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Impact analyses of riverscapes fragmentation on the conservation of bryophyte communities

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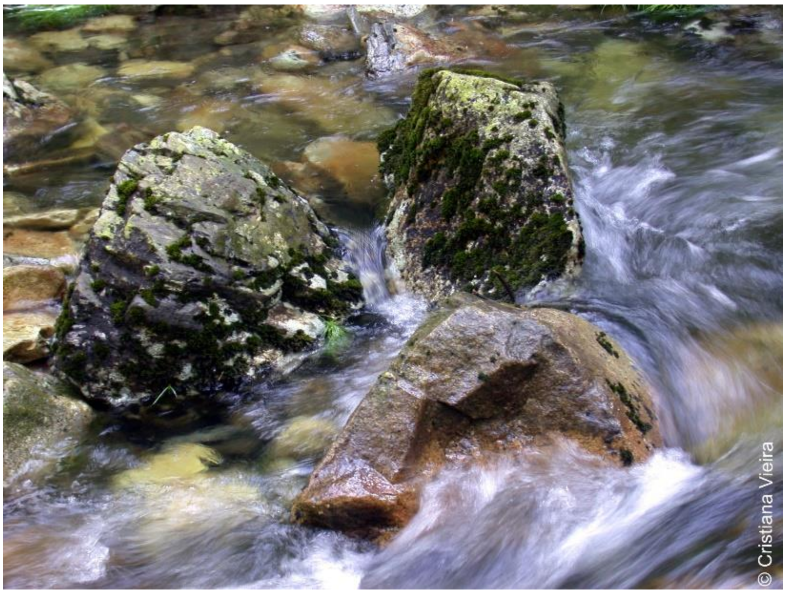
Ana Paula Senra Portela
Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto em
Ecologia, Ambiente e Território
2014



Impact analyses of riverscapes fragmentation on the conservation of bryophyte communities

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

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Acknowledgements

This work is part of project MoBia, funded by FEDER funds through the “Programa Operacional Factores de Competitividade – COMPETE” and through “Fundação para a Ciência e a Tecnologia” under the project “Monitorização da Biodiversidade em Avaliações Ambientais – MoBiA” (PTDC/AAC-AMB/114522/2009).

To my supervisor Doctor Rubim Almeida who encouraged the early start of this work and enabled me to work with extraordinary people.

To my co-supervisor Doctor Cristiana Vieira, my mentor for almost two years who dedicated so much of her time and effort to this work and me. I am very grateful for the warm welcome, for the enthusiastic scientific guidance, for the fascination for mosses you passed on, that certainly changed my perspective on the world and nature, and for the non-scientific breaks always filled with good conversation and laughter. Thank you for being such an inspiration.

To Helena Hespanhol for the valuable scientific advices and inputs and especially for the open armed, friendly welcome and inclusion.

To Joana Marques for scientific inputs and the warm friendly welcome into the group.

To Bruno Marcos, for the time helping me make sense of R code and making it work. Thank you for those detailed e-mails answering my sometimes vague questions.

To my colleagues at the laboratory of Botany with whom I have shared good, sometimes nonsensical, conversations and loud laughs. To keep my word, a special thanks to Filipe, a green colour scale does work better than a blue one for a conservation priority map.

To my friends Cláudia and Cristiana (the order is merely alphabetical), for your friendship and companionship throughout these five years, which cannot be condensed into this simple paragraph. Thank you for your support, for your help, for the team work, for the laughs and nonsense.

To my parents, I cannot thank you enough for your constant unwavering support and encouragement throughout the years that enabled me to accomplish many things including this thesis.

To my sister Filipa that so often had the difficult task of listening to my ecological/ moss related dwelling and never wavered in her interest.

Abstract and Keywords

Biodiversity conservation planning and the impact of human activities on biological diversity and landscapes are some of the most pressing issues in ecology nowadays. Freshwater biodiversity is among the most threatened worldwide, hence conservation planning is an urgent need.

The objective of this work was to provide an integrated assessment of anthropogenic impact and its implications for conservation planning and riverscapes' bryophyte diversity, in Northern Portugal.

To accomplish the sought integration and overcome the lack of spatial chorological data for fluvial bryophytes a community-level modelling approach was employed. This approach produced a set of four community types that constituted useful surrogates of regional bryophyte species presence in the conservation planning and management processes. The distribution of the four community types was modelled and projected for the study area using biomod2.

In order to assess the impact of energy production schemes (dams, small hydroelectrics and wind turbines) and transportation networks (railways and main roads), on fluvial bryophyte communities, spatial data on these elements and on respective areas of influence and magnitudes of impact was superimposed to the communities' potential distribution. In addition, a spatial conservation prioritization analyses using Zonation software was conducted to spatialize the different options priority of conservation areas chosen based on three bryophyte communities rich in species with conservation interest and different combinations of fragmentation restrictions.

We found that, although the total area of bryophyte communities potential presence impacted can be considered low, a considerable part of this impact is located within protected areas of the study area, which undermines their efficiency for the protection of fluvial bryophytes. In the spatial analysis, main roads were found to be the leading cause of impact across all communities. In fact, roads are known to be responsible for the alteration of streambed, margins, water quality and debris flow, so, consequently, the alteration of bryophyte community structure and a change in species diversity.

The Zonation analyses further reinforced the necessity of effective management strategies in protected areas, since the allocation of protection priority to these areas yielded some of the lowest values of protected distribution for the bryophyte communities.

This work, using fluvial bryophytic communities as a biological model for conservation studies, demonstrated that constraining protection of biodiversity solely to protected areas is not necessarily an effective strategy and that a more integrated management approach of a region and fragmentation elements should be considered in the overall conservation policies.

Keywords: bryophytes, riverscapes, impact, fragmentation, conservation

Resumo e Palavras-chave

O planeamento da conservação da biodiversidade e os impactos antropogénicos sobre a diversidade biológica são assuntos prementes em ecologia, atualmente. A biodiversidade fluvial é uma das mais ameaçadas ao nível mundial, sendo, por isso, o planeamento da sua conservação uma necessidade urgente.

O objetivo deste trabalho foi providenciar uma avaliação integrada dos impactos antropogénicos e as suas implicações para o planeamento da conservação da diversidade briofítica das paisagens fluviais do Norte de Portugal.

De forma a conseguir esta integração e ultrapassar a falta de informação corológica espacializada para as espécies de briófitas fluviais, foi empregue uma abordagem de modelação ao nível da comunidade. Esta abordagem produziu um conjunto de quatro comunidades tipo que constituem úteis indicadores de substituição da presença regional de diferentes espécies de briófitas em processos de gestão e planeamento da conservação. A distribuição destas quatro comunidades tipo foi modelada e projetada para a área de estudo utilizando biomod2.

Para avaliar o impacto de infraestruturas de produção de energia elétrica (barragens, mini-hídricas e aerogeradores) e redes de transportes (rede viária e ferroviária), nas comunidades briofíticas fluviais, a informação espacial relativa a estes elementos, às suas áreas de influência e às suas magnitudes de impacto, foi sobreposta à distribuição potencial das comunidades tipo.

Além disso, foi utlizado o software de priorização espacial de conservação Zonation para espacializar diferentes opções de áreas de conservação escolhidas com base nas três comunidades tipo mais ricas em espécies com interesse de conservação e em diferentes combinações de restrições de fragmentação.

Apesar da área total de impacto sobre a distribuição potencial das comunidades briofíticas poder ser considerada baixa, parte considerável deste impacto localiza-se em áreas protegidas da área de estudo, o que põe em causa a sua eficiência na proteção de briófitas fluviais.

Nesta análise espacial, a rede viária foi identificada como a principal causa de impacto em todas as comunidades. De facto, as estradas são responsáveis pela alteração do leito, margens, qualidade da água e fluxo de detritos, e como consequência pela alteração da estrutura das comunidades e riqueza específica.

As análises Zonation reforçaram a necessidade de estratégias de gestão eficazes nas áreas protegidas, uma vez que a alocação de prioridade de conservação a estas áreas deu origem a proporções de distribuição protegida mais baixas.
Utilizando comunidades briofíticas fluviais como modelo biológico para estudos sobre conservação, demonstrou-se que restringir a proteção da biodiversidade apenas a áreas protegidas não é necessariamente uma estratégia eficaz e que devem ser consideradas estratégias de gestão de fragmentação integradas aquando da elaboração de políticas de conservação à escala regional.

Palavras-chave: briófitas, paisagens fluviais, impacto, fragmentação, conservação

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List of Abbreviations

AUC – Area under the curve of the Receiver operating characteristic graph

DEM – Digital Elevation Model

LSC – Landscape Comparison analysis

ROC – Receiver operating characteristic

WFD - Water Framework Directive

CAZ – Core Area Zonation

List of Communications and Publications

The present thesis is also based in the preparation of the following communications and publications:

Oral Communications:

Vieira C.; Portela A.P.; Hespanhol H.; Marcos B; Honrado J.; "**Impact analysis of riverscapes fragmentation on the conservation of bryophyte communities**." XIX Simpósio de Botânica Criptogâmica, Gran Canaria, Spain (June 2013).

Vieira, C.; Portela, A.; Hespanhol, H.; Marcos, B., Honrado, J.; "**Assessing the impact of riverscapes fragmentation impact of energy and communication elements on the representativeness and structure of bryophyte communities at a regional level (North Portugal)**". XVII Congress of the Iberian Association of Limnology, Santander, Spain. (July 2014).

Portela, A; Vieira, C.; Hespanhol, H.; Marcos, B., Honrado, J.; "**Impact analysis of riverscapes fragmentation on the conservation of bryophyte communities: a conservation planning approach**". 3rd International Conference on Ecohydrology, Soil and Climate Change EcoHCC'14, Tomar, Portugal. (September 2014).

Poster Communications:

Portela, A; Vieira, C.; Hespanhol, H.; Marcos, B., Honrado, J.; Silva, A "**Análise de impacto da fragmentação da paisagem na distribuição de comunidades briofíticas fluviais**". IX Encontro Internacional de Fitossociologia ALFA. Parque Biológico de Gaia, Avintes, Gaia. (May 2013).

Portela, A; Vieira, C.; Hespanhol, H.; Marcos, B., Honrado, J.; Silva, A. "**Análise de impacto da fragmentação da paisagem na distribuição de comunidades briofíticas fluviais**". 4º Workshop Anual Bioplant - Programa Inter-Universitário de Doutoramento em Biologia de Plantas Fundamental e Aplicada. Faculdade de Ciências da Universidade do Porto, Porto. (July 2013).

Abstracts in Conference Proceedings:

Portela AP, Vieira C, Hespanhol H, Marcos B., Honrado J.. 2014. "**Impact analysis of riverscapes fragmentation on the conservation of bryophyte communities: a conservation planning approach**". In: C. Andrade (ed.), 3rd International Conference on Ecohydrology, Soil and Climate Change, 10-12 September 2014, Tomar, Portugal: 62.

Additional contributions:

Oral Communications:

Vieira C, Aguiar FC, Portela AP, Raven P, Holmes N, Cambra J, Flor-Arnau N, Chauvin C, Dorflinger G, Germ M, Manolaki P, Minciardi MR, Munné A, Papastergiadou E, Urbanik G, Ferreira MT. "**Bryophyte communities in hydrographic regions of Europe: distribution overview and conservation in global change scenarios**". 3rd International Conference on Ecohydrology, Soil and Climate Change, Tomar, Portugal. (September 2014).

Abstracts in Conference Proceedings:

Vieira C, Aguiar FC, Portela AP, Raven P, Holmes N, Cambra J, Flor-Arnau N, Chauvin C, Dorflinger G, Germ M, Manolaki P, Minciardi MR, Munné A, Papastergiadou E, Urbanik G, Ferreira MT. 2014. **"Bryophyte communities in hydrographic regions of Europe: distribution overview and conservation in global change scenarios**". In: C. Andrade (ed.), 3rd International Conference on Ecohydrology, Soil and Climate Change, 10-12 September 2014, Tomar, Portugal: 70-71.

1. General Introduction

1.1 Riverscapes heterogeneity and diversity

The terms riverscapes and riverine landscapes refer to a perspective which regards fluvial systems their patterns and processes as a whole. This view recognizes the fact that river channels are a part of a series of biotopes and environmental gradients that, together with the respective biotic communities, constitute fluvial ecosystems (Ward 1998).

Riverscapes are dendritic and hierarchical landscapes characterized by a downstream variation of geomorphological and hydrological patterns. The combination of substrate nature, morphology and stability with stream flow characteristics, such as magnitude of discharge, frequency, duration and timing, and natural disturbance regimes, generate a mosaic of different habitat patches (Poff & Ward 1989; Sidle & Onda 2004; Poole 2010).

Patterns and processes in riverscapes are strongly orientated to the direction of the water movement. This directionality determines the structure and ecological connectivity of the system along three vectors, the longitudinal (upstream-downstream linkages), lateral (channel-riparian and floodplain systems) and the vertical (running waters-contiguous groundwater) (Ward 1989).

Riverscapes also possess high spatio-temporal and hydrogeomorphological heterogeneity due to various environmental gradients and biotopes, natural disturbance regimes related to flow regimes and innate connectivity of the water column. The unique combination of processes and patterns acting at different spatial and temporal scales makes for a biologically diverse landscape (Fig. 1) (Ward 1998; Poole 2002; Wiens 2002).

Despite the heterogeneous nature of riverscapes, river and streams are mostly perceived as the epitome of connectivity, in what concerns the movement of water. In fact, water is an effective agent of linkage between landscape elements, both in time and space (Ward *et al.* 2002; Wiens 2002).

Community diversity in these landscapes is, therefore, promoted by spatial heterogeneity, which expand the resource gradient, and temporal heterogeneity, which increases the possibility for niche overlap (Ward *et al*. 2002). These conditions allow the persistence of several and diverse groups of organisms, among which are bryophytes.

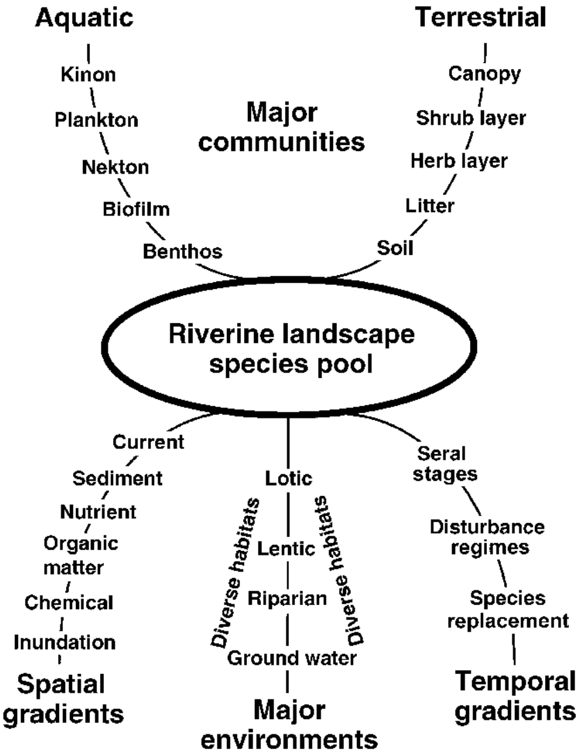

Fig. 1 Patterns and processes that influence the species pool of riverscapes. From Ward et al. (2002).

1.2 Regional conservation planning for fluvial biodiversity: Problems and approaches

Freshwater ecosystems and biodiversity face significant threats worldwide, constituting a component of biodiversity that is highly endangered (Abell 2002; Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010). Dudgeon *et al.* (2006) grouped major threats to freshwater biodiversity in five interacting categories: overexploitation, water pollution, flow modification, destruction or degradation of habitat and invasion by exotic species.

Fluvial systems in the Mediterranean region have a long history of human impact, which, during the past century, is mainly related to water and channel management, urbanization and alteration of practices and land use (Hooke 2006). The construction of dams, the implementation of small hydroelectric schemes, water flow regulation, channelization and deviation, extensively altered the hydrological regime and fluvial connectivity of many water courses (Jansson *et al.* 2000; Nilsson & Berggren 2000; Nilsson *et al.* 2005).

Additionally, transport infrastructures, such as railways and roads, and new energy production schemes, such as wind farms, have also played a role in the alteration of springs, river beds, margins and their surroundings (Wohl 2006; Perkin *et al.* 2013).

Human intervention has also led, inevitably, to aquatic and riparian habitats fragmentation, local extinction and community structure alteration (Hooke 2006; Prenda *et al.* 2006).

Riverscapes and associated biodiversity should be considered priorities in conservation planning due to their uniqueness and vulnerability. However, conservation plans are most commonly oriented for the conservation of terrestrial biodiversity, and fluvial ecosystems are often secondary concerns in the design and management of conservation areas (Nel *et al.* 2009a; Chessman 2013).

Only recently, the application of systematic conservation planning (Margules & Pressey 2000) to freshwater ecosystems has started gaining momentum (Nel *et al.* 2009b; Linke *et al.* 2011; Turak & Linke 2011). This type of framework usually involves the selection of biodiversity surrogates, definition of conservation goals and finding the solution with lesser costs and maximising the outcomes.

Systematic approaches require spatial data on biodiversity, and although available data is increasing, modelling techniques have proven useful in countering data needs. Statistical modelling techniques are powerful tools that enable modelling biological surrogates and extrapolating distributions across large regions. Although these techniques are mostly used for single species (Guisan & Thuiller 2005; Araújo & Guisan 2006; Guisan *et al.* 2006), new and more integrative approaches, using communities as biodiversity surrogates, are being implemented in the development of freshwater conservation plans at regional level (Olden 2003; Arponen *et al.* 2008; Leathwick *et al.* 2010). Community types with emblematic and representative species can act as surrogates for species diversity and fluvial integrity (Feio *et al.* 2012; Vieira *et al.* 2014).

These modelling approaches are generally designated as community-level modelling and can be implemented using three strategies (i) 'assemble first, predict later', (ii) 'predict first, assemble later' and (iii) 'assemble and predict together' (Fig. 2) (Ferrier & Guisan 2006). These approaches differ in the stage in which the data on multiple species is combined, usually by numerical classification (Ferrier et al. 2002; Ferrier & Guisan 2006).

'Assemble first, predict later' strategies involve some form of classification, ordination, aggregation of the biological data without any reference to environmental data, followed by modelling the previously obtained community-level entities as a function of environmental predictors (Ferrier *et al.* 2002) .

In 'predict first, assemble later' strategies, individual species are modelled one at a time and the resulting species distributions are then classified (Leathwick *et al.* 1996).

In 'assemble and predict together strategies', generating community level attributes and modelling of biological-environmental relationships are performed in one step, through the use of extended techniques species-level modelling (Olden 2003).

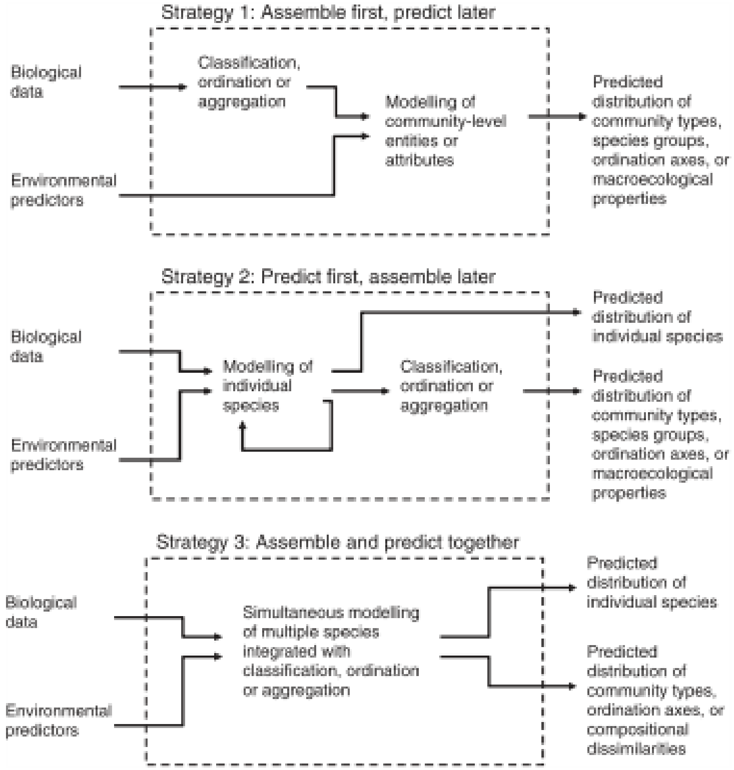

Fig. 2 Three approaches to modelling at the community-level, from Ferrier and Guisan (2006).

After the spatial data requirements are met, other concerns arise in spatial conservation planning for freshwater biodiversity. A regional conservation plan should promote a coherent network of reserve areas articulating land use, fluvial ecosystems and organisms. To accomplish this, it is necessary to implement new reserves that encompass the diversity of species and ecosystems associated with riverscapes, protect critical refuges, ensure hydrological connectivity, monitor human impact and management and also evaluate the efficiency of existing reserves for the conservation of freshwater biodiversity (Abell *et al.* 2007; Nel *et al*. 2009b; Piquer-Rodriguez *et al.* 2012; Scolozzi & Geneletti 2012).

1.3 Fluvial bryophytes: ecological role and conservation

Bryophytes are one of the most common group of macrophytes in riverscapes. Many species of bryophyte species are constrained in their distribution to moist habitats due to the lack of vascular system to transport water (Glime 2007). Riverscapes provide a wide range of wet conditions providing a highly suitable habitat for bryophytes depending on seasonal or permanently humid conditions (Slack & Glime 1985).

Bryophytes play a structural role in stream ecosystems, influencing community structure of stream fauna and competing for resources such as space, nutrients and light. These organisms partake in nutrient cycles, influencing nutrient uptake and retention, and are important primary producers in streams (Meyer 1979; Stream Bryophyte Group 1999). Also, bryophyte colonies provide refuge for fauna, supporting different invertebrate species assemblages (Suren 1993; Bowden *et al.* 1999; Stream Bryophyte Group 1999; Paavola *et al.* 2006).

Fluvial bryophytes distribution is influenced by microscale variables, such as substrate size, stream bed stability, stream slope and local flow type and also by mesoscale variables, such as geology, hydrology and water quality; the microscale set of variables influences their presence/absence and the mesoscale set the community type (Suren 1996; Suren & Ormerod 1998; Suren & Duncan 1999; Scarlett & O'Hare 2006; Leutner *et al.* 2012).

Bryophytes are recognized indicators of human impact, microhabitat heterogeneity and fluvial integrity, which determine the structure and composition of their communities (Zechmeister *et al.* 2003; Scarlett & O'Hare 2006; Fritz *et al.* 2009; Ceschin *et al.* 2012; Vieira *et al.* 2012). These organisms are already used as proxy of water quality and catchment environmental quality, for example, in the European Water Framework Directive (WFD) (Gecheva & Yurukova 2013; Luís *et al*. 2013; Vieira et al. 2014).

Portuguese bryoflora counts 40% of European bryophyte species, holds 65% of the Iberian Peninsula taxa and are a recognised group for the maintenance of the overall Iberian Peninsula’s biodiversity (Ros *et al.* 2007; Sérgio *et al.* 2007; Ros *et al.* 2013; Sérgio *et al.* 2013).

In Portugal fluvial bryophyte communities composition counts some rare, endemic species with conservation interest (Vieira et al. 2005; Vieira et al. 2012b; Vieira et al. 2012c), and are associated with many priority aquatic and semi-aquatic European habitats (Council of the European Communities 1992).

1.4 Aims and thesis layout

In this context the general aim of this thesis is to assess the impact of riverscapes fragmentation in the conservation of fluvial bryophyte communities in Northern Portugal.

Specific aims include:

(1) Establishing the main fluvial bryophyte community types at the regional level and obtaining spatialized information on their distribution, overcoming the existing lack of chorological information.

(2) Assessing, categorizing and summarizing the impact of regional fragmentation elements, such as energy production schemes and communication elements, on bryophyte communities.

(3) Analyse the effect of anthropogenic impacts in spatial conservation planning for fluvial bryophyte diversity.

(4) Discuss the spatial congruence of protection areas and the most promising areas for fluvial bryophyte communities' conservation in the studied region.

This thesis is organized in five chapters: (1) a general introduction, exploring the main concepts related to the subject of this thesis; (2) a general methods chapter, which contains the characterization of the study area, a description of the biological and environmental datasets employed and the general methodological framework; (3) a chapter named "Connecting riverscapes and bryophytes: a spatial conservation planning approach" following the organization of a manuscript submitted to a scientific journal, where detailed methods, results and discussion are presented together with other pertinent information to publish this thesis; (4) a general discussion chapter, exploring more exhaustively the main trends and results of this work; and (5) a concluding remarks chapter, summarizing the main findings and messages of this thesis .

All the references used are listed in the end of the respective chapter in a specific section.

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2. General Methods

2.1 Study area

The study area encompasses Northern Portugal as delimited by NUTSII administrative region (Fig. 3). This region possesses a temperate climate with mean annual temperature of 13ºC and average total annual precipitation of 1013 mm (Ninyerola M. *et al.* 2005). However, this is a climatically heterogeneous area, with a west *vs.* east differentiation in mean annual temperature and precipitation. The distance from the Atlantic Ocean and the interaction between the land relief and climate are responsible for this environmental differentiation. In a recent environmental classification this area was divided in three environmental zones (Fig. 4 A) that reflect the above mentioned differentiation (Metzger *et al.* 2005). The Lusitanian area is influenced by the proximity to the Atlantic Ocean, has mild and humid winters and high summer temperature with few months of drought. The Mediterranean Mountains area is influenced by continentality and the Mediterranean climate, but still retains some of the influence of mountainous climate. The Mediterranean North presents a characteristic Mediterranean summer drought.

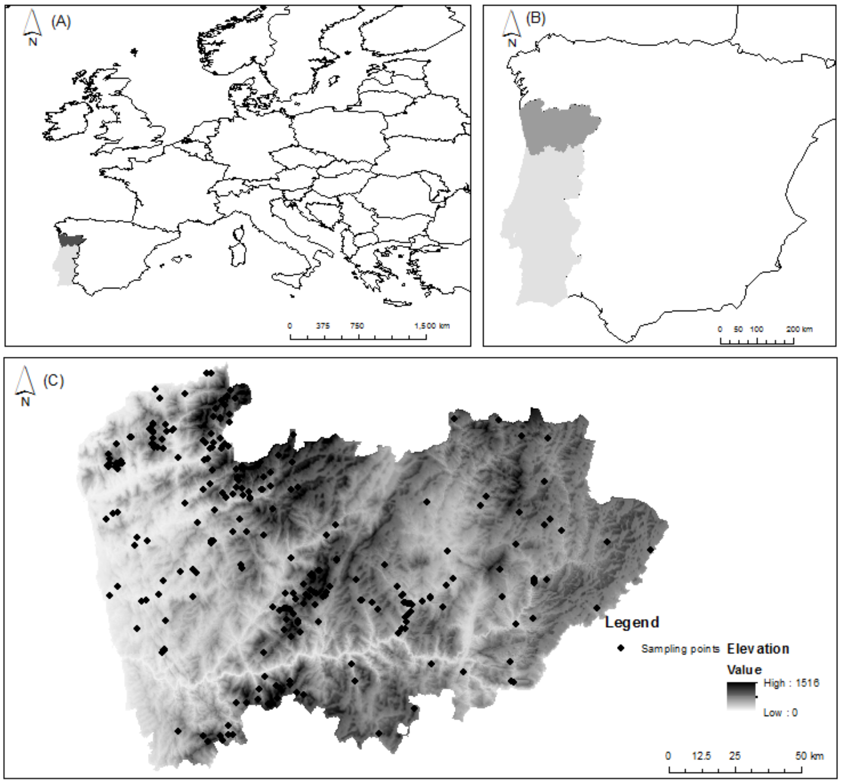

Fig. 3 Study area geographical context in Europe (A), the Iberian Peninsula (B) and the sampling points over an Digitial Elevation Model (DEM).

The main typologies of streams and rivers of the study area reflect the climatic differentiation and the land relief. These typologies include the mountainous rivers of the North, northern rivers of small to medium-large dimensions and the Alto Douro rivers of small to medium-large dimensions (Fig. 4 B) (INAG 2008). The mountainous rivers are steep watercourses, located at high altitudes, with small catchment areas (less than 100 km^2) and high average annual drainage (800-1400 mm), the annual average temperature is 11°C and annual average precipitation is 1900 mm. The northern rivers have an annual average temperature of 12 to 13 °C and annual average precipitation of 1200 mm, are located at a diverse range of altitudes and have little mineralization due to the siliceous lithology of the substrates. The Alto Douro rivers are characterized by the higher mean temperature (13°C) and decreased precipitation (600 mm average) typical of the Mediterranean region where they are located (INAG 2008).

Fluvial systems of the study area present high bryophyte species richness and, in the northwest territory, a total of 140 species has been reported (Vieira *et al.* 2005). Among these, 19 taxa are included in European or Iberian Red Lists, five taxa endemic to Europe and two endemic to the Iberian Peninsula (Vieira et al. 2005; Sérgio *et al.* 2013). Additionally, many species distribution is restricted to streams located in the west of the study area and, for other species, this region corresponds to the southern limit of their distribution (Vieira et al. 2005).

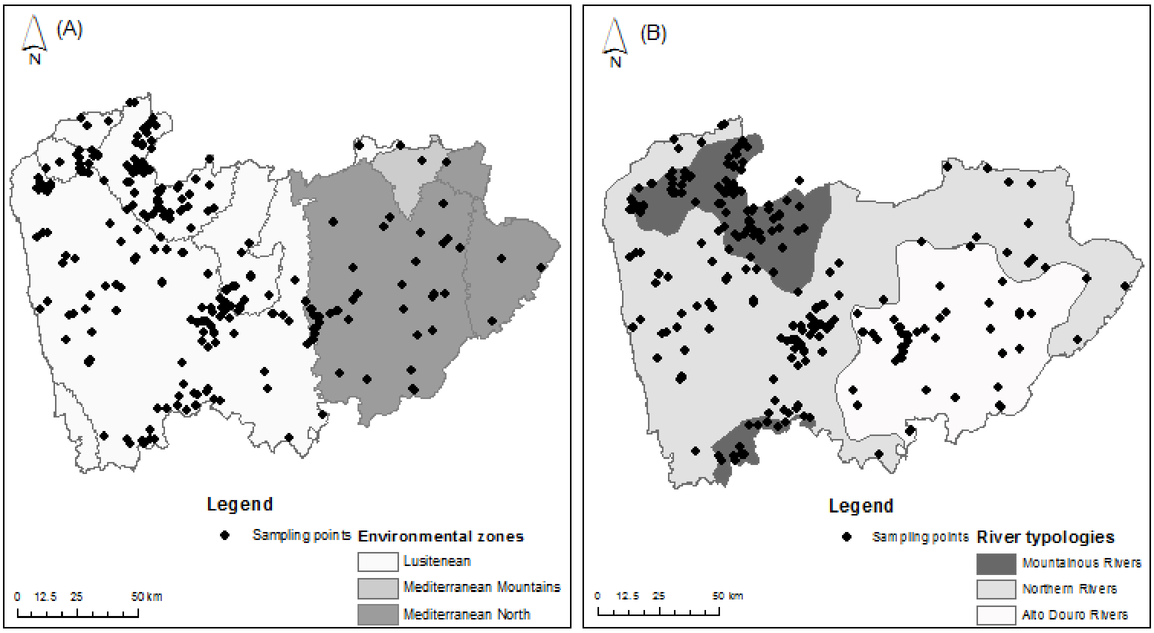

Fig. 4 Environmental zones according to Metzger *et al.* (2005) (A) and river typologies according to INAG (2008) (B) in the study area.

2.2 Methodological framework

In order to accomplish the aims laid out for this work we established a methodological framework (Fig. 5) that consisted of three stages:

(I) Community-level modelling: "assemble first, predict later" approach.

(II) Spatialization and summarization of impacts caused by regional fragmentation elements.

(III) Spatial conservation prioritization for the previously modelled community types.

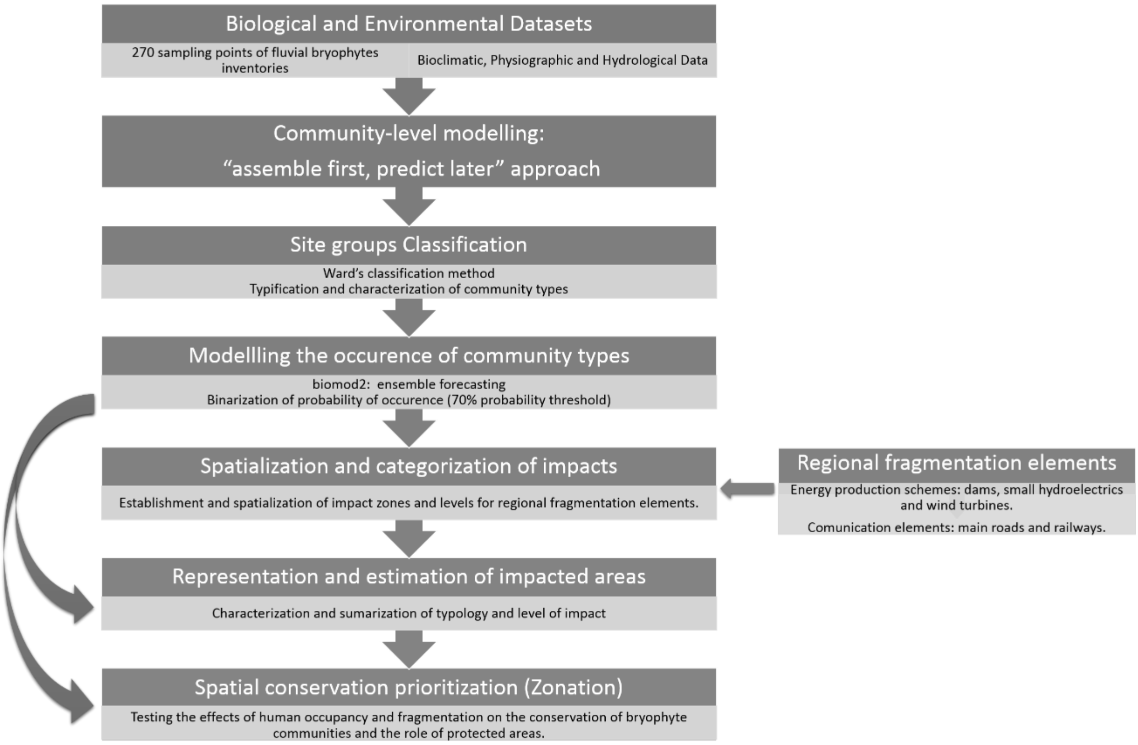

Fig. 5 Methodological framework employed in this thesis.

In the first stage the goal was to obtain spatialized information on bryophyte communities’ distribution. In order to achieve this, we applied a “assemble first, predict later” modelling strategy (Ferrier & Guisan 2006). The first step was to conduct a classification of the biological data (see 2.3 Species data) using, in this case, Ward’s classification method on a previously matrix calculated of Jaccard’s similarity between sites (Borcard *et al.* 2011).

The community types obtained by classification were then characterized according to species frequency and contribution for cluster similarity – a SIMPER analysis conducted in Community Analysis Package 1.52 (Hederson & Seaby 1999) .

The community types occurrence was then modelled for the study area using biomod2: Ensemble platform for species distribution modelling, in R environment (R Core

Team 2013; Thuiller *et al.* 2013). The predictions obtained for each algorithm that yielded area under the curve (AUC) of the receiver operating characteristic (ROC) values above 0.7 were combined to obtain an ensemble forecast of community types' distribution. The resulting probability map was binarized into a presence/absence map, using 70% probability as a threshold for occurrence.

In the following stage we compiled existing geographic information on regional fragmentation elements, including energy production schemes, such as dams, small hydroelectric schemes and wind turbines, and transportations networks, including main roads and railways. This information was obtained through "MoBia - Biodiversity Monitoring in Environmental Assessments" project, which main objective was to evaluate the effectiveness of the handling of biodiversity in environmental assessments and the contribution of associated monitoring programs for a global monitoring network (PTDC/AAC-AMB/114522/2009).

The geographic information available consisted of the location of different elements, for example, for wind turbines the information available corresponded only to point features. Understandably, this type of information merely depicts localized destruction, and does not take into account different magnitudes of impact associated with the implementation of the infrastructures and the alteration of the surrounding environment. In order to account for different magnitudes of impact, a set of buffers with different distances were established for each type of fragmentation elements (Table 1).

Table 1 Description of regional fragmentation elements spatial data and the different magnitude buffers established.

Regional fragmentation elements	*Number of elements in study area*	*General description of available geographic information*	*Impact levels and buffers definition*		
			1 (lower)	2 (medium)	3 (stronger)
Dams	24	Polygons corresponding to the reservoirs; All dams are already constructed.	n.a.	Buffer of 200 m around the reservoir + 1 km of buffer, 50 m wide, downstream of the reservoir	Intersection of community distribution with the area of the reservoir
Small hydroelectrics	34	Point features; 34 were being subjected to EIA at the time,4 already licensed, 2 in construction, 2 requiring EIA exemption and 1 project in execution	n.a.	Buffer of 500 m radius around the point	Intersection of point with community distribution
Wind farms	1054	Point features; all installed	Buffer of 500 m width around the area of impact level of two	Buffer of 250 m width around the area with strongest impact	Intersection of community distribution with the point feature and buffer of 100 m radius around the point
Railways	32	Line features; 619.08 km including deactivated lines such as the Tua Line, and remodelled lines such as the Póvoa Line	Buffer of 100 m width around the area of impact level of two	Buffer of 50 m width around the line.	Intersection of line community distribution
Main roads	278	Polygon features 5, 10, 15 m wide respectively in national and regional roads, main and complementary itineraries and highways. 1197.32 km of extent in total; 50.45 km of highways, 182.56 km of itineraries, 822.86 km national roads, 141.43 km of regional roads	Buffer of 100 m width around the area of impact level of two	Buffer of 50 m width around the road polygons	Intersection of polygon with community distribution

The assessment of impact was completed by superimposing the fragmentation elements and respective buffers to the binary potential distribution of bryophyte community types and summarizing impacted area for each community by fragmentation element and magnitude of impact in ArcMap 10.1 (ESRI 2012).

The last stage of the methodological approach consisted in the use of the conservation prioritization software Zonation 4.0.0 (Moilanen *et al.* 2014), to identify areas of potential conservation, assess the potential role of previously defined protected areas and test the effects of human occupancy and fragmentation on the conservation of bryophyte communities.

Zonation identifies areas that are important for retaining habitat quality and connectivity simultaneously for multiple species or other biodiversity features, providing a method for enhanced persistence of biodiversity on long term. This software produces a hierarchical prioritization of the landscape based on conservation value of the sites accounting for complementarity. The algorithm hierarchically removes least valuable cells from the landscape while minimizing marginal loss of conservation value, accounting for connectivity and priority given to biodiversity features. In this work we employed a Core Area Zonation (CAZ) removal rule, which selects as high priority cells those with high occurrence probability for highly weighted species, putting emphasis on rarity and conservation value of biodiversity features. The result of these analysis consists in a sequence of nested, highly connected structures with core areas that represent greatest conservation values (Lehtomäki & Moilanen 2013).

In this case, Zonation software produces a hierarchical prioritization of the landscape based on biological value of the sites (cells) accounting connectivity and the importance given to the biodiversity features (in this case fluvial bryophyte communities). This produces a spatial prioritization composed of a nested sequence of highly connected landscape structures with core areas that present the greatest conservation values (top fraction).

Restrictions to conservation, understood as the introduction of different fragmentations and human occupancy elements were considered in different Zonation analyses, both individually and combined (Table 2). The proportion of communities' distribution protected by the top fraction of the landscape was examined for each analyses conducted and compared. Additionally, it was conducted a landscape comparison analysis (LSC), which consists in a comparison between two solutions in order to evaluate how much do they overlap and their average difference in cell removal order. Finally, all solutions were compared to the one that only took into account the biodiversity features in the process of choosing the top fraction of the riverscapes.

Table 2 Spatial conservation prioritization analysis conducted in Zonation software with respective data inputs (restrictions) and aims of representation.

Analysis codes	*Data input*	*Representation*
BIO	Community types distributions modelled	"Pristine" conditions.
BIO+Pr	Community types distributions Mask Layer: All protected areas (Natura 2000 network, protected areas and Ramsar sites)	Force inclusion of protected areas in the choice of high priority conservation areas.
BIO+Urb	Community types distributions Condition Layer: Urban areas	Exclude urban areas from analysis.
BIO+Agr	Community types distributions Condition Layer: Agricultural areas	Exclude agricultural areas from analysis.
BIO+Frag	Community types distributions Condition Layer: Areas impacted by regional fragmentation agents	Exclude areas impacted by the regional fragmentation elements listed above.
BIO+Urb+Agr	Community types distributions Condition Layer: Urban and agricultural areas	Exclude both agricultural and urban areas to reflect human presence occupancy constraints.
BIO+Urb+Agr+Frag	Community types distributions Condition Layer: Urban, agricultural and impacted areas	Exclude urban, agricultural and areas impacted by regional fragmentation elements to reflect human occupancy and fragmentation constraints.
BIO+Urb+Agr+Frag+Pr	Community types distributions Condition Layer: Urban, agricultural and impacted areas Mask Layer: All protected	More realistic approach, reflecting not only the human presence and impact in the landscape, but also the constraints to the creation of new conservation areas.

2.3 Species data

Species data utilized in this work correspond to a compilation of databases of fluvial bryophytes inventories, that correspond to fieldwork carried out between 2000 and 2012 by Cristiana Vieira.

Bryophyte species were surveyed in all immerged or semi-immerged rock microhabitats found within 100 m of riverbed and margins, of a total of 270 sampling points. Bryophyte species presence/absence was registered using 0.25 m^2 sample plots placed in all recognizable hydrologic zones and microhabitats constantly or easily immerged, seasonally or several times a month, with discharges related to precipitation or dam releases and micro-habitats immerged only in extended periods of rain.

The species encountered and their conservation status according to the Red List of Threatened Bryophytes of Portugal (Sérgio et al. 2013) are listed in Table 3.

2.4 Environmental variables

The set of environmental variables employed in the modelling of community types distributions were chosen taking into account the environmental drivers of the communities' distribution and the available spatial data.

Table 3. List of bryophyte species in the study area, respective conservation status according to the Red List of Threatened Bryophytes of Portugal (Sérgio *et al*. 2013) and the number of sampling sites with registered presence. M – Moss; H – Liverwort; LC – Least concern; LC - att – Least concern, attention; LC-int – Least concern, introduced; NT – Near Threatened; VU- Vulnerable; DD – Data deficient; DD-n – Data deficient new; EN – Endangered.

Class	Species	Red List status	Number of sites
M	Andreaea rothii F. Weber & D. Mohr	LC	7
H	Aneura pinguis (L.) Dumort	LC	17
M	Atrichum undulatum (Hedw.) P. Beauv.	LC	20
M	Brachythecium rivulare Schimp.	LC	49
M	Brachythecium rutabulum (Hedw.) Schimp.	LC	11
M	Bryum alpinum Huds. ex With.	LC	33
M	Bryum argenteum Hedw.	LC	7
M	Bryum capillare Hedw.	LC	15
M	Bryum gemmiparum De Not.	LC	20
M	Bryum pseudotriquetrum (Hedw.) P.Gaertn. et al.	LC	47
M	Calliergonella cuspidata (Hedw.) Loeske	LC	6
H	Calypogeia fissa (L.) Raddi	LC	8
M	Campylopus introflexus (Hedw.) Brid.	LC-int.	3
M	Campylopus pilifer Brid.	LC	13
M	Campylopus pyriformis (Schultz) Brid.	NT	2
M	Ceratodon purpureus (Hedw.) Brid. subsp. purpureus	LC	6
H	Chiloscyphus polyanthos (L.) Corda	LC	43
M	Cinclidotus fontinaloides (Hedw.) P. Beauv.	LC	23
M	Cinclidotus riparius (Host ex Brid.) Arn.	VU	3
M	Cirriphyllum crassinervium (Taylor) Loeske & M.Fleisch.	LC	4
H	Conocephalum conicum (L.) Dumort.	LC	17
H	Corsinia coriandrina (Spreng.) Lindb.	LC	4
M	Dendrocryphaea lamyana (Mont.) P. Rao	LC	11
M	Dialytrichia mucronata (Brid.) Broth. var. mucronata	LC	14
M	Didymodon insulanus (De Not.) M.O.Hill	LC	21
M	Drepanocladus aduncus (Hedw.) Warnst.	NT	2
H	Dumortiera hirsuta (Sw.) Nees	VU	5
M	Epipterygium tozeri (Grev.) Lindb.	LC	3
M	Eurhynchium hians (Hedw.) Sande Lac. var. hians	LC	4
M	Eurhynchium pumilum (Wilson) Schimp.	LC	4
M	Fissidens bryoides Hedw. var. caespitans Schimp.	LC	48
M	Fissidens crassipes ssp. warnstorffi (Fleisch.) Brugg.- Nann.	LC	6
M	Fissidens dubius P.Beauv.	LC	5
M	Fissidens fontanus (Bach.Pyl.) Steud.	LC	3
M	Fissidens polyphyllus Wilson ex Bruch & Schimp.	LC	104
M	Fissidens pusillus (Wilson) Milde	DD	27
M	Fissidens serrulatus Brid.	LC	28
M	Fissidens taxifolius Hedw.	LC	2
M	Fissidens viridulus (Sw. ex anon.) Wahlenb. var. viridulus	LC	2
M	Fontinalis antipyretica Hedw.	LC	38
M	Fontinalis hypnoides Hartm. var. duriaei (Schimp.) Kindb.	LC	7
M	Fontinalis squamosa Hedw.	LC	67
H	Fossombronia angulosa (Dicks.) Raddi	LC	4
M	Funaria hygrometrica Hedw.	LC	5
M	Grimmia decipiens (Schultz) Lindb.	LC	7
M	Grimmia laevigata (Brid.) Brid.	LC	5
M	Grimmia lisae De Not.	LC	12
M	Grimmia meridionalis (Müll. Hal.) E. Maier	DD-n	4
M	Grimmia montana Bruch & Schimp.	LC	2
M	Grimmia ovalis (Hedw.) Lindb.	VU	9
M	Grimmia trichophylla Grev.	LC	4
M	Heterocladium wulfsbergii I.Hagen	DD	32
M	Hookeria lucens (Hedw.) Sm.	NT	6
M	Hygrohypnum ochraceum (Turner ex Wilson) Loeske	NT	35
M	Hyocomium armoricum (Brid.) Wijk & Margad.	LC	105
M	Isothecium holtii Kindb.	LC	51
M	Isothecium myosuroides Brid.	LC	3
H	Jungermannia gracillima Sm.	LC	14
H	Jungermannia hyalina Lyell	LC	26
H	Jungermannia obovata Ness	EN	2
H	Jungermannia pumila With.	EN	2
H	Jungermannia sphaerocarpa Hook.	LC	3
M	Kindbergia praelonga (Hedw.) Ochyra	LC	58
H	Lejeunea cavifolia (Ehrh.) Lindb.	LC	17

H	Lejeunea lamacerina (Steph.) Schiffn.	LC	5
M	Leptodictyum riparium (Hedw.) Warnst.	LC	18
M	Leskea polycarpa Hedw.	VU	2
H	Lophocolea bidentata (L.) Dumort.	LC	3
H	Lunularia cruciata (L.) Lindb.	LC	18
H	Marchantia polymorpha L.	LC	11
H	Marsupella emarginata (Ehrh.) Dumort.	LC	58
H	Marsupella sphacelata (Gieseke ex Lindenb.) Dumort.	LC	11
M	Mnium hornum Hedw.	LC	21
H	Nardia compressa (Hook.) Gray	NT	24
M	Orthotrichum rupestre Schleich. ex Schwägr. var. rupestris	LC	9
H	Pellia epiphylla (L.) Corda	LC	73
A	Phaeoceros laevis (L.) Prosk.	LC	3
M	Philonotis arnelli Husn.	LC	6
M	Philonotis caespitosa Jur.	LC	2
M	Philonotis fontana (Hedw.) Brid.	LC	25
M	Philonotis rigida Brid.	NT	3
H	Plagiochila porelloides (Torrey ex Nees) Lindenb.	LC	2
M	Plagiomnium affine (Blandow ex Funck) T.J.Kop.	LC	3
M	Plagiomnium undulatum (Hedw.) T. J. Kop.	LC	36
M	Plagiothecium denticulatum (Hedw.) Schimp.	LC	2
M	Plagiothecium nemorale (Mitt.) A. Jaeger	LC	16
M	Plagiothecium succulentum (Wilson) Lindb.	LC	9
M	Platyhypnidium lusitanicum (Schimp.) Ochyra & Bednarek-Ochyra	LC	108
M	Platyhypnidium riparioides (Hedw.) Dixon	LC	48
M	Pogonatum aloides (Hedw.) P. Beauv.	LC	13
M	Pohlia annotina (Hedw.) Lindb.	LC	4
M	Pohlia bulbifera (Warnst.) Warnst.	DD	5
M	Polytrichastrum formosum (Hedw.) G. L. Sm.	LC	3
M	Polytrichastrum formosum (Hedw.) G.L.Sm.	DD	6
M	Polytrichum commune Hedw.	LC	68
M	Polytrichum juniperinum Hedw.	LC	2
H	Porella pinnata L.	VU	6
M	Pseudotaxiphyllum elegans (Brid.) Z.Iwats.	LC	4
M	Racomitrium aciculare (Hedw.) Brid.	LC	119
M	Racomitrium affine (F.Weber & D.Mohr) Lindb.	LC	3
M	Racomitrium aquaticum (Dicks. ex Sw.) Bruch & Schimp.	LC	16
M	Racomitrium hespericum Sérgio, J. Muñoz & Ochyra	LC-att	11
M	Racomitrium heterostichum (Hedw.) Brid.	LC	6
M	Racomitrium lamprocarpum (Müll.Hal.) A.Jaeger	LC-att	49
M	Racomitrium lusitanicum Ochyra & Sérgio	LC-att	11
M	Rhizomnium punctatum (Hedw.) T. J. Kop.	LC	39
M	Rhynchostegium confertum (Dicks.) Schimp.	LC	3
H	Riccardia chamaedryfolia (With.) Grolle	VU	10
H	Riccardia multifida (L.) Gray	LC	13
H	Riccia huebeneriana Lindenb.	VU	2
H	Saccogyna viticulosa (L.) Dumort.	LC-att	10
H	Scapania compacta (A. Roth) Dumort.	LC	20
H	Scapania nemorea (L.) Grolle	LC	6
H	Scapania undulata (L.) Dumort.	LC	118
M	Schistidium apocarpum (Hedw.) Bruch & Schimp.	DD	18
M	Schistidium rivulare (Brid.) Podp.	VU	16
M	Sciuro-hypnum plumosum (Hedw.) Ignatov & Huttunen	NT	39
M	Scleropodium touretii (Brid.) L.F.Koch	LC	15
M	Scorpiurium deflexifolium (Solms) M. Fleisch. & Loeske	LC	23
M	Sphagnum auriculatum Schimp.	LC	38
M	Sphagnum capillifolium (Ehrh.) Hedw.	LC	3
M	Sphagnum subnitens Russow & Warnst.	LC	2
H	Targionia hypophylla L.	LC	2
M	Thamnobryum alopecurum (Hedw.) Gangulee	LC	42
M	Thamnobryum maderense (Kindb.) Hedenäs	VU	13
M	Trichostomum brachydontium Bruch	LC	6

The environmental predictors selected can be divided in three categories: climatic variables, physiographic and hydrologic (Table 4) (Suren 1996; Scarlett & O'Hare 2006). The climatic variables that influence the distribution of bryophytes are related to temperature and precipitation, their variation and seasonality (Hearnshaw & Proctor 1982; Proctor 1982; Arscott *et al.* 2000; Proctor 2000). The physiographic variables include elevation, slope, aspect and solar radiation (Suren 1996). The hydrologic variable used was flow accumulation since it is the spatial information related to hydrology that could be generated and became available for the entire study area.

Table 4 Environmental predictors considered in the bryophyte community modelling process and respective sources.

Type of variable	*Variable*	*Source*
Climatic	Annual Average Temperature	Digital Iberian Climatic Atlas (Ninyerola M. *et al*. 2005)
	Temperature annual range	Derived from Digital Iberian Climatic Atlas using R package “dismo” (Hijmans *et al.* 2013)
	Thermicity index	Derived from Digital Iberian Climatic Atlas using R package “dismo” (Hijmans *et al*. 2013)
	Annual Average Precipitation	Digital Iberian Climatic Atlas (Ninyerola M. *et al*. 2005)
	Precipitation of the driest month	Derived from Digital Iberian Climatic Atlas using R package “dismo” (Hijmans *et al*. 2013)
	Precipitation of the warmest quarter	Derived from Digital Iberian Climatic Atlas using R package “dismo” (Hijmans *et a*l. 2013)
Physiographic	Elevation	Consortium for Spatial Information (CGIAR-CSI) SRTM Database (Jarvis *et al.* 2008)
	Slope	Derived from Digital Elevation Model (DEM) using ArcMap 10.1 ™ (ESRI 2012)
	Aspect	Derived from DEM using ArcMap 10.1 ™ (ESRI 2012)
	Solar radiation	Derived from DEM using ArcMap 10.1 ™ (ESRI 2012)
Hydrologic	Flow Accumulation	Derived from DEM using ArcMap 10.1 ™ (ESRI 2012)

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3. Connecting riverscapes and bryophytes: a spatial conservation planning approach

The following text will be submitted to the journal "Conservation Biology".

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Abstract: Biodiversity conservation planning and the impact of human activities on biological diversity and landscapes are some of the most pressing issues in ecology nowadays. Freshwater biodiversity is among the most threatened worldwide, hence conservation planning is an urgent need.

The objective of this work was to provide an integrated assessment of anthropogenic impact and its implications for conservation planning and riverscapes' bryophyte diversity, in Northern Portugal.

To accomplish the sought integration and overcome the lack of spatial chorological data for fluvial bryophytes a community-level modelling approach was employed. This approach produced a set of four community types that constituted useful surrogates of regional bryophyte species presence in the conservation planning and management processes. The distribution of the four community types was modelled and projected for the study area using biomod2.

In order to assess the impact of energy production schemes (dams, small hydroelectrics and wind turbines) and transportation networks (railways and main roads), on fluvial bryophyte communities, spatial data on these elements and on respective areas of influence and magnitudes of impact was superimposed to the communities' potential distribution. In addition, a spatial conservation prioritization analyses using Zonation software was conducted to spatialize the different options priority of conservation areas chosen based on three bryophyte communities rich in species with conservation interest and different combinations of fragmentation restrictions.

We found that, although the total area of bryophyte communities potential presence impacted can be considered low, a considerable part of this impact is located within protected areas of the study area, which undermines their efficiency for the protection of fluvial bryophytes. In the spatial analysis, main roads were found to be the leading cause of impact across all communities. In fact, roads are known to be responsible for the alteration of streambed, margins, water quality and debris flow, so, consequently, the alteration of bryophyte community structure and a change in species diversity.

The Zonation analyses further reinforced the necessity of effective management strategies in protected areas, since the allocation of protection priority to these areas yielded some of the lowest values of protected distribution for the bryophyte communities.

This work, using fluvial bryophytic communities as a biological model for conservation studies, demonstrated that constraining protection of biodiversity solely to protected areas is not necessarily an effective strategy and that a more integrated management approach of a region and fragmentation elements should be considered in the overall conservation policies.

Keywords: bryophyte; community; riverscapes; modelling; conservation; Zonation

3.1 Introduction

The uniqueness and vulnerability of riverscapes and associated biodiversity should enhance their priority in conservation planning. Nevertheless, fluvial ecosystems are often secondary concerns in the design and management of conservation areas (Chessman 2013). Riverscapes possess high spatio-temporal and hydrogeomorphological heterogeneity due to various environmental gradients and biotopes, natural disturbance regimes related to flow regimes and innate connectivity of the water column. This unique combination of processes and patterns acting at different spatial and temporal scales makes for a biologically diverse landscape (Ward 1998; Wiens 2002).

The need for systematic conservation planning applied to the specificities of riverscapes becomes evident, yet the application of landscape level spatial prioritization to freshwater conservation is a relatively recent concern (Nel *et al.* 2009b; Linke *et al.* 2011).

The Mediterranean region has a long history of human impact on fluvial systems, which, during the past century, were mainly related to water and channel management, land use and practices changes and urbanization (Hooke 2006). Dams, small hydroelectric schemes, flow regulation, channelization and diverting water flow have, extensively, altered the hydrological regime and fluvial connectivity of many water courses (Nilsson & Berggren 2000; Nilsson *et al.* 2005). Railways and roads and, in recent times, wind farms have played an additional role in the alteration of springs, river beds, margins and their surroundings (Perkin *et al.* 2013). Human intervention has also led, inevitably, to aquatic and riparian habitats fragmentation, local extinction and community structure alteration (Hooke 2006; Prenda *et al.* 2006).

At regional scales, human impact needs monitoring and management to ensure connectivity between protected areas (Piquer-Rodriguez *et al.* 2012; Scolozzi & Geneletti 2012). A regional conservation planning approach should promote a coherent network of reserve areas articulating proper land use, fluvial ecosystems and organisms. In order to achieve this, it is necessary to implement new reserves that encompass the diversity of species and ecosystems associated to riverscapes, protect critical refuges and also evaluate the efficiency of existing reserves for the conservation of freshwater biodiversity (Abell *et al.* 2007; Nel *et al.* 2009a).

Bryophytes, as one of the most common group of macrophytes in riverscapes, are recognized indicators of human impact, microhabitat heterogeneity and fluvial integrity, which is reflected in the structure and composition of their communities (Zechmeister *et al.* 2003; Scarlett & O'Hare 2006; Fritz *et al.* 2009; Ceschin *et al.* 2012; Vieira *et al.* 2012c). Bryophytes also play a structural role in water courses partaking in nutrient cycles and providing refuges for invertebrates (Stream Bryophyte Group 1999). In Portugal this distinctive communities count some rare, endemic species with conservation interest in their composition (Vieira *et al.* 2005; Vieira *et al.* 2012b; Vieira et al. 2012c), and are associated with many priority aquatic and semi-aquatic European habitats (Council of the European Communities 1992)

The difficulties associated with identifying certain bryophyte taxa or incomplete knowledge on species distributions hinder their inclusion in some management plans (Tremp *et al.* 2012). Nevertheless, bryophytes are already used as proxy of water quality and catchment environmental quality, for example, in the European Water Framework Directive (WFD) (Gecheva & Yurukova 2013; Luís *et al.* 2013; Vieira *et al.* 2014). Moreover, conservation planning deals not only with human impact on landscapes but also with the challenges related to data collection and selection criteria, since data collection is many times confined to a small set of survey sites. Statistical modelling of species distributions is a powerful tool that enables us to extrapolate species distributions across large regions. However, their use in conservation planning and in general has focused mainly on individual species modelling (Araújo *et al.* 2004; Guisan & Thuiller 2005; Guisan *et al.* 2006). New and more integrative approaches, such as community-level modelling, have addressed biodiversity as a whole, using large datasets, numerical classification and statistical modelling to generate effective regional conservation plans (Olden 2003; Arponen *et al.* 2008; Leathwick *et al.* 2010). In this context, species assemblages, *i.e.,* community types with emblematic and representative species can be even more useful recognizable management units, acting as surrogates for species diversity and fluvial quality (Feio *et al.* 2012; Vieira et al. 2014).

Three broad modelling strategies can be used in community-level modelling: (i)'assemble first, predict later', (ii) 'predict first, assemble later' and (iii) 'assemble and predict together' (Ferrier & Guisan 2006). These strategies differ essentially in the stage in which numerical classification of the communities is undertaken (Ferrier *et al.* 2002; Ferrier & Guisan 2006). In this work we used a "assemble first, predict later" community level approach to model the occurrence of fluvial bryophyte communities in Northern Portugal and a spatial conservation prioritization approach to assess the impact of regional fragmentation elements and validate protection areas and explore conservation management options.

3.2 Methods

3.2.1 Study area

The study area encompasses the Northern region of Portugal, delimited, for this purpose, by the NUTS II administrative region (Fig. 6). The climate is temperate, with mean annual temperatures of 13 °C and an average total annual precipitation of 1013 mm (Table 5). There is, however, a climatic differentiation between the west and the east of the area that results from the decreasing influence of the Atlantic Ocean and the interaction of the climate with land relief. Metzger *et al.* (2005) divided the study area in three environmental zones, Lusitanian, Mediterranean Mountains and Mediterranean North (Fig. 6 D). The Lusitanian area is Atlantic with high summer temperatures, some dry months and mild and humid winters.

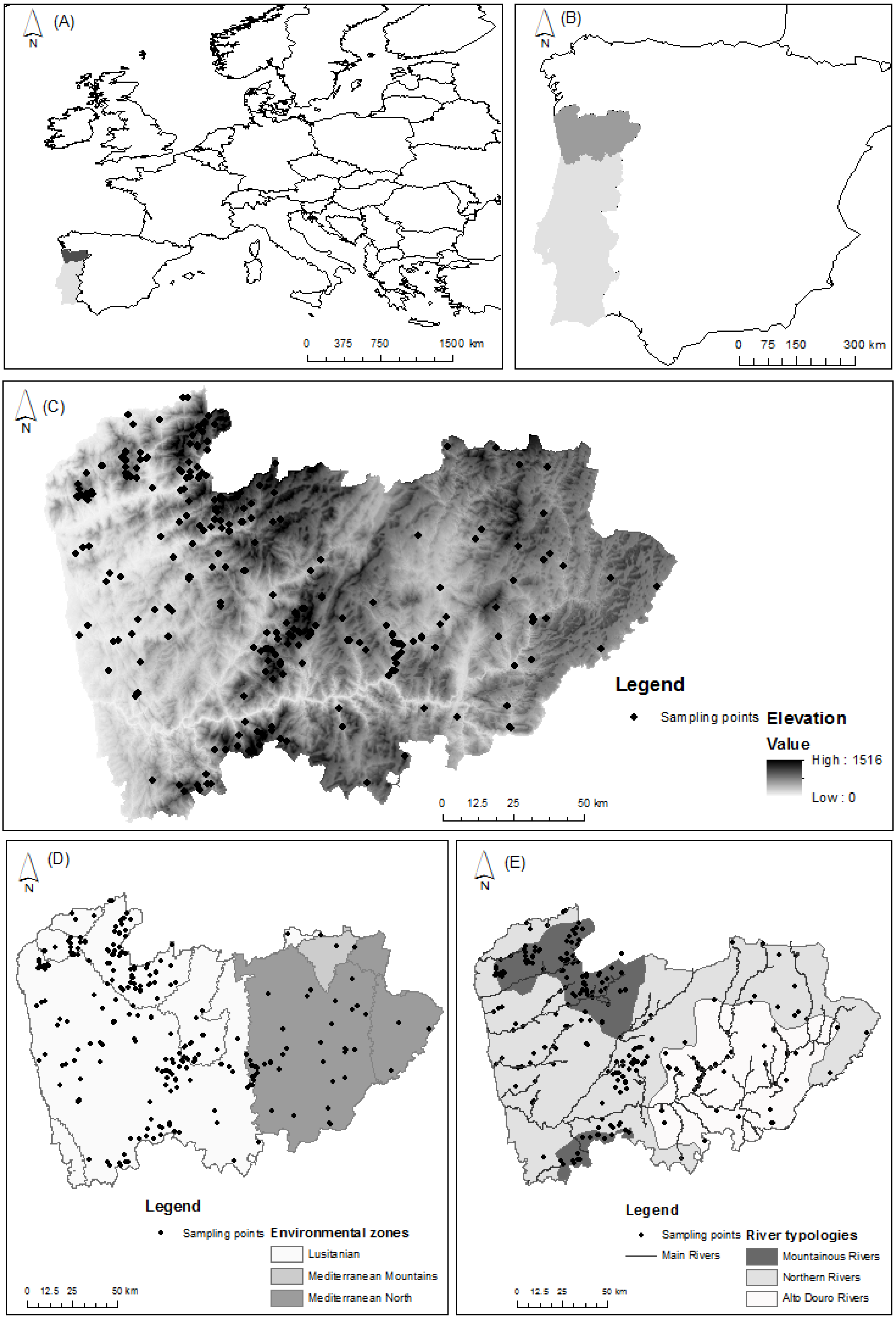

Fig. 6 Geographical context of the study area in Europe (A) and the Iberian Peninsula (B), and sampling points symbolized over a Digital Elevation Model (C), environmental zones (D) and river typologies (E) in the study area.

The Mediterranean Mountains are influenced by the Mediterranean climate, but still retain the influence of mountainous climate. The Mediterranean North presents the characteristic Mediterranean summer drought.

Water courses in the study area were typified into three major categories mountainous rivers of the north, northern rivers of small to medium-large dimensions and small to medium-large rivers of the Alto Douro region (Fig.6 E) (INAG 2008). The mountainous rivers are steep watercourses, located at high altitudes, with small catchment areas (less than 100 km^2) and high average annual drainage (800-1400 mm), the annual average temperature is 11°C and annual average precipitation is 1900 mm. The northern rivers have an annual average temperature of 12 to 13 °C and annual average precipitation of 1200 mm, are located at a diverse range of altitudes and have little mineralization due to the siliceous lithology of the substrates.

Table 5 Environmental characterization of study area and respective environmental zones (See Fig.6).

Environmental factor		*Lusitanian*	*Mediterranean Mountains*	*Mediterranean North*	*Study Area*
Aspect(°)	Min	0	0	0	0
	Max	359.912	359.8472	359.8824	359.912
	Mean	190.7171	183.5685	181.116	187.2565
Mean annual temperature (ºC)	Min	7.866667	7.825	9.354167	7.825
	Max	16.27083	13.3375	17.87917	17.87917
	Mean	13.46179	11.03067	13.2203	13.32059
Total annual precipitation (mm)	Min	572.3	861.2	391.7	391.7
	Max	1704.4	1375.5	1189.8	1704.4
	Mean	1155.707	1081.426	745.7499	1013.1
Precipitation of the driest month (mm)	Min	0.9	11.1	0.1	0.1
	Max	38.9	28.3	29.5	38.9
	Mean	18.98596	19.23736	12.65394	16.81683
Precipitation of warmest quarter (mm)	Min	73.1	121.2	48.2	48.2
	Max	247.6	197.2	186.9	247.6
	Mean	148.972	197.2	109.4509	135.4713
Temperature annual range	Min	23.3	26.6	26	23.3
	Max	30.3	28.9	33.1	33.1
	Mean	26.94553	27.5434	28.42655	27.46861
Elevation (m)	Min	0	414	75	0
	Max	1510	1472	1306	1510
	Mean	460.7525	865.4753	564.4825	505.96
Slope (%)	Min	0	0.176777	0	0
	Max	90.55695	60.39919	104.4723	104.4723
	Mean	13.69104	14.17088	11.73911	13.03359
Thermicity	Min	202.4849	200.4849	215.4849	200.4849
	Max	291.4849	253.4849	300.4849	300.4849
	Mean	255.6382	231.7186	255.4841	255.0127
Flow Accumulation (km^2)	Min	0	0	0	0
	Max	2621.4	1	2621.4	2621.4
	Mean	10.92293	0.022817	12.90601	11.34084
Solar Radiation (MWH/m^2)	Min	579852.1	810326.8	677851.6	579852.1
	Max	1552029	1483440	1486186	1552029
	Mean	1216340	1288967	1239494	1226009

The Alto Douro rivers are characterized by the higher mean temperature (13°C) and decreased precipitation (600 mm average) typical of the Mediterranean region where they are located (INAG 2008). The water courses of the study region support a high bryophyte species richness and previous studies reported 140 fluvial bryophyte species for the northwest territory. A total of 19 taxa are included in the European or Iberian Peninsula Red List (Sérgio *et al.* 2013). Four mosses and three liverworts endemic to Europe and two species are endemic to the Iberian Peninsula (*Racomitrium hespericum* and *R. lusitanicum*) and can be found in the study area.

Furthermore, many of the atlantic bryophytes (*e.g.*, *Isothecium holtii*, *Fissidens polyphyllus*, *Heterocladium wulfsbergii*, *Amphidium mougeotii*, *Fontinalis squamosa* var. *dixonii*, *Grimmia lisae*, *Plagiothecium succulentum*, *Platyhypnidium lusitanicum*, *Hyocomium armoricum*, *Saccogyna viticulosa*, *Dumortiera hirsuta*, *Riccardia chamedryfolia*, *Racomitrium hespericum*, *Nardia compressa*, *Lejeunea lamacerina*, *Radula holtii*) are specially important since their suitable habitat is restricted to mainland northwestern streams. These species are also among the most threatened and responsive to thermal conditions and hydrological regime changes, and northern Portugal region corresponds to their southern European limit distribution limit (Vieira *et al*. 2005; Vieira *et al*. 2012b).

3.2.2 Species Data

We utilized a compilation of databases on bryophytic communities from field campaigns undertaken from 2000 to 2012, in a total of 270 sampling points in northern Portugal. Inventories correspond to fieldwork carried out by Cristiana Vieira following WFD methodologies during the implementation of this Directive, in Environmental Impact Assessment studies and PhD sampling. Bryophytes were surveyed in all immerged or semi-immerged microhabitats found within the 100 m of riverbed and margins. Sampling focused in the rocky substrates. Bryophyte species presence/absence was registered using 0.25 m^2 (0.5 m x 0.5 m) sample plots placed in all recognizable hydrologic zones and microhabitats constantly or easily immerged, seasonally or several times a month, with discharges related to precipitation or dam releases, and micro-habitats immerged only in extended periods of rain.

3.2.3 Community analysis: ordination and classification

A species *vs.* sites Jaccard dissimilarity matrix was subjected to Ward’s hierarchical clustering using R’s vegan package to obtain community types by group of sites (clusters) (Oksanen *et al.* 2013; R Core Team 2013). Species composition of site clusters was then analysed and the dominant species and their frequencies calculated. The contribution of each species for intra-cluster similarity was calculated with SIMPER analysis ('Similarity Percentages – Species Contributions') in the Community Analysis Package 1.52 for each site group (Hederson & Seaby 1999).

3.2.4 Environmental Predictors

The environmental predictors used in the distribution modelling of the community types and the respective sources of information are listed in (Table 6). The higher resolution rasters available for environmental predictors were resampled to match the 200*200 m climatic data resolution. To ensure each cluster responsiveness to the environmental factors, we analysed the differences in environmental factors between clusters, by conducting a PERMANOVA analysis using PAST (PAleontological STatistics) 3.01 (Hammer *et al.* 2001), to test the significance of differences in environmental conditions between clusters.

Table 6. Environmental predictors (spatial information) used to model community types distributions.

Type of variable	*Variable*	*Code (used in Table 9)*	*Source*
Climatic	Annual Average Temperature	bio1	Digital Iberian Climatic Atlas (Ninyerola M. *et al.* 2005)
	Temperature annual range	bio7	Derived from Digital Iberian Climatic Atlas using R package "dismo" (Hijmans *et al.* 2013)
	Thermicity index		Derived from Digital Iberian Climatic Atlas using R package "dismo" (Hijmans et al. 2013)
	Annual Average Precipitation	bio12	Digital Iberian Climatic Atlas (Ninyerola M. et al. 2005)
	Precipitation of the driest month	bio14	Derived from Digital Iberian Climatic Atlas using R package "dismo" (Hijmans et al. 2013)
	Precipitation of the warmest quarter	bio18	Derived from Digital Iberian Climatic Atlas using R package "dismo" (Hijmans et al. 2013)
Physiographic	Elevation	dem	Consortium for Spatial Information (CGIAR-CSI) SRTM Database (Jarvis *et al.* 2008)
	Slope	slope	Derived from Digital Elevation Model (DEM) using ArcMap 10.1 ™ (ESRI 2012)
	Aspect	aspect	Derived from DEM using ArcMap 10.1 ™ (ESRI 2012)
	Solar radiation	solarrad	Derived from DEM using ArcMap 10.1 ™ (ESRI 2012)
Hydrologic	Flow Accumulation	flowaccumulation	Derived from DEM using ArcMap 10.1 ™ (ESRI 2012)

3.2.5 Modelling techniques

Bryophyte community distribution was predicted using ten models available in "biomod2: Ensemble platform for species distribution modelling" (Thuiller *et al.* 2013) in

R environment (R Core Team 2013). The models included were Generalized Linear Models (GLM), Generalized Boosted Models (GBM), Generalized Additive Models (GAM), Classification Tree Analysis (CTA), and Artificial Neural Networks (ANN), Surface Range Envelop also known as BIOCLIM (SRE), Flexible Discriminant Analysis (FDA), Multiple Adaptive Regression Splines (MARS), Random Forests (RF) and Maximum Entropy (MAXENT). For all models biomod2 default parameters were used (Thuiller et al. 2013). These models were then combined and an ensemble forecast was generated for each community type.

The available data for community type occurrences was presence-only, *i.e.*, no confirmed absences were available. A set of random pseudo-absences (corresponding in number to 20% of the study area) was generated in order to use presence-absence models which tend to perform better than presence-only models (Elith *et al.* 2006; Barbet-Massin *et al.* 2012).

Distribution models were calibrated using 80% of the species data and pseudo-absences selected randomly. The remaining 20% were used to evaluate model performance. Model evaluation was performed calculating the area under the curve (AUC) of receiver operating characteristic (ROC). Models with AUC values of < 0.5 were considered no better than random, 0.5-0.7 were considered poor, 0.7-0.9 useful and > 0.9 excellent (Swets 1988; Manel *et al.* 2001).

Only the models that presented AUC values > 0.7 were included in the ensemble forecast of communities' distribution models. We tested several consensus methods (mean, weighted mean, median, confidence interval, coefficient of variation and committee averaging) and kept the prediction that presented greater AUC values. The map binarization was completed in ArcMap 10.1^{TM} (ESRI 2012) using a 70% probability of occurrence as a threshold for presence of community in a pixel.

3.2.6 Fragmentation analysis

The regional fragmentation elements included in the spatial analysis were dams, small hydroelectric schemes, wind turbines, main roads and railways (Table 7). The fragmentation elements were obtained through "MOBIA-Biodiversity Monitoring in Environmental Assessments" project, which main objective was to evaluate the effectiveness of the handling of biodiversity in environmental assessments and the contribution of associated monitoring programs for a global monitoring network (PTDC/AAC-AMB/114522/2009).

We created a set of buffers around each fragmentation element in order to depict and account for different magnitudes of its impact. The dimensions established for the different magnitudes of buffers are described in Table 7.

Table 7 Regional fragmentation elements and the respective buffer areas of impact proposed in the study. (n.a. not applicable).

Regional fragmentation elements	*Number of elements in study area*	*General description of available geographic information*	*Impact levels and buffers definition*		
			1 (lower)	2 (medium)	3 (stronger)
Dams	24	Polygons corresponding to the reservoirs; All dams are already constructed.	n.a.	Buffer of 200 m around the reservoir + 1 km of buffer, 50 m wide, downstream of the reservoir	Intersection of community distribution with the area of the reservoir
Small hydroelectrics	34	Point features; 34 were being subjected to EIA at the time,4 already licensed, 2 in construction, 2 requiring EIA exemption and 1 project in execution	n.a.	Buffer of 500 m radius around the point	Intersection of point with community distribution
Wind farms	1054	Point features; all installed	Buffer of 500 m width around the area of impact level of two	Buffer of 250 m width around the area with strongest impact	Intersection of community distribution with the point feature and buffer of 100 m radius around the point
Railways	32	Line features; 619.08 km including deactivated lines such as the Tua Line, and remodelled lines such as the Póvoa Line	Buffer of 100 m width around the area of impact level of two	Buffer of 50 m width around the line.	Intersection of line community distribution
Main roads	278	Polygon features 5, 10, 15 m wide respectively in national and regional roads, main and complementary itineraries and highways. 1197.32 km of extent in total; 50.45 km of highways, 182.56 km of itineraries, 822.86 km national roads, 141.43 km of regional roads	Buffer of 100 m width around the area of impact level of two	Buffer of 50 m width around the road polygons	Intersection of polygon with community distribution

The impacted area of different levels of magnitude caused by each fragmentation element was calculated over the binary distribution of the community types using ArcMap 10.1^{TM} Zonal Statistics tool (ESRI 2012). These results were combined to calculate the proportion of impact caused by fragmentation elements typology to obtain spatial statistics of impact (total and partial areas).

3.2.7 Zonation analysis

In order to understand how the impact of human presence affects the conservation of fluvial bryophyte communities and their regional representation and connectivity we set out for a series of exercises using conservation planning software Zonation 4.0.0 (Moilanen *et al.* 2005; Moilanen *et al.* 2014).

Zonation software produces a hierarchical prioritization of the landscape based on biological value of the sites (cells) accounting for complementarity. The algorithm sequentially removes the least valuable cells while minimizing marginal loss of conservation value, accounting for connectivity and the importance given to the biodiversity features (in this case fluvial bryophyte communities). This produces a spatial prioritization composed of a nested sequence of highly connected landscape structures with core areas that present the greatest conservation values (top fraction).

In this analysis we used the 200*200 m rasters with the probability of occurrence obtained for the community types with the highest conservation value through the modelling procedure and weighted them according to conservation importance (1, 0.5 and 2 respectively). We selected Core Area Zonation (CAZ) removal rule, which selects as high priority cells the ones with a high occurrence probability for highly weighted species, enabling emphasis on rarity and conservation value of certain community assemblages. This analysis was repeated with the same parameters for the biodiversity features (BIO) and different condition and mask layers were added, the first to exclude unsuitable areas (*e.g.,* agricultural and urban areas) from the selection and the second to force the inclusion of others (*e.g.*, protected areas) in the high priority cells. The different analysis performed are described in the Table 8. We also performed a landscape comparison (LSC) as part of the post-processing analysis in order to compare the overlap and average difference in cell removal order of the top 25% fraction of the landscape between the "biodiversity features only" solution (BIO) and all other solutions.

Table 8 Zonation analyses coding, description and data inputs

Analysis codes	*Data input*	*Representation*
BIO	Community types distributions modelled	"Pristine" conditions.
BIO+Pr	Community types distributions Mask Layer: All protected areas (Natura 2000 network, protected areas and Ramsar sites)	Force inclusion of protected areas in the high priority conservation areas.
BIO+Urb	Community types distributions Condition Layer: Urban areas	Exclude urban areas from analysis.
BIO+Agr	Community types distributions Condition Layer: Agricultural areas	Exclude agricultural areas from analysis.
BIO+Frag	Community types distributions Condition Layer: Areas impacted by regional fragmentation agents	Exclude areas impacted by the regional fragmentation elements listed above.
BIO+Urb+Agr	Community types distributions Condition Layer: Urban and agricultural areas	Exclude both agricultural and urban areas to reflect human presence occupancy constraints.
BIO+Urb+Agr+Frag	Community types distributions Condition Layer: Urban, agricultural and impacted areas	Exclude urban, agricultural and areas impacted by regional fragmentation elements to reflect human occupancy and fragmentation constraints.
BIO+Urb+Agr+Frag+Pr	Community types distributions Condition Layer: Urban, agricultural and impacted areas Mask Layer: All protected	More realistic approach, reflecting not only the human presence and impact in the landscape, and also the constraints to the creation of new conservation areas.

3.3 Results

3.3.1 Community types characterization

Using the classification tree with Ward's method we obtained four clusters of sites corresponding to four different community types (A, B, C and D) shown in Fig. 7.

Community A species assemblage was dominated by *Scorpiurium deflexifolium*, *Platyhypnidium riparioides* and *Cinclidotus fontinaloides,* essentially Mediterranean species (Table 9).

Community B is dominated by *Kindbergia praelonga*, *Chiloscyphus polyanthus* and *Brachythecium rivulare* species that appear in the most atlantic river valleys. Community C is dominated by *Fontinalis antipyretica* and *Leptodyctium riparium* characteristic of more mineralized, if not polluted, rivers and streams. Community D is characterized by *Scapania undulata*, *Hyocomium armoricum* and *Racomitrium aciculare,* high altitude Atlantic streams species.

All the species assemblages obtained contain one or more taxa listed in the Red List of Threatened Bryophytes of Portugal (Sérgio *et al*. 2013) as vulnerable or endangered.

Community A includes four species listed as vulnerable and two as near threatened. In community B there are seven taxa considered vulnerable and an additional six as near threatened. Community C counts only one taxa listed as near threatened. Community D contains two endangered species, four listed as vulnerable and six listed as near threatened. When considering only endangered or vulnerable taxa, they correspond to 6.7%, 7.1% and 5.94% of the species present in community A, B and D respectively.

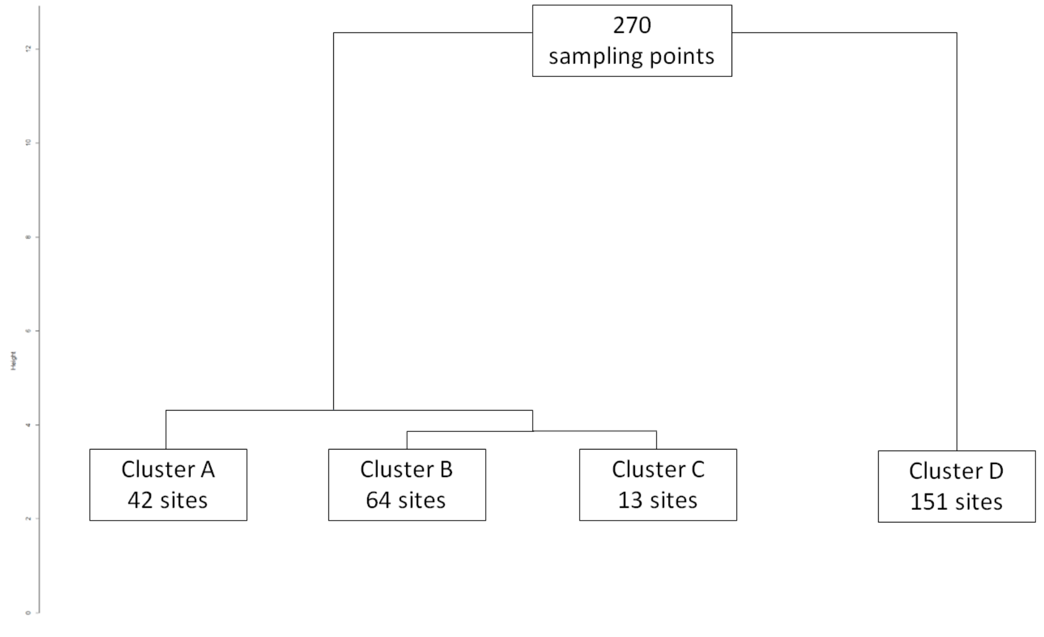

Fig. 7 Ward's hierarchical classification dendrogram of the sampled sites and the bryophytic community types obtained.

Table 9 Results of classification, modelling and fragmentation analysis aggregated by community types. Community C was not included in the modelling step, therefore it shows no results (n.a. - not applicable) for spatial modelling and fragmentation analysis.

Community type		**A**		**B**		**C**		**D**	
Classification	Number of sites	42		64		13		151	
	Species and Frequency (%) /SIMPER percentage of contribution for group similarity (Only species with frequency > 20% are presented; Dominant species in grey)	*Scorpiurium deflexifolium* (Solms) M.Fleisch. & Loeske	54.76/ 22.36	*Kindbergia praelonga* (Hedw.) Ochyra	39.06/ 14.33	*Fontinalis antipyretica* Hedw.	76.92/ 59.55	Scapania *undulata* (L.) Dumort.	70.20/ 14.72
		Platyhypnidium riparioides (Hedw.) Dixon	52.38/ 27.28	*Chiloscyphus polyanthos* (L.) Corda	37.50/ 11.37	*Leptodictyum riparium* (Hedw.) Warnst.	53.85/ 32.78	*Hyocomium armoricum* (Brid.) Wijk & Margad.	67.55/ 12.56
		Cinclidotus fontinaloides (Hedw.) P. Beauv.	45.24/ 14.55	*Brachythecium rivulare* Schimp.	34.38/ 9.16	*Platyhypnidium riparioides* (Hedw.) Dixon	30.77	*Racomitrium aciculare* (Hedw.) Brid.	66.89/ 13.15
		Dialytrichia mucronata (Brid.) Broth. var. *mucronata*	28.57/ 4.27	*Fontinalis squamosa* Hedw.	32.81/ 9.55			*Fissidens polyphyllus* Wilson ex Bruch & Schimp.	63.58/ 11.79
		Scleropodium touretii (Brid.) L.F.Koch	28.57/ 9.10	*Bryum pseudotriquetrum* (Hedw.) P.Gaertn. et al.	20.31/ 3.07			*Platyhypnidium lusitanicum* (Schimp.) Ochyra & Bednarek-Ochyra	62.91/ 11.74
		Lunularia cruciata (L.) Lindb.	23.81/ 3.92	*Platyhypnidium lusitanicum* (Schimp.) Ochyra & Bednarek-Ochyra	20.31/ 3.17			*Pellia epiphylla* (L.) Corda	43.05/ 5.41
		Schistidium apocarpum (Hedw.) Bruch & Schimp.	23.81/ 2.34	*Platyhypnidium riparioides* (Hedw.) Dixon	20.31/ 2.63			*Polytrichum commune* Hedw.	42.38/ 5.16
				Racomitrium aciculare (Hedw.) Brid.	20.31/ 3.02			*Marsupella emarginata* (Ehrh.) Dumort.	37.09/ 3.15
								Isothecium holtii Kindb.	31.13/ 2.33
								Racomitrium lamprocarpum (Müll.Hal.) A.Jaeger	30.46/ 2.67
								Fontinalis squamosa Hedw.	29.80/ 2.12
								Fissidens bryoides Hedw. var. *caespitans* Schimp.	23.18/ 1.05
								Sphagnum auriculatum Schimp.	23.18/ 1.05
								Bryum pseudotriquetrum (Hedw.) P.Gaertn. et al.	21.19/ 1.06
								Heterocladium wulfsbergii I.Hagen	21.19/ 0.92
								Sciuro-hypnum plumosum (Hedw.) Ignatov & Huttunen	20.53

Table 9 continued.

Spatial Modelling	Modelling Algorithms that yielded AUC values ≥ 0.7 (see Methods for modelling techniques codes)	GAM 1.00 GBM 0.80 RF 0.79 GLM 0.77 FDA 0.76 MAXENT 0.75	GAM 0.86	n.a	GAM 1.00 GBM 0.85 MAXENT 0.85 RF 0.85 GLM 0.82 FDA 0.81 ANN 0.80 CTA 0.79 MARS 0.79
	Variable Importance (decreasing order)	flowaccumulation; aspect; bio7 slope solarrad; bio14; dem; bio1; thermicity; bio18; bio12	bio18; flowaccumulation solarrad; bio7 slope; bio14; aspect; bio12; dem; bio1 thermicity		aspect; solarrad; flowaccumulation; bio7; slope; bio14; bio1; bio18; bio12;thermicity; dem
	Best ensemble	Weighted mean	Mean		Mean
	Area of potential occurrence (after binarization)	839 km^2	3053.64 km^2		3614.68 km^2
Fragmentation analysis	Total impacted area / Percentage of occurrence impacted all magnitudes included (all magnitudes included)	150.56 km^2 / 17,95%	468.04 km^2 / 15,33%	n.a.	379.56 km^2 / 10,5%
	Percentage of impact caused by fragmentation element in relation to total impacted area.	Dams: 19.18% Small hydroelectric: 1.33% Wind Farms: 0.00% Main roads: 52.28% Railways: 27.21%	Dams: 14.65% Small hydroelectric: 0.62% Wind Farms: 0.50% Main roads: 70.49% Railways: 13.75%		Dams: 8.09% Small hydroelectric: 1.09% Wind Farms: 38.43% Main roads: 48.88% Railways: 3.51%

3.3.2 Community types distribution modelling

Community type C was excluded from the modelling step and posterior analysis since only 13 presence points were available, a number considered insufficient to generate a reliable distribution model (Franklin 2009). The models that obtained AUC values greater than 0.7 are listed in Table 9. In general, the best performing algorithms were GAM, GBM, MAXENT, CTA and RF. For community types B and D the best performing consensus method was the mean, and for community type A the weighted mean.

The spatial ensemble forecasts obtained for each community type, both as probability of occurrence and presence/absence maps obtained with the binarization process, are presented in Fig. 8 and Fig. 9, respectively. The potential area of occupancy calculated after the binarization is presented in Table 9, with type D with the biggest potential area, 3614.68 km^2, and type A with the smallest potential area, 839 km^2.

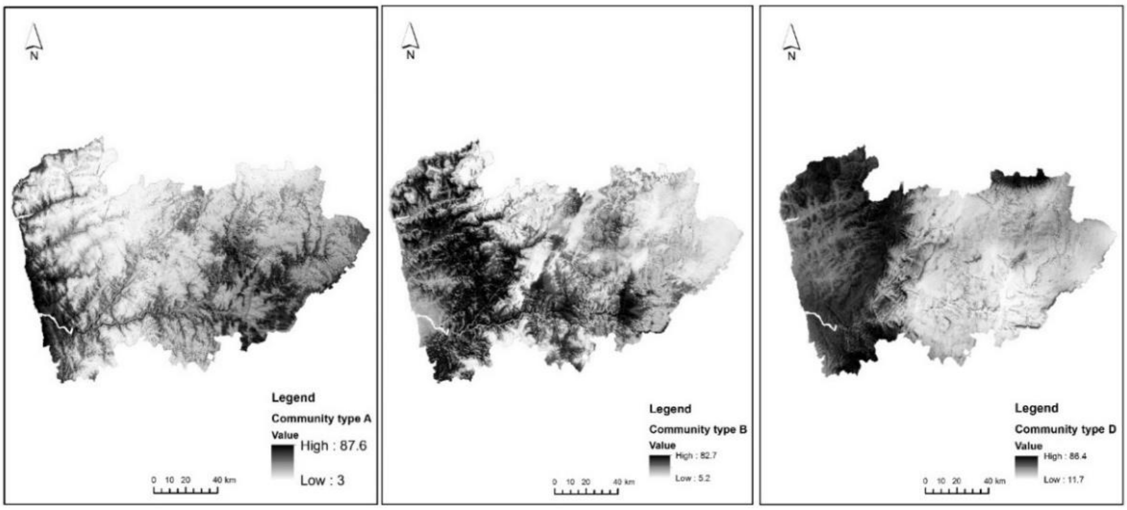

Fig. 8 Probability of occurrence obtained for each community type through the biomod2 spatial modelling. Community C was not included in the modelling step, hence no distribution map is presented.

3.3.3 Fragmentation analysis

A total of 2904.149 km^2 of area with some potential occurrence of bryophytic communities are impacted in some order of magnitude by the regional fragmentation elements. This corresponds to 12.8 % of the total potential area of occurrence for all the communities. For each community the area of potential occurrence affected varies from 10.5% for community type D to 17.95% for type A (Fig. 10 A). Most of the impacted area for community type A and B has a level of magnitude of level two. For community D the majority of impact is of magnitude of level one (Fig. 10 B).

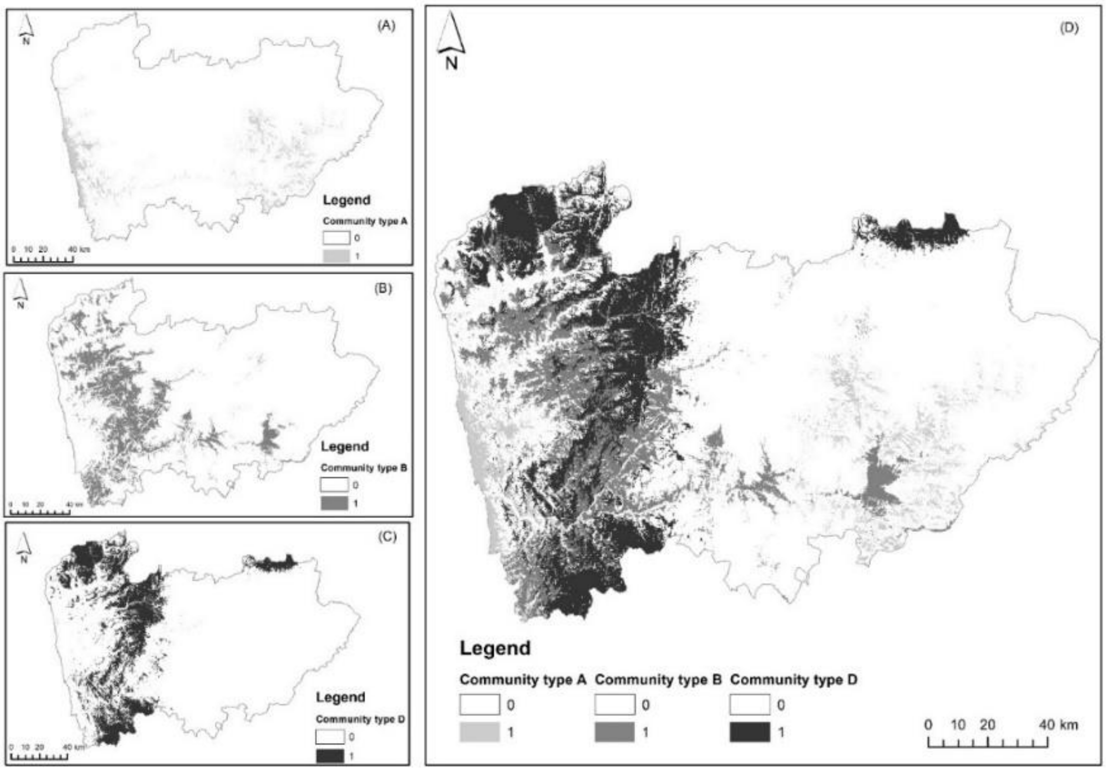

Fig. 9 Binarization of potential modelled occurrence for each community type separately (A, B, C) and the collective potential modelled occurrence (D).

Main roads were responsible for 59.53% of the total impact caused on all community types, followed by wind farms, responsible for 14.85% of the impact, while dams and railways are responsible for 12.84% and 11.89% of impact, respectively.

When each community type is analysed separately, main roads are still the leading cause of impact only differing in percentage. The second cause of impact is different for all 3 community types: railways for type A, dams for type B and wind farms for type D (Table 9).

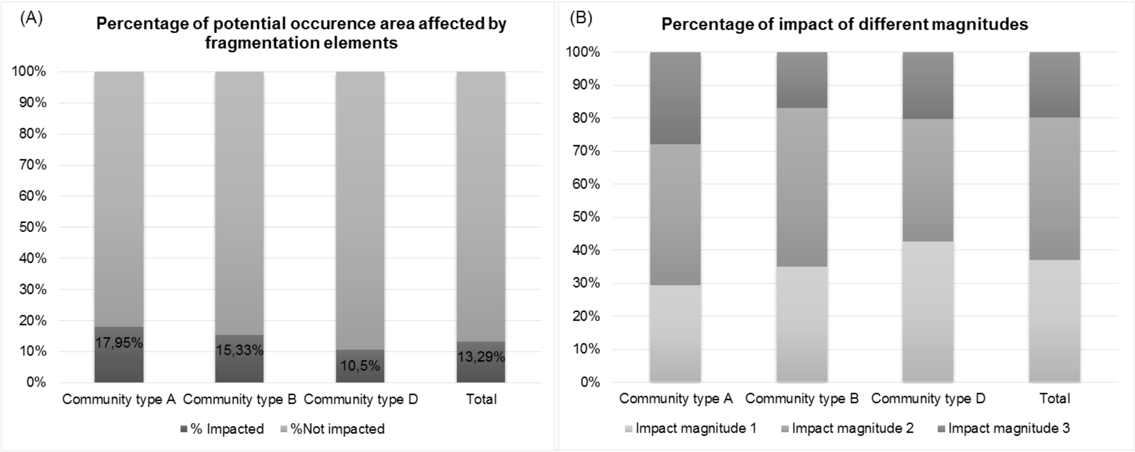

Fig. 10 Percentage of community types' potential occurrence impacted by the regional fragmentation elements (A), and percentage of impact of different magnitudes (B).

3.3.4 Zonation analysis

The spatial prioritization maps, showing the priority rank for the study area, obtained for each analysis conducted using Zonation are presented in Appendix A.

In general, the selected areas for conservation, when taking into account only the biodiversity features (Fig. 11 A), coincide with areas already protected (as National Parks, Natura 2000 Sites, and Special Protection). However, it becomes apparent that the top fraction of the landscape chosen by Zonation is smaller and spatially more fragmented as we include in the analysis more constraints that highlight human presence in the territory (such as the urban and agriculture mosaics as surrogates of human occupation) (Fig. 11 B and 11 C).

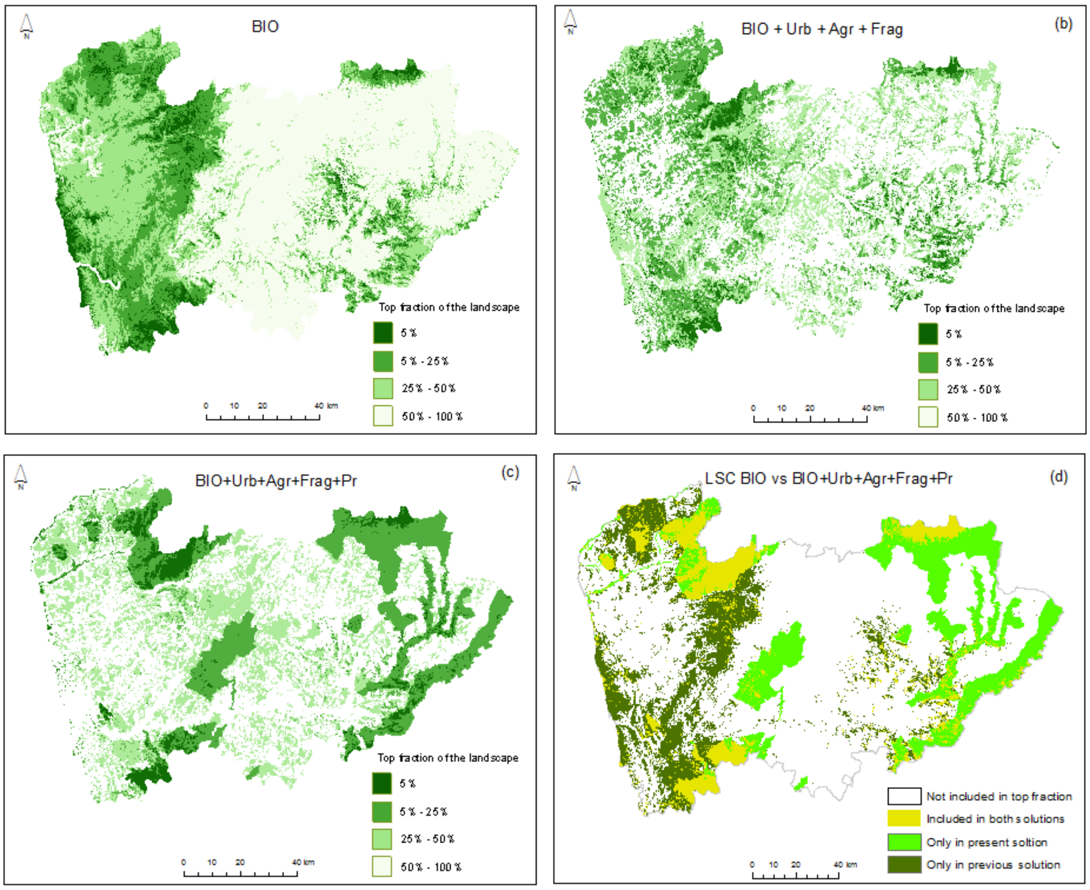

Fig. 11 Spatial conservation prioritization obtained for the BIO analysis (A), BIO+Urb+Frag analysis (B) and BIO + Urb+Agr+Frag+Pr analysis (C). Also the spatial output of the landscape comparison post-processing analysis between Bio and Bio+Urb+Agr+Frag+Pr (D). See Table 8 for Zonation analyses coding and description.

As expected, in all the solutions constrained both by human occupation (Urb+Agric) and fragmentation elements (Frag), the proportion of the communities protected by the top 25% of landscape is smaller than in the BIO solution (Fig. 12). The forced inclusion of protected areas (BIO+Pr) in the selection process of the top fraction of the landscape is one of the solutions which allows the protection of a smaller proportion of the potential distribution of all three community types, exceeded only by the solution with most restrictions - BIO+Urb+Agric+Frag+Pr, which allows the protection of the smallest proportion. Community type D retains a bigger proportion of its distribution across all Zonation analyses conducted.

The LSC analysis (Fig. 11 D), comparing the BIO solution with the BIO+Urb+Agr+Frag+PR solution, reveals a small coincidence between the two solutions (only 2032.48 km^2, corresponding to 9.6% of total area coincides between the two analysis). Additionally, there is a loss of 3263.24 km^2 that are no longer selected in the BIO+Urb+Agr+Frag+PR solution, and are mostly concentrated in the Atlantic region of the study area. On the other hand, another 3263.24 km^2, mostly located in the Mediterranean region, are only selected in the BIO+Urb+Agr+Frag+PR solution. A total of 6526.48 km^2 (30.81 %) of divergence in the top 25% of the landscape is found between the two options. Other LSC analyses are presented in Appendix B.

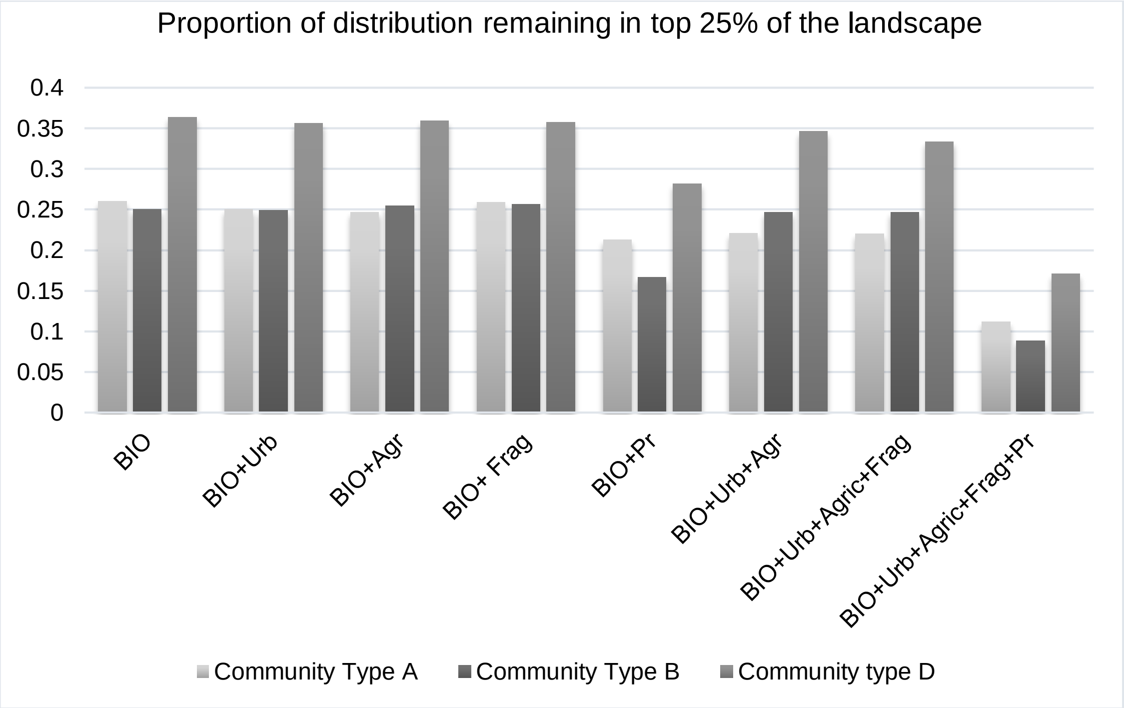

Fig. 12 Proportion of community types' distribution protected by the top 25% of the landscape in each Zonation analysis.

3.4 Discussion

Our approach is based on a hierarchical classification in community types, and hence an "assemble first, predict later" methodology. A few shortcomings were pointed to this approach, such as not describing community variation and predicting non existing co-occurrence of communities (Baselga & Araújo 2010). However, the classification and spatialization of community types we obtained are in line with field observations and literature (Vieira *et al.* 2012a; Vieira et al. 2012b; Vieira et al. 2014). Furthermore, in biological groups such as bryophytes which face some negative bias in terms of field reconnaissance, community types with flagship species are useful and recognisable surrogates for planning and management of riverscapes' biodiversity (Leathwick *et al*. 2010; Turak *et al.* 2011).

The community types and its species assemblages obtained are consistent with previous fluvial bryophytes community classifications for this region (Vieira *et al*. 2012a; Vieira *et al*. 2014). The community types obtained resemble the major groups obtained in the cited studies, reflecting a differentiation in Atlantic (B and D) and Mediterranean assemblages (A), and also separating assemblages that occur in more mineralized rivers (C). Moreover, each community type is characterized by a small set of frequent species that present the highest percentage of contribution for group cohesion (see Table 9).

The modelled potential distribution obtained for the three community types is concordant with the chorological patterns of the corresponding core species. The distributions present a spatial overlap. Yet this is not unlikely to occur, since in the same river segment we can observe the co-occurrence of different communities due to the mosaic of hydrogeomorphologic features that can be found within the 100 meters of reach surveyed (in 15487 pixels, which correspond to 8.25% of total pixels modelled, co-occurrence of bryophyte communities was predicted). In our case, the most frequent situation of co-occurrence is between community types B and D. The specific potential co-occurrence of communities B and D can be explained by the lack of spatial information on water quality for the total area modelled. In fact, previous studies, shown that community B substitutes community D whenever water becomes less acidic and more mineralized (Vieira *et al*. 2012b). Nevertheless, since this information is not included in the models and both communities overlap in part of their substrate and macroclimatic niche, they are modelled as co-occurring in the same pixels.

In general, the impact of regional fragmentation elements (Table 9, Fig. 10) on fluvial bryophyte communities can be considered low (13.29% of total area corresponds to impacted area). However, the analysis of specific impacts and the occurrence of impacts within protected areas reveals other trends. For example, almost 13% of the total impact occurs within protected areas. For community type B, 18% of its protected occurrence is impacted. This raises questions about protected areas management and human impact within these areas.

On the other hand community types A, B and D richer in threatened taxa (more than 5% of the species in each community type) are impacted in more than 10% of their distribution. This situation translates into a reduction in the potential distribution of species with conservation interest, further endangering taxa with restricted distributions.

The most impacted community type is community A, which is the least represented community in the study area, but the one with greater proportion of impact in its occurrence. Community type D is the less potentially impacted, what is probably related to the fact that it occurs in mountainous areas with less access and less human occupation.

Regarding the typology of the impact, roads are the leading cause of impact across all communities, which is related to the widespread and dense presence of this elements in the analysed territory. In the study area, as in many parts of the world, roads are an ubiquitous presence in the landscape (Girvetz *et al.* 2008). Roads alter water flow regimes and debris transport in watercourses through the modification of the river margins and also the streambed, they are also related to increased nutrient input and the introduction of chemicals due to water runoff (Jones *et al.* 2000; Trombulak & Frissell 2000). Moreover this impact is often cumulative and far reaching, as roads are network infrastructures. In addition, riverscapes are hierarchical by nature propagating these effects (Jones et al. 2000; Coffin 2007). For fluvial organisms, including bryophytes, this translates to habitat destruction and habitat deteoration and ultimatly loss of connectivity between habitat patches and riverscapes fragmentation (Auerbach *et al.* 1997; Forman & Alexander 1998; Coffin 2007).

The second cause of impact is for the most part related with the coincidence of distribution of community types and the fragmentation elements in the territory. For instance, community D second cause of impact are wind farms, which are installed in the mountain tops where this community is expected to occur. Community A second cause are railways that, in the Mediterranean part of the territory, roughly coincide the main water courses trajectory.

Despite the fact that main roads are the main cause of impact across communities, this analysis reveals the diversity of impacts and protection necessities that can be encountered in a relatively small, but diverse, territory. Moreover this reflects the need for integrated systematic conservation planning and management and to evaluate the effects on biodiversity of current management practices.

The proportions of protected distribution afforded by the “biodiversity features only” (BIO) solution and those including humans constraints considered individually are similar, nevertheless, when Zonation software combines biodiversity values and constraints (human occupancy and fragmentation elements), there is a decrease in the proportion of biodiversity that can be successfully protected. As we include human constraints in the analyses the top fraction of the landscape becomes smaller and more fragmented, disrespecting the needs of connectivity essential for effective watershed management (Ward *et al.* 2002; Pringle 2003; Jansson *et al.* 2007)

When forcing the allocation of the top fraction of the landscape to existing protected areas, we obtained the worst scenario for bryophytic conservation in terms of protected proportion of distribution. Moreover, the BIO solution and the BIO+Urb+Agr+Frag+Pr solution present a disparity of about 6000 km^2.This disparity translates into losses in the protection of the distribution of the atlantic communities (communities B and D) that are less represented at the regional and also at national level.

This analyses demonstrated that constraining the conservation of fluvial bryophytic communities to protected areas is not necessarily an effective strategy. The protection of bryophytic fluvial diversity in the study area depends greatly on the conservation of atlantic streams and headwaters, that are, for the most part but not only, included within protected areas. Additionally, protected areas are also part of the top fraction of the landscape that is fragmented due to human occupation and fragmentation elements that utilize mountains hydraulic and eolic energy (wind farms and dams).

Methodological options and constraints, such as the number of clusters chosen, the spatial distribution of the sampling points and the lack of spatialized information on water quality and micro-scale variables, affect the spatialization of communities and consequently the spatial conservation prioritization.

The results put emphasis on the necessity of studying the cumulative effects of regional fragmentation elements and land use, especially in protected areas, and also the need to account for these interactions in the elaboration of environmental impact studies and strategic environmental assessments.

Riverscapes' biodiversity strategies at the regional level will have to consider the cumulative effects mentioned above but also the role and effectiveness of existing protected areas.

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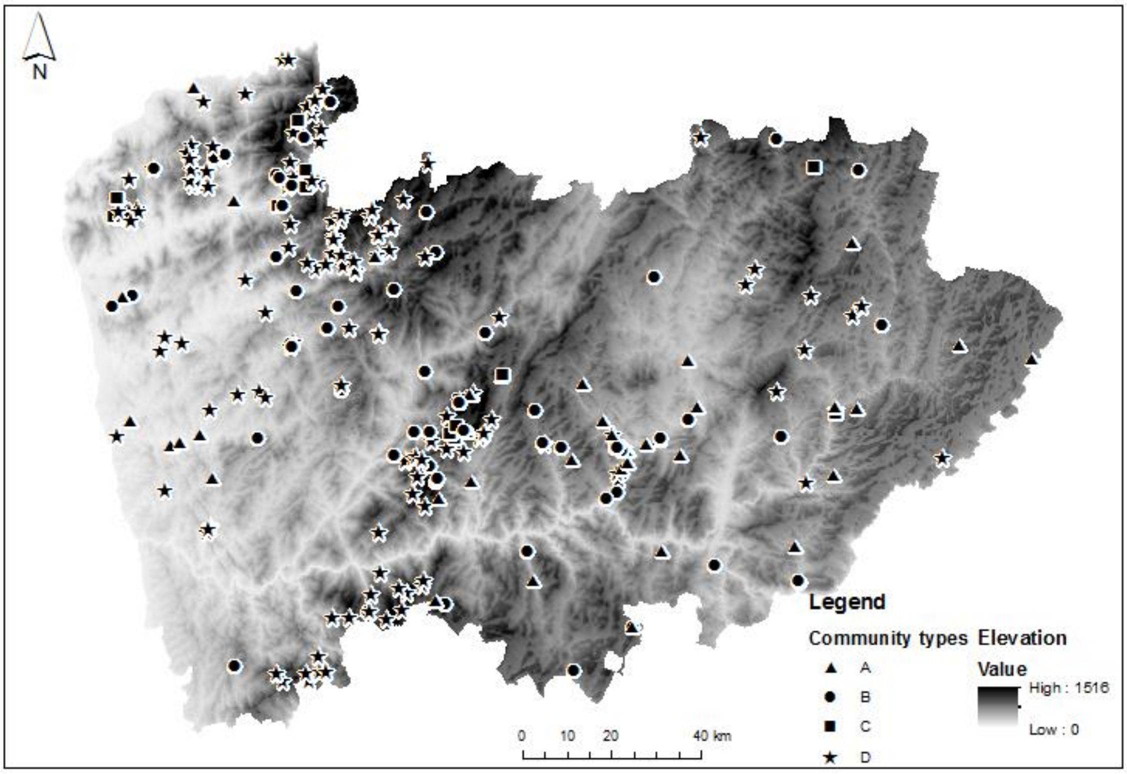
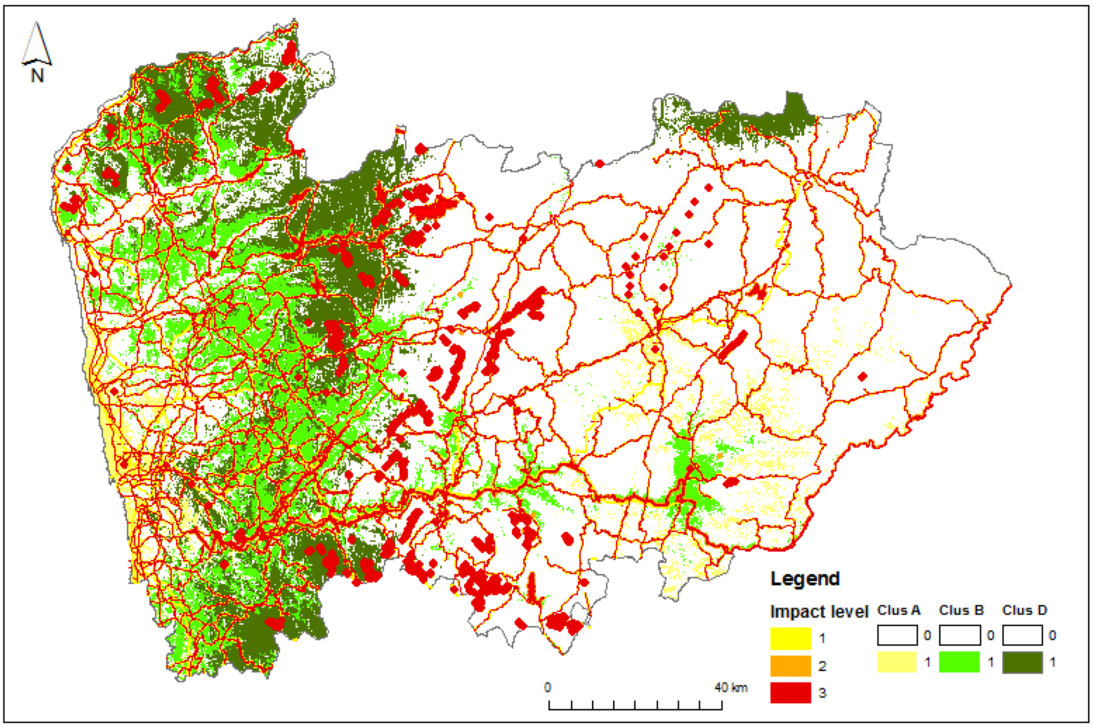
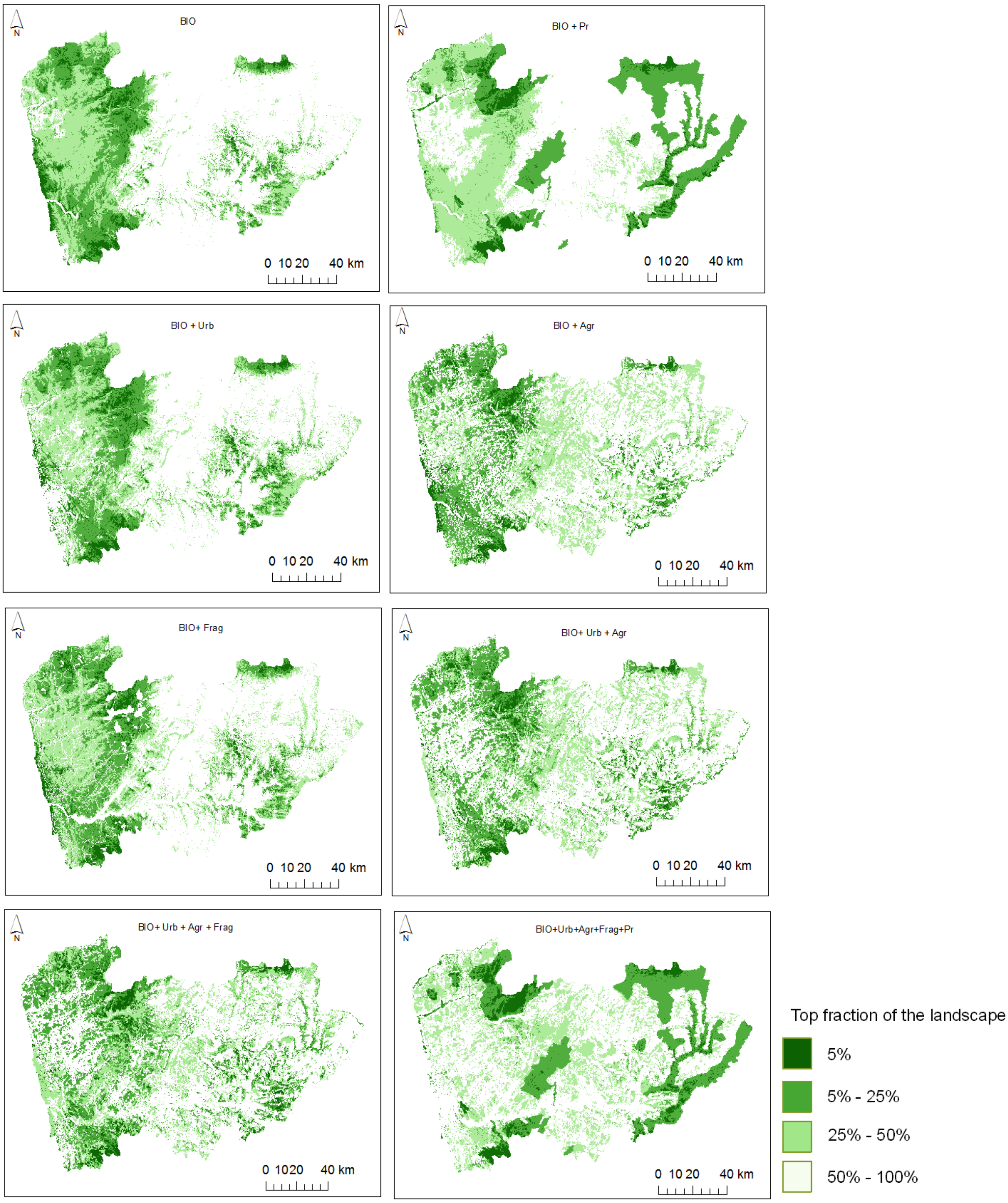
3.6 Appendix A: Sampling points symbolized by attributed community types.



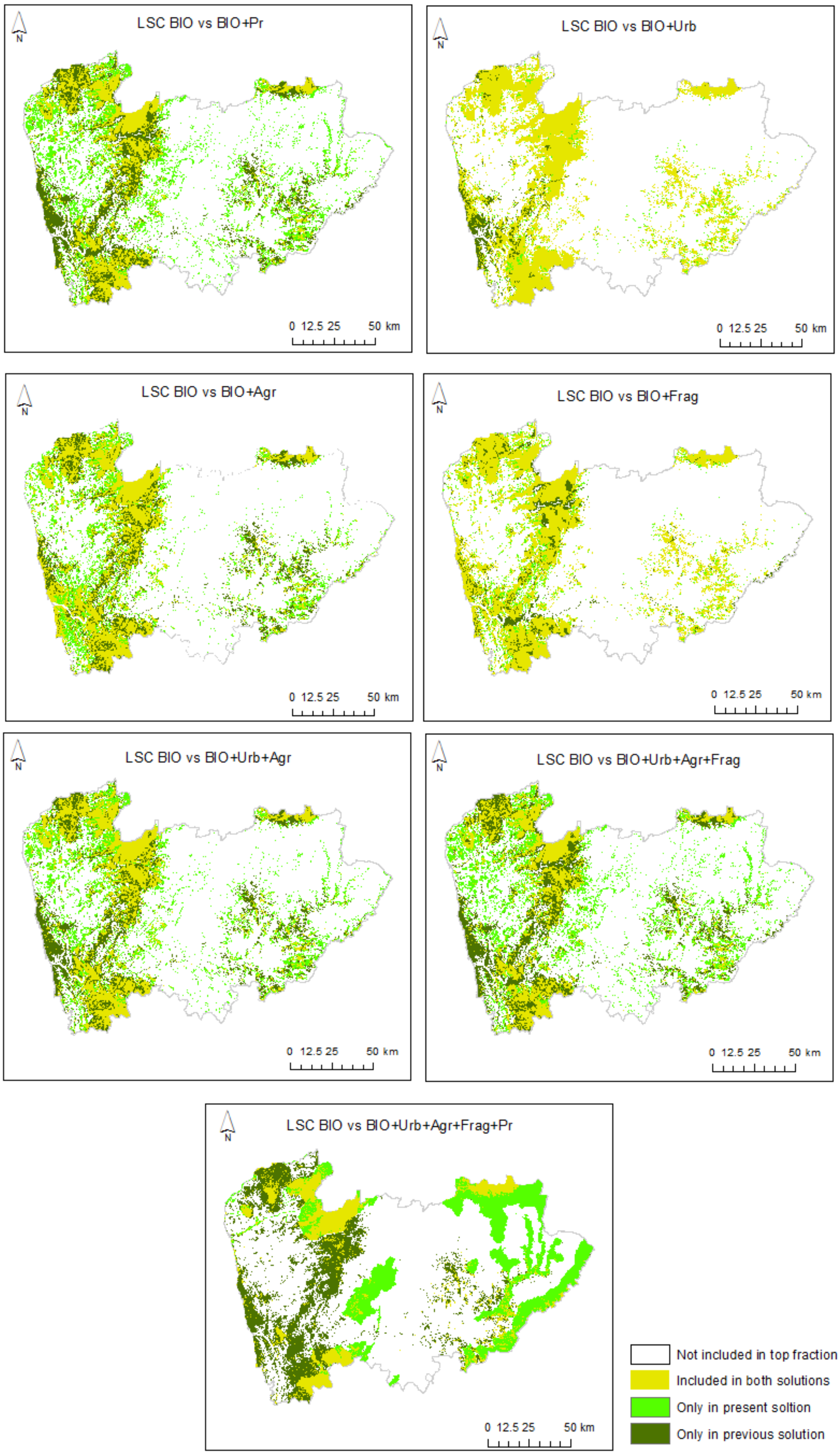
3.7 Appendix B: Buffers of impact for the regional fragmentation elements superimposed to the community types distributions.



3.8 Appendix C: Spatial conservation prioritization obtained for all the Zonation analyses.



3.9 Appendix D: Comparison of the top 25% fraction of the landscape of all solutions with the “biodiversity features only” solution.



4. General Discussion

Anthropogenic impacts on biodiversity, landscape fragmentation and conservation planning are among some of the most pressing issues in ecology nowadays. This work intent was to provide an integrated assessment of anthropogenic impact and its implications for conservation planning, for an ecosystem that, only recently, has been the subject of directed conservation planning studies, and for a group of organisms often overlooked in this type of study.

4.1 Application of community-level approaches

In order to provide the sought integration and overcome the lack of spatial chorological information for fluvial bryophyte species, we employed a community level modelling approach that yielded a set of four community types that constitute useful surrogates for the planning and management of riverscapes' biodiversity. The community types obtained are consistent with previous works and reflect the main ecological drivers of differentiation between these communities at a regional scale (Vieira *et al.* 2012; Vieira *et al.* 2014). Despite the few shortcomings appointed to this approach (*e.g.*, not describing community variation) this approach allowed the spatialization of community types that include flagship species and/or species with a low number of documented presences, and also counter some of the negative bias related to the field identification of these organisms at the species level. Moreover the potential distribution of the community types obtained is concordant with the chorological patterns of the corresponding core species.

4.2 Regional impact assessment

The impact of fragmentation on the community types can be considered low, however a considerable part of this impact is located within protected areas of the study area. This fact undermines the efficiency of protected areas for the conservation of fluvial bryophytes as one of requisites for their efficacy is the implementation of adequate management strategies. Despite the importance of the creation of protected areas, the efficiency of protection depends on land use and human impact within these areas (Mancini *et al.* 2005; Chessman 2013).

Main roads are the leading cause of impact across all communities, these are in fact abundant structures in the study area. For fluvial bryophyte diversity the alteration of streambed, margins and water and debris flow in the rivers mean alteration in the community structure and a decreased species diversity (Jones *et al.* 2000). The fact that roads are network infrastructures implies that these effects are often cumulative. Additionally the hierarchical nature of riverscapes has the potential to propagate these effects. The joint effect of these two components results in habitat deterioration, loss of connectivity and landscape fragmentation (Forman & Alexander 1998; Trombulak & Frissell 2000).

In spite of the transversal impact of roads, the remaining fragmentation elements vary in the impact they have in each communities' distribution, which reveals the variety of impacts, and management practices needed.

4.3 Spatial conservation prioritization

The spatial conservation prioritization analyses employed in this work further reinforced the necessity of effective management strategies in protected areas. For fluvial bryophyte diversity, forced allocation of conservation priority yielded some of the lowest values of protected distribution. Moreover, the inclusion of constraints to conservation such as land use and landscape fragmentation elements produces a smaller and more fragmented top fraction of the landscape, disrespecting the needs of connectivity of protected areas (Pringle 2003; Roux *et al.* 2008).

Furthermore, there is a disparity in the spatial location of the top fraction of the landscape between the solutions that take into account only the biodiversity features and those that include human occupancy and fragmentation constraints. In the latter solution the Mediterranean territory is privileged in detriment of the Atlantic territory that would be selected if only biodiversity features were taken into account. This translates into losses in the protected distributions of the Atlantic communities that are less represented at the national level and include the most important taxa for conservation.

This work demonstrated that constraining conservation of fluvial bryophytic communities solely to protected areas might not necessarily be an effective strategy. In the study area the protection of fluvial bryophyte diversity depends greatly on the conservation of atlantic streams and headwaters that are for the most part, but not only, included in protected areas. However, protected areas in the study area correspond to a top fraction of the landscape that suffers from the effects of human occupation and fragmentation elements, and their management should be more thoroughly planned, especially in the cases where the human needs (eg. power facilities or road construction) interfere with fluvial conservation.

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4. Concluding Remarks

Our results put emphasis on the necessity of studying the cumulative effects of regional fragmentation elements and land use on biodiversity and riverine ecosystems. Future studies should focus on better understanding the joint effect of land use and fragmentation elements on biodiversity and the implications for riverscapes diversity and connectivity.

Furthermore it becomes evident the need to evaluate the efficacy of existing protected areas for the protection of freshwater biodiversity which is rapidly declining worldwide. In this context, it becomes apparent the need to develop regional management strategies oriented for the conservation of freshwater biodiversity and to continue the application of systematic conservation planning to freshwater biodiversity acknowledging the specificities of the habitat.

Important management strategies for the conservation of fluvial bryophyte flora ought to include the protection of headwaters, the idealization of microreserves, and the overall conservation of river ecosystems integrity.