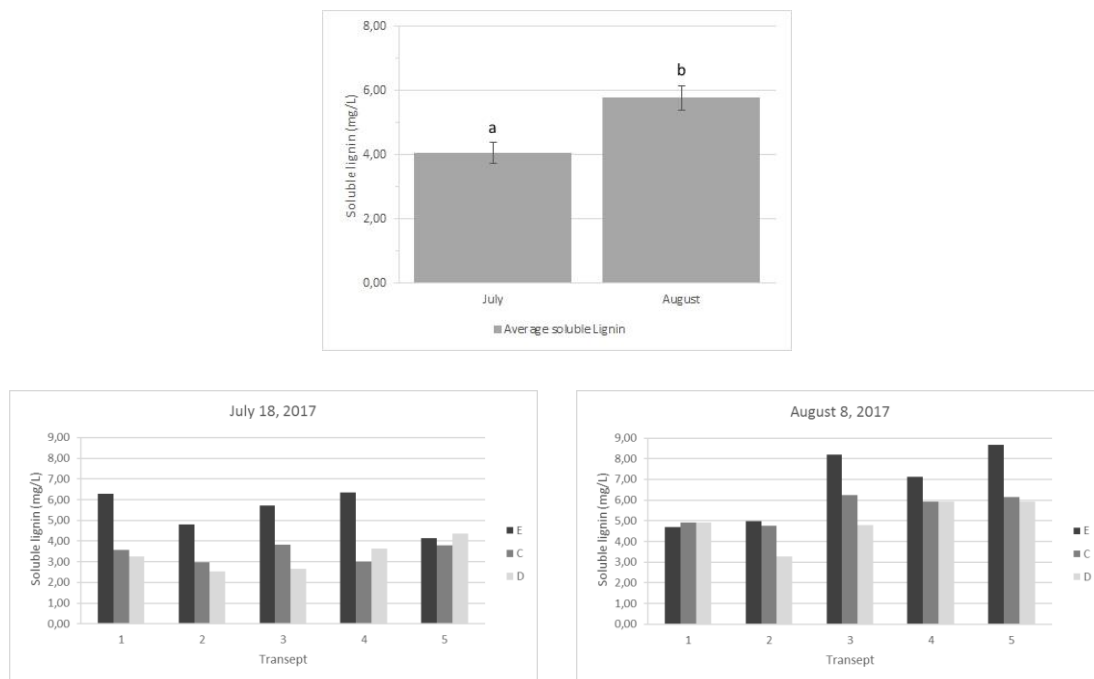


Figure 69. Top: soluble lignin levels on both sampling dates. The vertical bars are standard errors. Different letters indicate significant differences between parameter levels. Bottom: soluble lignin levels (mg L^{-1}) along the 5 transects. L - left margin (L); M - middle of the channel; R - right margin.

3.2 Additional data

To perceive the historical evolution of some parameters, the results obtained in this sampling campaign were compared with additional water quality information collected in the SNIRH database. It should be noted that the monitoring stations with historical water quality data are at some distance from the evaluated river section and are subject to the influence of other sources of pollution.

The average pH value from the current sampling is within historical values for the period where data exists (Figure 70). The parameters total nitrogen, total phosphorus, nitrates, total suspended solids, dissolved zinc and dissolved arsenic have mean values below the historical annual values (Figures 71 to 76). In contrast, the dissolved nickel and dissolved organic carbon parameters presented average values higher than the historical ones (Figures 77 and 78).

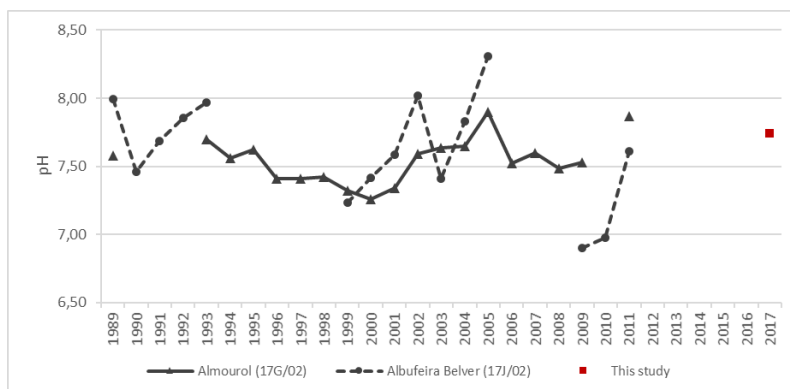


Figure 70. Historical evolution of pH levels in the river sections adjacent to the study area (Source: APA, 2017b).

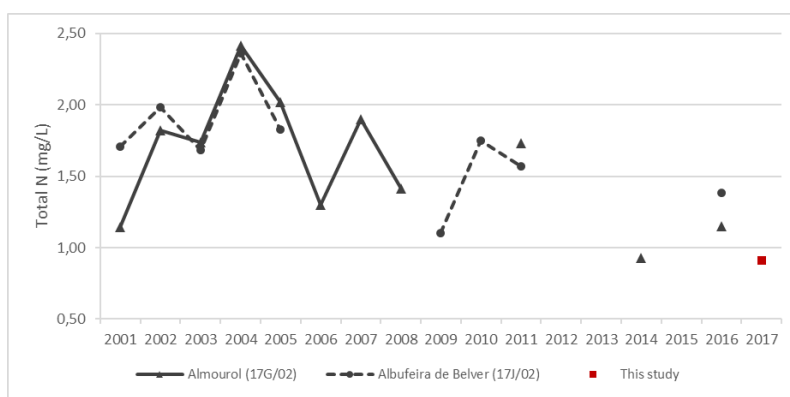


Figure 71. Historical evolution of the total nitrogen levels in the river sections adjacent to the study area (Source: APA, 2017b).

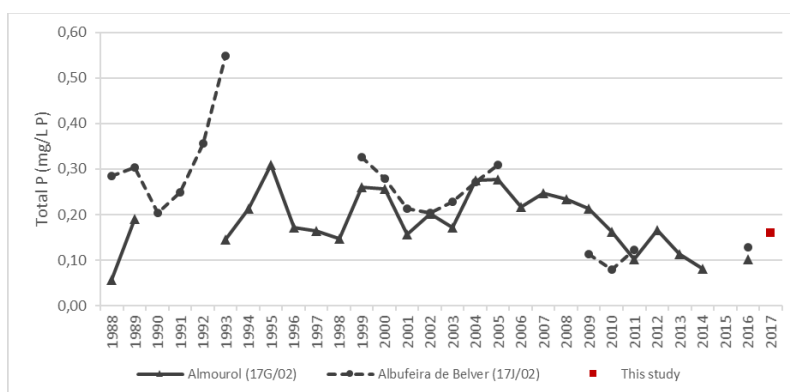


Figure 72. Historical evolution of the total phosphorous levels in the river sections adjacent to the study area (Source: APA, 2017b).

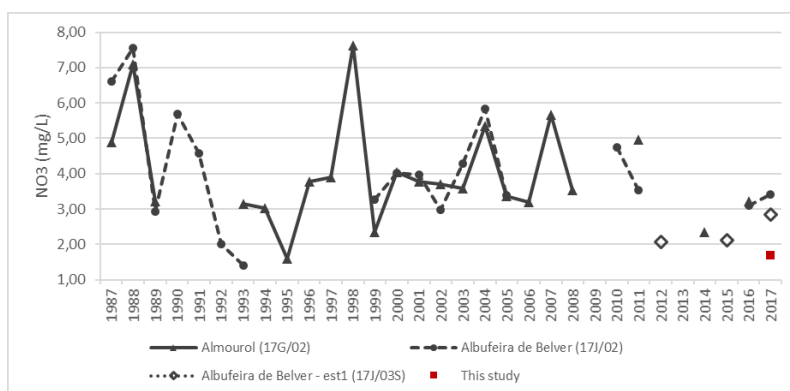


Figure 73. Historical evolution of the nitrate levels in the river sections adjacent to the study area (Source: APA, 2017b)

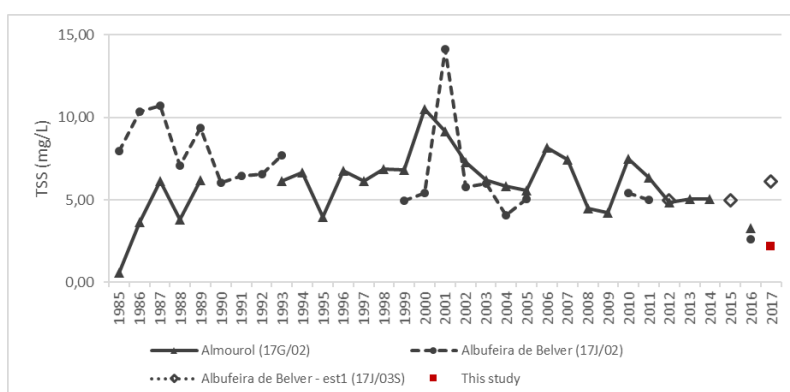


Figure 74. Historical evolution of the total suspended solids levels in the river sections adjacent to the study area (Source: APA, 2017b)

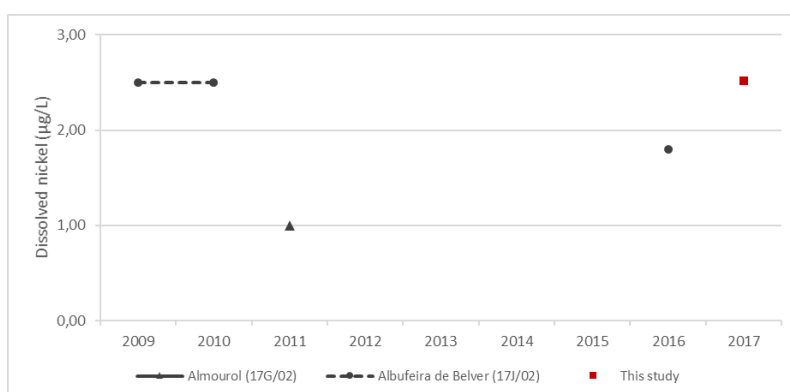


Figure 75. Historical evolution of the dissolved nickel levels in the river sections adjacent to the study area (Source: APA, 2017b)

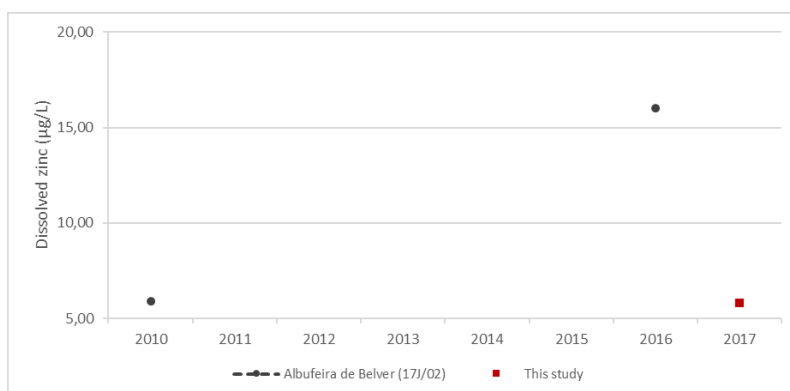


Figure 76. Historical evolution of the dissolved zinc levels in the river sections adjacent to the study area (Source: APA, 2017b)

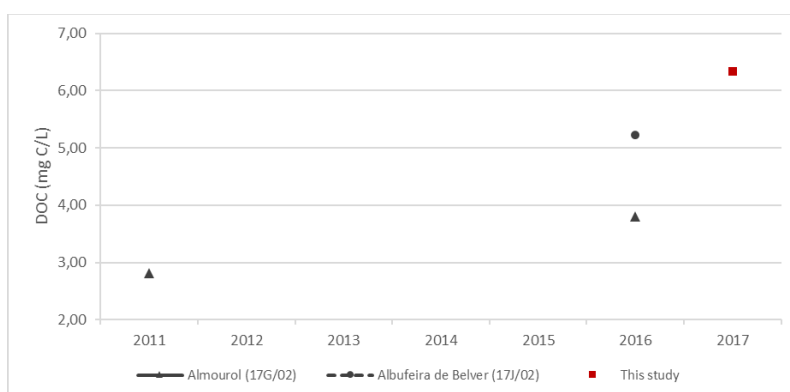


Figure 77. Historical evolution of the dissolved organic carbon levels in the river sections adjacent to the study area (Source: APA, 2017b)

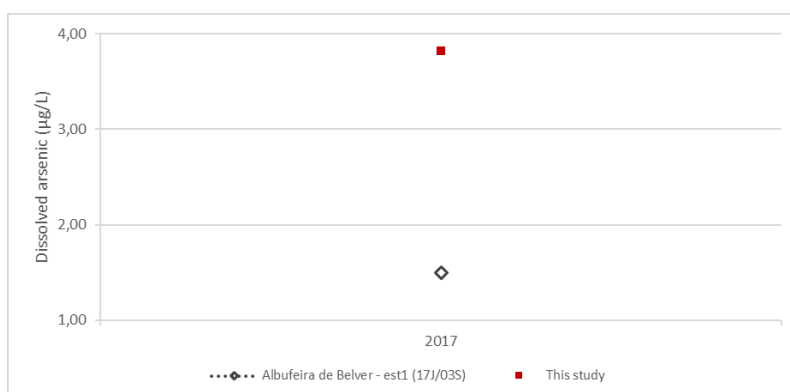


Figure 78. Historical evolution of the dissolved arsenic levels in the river sections adjacent to the study area (Source: APA, 2017b)

4. DISCUSSION

The pH levels from our study are similar to those present in some European rivers subject to effluent discharges from pulp mills (e.g. Brodnjak-Vončina *et al.*, 2002). The pH of water is indicative of its microbial and chemical characteristics. A shift in the pH raises phosphorous mobilization from most sediments, which may contribute to harmful algal blooms (Boström, 1984; Huang *et al.*, 2005). Additionally, the aqueous ammonia equilibrium depends of the pH of the solution. Thus, as pH increases, increasing the hydroxide ion concentration, the equilibrium shifts towards the NH_3 species. This un-ionized form of ammonia is toxic to fishes even at low concentrations, and an increase of one pH unit (e.g. from 7 to 8) increases the NH_3 concentration 10 times (Downing & Merckens, 1955; Warren, 1962; Report, 1970). Therefore, considering that average pH levels were significantly higher in July, average total nitrogen results were, as expected, also significantly higher on that sampling date. Additionally, high pH of the river water may result in the reduction of heavy metal toxicity (Dean-Ross & Mills, 1989).

Total phosphorous levels in July exceeded the 0.13 mg P L^{-1} limit for the Good Ecological State (INAG, 2009) in the left bank, downstream of the CAIMA emissary. In August the total phosphorus levels were above the limit considered for the definition of Good Ecological Status in all transects, with a peak in the left bank downstream of the CAIMA emissary. It should be noted, however, that this was a one-off irregular sampling and that the limit value for Good Ecological Status is calculated through annual average (INAG, 2009). Nevertheless, as mentioned previously, higher phosphorous levels increase the probability of algal blooms.

Statistical analysis showed that there were no significant differences between the nitrate values for the two sampling seasons. Nitrate usually enters the aquatic ecosystems from non-point sources (Ongley, 1996) and is not usually present in pulp mill effluents. High concentrations of nitrate in the water have negative effects in health and are associated with diseases such as stomach cancer and cardiac disease (Townsend *et al.*, 2003). Therefore, a limit for nitrate concentration in potable water of $50 \text{ mg NO}_3^- \text{ L}^{-1}$ was adopted in the European

Union (Drinking Water Directive 98/83/EC). Recorded nitrate levels in our study ranged from 0.77 mg L⁻¹ to 2.60 mg L⁻¹, much lower than the legal limit.

High suspended solids levels are a threat to freshwater mussels and many other aquatic organisms (Richter *et al.*, 1997; Gascho Landis *et al.*, 2013). Total suspended solids from the CAIMA pulp mill effluent do not appear to have a strong impact on the Tagus River water quality, with this parameter levels ranging from below the limit of quantification (2 mg L⁻¹) to 3.2 mg L⁻¹. These values are lower than the mean levels reported in the literature for other comparable rivers (Dassenakis *et al.*, 1998; Xue *et al.*, 2000; Alonso *et al.*, 2004).

Inorganic arsenic is a toxic carcinogen and is a significant chemical contaminant in drinking-water globally (IPCS, 2001). The mean level of arsenic in natural waters usually ranges between 1 and 2 µg/l (Hindmarsh *et al.*, 1986), sometimes even less (Martin *et al.*, 1993). In our study, mean dissolved arsenic levels were low, although higher than the mean levels reported for the rivers Marne and Seine, in France (Elbaz-Poulichet *et al.*, 2006).

Average dissolved zinc levels from our study are higher than the ones present in unpolluted rivers (Shiller & Boyle, 1985), in the River Kleine (Xue *et al.*, 2000) or in the Guadalquivir River (Mendiguchía *et al.*, 2007). However, they are lower than the mean levels present in many industrialized areas of Europe (Grimshaw *et al.*, 1976; Duinker & Kramer, 1977; Burrows & Whitton, 1983; Schuhmacher *et al.*, 1995; Neal *et al.*, 2006; Milovanovic, 2007), United States (Hem, 1972) and rest of the world (Ntengwe & Maseka, 2006; Aktar *et al.*, 2010; Reza & Singh, 2010). Agricultural activities may also contribute to increase zinc levels in the river water (Xue *et al.*, 2000)

Average dissolved nickel levels from our study are similar to the ones present in the Guadalquivir River (Mendiguchía *et al.*, 2007), and lower than the ones present in the Thame River (Neal *et al.*, 2006). Agricultural activities may also contribute to increase nickel levels in the river water (Dassenakis *et al.*, 1998).

Although there were differences between sampling seasons in the dissolved nickel and dissolved zinc parameters, those were not statistically significant. The Wilcoxon signed-rank test for paired data tests the null hypothesis that the median difference between pairs of observations is zero (McDonald, 2014). That

may explain the above-mentioned results because, although those parameters means are quite different, the medians are not. Also, the use of non-parametric tests carries an increased risk of Type II errors (accepting that there are no differences between pairs when they exist) (Gaur & Gaur, 2009; Maroco, 2010). Thus, these statistical results should be analyzed with some caution.

The dissolved organic carbon (DOC) levels from our study are within the expected mean levels for the Mediterranean climate (Thurman, 1985). However, current levels are higher than the past recorded levels. One possible explanation is that natural DOC levels in rivers vary with the size of the river, the climate, the season of the year and the vegetation in the river basin (Thurman, 1985). In fact, riparian soil organic carbon may dominate the natural DOC flux in a stream (Dosskey & Bertsch, 1994). Further, DOC levels may also be related with anthropogenic contamination (Noacco *et al.*, 2017). Excessive DOC can enhance the water solubility of hydrophobic organic pollutants (Warren *et al.*, 2003), thus facilitating their transport and bioavailability (Gao *et al.*, 1998). Because the historical data is composed of only a few records it is difficult to assess if the current levels are the result of anthropogenic pollution or just the natural DOC variation of the river system. Nevertheless, the July sampling levels seem to have some relation with the CAIMA effluent.

The total solids content levels were higher than the ones sampled in a upstream river section by Ferreira *et al.* (2017). They were also slightly higher than the mean values found in the bibliography for quasi-pristine locations, but similar or lower than the values for anthropogenic affected river sections (Alberto *et al.*, 2001; Sliva & Dudley Williams, 2001; Singh *et al.*, 2004).

Cellulose levels were higher than the ones sampled upstream by Ferreira *et al.* (2017). The July cellulose levels were relatively constant along the sampled river section, indicating that most of the cellulose came from upstream sources, but the August sampling levels seem to have some relation with the CAIMA effluent.

The river water dissolved lignin levels from our study are much higher than in some European, American and Arctic rivers (Cotrim da Cunha *et al.*, 2001; Ward *et al.*, 2012; Feng *et al.*, 2017). Although the July soluble lignin levels were relatively constant along the sampled river section, indicating that the majority of

lignin came from upstream sources, the August sampling levels seem to have some relation with the CAIMA effluent. Nevertheless, dissolved lignin levels are lower than the ones sampled upstream by Ferreira *et al.* (2017). Dissolving pulp is a high-grade cellulose pulp, with low contents of hemicellulose, lignin, and resin (Bajpai, 2014). To achieve this low lignin content, more residual lignin is removed in the bleach step. Thus, the organic load to be treated in the waste water treatment plant is higher, which translates into higher lignin emissions to water bodies (Suhr *et al.*, 2015).

The profile of the channel, more silted on the right bank and center of the river, and deeper on the left bank, directs much of the upstream flow to the latter margin. This results in a higher flow velocity and in the concentration of substances in this zone. These pollutants are then subjected to advection and mixing processes that facilitate their transport downstream. Thus, in general, the left margin presented higher levels of the different parameters.

5. CONCLUSIONS

The monitoring of spatial and temporal characteristics of industrial effluents is important to assist stakeholders committed to water resources management. The type of sampling performed, a non-systematic first approach, limited in time, did not allow for the establishment of a profile of spatial-temporal evolution of the sampled parameters, but only to characterize the current situation. In fact, emissions to water for different reference periods vary over time for a given pulp mill (Brodnjak-Vončina *et al.*, 2002; Suhr *et al.*, 2015). Thus, more sampling dates and greater spatial coverage would be required for more detailed and comprehensive conclusions. Also, although the sampling took place in a heavily regulated river (with more constant flows), pollutants levels are very dependent of changes in river flow (Dassenakis *et al.*, 1998; Xue *et al.*, 2000; Neal *et al.*, 2006; Ltifi *et al.*, 2017). Therefore, it is of paramount importance that sampling encompasses a wide range of weather and flow conditions. It would also be important to sample the final section of the Zêzere River, an important Tagus tributary with influence in the sampled river section, in order to determine its influence on the parameters sampled in transects 2 to 5.

The variation of the levels of pH, total phosphorous, total suspended solids, dissolved nickel, dissolved organic carbon, dissolved zinc, soluble lignin and cellulose seems to have some relation with the CAIMA effluent outflow. However, although there are some parameters with relevant levels, like total phosphorous and dissolved lignin, our results did not show particularly high levels of pollution. Changes in total phosphorus, total nitrogen, nitrate and total solids levels were important, but not necessarily directly related to the manufacturing process, since the CAIMA effluent is mixed with domestic effluents. High total phosphorus levels are also present upstream of the effluent outflow, meaning that the Tagus River is subject to nutrient pollution even before the sampled river section. Nevertheless, the existence of consistently higher levels of total phosphorous, dissolved lignin, cellulose, pH, total nitrogen and dissolved zinc downstream of the emissary advise the setting up of a monitoring station integrated in the monitoring network of the Tagus River Basin.

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CHAPTER 7

Alternative organic substrates for nitrate removal from water

1. INTRODUCTION

Non-point source pollutants, like nitrogen, are transported by rainwater and melting snow overland and through the soil, ultimately finding their way into groundwater and aquatic ecosystems (Ongley, 1996). The impact of these pollutants ranges from simple nuisance substances to severe ecological impacts, such as acidification of freshwater bodies, eutrophication and associated hypoxic zones, adverse health effects on aquatic organisms, and N₂O production, a greenhouse gas (Ongley, 1996; Vitousek *et al.*, 1997; Howarth *et al.*, 2000; Rabalais, 2002; Camargo & Alonso, 2006; Phoenix *et al.*, 2006), as well as potential impacts on human health (Townsend *et al.*, 2003). Therefore, eutrophication is a worldwide problem which, together with oxygen depletion, is probably the most serious pollution issue of aquatic ecosystems (Jørgensen *et al.*, 2013). The eutrophication of aquatic ecosystems has risen rapidly in recent years due to urbanization and fertilizer application, which results in the increasing of nutrient discharge to watercourses (Vitousek *et al.*, 1997; Galloway & Cowling, 2002; Jørgensen *et al.*, 2013).

Ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) are the most common reactive forms of dissolved inorganic nitrogen in aquatic ecosystems (Rabalais, 2002). Nitrogen mineralization, i.e. the conversion of organic to inorganic forms, is a bottleneck biogeochemical process of ecosystems that influences standing stocks of nutrients and nutrient availability to primary producers (Noe *et al.*, 2013). Nitrogen mineralization rates in all ecosystems are determined by the abundance of nitrogen, the lability of organic matter, and microbial activity (Binkley & Hart, 1989). Excessive amounts of nitrogen fertilizer increase the potential for nitrate leaching (Cameira *et al.*, 2003; Long *et al.*, 2011). The movement of nitrate out of the terrestrial plant root zone depends on the soil hydraulic properties, the amount of irrigation and/or precipitation, the quantity of N applied, the N chemical form in the fertilizer and the time of the application (Cameira *et al.*, 2003).

In the last decades, the recognition of the influence of riparian zone processes on water quality has led to a growing interest in the use of riparian buffer zones along river corridors to mitigate the effects of non-point source pollution (Hill, 1996). Riparian zones function as transition areas between terrestrial and aquatic

environments, and are characterized by the moving of large flows of energy and nutrients between them (Mitsch & Gosselink, 2015). These areas are spatially and temporally heterogeneous with respect to hydrology (Lowrance *et al.*, 1997; Ocampo *et al.*, 2006), soil characteristics (Murray *et al.*, 1995; Jacinthe *et al.*, 1998), and biogeochemical processes (Hill *et al.*, 2000; Mitsch & Gosselink, 2015). Such variability affects the rate of nitrate removal in the riparian zone because the major pathway for nitrate movement is through subsurface flow (Hill, 1996). Thus, the removal capacity of riparian zones is controlled by the water residence time and degree of contact between soil and groundwater (Gold *et al.*, 1998; Ocampo *et al.*, 2006; Noe *et al.*, 2013), and also by plant uptake and denitrification (Groffman *et al.*, 1992, 1996; Aguiar Jr. *et al.*, 2015). The relative influence of these factors depends on soil characteristics (Groffman *et al.*, 1992; Flite III *et al.*, 2001; Sabater *et al.*, 2003) and nitrogen input to the riparian zone (Hanson *et al.*, 1994). Consequently, nitrogen containing molecules applied to the landscape can interact with many different biological components, sometimes in close proximity or separated by great distances in time and space (Schmidt & Clark, 2012).

Several processes to reduce the concentration of nitrate in water have been described in literature (e.g. Kesore *et al.*, 1997; Huang *et al.*, 1998; Pintar *et al.*, 2001; Shrimali & Singh, 2001; Schoeman & Steyn, 2003; Hassan *et al.*, 2010; Jaya *et al.*, 2015; Zahrim *et al.*, 2015). Well-established water treatment processes, such as filtration, are not suitable to remove nitrate from water because it is a stable and highly soluble ion with low potential for adsorption or co-precipitation (Heredia *et al.*, 2006). Conversely, physical and chemical processes are highly efficient in removing nitrate, but are expensive and technologically complex when compared to biological nitrate removal (Della Rocca *et al.*, 2007). Thus, there is a trend towards using wood based solid carbon sources or biodegradable polymers that simultaneously serve as a biofilm carrier and as a source of organic carbon for denitrification (Schipper & Vojvodić-Vuković, 1998; Greenan *et al.*, 2009; Rodrigues *et al.*, 2011, 2013).

Denitrification is an anaerobic process in which NO_3^- and NO_2^- are reduced to N_2O and N_2 (Tiedje, 1988). It occurs when the following conditions are met (Firestone & Davidson, 1989): presence of bacteria capable of nitrate reduction,

availability of an electron donor (like microbially available carbon), low levels of oxygen and supply of electron acceptors (like nitrate or nitrite). This process can be limited by temperature, carbon (C) availability, pH, NO_3^- and/or dissolved oxygen concentrations (Tiedje, 1988; Seitzinger *et al.*, 2006). In areas where the water table is relatively close to the soil surface, there is an opportunity for an enhancement of denitrification by increasing the contact between shallow groundwater and carbon-rich areas that can support denitrification (Schmidt & Clark, 2012). Denitrification can be enhanced by placing woodchips or sawdust in contact with agricultural effluent, in what are termed bioreactors (Schipper *et al.*, 2010b). There are many different techniques available, including containerized treatment systems of woodchips to treat concentrated discharges (denitrification beds) and traditional permeable reactive barriers (denitrification walls) where an organic carbon source is usually mixed within the soil structure to treat diffuse groundwater flowing perpendicularly through the wall (Schipper *et al.*, 2010b; a). Such techniques are characterized by the use of organic media that act as a slow release carbon source. Some of the most common media are wood-based, like sawdust, softwood or hardwood woodchips, bark and mulch (Gibert *et al.*, 2008; Robertson *et al.*, 2008; Capodici *et al.*, 2014). Sometimes those media are chemically modified in order to improve nitrate removal efficiency (Orlando *et al.*, 2002; Keränen *et al.*, 2013, 2015). Other solid carbon sources used as nitrate retention media are cornstalks, maize cobs, wheat straw, cardboard fibers, crab-shell chitin and cotton (Della Rocca *et al.*, 2005; Greenan *et al.*, 2006; Robinson-Lora & Brennan, 2009; Schipper *et al.*, 2010b). Denitrification walls have been proven to be sustainable, with nitrate reductions from 60 to 90% for at least 15 years with no maintenance (Robertson *et al.*, 2008; Moorman *et al.*, 2010; Robertson, 2010; Long *et al.*, 2011; Schmidt & Clark, 2012).

The design of a denitrification wall should contemplate the execution of laboratory feasibility tests, whose main objectives are the selection of a viable media for the wall and the evaluation of its capacity for removing the contaminant of interest (Gibert *et al.*, 2008). Usually, this knowledge is achieved in a first step by using batch and column experiments (Gavaskar, 1999). Therefore, there is a need to

test the feasibility of readily available and cheap wood-based media under Mediterranean conditions.

Portugal is responsible for 49.6% of the world cork production (APCOR, 2018), with several cork products, like granulated or waste cork, easily available on the market. Portugal is also one of the main world producers of stone pine nut, with 344 tons of shelled nut exported in 2014 (INC, 2016). Thus, there is an opportunity to find an economic use for the pine nut shells. Regarding the Tasmanian blue gum, pulp production from this species in Portugal represents 7.3% of the Confederation of European Paper Industries (CEPI) members pulp production in 2016 (CEPI, 2017). Currently, the bark from this species is used by the pulp industry as fuel for energy production, being interesting to study alternative uses for this material. Silver wattle is a widespread exotic invader in Portugal (Almeida & Freitas, 2006). Its management is difficult and costly, so there is some interest in researching new uses that may provide added value in the control of this species.

In this context, it is of utmost importance to test alternative wood-based media, namely cork, pine nut shells, Tasmanian blue gum bark and silver wattle bark, as carbon sources to enhance biological denitrification. Therefore, a microcosm batch test was developed to investigate whether the aforementioned organic substrates are useful for denitrification enhancement in soil environment.

2. MATERIALS AND METHODS

2.1 Materials

The following organic substrates were used in this study: cork oak outer bark (phellogen) (*Quercus suber* L.), hereinafter referred to as cork, pine nut shell (*Pinus pinea* L.), silver wattle bark (*Acacia dealbata* Link) and Tasmanian blue gum bark (*Eucalyptus globulus* Labill.). Cork was provided by Amorim Cork Composites, pine nut shells were stored in the Forestry Research Centre warehouse and are from unknown origin, silver wattle bark came from the Sintra Mountain (20 km west of Lisbon) and Tasmanian blue gum bark came from a Portuguese pulp and paper mill (Portucel Soporcel Group).

Additionally, a soil substrate (from a Fluvisol, *sensu* WRB2014) collected in the river Sorraia alluvial plain (Torrinha Estate, Biscainho, Coruche, N 38.941066°; W -8.662611°) was used as control and mixed with the organic substrates for the batch denitrification study. The soil material was collected in the 10 – 100 cm depth range.

2.2 Characteristics of the substrates

The main characteristics of the evaluated substrates are given in the Tables 23 and 24. Cork presented the highest organic C content and the Tasmanian blue gum bark the lowest. The nitrogen content of the silver wattle bark was much higher than in the other organic substrates. The soil material presented a loam texture (Atterberg Scale).

Table 23. Composition of the organic substrates: organic C (Org C), total N, P, Ca, Mg, K and Na.

Organic substrates	Org C	N	P	Ca	Mg	K	Na
	g kg ⁻¹						
Cork	534.90	4.42	0.2366	3.76	0.23	2.74	0.27
Pine nut shell	439.88	6.66	1.0660	1.78	1.12	2.32	0.52
Silver wattle bark	490.68	13.74	0.6472	6.33	0.99	5.68	0.36
Tasmanian blue gum bark	438.36	2.95	0.5525	26.29	2.41	4.59	1.06

Table 24. Main characteristics of the soil material used in the experiment: concentrations of organic C (Org C), total N, extractable P (P_{ext}) and K (K_{ext}) and non-acid cations, pH and particle-size distribution.

Org C	N	P_{ext}	K_{ext}	Ca^{2+}	Mg^{2+}	K^+	Na^+	pH		Particle-size distribution (g kg ⁻¹)			
g kg ⁻¹		mg kg ⁻¹		-----	cmol _c kg ⁻¹	-----		H ₂ O	KCl	Coarse sand	Fine sand	Silt	Clay
11.65	0.97	69.28	96.63	4.79	2.46	0.34	0.30	5.46	4.08	403.3	213.3	216.7	166.7

2.3 Experiments

- Leaching tests

Leaching tests were carried out to determine the leachable nitrogen of the organic substrates. Two grams of each substrate were placed into a 50 mL centrifuge

tube and 45 mL of deionized water were added. There were three replicas for each substrate. The tubes were placed in a rotation incubator (Fröbel Labortechnik CMV-ROM) for 66 hours at 132 rpm. The supernatant was filtered through a 0.45 µm syringe filter, stored at -18 °C and analyzed for NO₃⁻-N, NO₂⁻-N, NH₄⁺-N and total organic carbon (TOC).

- Batch denitrification study

A batch microcosm experiment was conducted in 1.8 dm³ glass flasks. In each treatment the flasks were filled with 0.9 dm³ (bulk volume) of a 4:1 mixture of organic substrate (<2 mm in diameter) and soil substrate. In the control treatment the flasks were filled with 0.9 dm³ of soil substrate. The remaining volume was filled up to the lid with an amended distilled water solution (50 mg L⁻¹ NO₃⁻-N + 2mg L⁻¹ PO₄⁺-P), thus avoiding headspace formation. Potassium hydrogen phosphate was added to the solution to avoid any phosphorus limiting effect on bacteria metabolism (White & Reddy, 1999; Capodici *et al.*, 2014). The flasks were sealed to create anaerobic conditions and covered with aluminum foil to simulate light conditions encountered in an aquifer. All experiments were conducted at 24 ± 2 °C. Samples (85 mL) were taken periodically. The extracted volume was replaced with new nitrate-amended water solution. At the end of the sampling procedure the flasks were shaken to homogenize the content. Collected samples were filtered through a 0.45 µm syringe filter. Part of the sampled volume (55 mL) was analyzed for dissolved oxygen (DO), pH and temperature. The remaining volume (30 mL) was stored at -18 °C and later analyzed for NO₃⁻-N, NO₂⁻-N, NH₄⁺-N and total organic carbon (TOC).

The DO loss rate through time (K) of the amended distilled water solution was calculated using the following equation (Olson, 1963):

$K = -\ln\left(\frac{X}{X_0}\right)$, where X is the DO concentration at a given time and X_0 is the DO concentration at the start of the experiment.

The denitrification rates were calculated from the nitrate concentration versus time experimental points (Figure 81a). The selected points for this calculation were those unaffected by the release of high amounts of nitrates (Gibert *et al.*, 2008). Thus the 6 hours experimental points were excluded from the

calculations, as well as the silver wattle 1-day experimental point. Nitrate concentrations for denitrification rate calculations were corrected for both ammonium and nitrite concentrations. Additionally, the calculated volumetric rates were normalized on a substrate mass basis to ease the comparison with relevant literature.

Nitrate removal through denitrification was calculated as the overall nitrate removal minus the net production of ammonium after 16 days of experiment (Gibert *et al.*, 2008). Volatilization loss of ammonium was ignored because the pH in the flasks was lower than 8 (Vlek & Stumpe, 1978). Nitrate immobilization was presumed negligible, as reported in similar studies (Schipper & Vojvodić-Vuković, 2000; Greenan *et al.*, 2006).

2.4 Analytical methods

- Soil substrate

Soil properties were determined on air-dried samples. Particle size fractions were determined as described by Póvoas & Barral (1992). Organic C was determined by wet oxidation (De Leenheer & Van Hove, 1958), total N using Kjeldhal digestion (Digestion System 40, Kjeltex Auto 1030 Analyzer) (Bremner & Mulvaney, 1982) and extractable P and K by the Egnér-Riehm test (Egnér *et al.*, 1960), followed by determination with the molybdate-blue method (Murphy & Riley, 1962). The soil exchangeable non-acid cations were extracted by the standard method (1 M NH₄OAc, adjusted at pH 7.0; Chapman, 1965) and measured by atomic absorption spectrometry (Aanalyst 300, Perkin Elmer). Soil pH was determined potentiometrically in distilled water and KCl 1 M (soil/solution ratio, 1:2.5).

- Organic substrates

The organic substrates were fractionated and separated by particle size using a knife mill (Retsch SM 2000) with an output sieve of 6 x 6 mm and screened using sieves with the following mesh sizes: 15 (1 mm) and 10 (2 mm). The analysis was done using a granulometric fraction of ≤ 1 mm. Organic C and Total N were determined as in the soil samples. The content of Ca, Mg, K, Na and P were determined using wet oxidation in HClO₄ and HF (1:1, heated in a sand bath at

80°C), followed by solubilization with HCl 3M and filtration. They were then measured by atomic absorption spectrometry (AAnalyst 300, Perkin Elmer).

- Experiments

Potassium nitrate (KNO_3) (> 99% purity, LabChem Inc.) and potassium hydrogen phosphate (K_2HPO_4) (\geq 98% purity, Sigma-Aldrich) were used to prepare the amended distilled water solution used in the study.

Dissolved oxygen and temperature were measured with a Hanna HI-98193 portable dissolved oxygen meter. The pH measurements were done using a Thermo Electron Corporation Orion 410 A+ Basic pH meter. The NO_3^- -N, NO_2^- -N and NH_4^+ -N concentration in the samples was determined using an automated segmented flow analyzer (Skalar, San Plus System, Netherlands). The NO_3^- -N and NO_2^- -N measurements were made in the 540 nm wavelength. The NH_4^+ -N measurements were made in the 660 nm wavelength. It was not possible to analyze the TOC concentration because the samples stored for this purpose were lost due to a malfunction in the cold storage unit.

2.5 Data analysis

Data analysis was made using the integrated development environment RStudio (version 1.1.383) (RStudio Team, 2017) and R (version 3.4.3) (R Core Team, 2017) statistical software. The distribution of the residuals was assessed using the One-sample Kolmogorov-Smirnov test and visually, using histograms and normal Q-Q plots. Homoscedasticity was assessed by the Brown-Forsythe test (Brown & Forsythe, 1974) using the lawstat R Package (version 3.2) (Gastwirth *et al.*, 2017) and visually through residuals vs. fitted values plots.

- Leaching tests

Data was analyzed using non-parametric tests, because the normality of residuals and homoscedasticity assumptions were not met. A Kruskal-Wallis test was performed to establish if the leached nitrogen distribution was the same between the different substrates. In order to compare concentration differences between substrates, a Dunn multiple comparisons test using rank sums was performed (Dunn, 1964; Zar, 2010). This was done using the FSA R Package (version 0.8.19) (Ogle, 2018).

- Batch denitrification study

The normality of residuals and homoscedasticity assumptions were met after transforming the removal rate data (base-10 log transformation) and removal efficiency data (arcsine transformation). Fixed effect (Type I) one-way ANOVAs were performed to ascertain if the mean NO_3^- -N removal rate and mean NO_3^- -N removal efficiency were the same between the different substrates. This was done using the Companion to Applied Regression R Package (version 3.0-0) (Fox *et al.*, 2018). In order to compare the mean NO_3^- -N removal rate differences between substrates, a Tukey HSD test was performed (Tukey, 1949) using the Statistical Procedures for Agricultural Research R Package (version 1.2-8) (Mendiburu, 2017).

3. RESULTS

3.1 Leaching tests

The results regarding the leaching tests are shown in Table 25. The highest content of NH_4^+ -N leached by the silver wattle (0.12 mg g^{-1}) is noteworthy. None of the substrates released detectable contents of NO_3^- -N ($< 0.0003 \text{ mg g}^{-1}$).

Table 25. Average leachable nitrite, nitrate and ammonium of the evaluated substrates, with standard error ($n=3$). Substrates with different letters are significantly different according to Dunn's test ($p<0.05$).

Substrate	Leachable amounts ($\text{mg g}^{-1}_{\text{sub}}$)		
	NO_3^- -N	NO_2^- -N	NH_4^+ -N
Soil	$< 0.0003 \pm 0.00^a$	$< 0.0003 \pm 0.00^a$	$< 0.0003 \pm 0.00^a$
Cork	$< 0.0003 \pm 0.00^a$	$< 0.0003 \pm 0.00^a$	$< 0.0003 \pm 0.00^a$
Pine nut shell	$< 0.0003 \pm 0.00^a$	0.0002 ± 0.00^a	0.0012 ± 0.00^a
Silver wattle bark	$< 0.0003 \pm 0.00^a$	0.0004 ± 0.00^a	0.1234 ± 0.01^b
Tasmanian blue gum bark	$< 0.0003 \pm 0.00^a$	$< 0.0003 \pm 0.00^a$	0.0288 ± 0.03^a

It was not possible to analyze the TOC content because the samples stored for this purpose were lost due to a malfunction in the cold storage unit.

3.2 Batch denitrification study

- pH

The starting pH in all treatments varied between 7.1 and 7.7, with a decrease to between 5.3 and 5.8 after 6 hours. Over time, the pH values increased, with only small differences in the observed values between treatments, which ranged between 6.0 and 7.0 (Figure 79). The exception was the silver wattle, which showed a lower pH (averaging 5.67 ± 0.07) during the study period (Figure 79).

- Dissolved Oxygen (DO)

The measured values showed a marked decrease in the DO levels after 24 hours, followed by a slower decrease over time towards hypoxic conditions ($< 2 \text{ mg L}^{-1}$) (Figure 80). The exceptions were the cork treatment, in which the DO decrease trend was slower, and the control treatment, where there was an increase in the DO levels between the 5th and the 8th day (Table 26). Overall, the DO levels remained at or near hypoxic conditions for most of the study timeframe (Figure 80).

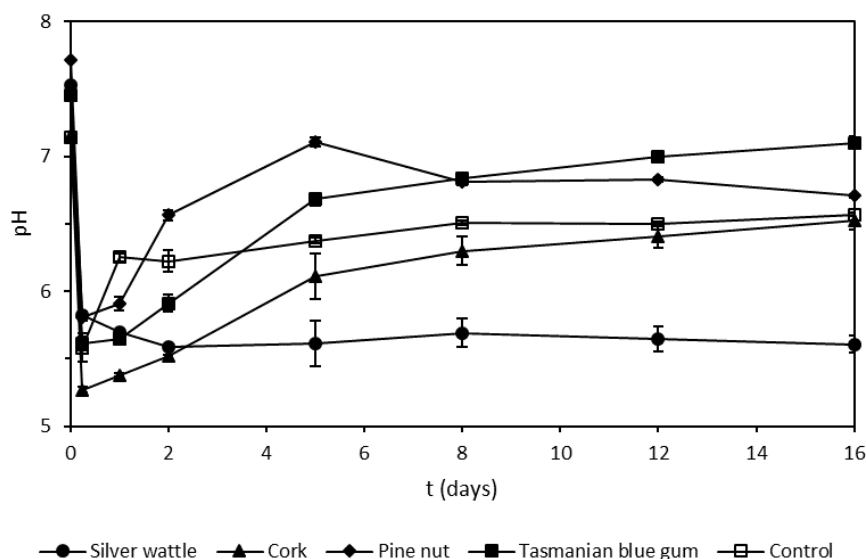


Figure 79. Change in pH over time for the tested organic substrates. Data points represent averages from three replicas. The vertical bars are standard errors ($n=3$).

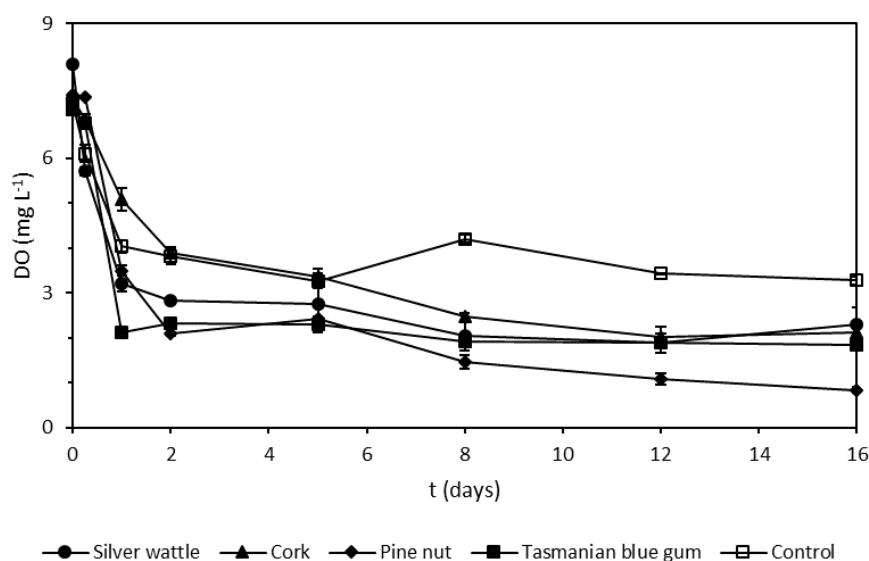


Figure 80. Change in DO over time for the tested organic substrates. Data points represent averages from three replicates. The vertical bars are standard errors ($n=3$).

- Nitrate, nitrite and ammonium

The pine nut, Tasmanian blue gum and cork treatments removed all the nitrate to residual (Cork, $0.29 \pm 0.22 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$) or below detection levels ($< 0.01 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$) by the end of the study (Figure 81a). However, pine nut presented the fastest nitrate decrease, reaching residual levels 2 days after the start of the study (Tasmanian blue gum needed 8 days to achieve a similar decrease, and cork needed 16 days). The silver wattle and the control treatments exhibited a different behavior. In the former, nitrate rose to $55.66 \pm 0.33 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$ after 6 hours, maintaining similar levels for 2 days, after which nitrate gradually decreased until reaching a concentration of $18.05 \pm 8.71 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$. Regarding the control treatment, there was a strong increase in the nitrate concentration 6 hours after the start of the trial ($76.62 \pm 5.58 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$), with a gradual decrease up to the end of the trial ($27.32 \pm 0.52 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$).

Nitrite was present in all treatments (Figure 81b). It occurred in small concentrations in the Tasmanian blue gum, cork and pine nut treatments, although with a $20.35 \pm 1.93 \text{ mg L}^{-1} \text{ NO}_2^- \text{-N}$ peak in the latter. In the silver wattle treatment nitrite was observed only at day 5 of the experiment, also in small concentrations. In the control treatment nitrite ranged between 0.26 ± 0.06 and $6.99 \pm 1.00 \text{ mg L}^{-1} \text{ NO}_2^- \text{-N}$ during the 16 days of the trial.

Table 26. Fractional DO loss rate through time (K) of the amended distilled water solution.

Time (d)	Silver wattle	Cork	Pine nut	Tasmanian blue gum	Control
0	0,00	0,00	0,00	0,00	0,00
6 hours	0,15	0,03	0,00	0,02	0,07
1	0,40	0,16	0,33	0,52	0,25
2	0,46	0,27	0,55	0,49	0,28
5	0,47	0,34	0,48	0,49	0,35
8	0,60	0,47	0,71	0,57	0,24
12	0,63	0,56	0,84	0,57	0,32
16	0,55	0,54	0,95	0,59	0,34

The silver wattle treatment presented a sustained ammonium increase throughout the experiment, with a peak of $10.61 \pm 0.16 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$ after 16 days (Figure 81c). The pine nut treatment also showed moderate levels of ammonium, although with a slightly different trend: the concentration peaked at $11.86 \pm 0.36 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$ after 1 day, then decreased to $6.00 \pm 0.19 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$ after 5 days, followed by a slow increase trend towards the end of the experiment (Figure 81c). There were negligible amounts of ammonium in the Cork and Tasmanian blue gum treatments, with small concentration peaks of $1.42 \pm 1.33 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$ after 1 day for the former, and of $1.13 \pm 1.13 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$ after 2 days for the latter. On both treatments the concentration levels decreased sharply after peaking. Ammonium concentration in the control treatment was also low, although it peaked at $2.49 \pm 1.09 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$ after 16 days (Figure 81c).

The Tasmanian blue gum treatment presented the highest denitrification rate ($0.0709 \pm 0.001 \text{ mg NO}_3^-\text{-N L}^{-1} \text{ d}^{-1} \text{ g}^{-1} \text{ sub}$) and the control treatment the lowest one ($0.0026 \pm 0.000 \text{ mg NO}_3^-\text{-N L}^{-1} \text{ d}^{-1} \text{ g}^{-1} \text{ sub}$). There were significant differences between the average denitrification rates of the tested substrates (one-way ANOVA, $F_{4, 10}=162.92$, $p=4.671 \times 10^{-9}$). There were significant differences between all the tested substrates, except for the pine nut shell ($0.0070 \pm 0.000 \text{ mg NO}_3^-\text{-N L}^{-1} \text{ d}^{-1} \text{ g}^{-1} \text{ sub}$) and the control treatment (Figure 82). The average denitrification rate in the Tasmanian blue gum treatment was significantly higher than in the other substrates.

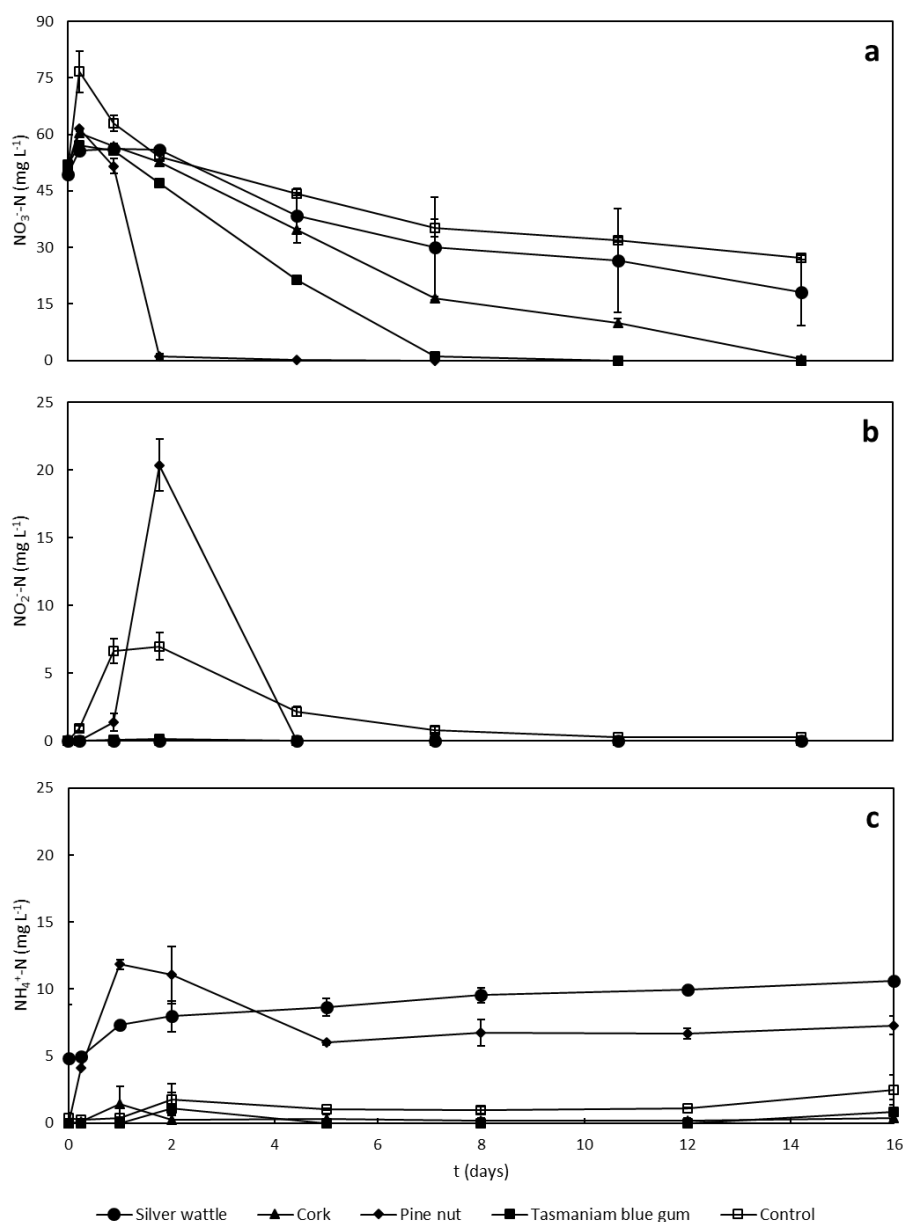


Figure 81. Change in nitrate (NO_3^- -N), nitrite (NO_2^- -N) and ammonium (NH_4^+ -N) (mg L^{-1}) over time for all the tested substrates. The scale of the y-axis varies between figures. The vertical bars are standard errors ($n=3$).

There were significant differences between the mean denitrification efficiency of the tested substrates (one-way ANOVA, $F_{4, 10}=8.44$, $p=0.0030$). Cork (98.5 ± 0.62 %) and Tasmanian blue gum (95.5 ± 3.73 %) mean denitrification efficiency was significantly higher than that of the silver wattle (45.3 ± 21.88 %) and control (41.3 ± 3.08 %) treatments (Figure 83). There were no significant differences between the pine nut treatment (81.9 ± 0.85 %) and the other substrates (Figure 83).

The dissimilatory nitrate reduction to ammonium (DNRA) contributed 19.5 ± 4.47 % of the nitrate removal for silver wattle, 16.0 ± 1.53 % for pine nut, 5.2 ± 2.59 % for the control and less than 2 % for each of the remaining tested substrates (Figure 83).

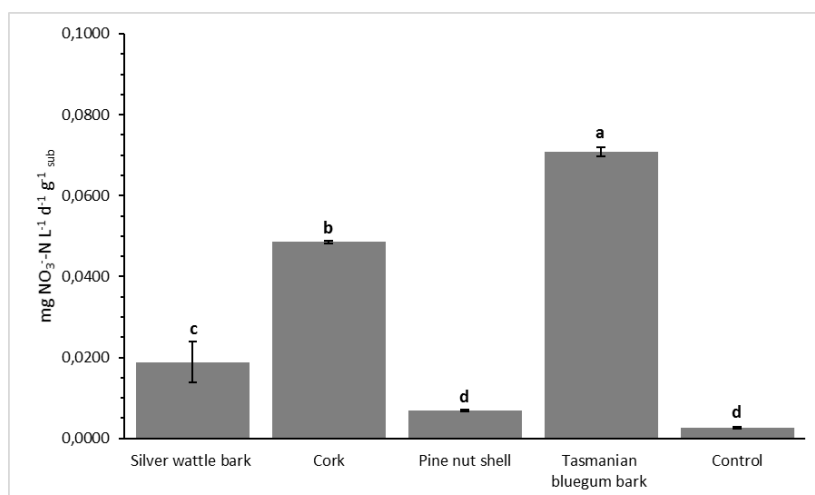


Figure 82. Mean daily denitrification rates per mass of tested substrate. The vertical bars are standard errors ($n=3$). Bars with different letters are significantly different according to Tukey's HSD test ($p<0.05$).

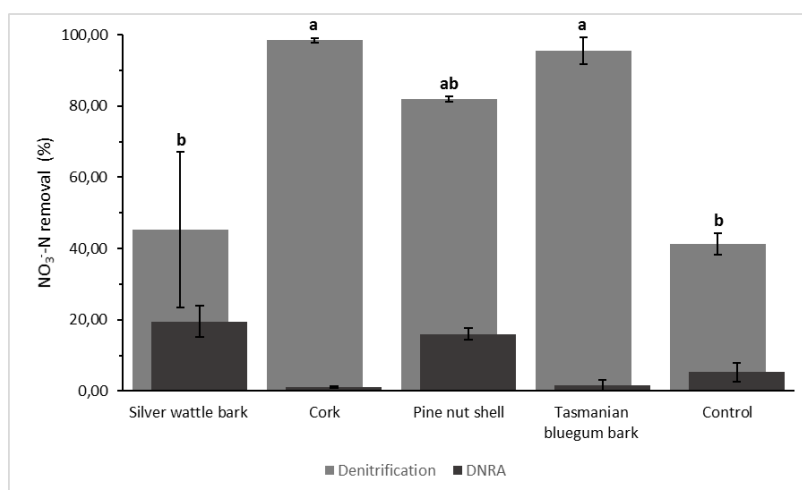


Figure 83. Average denitrification and DNRA NO₃-N removal efficiency. The vertical bars are standard errors ($n=3$). Bars with different letters are significantly different according to Tukey's HSD test ($p<0.05$).

4. DISCUSSION

With the exception of the silver wattle treatment, the pH levels remained near or at the optimal range for denitrifiers (Knowles, 1982). The low pH levels in the silver wattle treatment may be explained by the leaching of the bark tannin content. Although less likely due to the low oxygen levels in the flasks, the pH decrease could also be the result of the production of hydronium ions (H_3O^+) during the initial nitrification period (2 days).

The neutral pH and the rapid decrease of the DO levels towards hypoxic conditions ($< 2 \text{ mg L}^{-1}$) indicates that the denitrifying bacteria populations could develop in all flasks without limitations in relation to these parameters. Nevertheless, denitrification seemed to occur even at higher DO levels, although at a much slower rate. The seemingly low sensitivity to DO levels in the denitrification process was reported by other authors (e.g. Gómez *et al.*, 2002; Healy *et al.*, 2006; Robertson *et al.*, 2009; Warneke *et al.*, 2011), and may be explained by the non-homogeneity of the substrate mixture, which can create anaerobic micro niches where denitrification takes place (Robertson & Kuenen, 1984).

The inability to analyze the TOC content of the flasks over time due to the destruction of the samples was a major setback. That fact did not allow for the assessment of the organic carbon levels available to the micro-organisms. Thus, it was not possible to know if the availability of organic carbon was a limitation of the denitrification process during the experiment timeframe.

Nitrate removal was observed in all treatments, although at different rates and extent. All treatments presented an initial nitrification phase (6 hours after the start of the experiment), followed by the start of the nitrate removal process (except in the silver wattle treatment). The steadily decrease in nitrate concentrations towards depletion in the Tasmanian blue gum, cork and pine nut treatments, together with the pH increase (probably from the generation of hydroxyl ions during denitrification) and the fast consumption of the DO, indicated that denitrification conditions developed quickly in the flasks. The silver wattle and control treatments did not manage to remove nitrate to values below the maximum permissible concentration set by the European Nitrates Directive (11.3

mg L⁻¹ NO₃⁻-N) (European Commission, 1991). In the silver wattle treatment, the nitrification phase was longer (lasted for 48 hours), followed by a continuous nitrate decrease until reaching the concentration of 18.05±8.71 mg L⁻¹ NO₃⁻-N at the end of the experiment. The lower nitrate removal capability may be related with the inhibitory effect of silver wattle bark tannins on denitrification, as reported by Matsubara & Ohta (2015) for *Acacia mangium* Willd bark. Additionally, the low average pH of this treatment (5.67±0.07) may have slowed the denitrification rate (Lance, 1972). Denitrification is positively correlated with the organic matter content of alluvial soils (e.g. Brettar *et al.*, 2002; Baker & Vervier, 2004; Hernandez & Mitsch, 2007; Gift *et al.*, 2010), so the slow decrease in nitrate concentration in the control treatment was expected. Nevertheless, our results show that, in spite of the relatively low organic matter content (Table 24), the studied soil substrate was able to support denitrification and remove some nitrate (Puckett & Cowdery, 2002).

The low concentrations of observed nitrites in the cork, pine nut, silver wattle and Tasmanian blue gum treatments is the result of their fast reduction to N₂O or N₂, which suggests that the inhibition of NO₂⁻ reductase due to high NO₃⁻ concentrations was small or non-existent (Kornaros *et al.*, 1996). The nitrite spike in day 2 of the pine nut treatment resulted from the incomplete reduction of nitrate and may be explained by the very fast denitrification rate that took place between samplings (NO₃⁻-N concentration decrease from 51.59±1.98 mg L⁻¹ to 1.08±0.59 mg L⁻¹ in 24 hours). The accumulation of nitrites was more evident in the control treatment and it is probably related to the higher average DO concentration (4.42±0.11 mg L⁻¹ O₂) in this treatment. High oxygen concentration decreases nitrate removal rate and increases the accumulation of nitrites (Gómez *et al.*, 2002). Nitrate accumulation may also be related with the C source, C/N ratio of the substrate and the pH (Wang *et al.*, 2015; Rocher *et al.*, 2015).

The low to moderate sustained ammonium increases observed in the silver wattle, pine nut and control treatments suggest that dissimilatory nitrate reduction to ammonia (DNRA) was taking place in these treatments. DNRA is a anaerobic reaction in which nitrate is reduced to ammonium (Tiedje, 1988), instead of being converted to N₂, as in denitrification. DNRA and denitrification compete for NO₃⁻ under hypoxic or anaerobic conditions (Schipper & Vojvodić-Vuković, 2000;

Greenan *et al.*, 2006). Some of the main factors that control this competition are labile organic carbon, nitrate availability and C/N ratio (Yin *et al.*, 2002; Burgin & Hamilton, 2007; Kraft *et al.*, 2014; Smith *et al.*, 2015; Liu *et al.*, 2016; Shan *et al.*, 2016). Therefore, it is probable that the suspected DNRA activity was the result of a high C/NO₃⁻ ratio, especially because in a batch system there is a tendency for DOC accumulation over time (Ovez, 2006). However, the lack of TOC data does not allow for the confirmation of this assumption.

The average daily denitrification rates per mass of substrate obtained in this experiment are lower than or comparable to those which have been previously reported for other substrates in batch tests (Table 27). Nevertheless, our results from the Tasmanian blue gum treatment are higher than those reported by Gibert *et al.* (2008) for mulch (0.066 mg NO₃⁻-N L⁻¹ d⁻¹ g⁻¹ sub) and for hardwood (0.035 mg NO₃⁻-N L⁻¹ d⁻¹ g⁻¹ sub). Regarding the cork treatment, our results are 5.8 times lower than those obtained by Capodici *et al.* (2014) for the same substrate (0.28 mg NO₃⁻-N L⁻¹ d⁻¹ g⁻¹ sub). Although pine nut shell presented the second highest NO₃⁻-N removal at the end of the study, the daily denitrification rate per mass of substrate was strongly influenced by its higher density. The wide range of rate values in the literature is probably related with different test parameters, like the type of organic substrate and inoculum (if any), the presence or absence of a nutrient medium, the inclusion of a easily degradable organic compound, the solid – liquid ratio, the initial nitrate concentration, the operational conditions of the batch reactors (agitation of the flasks, constant addition of nitrate) and temperature (Gibert *et al.*, 2008).

The nitrate removal efficiency varied with the type of substrate used. Also, silver wattle results were strongly influenced by the low denitrification performance of one of the replicas. The percentage of NO₃⁻ removed through denitrification was higher than 95% for cork and Tasmanian blue gum. On the contrary, in the silver wattle and pine nut shell treatments DNRA contributed with more than 19% of NO₃⁻ removal in the former and a little more than 16% in the latter. Our results show a predominance of denitrification over DNRA in nitrate removal, which is in accordance with results reported by other authors (e.g. Schipper & Vojvodić-Vuković, 2000; Greenan *et al.*, 2006; Gibert *et al.*, 2008; Capodici *et al.*, 2014).

Table 27. Summary of key parameters and results in batch denitrification experiments with natural organic substrates without any supplement of easily assimilable organic compounds reported in the literature (Adapted from Gibert *et al.*, 2008).

Organic substrate	Nutrient medium provided?	Initial NO ₃ ⁻ -N (mg L ⁻¹)	Overall NO ₃ ⁻ removal (%)	Denitrification rate (mg NO ₃ ⁻ -N L ⁻¹ d ⁻¹ g ⁻¹ sub)	Temperature (°C)	Reference
Cotton burr compost	No	20	> 99	0.91 ^a	23	Su & Puls (2007)
Giant reed	Yes	100	> 99	3.33	20	Ovez (2006)
Liquorice	Yes	100	> 99	6.20	20	Ovez (2006)
Seaweed	Yes	100	> 99	13.13	20	Ovez (2006)
Woodchips	No	100 ^b	85.4 ^c	0.45	20	Greenan <i>et al.</i> (2006)
Woodchips + Soybean oil	No	100 ^b	80.1 ^c	0.76	20	Greenan <i>et al.</i> (2006)
Cardboard	No	100 ^b	95.8 ^c	1.05	20	Greenan <i>et al.</i> (2006)
Cornstalks	No	100 ^b	91.7 ^c	2.88	20	Greenan <i>et al.</i> (2006)
Softwood	No	32.2	98.7	0.067	22	Gibert <i>et al.</i> (2008)
Hardwood	No	32.2	98.7	0.035	22	Gibert <i>et al.</i> (2008)
Coniferous	No	32.2	95.1	0.048	22	Gibert <i>et al.</i> (2008)
Mulch	No	32.2	89.7	0.066	22	Gibert <i>et al.</i> (2008)
Willow	No	32.2	86.3	0.056	22	Gibert <i>et al.</i> (2008)
Compost	No	32.2	92.7	0.026	22	Gibert <i>et al.</i> (2008)
Leaves	No	32.2	93.9	0.217	22	Gibert <i>et al.</i> (2008)
Mixture	No	32.2	98.3	0.048	22	Gibert <i>et al.</i> (2008)
Soil	No	32.2	73.2	0.001	22	Gibert <i>et al.</i> (2008)
Pine bark	No	60	59.7	0.09	21	Capodici <i>et al.</i> (2014)
Olive pomace	No	60	60.3	0.05	21	Capodici <i>et al.</i> (2014)
Sawdust	No	60	77.7	0.28	21	Capodici <i>et al.</i> (2014)
Cork	No	60	80.8	0.37	21	Capodici <i>et al.</i> (2014)
Soil substrate	No	50	42.4	0.0026	24	This study
Cork	No	50	98.7	0.0485	24	This study
Pine nut shell	No	50	85.9	0.0070	24	This study
Silver wattle bark	No	50	52.5	0.0189	24	This study
Tasmanian blue gum bark	No	50	98.4	0.0709	24	This study

Note:

^a Rate value roughly estimated from Figure 2.

^b NO₃⁻ spiked when concentration was lower than 10 mg L⁻¹.

^c Calculated as the difference between total NO₃⁻ added and total NO₃⁻ recovered in Table 2.

Although the soil used in the batch test presented a non-neglectable nitrate removal capability, it is not representative of the aquifer material. It was collected from the upper soil layers, which have a higher organic matter content than the coarser material in the aquifer. Denitrification in soils under anaerobic conditions

increases in the presence of higher soil organic matter content (Burford & Bremner, 1975; Brettar *et al.*, 2002).

5. CONCLUSIONS

All the tested substrates were capable of enhancing biological denitrification, although at different extents. To our knowledge, this was the first time that the Tasmanian blue gum bark, silver wattle bark and pine nut shell were tested as a carbon source to enhance biological denitrification. Nitrate reduction occurred in connection with some ammonium production, indicating that DNRA was also taking place. The best substrates in terms of denitrification efficiency were cork and Tasmanian blue gum, with a nitrate removal through denitrification of 98% in the former and 96% in the latter; the denitrification rate was $0.0485 \text{ mg NO}_3\text{-N L}^{-1} \text{ d}^{-1} \text{ g}^{-1} \text{ sub}$ for cork and $0.0709 \text{ mg NO}_3\text{-N L}^{-1} \text{ d}^{-1} \text{ g}^{-1} \text{ sub}$ for Tasmanian blue gum. Silver wattle and pine nut shell were considered unsuitable due to insufficient nitrate removal (in the former) and excessive reduction of nitrate to ammonium (on both). The microcosm batch experiment allowed for the selection of the best substrate for further studies. Thus, future research will focus on testing Tasmanian blue gum bark in a continuous column apparatus.

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SECTION V

GENERAL CONCLUSIONS

CHAPTER 8

Conclusions

1. CONCLUSIONS

The objectives and the conclusions achieved in this study are presented as follows. Resulting ideas of future work are also listed.

1.1 Objective a. To analyze how restoration standards in Europe can be improved, through soft law and reinforcement mechanisms recommendations

Although nature restoration legislation already exists in the European Union for 39 years (the 1979 Birds Directive), the freshwater ecosystems of the member countries are some of the most degraded and fragmented in the world. Thus, significant restoration efforts are still expected to occur under existing legislation (European Commission, 2011). However, recent European experience regarding the implementation of the Water Framework Directive (WFD) and Nature Directives shows how difficult it can be to achieve ambitious goals. Moreover, results from the implementation of the WFD in these past eighteen years indicate that by 2015 slightly less than half of the Member States water bodies complied or were expected to comply with the good ecological status target (EEA, 2012). Thus, the creation of new/further ecosystem restoration soft law and reinforcement mechanisms related with governance, quality, stakeholders, publicity and research is highly recommended to improve freshwater restoration success in Europe. Likewise, the creation of mechanisms of cooperation and joint sharing of knowledge (national or supra-national) may help to improve restoration success and address legal framework failures.

1.2 Objective b. To assess the impacts that the combined effects of climate change and management practices may have on nitrate concentrations in the water of an agricultural river basin with crop irrigation and water abstraction problems

In this work we proposed to predict the nitrate behavior in the Sorraia river basin using three different storylines, which combine alternative management practices, including different environmental measures, with climate change scenarios. One of the scenarios (STL2) simulates fewer pressures in the basin and includes environmental conservation measures, like increasing the riparian buffer width. However, the simulation results do not show a relevant improvement

of the river nitrogen concentrations when compared with the other storylines. Also, the water quality in the Sorraia basin is expected to deteriorate in the modeled timeframe, with nitrogen concentrations likely to increase up to 101% by 2060. The nitrate increase seems to be related with land use and agricultural practices, with higher concentrations of nutrient pollution particularly in scenarios where there is agricultural expansion and an increase in fertilization. Furthermore, climate change will also result in a strong reduction of annual mean streamflow in the Sorraia river, a decrease of the river's dilution capability, and an increase of nutrient concentration. Thus, there is a joint effect of climate change and land use on the river water quality. Accordingly, intensive agricultural practices may aggravate the negative impacts of climate change in the ecological quality of rivers. Also, the proposed environmental conservation measures may be too conservative to have a significant effect in the river nitrogen concentration, particularly in a climate change context. These results highlight the importance of implementing adaptative management solutions that contemplate both climate and land use changes. Moreover, there is a need for further research on river basin management and its effects on river water quality in a climate change context, to improve the ecological quality of our river systems and fulfill the Water Framework Directive obligations.

1.3 Objective c. To assess the results of a wetland restoration

The plantation of riparian forest species seedlings produced through classical forest nursery methods, together with the application of soil bioengineering techniques, allowed for the successful restoration of the area submitted to intervention. Currently the site presents a more complex plant community structure, with abundant natural regeneration and the presence of multiple feeding, breeding and shelter habitats for waterfowl. The restoration of this pilot area provided a good insight into the needs and problems related with this type of intervention in Mediterranean wetlands, notably the ones concerning plant survival. It is also clear that riparian restoration is a long-term process and that it needs continuous monitoring to guide adaptive corrections. Tree survival and growth were satisfactory, although it is unclear if this restoration effort restored all of the ecological functions associated with the native wetland ecosystem (Avera *et al.*, 2015). Moreover, cattle grazing, and other types of human

disturbance may endanger what was achieved so far. Thus, local population awareness and participation are as essential as water table levels and tree installation techniques for wetland restoration success.

1.4 Objective d. To assess the results of habitat restoration in selected river sections of a regulated intermittent Mediterranean river

Several river segments impacted by intensive permanent agricultural crops and flow modification were selected for an environmental restoration intervention. Although the elapsed time is still short for definitive conclusions, natural riparian vegetation cover has improved in the restored areas and riverbank stability was enhanced, particularly in Sectors I and M. The control of the exotic invader *A. donax* was less successful, with a slow but steady increase of the number of patches of this species. Also, although native fish habitat heterogeneity and quality has improved, it was not followed by an increase in *S. aradensis* and *I. almacai* populations, probably because the populations in the area are too impoverished to respond to such short period and limited area of restoration. Riverbank restoration in Mediterranean areas using soil bioengineering techniques needs careful management in the early years, particularly regarding plant water stress more so in the view of future climatic changes. This study showed the likely need to irrigate and control invasive weeds in the years following restoration. Anthropogenic factors, like livestock grazing and organic pollution are other major threats to the success of this type of restoration project. The implementation of an ecologically effective restoration should have enough flexibility to adjust to changing climate and societal priorities, retaining simultaneously the capacity to integrate information from new technologies into site assessment and restoration planning (Kondolf *et al.*, 2011).

1.5 Objective e. To assess the impacts of the liquid effluent, notably nutrients, of an acid bisulfite pulp mill on the river water of a major Iberian river

The monitoring of spatial and temporal characteristics of industrial effluents is important to assist stakeholders committed to water resources management. However, the type of sampling performed, a non-systematic first approach, limited in time, did not allow for the establishment of a profile of spatial-temporal evolution of the sampled parameters, but only to characterize the current

situation. Thus, more sampling dates and greater spatial coverage would be required for more detailed and comprehensive conclusions. Moreover, pollutants levels are very dependent of changes in river flow (Dassenakis *et al.*, 1998; Xue *et al.*, 2000; Neal *et al.*, 2006; Ltifi *et al.*, 2017). Hence, it is necessary that sampling encompasses a wide range of weather and flow conditions. Furthermore, it would also be important to sample the final section of the Zêzere River, an important Tagus tributary with influence in the sampled river section, to determine its influence on the parameters sampled in transects 2 to 5.

The variation of the levels of pH, total phosphorous, total suspended solids, dissolved nickel, dissolved organic carbon, dissolved zinc, soluble lignin and cellulose seems to be at least partially related with the CAIMA effluent outflow. Still, despite the somewhat relevant levels of total phosphorous and dissolved lignin, our results did not show particularly high levels of pollution. Changes in total phosphorus, total nitrogen, nitrate and total solids levels were important, but not necessarily directly related to the manufacturing process, since the CAIMA effluent is mixed with domestic effluents. Furthermore, phosphorus levels in the Tagus River are also relatively high upstream of the effluent outflow, meaning that the river suffers from nutrient pollution even before the sampled river section. Nevertheless, the existence of consistently high levels of total phosphorous, dissolved lignin, cellulose, pH, total nitrogen and dissolved zinc downstream of the emissary advise the setting up of a monitoring station integrated in the monitoring network of the Tagus River Basin.

1.6 Objective f. To study the nitrate removal capability of several alternative denitrification substrates in laboratory batch tests

The tested substrates can enhance biological denitrification, although at different extents. To our knowledge, this was the first time that the Tasmanian blue gum bark, silver wattle bark and pine nut shell were tested as a carbon source to enhance biological denitrification. Nitrate reduction occurred in connection with some ammonium production, indicating that DNRA was also taking place. The best substrates in terms of denitrification efficiency were cork and Tasmanian blue gum bark, with a nitrate removal trough denitrification of 98% in the former and 96% in the latter. Also, the denitrification rate was $0.0485 \text{ mg NO}_3\text{-N L}^{-1} \text{ d}^{-1}$

$\text{g}^{-1}_{\text{sub}}$ for cork and $0.0709 \text{ mg NO}_3^- \text{-N L}^{-1} \text{ d}^{-1} \text{ g}^{-1}_{\text{sub}}$ for Tasmanian blue gum bark. Silver wattle bark and pine nut shell were considered unsuitable due to insufficient nitrate removal (in the former) and excessive reduction of nitrate to ammonium (on both). The microcosm batch experiment allowed for the selection of the best substrate for further studies.

1.7 Future research

Periodical and detailed nutrient data sources are still inadequate in the Sorraia basin. Thus, to better assess the impacts that the combined effects of climate change and management practices have on nitrate behavior in the Sorraia river basin, future research should focus on improving the model validation and calibration using more accurate flow and nutrient measured data. Moreover, to estimate the denitrification rates of the main soil units and land uses present at the basin, batch laboratory studies should also be done.

Regarding the material interventions in the ecological restoration of Mediterranean type riparian systems, future studies should give special consideration to the control of exotic plant invaders in riparian and wetland environments, including the development of effective eradication measures. Moreover, future research should also focus on the use of a more holistic (*i.e.*, addressing the whole river ecosystem) framework for environmental flows determination (e.g. Rivaes *et al.*, 2017). Also, the development of novel strategy approaches for landowner engagement should be researched and tested.

Definitive conclusions regarding the influence of the CAIMA paper mill on the Tagus river water quality are still lacking. Therefore, future studies should focus on a systematic spatially wider sampling, encompassing a range of weather and flow conditions. That approach will allow for a better understating of the local influence of the pulp mill effluents on the river water quality.

Concerning the testing of alternative organic substrates for nitrate removal from water, future research should focus on testing the Tasmanian blue gum bark (the substrate with the best denitrification rate) in a continuous column apparatus and on a field scale removal test using a lysimeter test station.

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