An integrated approach to support a river ecological network: A case study from the Mediterranean

Land Use Policy

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Abstract:	Riverine ecosystems are among the most impacted ecosystems worldwide since they are exposed to multiple stressors. Land Use/Land Cover (LULC) changes is the main human imprint on those ecosystems whose spatiotemporal habitat destructions pose a threat to biodiversity, ecosystem integrity and ecological processes. The most important statutory instruments for riverine ecosystem protection, conservation and restoration in the European Union are the Water Framework Directive (WFD) and the Habitats and Birds Directive (HBDs). In this study, we develop a methodological framework to spatially link the ecological integrity of river sub-basins with the protected areas therein, taking into account the influence of land use as expressed in the WFD. We combined a multi-criteria evaluation approach using six of the most frequently applied criteria for conservation evaluation to assess ecological integrity index (S) at the sub-basin level, and used as a proxy for functional connectivity categories. In addition, we used the distance of every sub-basin from the surrounding Natura 2000 sites as a measure of structural connectivity. Using ecological network design principles (i.e. Core areas; Corridors; Stepping Stones; Buffer areas; and Restoration areas), we incorporated the two aspects of connectivity into a framework, which links river management at the basin level with the site level assessment as dictated by the HBDs. We implemented this framework in a Mediterranean river basin located in Southern Tuscany which is part of the Natura 2000 network. Six of the sub-basins (20%) have high functional connectivity, 14 sub-basins (47%) medium and 10 sub-basins (33%) low functional connectivity. Structural connectivity of the study area followed the same tendency as that of functional connectivity, with the majority of the sub-basins having medium connectivity (57%; 17 sub-basins), and 23% (7 sub-basins) and 20% (6 sub-basins) high and low structural connectivity respectively. As a result six of the sub-basins were characterised as corridor areas while the majority of the sub-basins were identified as buffer areas (57%). Two sub-basins were characterised restoration areas and one as stepping stone (SS). Our approach is one of many plausible ecological networks which although analytically simple, can be enriched with data on species and stakeholders' involvement.
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Editor and Reviewer comments:

Dear Editor

We would like to thank the reviewers for their constructive feedback. We have now addressed all comment by the reviewers. We believe that with the reviewers help this has resulted in a much improved manuscript. Please see our reply to the reviewers, point by point below in blue. In addition significant changes are shown in red in the revised text.

Looking forward to hearing from you.

the authors

Reviewer #1:

General comments

Dear authors,

I regret to have the opinion that your work is not suitable for publication in Land Use Policy, on the current form.

We would like to thank the reviewer for her/his constructive criticisms. We have attempted to address all her/his concerns point by point below.

- The main reasons **relate to the approach of the paper**, **which does not integrate all necessary components for the prioritization of restoration of conservation areas**.

Perhaps our approach has led to confusion and misinterpretation. Prioritization since the early days of Ratcliff's *Nature Conservation review* (1977) to *Systematic Conservation Planning* (Margules and Pressey 2000) includes many diverse criteria and many techniques to pursuit them (from simple scoring, Analytic Hierarchical Process, to Marxan/Zonation). The output always dependent on the choice of criteria and methods.

In order to avoid this confusion, we have refocused on ecological integrity (EI) rather than conservation evaluation and the use of ecological connectivity as a means to ensure EI and increase resilience at the basin level. Ecological Connectivity is explicitly linked with EI while clarifying the ecological network proposed through the use of connectivity was requested by the 3rd reviewer. We used the same parameters as in the initial submission which are among the most widely used for assessing ecological integrity (EI). Therefore, with a parsimonious number of widely employed criteria, without recourse to complex and computer intensive methodologies, we provide a simple approach far from technical complexities which often deter planner and policy makers.

We have also changed the title to reflect these changes.

- It **lacks the relevance of the spatio-temporal scales, as it is static in time** (does not incorporate **climate change issues for instance**) and **broader in spatial scale**, without taking into consideration **local issues**.

Climate change was beyond the scope of the paper which does not make our approach less valid [please see a detailed response also below]. In addition, the scale of the study was set at the river basin level since our idea was to link processes at the wider scale to changes in the conservation status of the N2K sites as well as the ecological status of the river ecosystem as suggested by the two Directives (WFD and HBDs). The wider applicability/uptake of this methodology is already discussed in the discussion section.

- I find the approach **risky**, as it can prioritize areas to restore without changes across time in conservation areas and goals. For instance, climate change issues are not included as well as dynamics or scenarios of LULC change. In addition, there is a need to better incorporate a larger perspective of the integration of the EU water and conservation legislation and other legal acts, recommendations, and transnational frameworks. This is important for Land Use Policy readership.

We appreciate the reviewer's concern. Any type of approach which cannot rely on evidence but projections entail an element of uncertainty. Any "prediction" of socio-economic or environmental/climatic futures, needs to appraise notions of connectivity, such as human–landscape interactions, or interactions within the landscape itself. In that sense what we attempt herein is to highlight the importance of catchment connectivity as a key aspect in environmental planning and management.

Although directly the paper does not deal with climate change, recognizing larger connected areas (parts of an ecological network) is one of the ways to create climate wise connectivity networks (senso Keeley et al. 2018 Env. Res. Letters) even if climate change is not explicitly included. Such approaches are no less valid and have been employed many times for land use planning (e.g. Jongman et al. 2011; Landscape Ecology; Vos et al. 2010 Landscape Ecology, Morecroft et al. 2010 Journal of Applied Ecology).

We agree with the reviewer and are well aware about the importance of temporal aspects in such a framework. Therefore, prior to submission we had analysed LULC changes using the Corine land cover dataset, the *only dataset available* which gives in a consistent manner (thematically and spatially) a complete picture about the area over time. We found that the area remained remarkably stable over the past 12 years [please see enclosed Tables at the end of this letter]. Had the LULC changes been significant over time this could have been incorporated under the criterion threat expressed as land take from semi-natural to urban uses. However, this is not the case since there is limited anthropogenic impact in the area. The inclusion of LULC dynamics is now discussed in the revised manuscript when commenting on the potential of the framework's application elsewhere.

We are well aware and we can think of several policy documents which are relevant to our work e.g. the EU flood and Soil Directives, the Biodiversity Strategy to 2030 and the revised CAP (in particular Green architecture). However, one of the objectives was to develop and apply a methodological framework to spatially link the WFD with HBDs which is a distinct gap hampering integrated conservation in riverscapes. We now refer to possible synergies with other policies in the discussion section.

- The structure of the paper **resembles a technical report** and **not a research article**, we can notice that there is an application of an approach to solving a problem at a broad scale, but do not have a specific research objective. This has to be better thought.

Following the reviewer's comments, we have revised the aim and objectives and restructured the paper to reflect a research article as pointed out by the reviewer. The latter includes a restructure of the methodology including a section on Framework development, an additional analytical step on connectivity and a complete rewrite of the discussion section.

- Finally, you have included more **than one hundred and twenty references**, this is too much for a standard article, and especially because many of the references do not completely make their work, that is, many references are not adapted to communicate the state-of-art or to support the discussion.

This was an oversight which has been now rectified in the revised manuscript to reflect in an accurate way the state of art and back up our own results.

- For the improvement of your work, the references list has to be more directed to the main goal of the paper, and also updated for a better contextualization of the state-of-art.

We have now corrected the list as instructed.

Other:

- some figure captions cannot be read independently, for instance, Figure 2 do not include the explanation of acronyms, Figure 3 does not include the designation of basins (at least it should be informed where we can find the designations), the same for F,T, R,N, D SP,S...

Figures have now been improved and changed in the revised MS as suggested. Figure 2 caption now explains the acronyms. We assume that the reviewer refers to Figure 4 in the submitted manuscript. In any case we have now replaced Figure 4 following the additional methodological step as a result of comments by Reviewer 3.

- there is a need for revision of references for standardization and typos across the text

We have standardized the references throughout the text.

- usage of the English language could be improved

The manuscript has been now reviewed for language by a native speaker

- Figure 2 is not informative

We have revised Figure 2 providing the acronyms as requested in a previous comment above by the reviewer.

- line 50: aquatic ecosystem ecological status is a wordy expression, change to aquatic ecological status

We have revised as requested

- line 81 - recent review (2014)... remove recent

We have revised as requested

- legislation has to be included on references (even when they are largely known, as readers are not only from Europe)

We have now included relevant references as requested

I encourage the improvement of your work, especially by incorporating more local criteria, better validation and discussing the interconnection with other legislative or EU and other policy guidelines.

We are not sure about what the reviewer means by local criteria since the context of all the criteria used were informed by a) local reality and datasets b) evaluated by, among others, local experts. In that sense we ensure that the methodology is repeatable elsewhere. Validation of such a framework can be carried out in a different geographical context and this is certainly a future step of this work.

As we have mentioned above in a similar comment we are well aware of other policy instruments but we think that integrating the Habitats and Birds Directive together with the Water Framework Directive remains a challenge for large scale conservation in riverscapes which we have tried to address. We now refer to possible synergies with other policies in the discussion section.

We hope we have addressed adequate your concerns and comments. Thank you for your suggestions.

Reviewer #2:

The manuscript is well prepared and interesting for many researchers worldwide. The methods and data are appropriate, however many different approaches are also available for such studies. I think that the manuscript can be published in the Land Use Policy journal.

We would like to thank the reviewer for his encouraging comments.

Reviewer #3

This study provided a methodology to link spatially the conservation value of a river catchment with the protected areas therein, which takes into account the influence of land cover as expressed in the WFD. As the author says, it is analytically simple but can be enriched with data on species and stakeholders' involvement.

However, as far as I know, **the corridors of the ecological network should involve flow, process, or connectivity**. It is not sufficient to rely solely on conservation value and whether it overlaps with existing protected areas as the basis for the design of each component of the ecological network. The study provided a simple way of evaluating and comparing conservation significance, but the technical details and method system still need to be considered.

We agree with the reviewer and have now included a connectivity analysis using both aspects of connectivity (functional and structural) which allows the quantification of the elements of ecological network.

1. As mentioned in the study, land Use/Land Cover (LULC) changes is a reflection of human activities on riverine ecosystems and its importance in the assessment of ecological conservation areas. However, only **one period of static land cover ecological conservation value evaluation in the study is not enough to explain the role of natural and human activities in this evaluation**. It should be based on **dynamic land use assessment**. As far as I know, there are some such studies involving historical evolution.

Current status is the reflection of previous land use practices in the area since the effect of changes in addition to an immediate effect are also demonstrated as time lagged responses (e.g. Ziter et al. 2017 Landscape Ecology; Lindborg and Eriksson 2004 Ecography). We had actually analysed LULC changes prior to submission using the Corine land cover dataset, the *only dataset available* which gives in a consistent manner (thematically and spatially) a complete picture about the area over time. We found that the area remained remarkably stable over the past 12 years [please see enclosed Tables at the end of this letter]. Had the LULC changes been significant over time this could have been incorporated under the criterion threat expressed as land take from semi-natural to urban uses. However, this is not the case since there is limited anthropogenic impact in the area. The inclusion of LULC dynamics is now discussed in the revised manuscript when commenting on the potential of the framework's application elsewhere.

2. The meaning of each aspect （LULC composition, impact and statutory protection, it is recommended to be clear.

LULC composition refers to the naturalness, diversity and richness of land cover types within sub-basins. Impact on threat and fragmentation while statutory protection refers to the designation of Natura 2000 sites. We have revised the manuscript and clarified these aspects in the methods section.

3. It is necessary to clarify what an ecological network is (I saw the concept adopted by the author in the last paragraph of the discussion of the paper, but even so, I don't think that the division of authors can be called an ecological network). In addition, the role played by each sub-basin in the ecological network and the basis for the classification of various types are not clearly stated. At the same time, it is recommended to intuitively reflect the various components of the ecological network.

We now clarify it. In addition given the new piece of analysis we are now confident that it complies with the terminology used for ecological networks. Every term is now explained in the Methodology section and subsequently is shown in the Results section (and map).

4. The meaning expressed in Line 321 is not shown in Figure 2; The legend of Figure 4 does not have the description of R; The watershed boundary in Figure 4 is recommended to be redrawn, and the map design is not friendly enough, especially for the classification standard set by the user, the ecological network.

We have revised these figures to make the clearer for the reader as requested. Figure 4 is now revised following the new piece of analysis.

The result is simple and the discussion part seems lengthy.

We have now shortened the discussion as requested.

FIORA RIVER BASIN LULC Changes from 2006-2012											
	2012					2006					
Sub-basin ID	% of Natura 2000 in sub-basin	Area of sub-basin (ha)	CLC 2012	Area of CLC 2012 per sub-basin (ha)	% CLC 2012 per sub-basin	CLC 2006	% of Natura 2000 in sub-basin	Area of sub-basin (ha)	Area of CLC 2006 per sub-basin (ha)	% CLC 2006 sub-basin	change in % of CLC coverage per sub-basin
3	40,3	3232,92	243	585,53	18,1	243	40,3	3232,9	590,5	18	0,153
3	40,3	3232,92	311	1503,52	46,5	311	40,3	3232,9	1498,6	46	-0,154
3	40,3	3232,92	321	74,79	2,3	321	40,3	3232,9	166,0	5	2,822
3	40,3	3232,92	333	91,23	2,8	333		3232,9	0,0	0	-2,822
4	50,3	864,26	231	164,80	19,1	231	50,3	864,3	174,7	20	1,144
4	50,3	864,26	311	543,26	62,9	311	50,3	864,3	533,4	62	-1,144
6	18,0	1270,57	311	555,08	43,7	311	18,0	1270,6	561,4	44	0,500
6	18,0	1270,57	324	45,02	3,5	321	18,0	1270,6	1,8	0	-3,399
6	18,0	1270,57	331	1,27	0,1	324	18,0	1270,6	38,7	3	2,943
6	18,0	1270,57	333	1,83	0,1	331	18,0	1270,6	1,3	0	-0,044
7	45,0	1055,57	211	541,79	51,3	211	45,0	1055,6	548,7	52	0,655
7	45,0	1055,57	331	49,42	4,7	331	45,0	1055,6	42,5	4	-0,656
10	7,4	1942,87	242	317,84	16,4	242	7,4	1942,9	311,9	16	-0,304
10	7,4	1942,87	311	696,26	35,8	311	7,4	1942,9	702,2	36	0,304
10		1942,87	321	0,00	0,0	321	7,4	1942,9	81,6	4	4,199
10	7,4	1942,87	333	81,58	4,2	333		1942,9	0,0	0	-4,199
12		2261,20	242	423,68	18,7	242		2261,2	415,8	18	-0,350
12		2261,20	311	1106,80	48,9	311		2261,2	1114,7	49	0,350
13		1084,24	242	165,52	15,3	242		1084,2	160,5	15	-0,466
13		1084,24	311	289,39	26,7	311		1084,2	294,4	27	0,466

14		85,20	242	42,35	49,7	242		85,2	42,4	50	-0,001
14		85,20	324	3,34	3,9	324		85,2	3,3	4	0,001
22	66,0	2111,18	211	85,75	4,1	211	66,0	2111,2	85,7	4	-0,001
22	66,0	2111,18	321	57,36	2,7	321	66,0	2111,2	125,4	6	3,221
22	66,0	2111,18	333	68,01	3,2	333		2111,2	0,0	0	-3,221
25	31,1	895,16	243	128,57	14,4	243	31,1	895,2	130,4	15	0,200
25	31,1	895,16	311	464,58	51,9	311	31,1	895,2	462,8	52	-0,200

FIORA RIVER BASIN LULC Changes from 2012-2018											
Sub-basin ID	2018						2012				
	Area of sub-basin (m^2)	% Natura 2000	CLC 2018	Area of CLC 2018 (m^2)	% of CLC 2018 in sub-basin	% Natura 2000	CLC 2012	Area of CLC 2012 (m^2)	% of CLC 2012 in sub-basin	change in area (m^2)	change in area (%)
26	1411027,3	24,5	211	426544,9	30,23	24,5	211	348201,22	24,68	-78343,7	-5,6

Highlights

An integrated framework can link spatially the WFD with HBDs

Ecological network design may build resilience at the river basin level

Resilient riverscapes increase coherence of protected areas network

HBDs and WFD may act complementary on river basin management

An integrated approach to support a river ecological network: a case study from the Mediterranean

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An integrated approach to support a river ecological network: a case study from the Mediterranean

Abstract

Riverine ecosystems are among the most impacted ecosystems worldwide since they are exposed to multiple stressors. Land Use/Land Cover (LULC) changes is the main human imprint on those ecosystems whose spatiotemporal habitat destructions pose a threat to biodiversity, ecosystem integrity and ecological processes. The most important statutory instruments for riverine ecosystem protection, conservation and restoration in the European Union are the Water Framework Directive (WFD) and the Habitats and Birds Directive (HBDs). In this study, we develop a methodological framework to spatially link the ecological integrity of river sub-basins with the protected areas therein, taking into account the influence of land use as expressed in the WFD.

We combined a multi-criteria evaluation approach using six of the most frequently applied criteria for conservation evaluation to assess ecological integrity index (S) at the sub-basin level, and used as a proxy for functional connectivity categories. In addition, we used the distance of every sub-basin from the surrounding Natura 2000 sites as a measure of structural connectivity. Using ecological network design principles (i.e. Core areas; Corridors; Stepping Stones; Buffer areas; and Restoration areas), we incorporated the two aspects of connectivity into a framework, which links river management at the basin level with the site level assessment as dictated by the HBDs. We implemented this framework in a Mediterranean river basin located in Southern Tuscany which is part of the Natura 2000 network.

Six of the sub-basins (20%) have high functional connectivity, 14 sub-basins (47%) medium and 10 sub-basins (33%) low functional connectivity. Structural connectivity of the study area followed the same tendency as that of functional connectivity, with the majority of the sub-basins having medium connectivity (57%; 17 sub-basins), and 23% (7 sub-basins) and 20% (6 sub-basins) high and low structural connectivity respectively. As a result six of the sub-basins were characterised as corridor areas while the majority of the sub-basins were identified as buffer areas (57%). Two sub-basins were characterised restoration areas and one as stepping stone (SS). Our approach is one of many plausible ecological networks which although analytically simple, can be enriched with data on species and stakeholders' involvement.

Keywords: connectivity, ecological network, ecological integrity, environmental policy integration, Habitats and Birds Directive, Multi-Criteria Evaluation, Natura 2000, Water Framework Directive

Introduction

Riverine ecosystems are among the most fragmented, degraded and threatened ecosystems in the world (Millennium Ecosystem Assessment, 2005; Tockner et al., 2010), subjected to multiple impacts at a range of spatial scales from catchment to instream level (Boon, 2000). Human activities have changed river ecosystem processes, biotic composition, and structure, making the conservation, management and monitoring of the riverine ecosystem condition challenging (Stella et al., 2013). Maintaining riverine ecosystem integrity is essential in supporting human societal existence through the provided services (Arthington et al., 2010). Therefore, recognizing the urgent need to mitigate the intensive anthropogenic pressure that riverine ecosystems have faced in the past decades, has prompted worldwide initiatives and actions for their preservation and management (Bernhardt et al., 2005; Bernhardt and Palmer, 2011). At the European level, this effort led to the adoption of an innovative strategy in the sector of water management and policy, the EU Water Framework Directive (WFD), in which the approach to water management has radically changed (Directive 2000/60/EC). WFD targets the evaluation of the ecosystem condition and introduces the term *good ecological status* as a measure of ecosystem integrity and biodiversity (Grizzetti et al. 2019). Also, under the WFD a river basin management plan is required for each River Basin District (RBD) recognizing thus, the strong influence of land use/land cover (LULC) properties in the catchment area.

Therefore, aquatic ecological status and integrity has been holistically monitored taking into account the remote, often cumulative, impacts within the river basin area (hydrological alterations, fragmentation, land-use, etc.) that affect the biotic communities and ecosystem processes and functions. Hence, approaches that facilitate multiple scale assessment, the implementation of broad-scale ecosystem health information (ecological integrity) and the development of indicators of resource distribution, status, and condition (i.e. Faber-Langendoen et al., 2019; Walston and Hartmann, 2018) for natural ecosystems management are continuously developed (Reza and Abdullah, 2011). During these efforts, adopting landscape ecology principles provide a means for practical approximation of the ecological integrity of streams on a large scale and important insights to the study of riverine ecosystems (Wiens, 2002).

In addition to the WFD, river conservation in the European Union (EU), has also benefited from the Habitats and Birds Directives (HBDs) (Council of the European Communities 1992; European Parliament and Council of the European Communities 2009). Both WFD and HBDs contribute directly or indirectly to biodiversity conservation, the first by following a multi-species approach (biological quality elements, BQEs) and the second through species-focused approaches for the protection of endangered species or habitats. The implementation of HBDs resulted in perhaps the largest network of protected areas in the world, the 'Natura 2000' network (N2K; Evans 2012). Many of these sites can be found in rivers, usually occupying a relatively small area of the riparian zone and river bed. The linear form of rivers means that they traverse long distances and large areas, and are subject to many impacts which usually originate at the catchment level and are "carried" along longitudinally but also laterally. Therefore, sites which are part of the rivers are disproportionately affected by these impacts compared to other sites comprising purely terrestrial habitats.

In Italy, N2K sites in rivers include all the 26 freshwater habitats listed in Annex I, which are among the most threatened both at national and European level (Gigante et al., 2018; Janssen et al., 2016) while in some regions like Tuscany, rivers represent more than 10% of the total Special Areas of Conservation[1]. It is clear, therefore, that the diversity of rivers and associated species cannot be adequately represented in the list of protected areas currently selected under the HBDs (Boon and Lee, 2005). The function of rivers as biodiversity corridors, providing connections between protected areas (Fremier et al., 2015) and buffering capacity for climate change (Beier, 2012) is well documented, and has shaped EU policy regarding green (and blue) infrastructure (EEA 2014; Jongman et al., 2011). A review by Palmer et al. (2014) concluded that projects implementing actions in the river basin level were few compared to reach scale projects, despite the fact that catchment-scale actions known to lead to significant improvements in the ecological health. In addition, the difficulty of trying to squeeze rivers into the traditional mold of terrestrial site protection has long been recognized, because legal designations rarely extend to entire catchments and often stop at the top of the river bank (Nel et al., 2007).

Similarly, and although the WFD has much to offer to river habitat conservation, in some cases its aims are not completely congruent with the exclusively ecological and/or conservational focus (Janauer et al., 2015). This is because it is not a nature conservation directive per se, and the

[1] http://www.regione.toscana.it/-/la-carta-degli-habitat-nei-siti-natura-2000-toscani

concept of ecological status enshrined within the WFD is not synonymous with conservation status in HBDs (Boon and Lee, 2005). A departure from naturalness, which lies at the heart of the HBDs and assessed in terms of reference conditions, is an important component (perhaps the most important component) of conservation status but it is not the only one (Ratcliffe, 1977; Boon, 2000; Dunn, 2004). Enhancing ecological coherence in the ground should be underpinned by coherence of directives at a policy level. WFD and HBDs are perhaps two of the best examples where this is urgently needed (Weigelhofer et al., 2020). While both the HBDs and the WFD have an invaluable role to play in furthering river conservation in Europe, this role remains limited. Assessing the conservation value of rivers only to meet the requirements of EU legislation will lead to either the protection of part of the catchment area or to the management of the broader river basin area but without the provision of conservation purpose. There is a need to rethink nature conservation at the river basin level taking into account both habitat conservation and water sustainable management as a way of securing the provision of all river-related ecosystem services. In that respect, the role of protected areas in future strategies for river conservation is a topic ripe for debate and action.

In addition to differences in the existing paradigms, rivers conservation is often hampered by the lack of data at a detailed habitat level which requires the development of different approaches in evaluating nature conservation value. In the case of rivers there is a threefold significance: 1) Processes in the river basin takes places at the watershed scale; 2) The extent of protected areas, as well as Natura 2000 sites, often includes only the bed, banks and riverside space of reaches of rivers and streams, while -river basin management maintenance are indispensable strategies for riverscape biodiversity conservation (Abellán et al., 2007); 3) To ensure the long-term future of N2K sites across Europe, effective techniques are required for evaluating and monitoring their conservation significance taking into account indicators/factors both inside and outside protected areas (Gigante et al., 2016; Vogiatzakis et al., 2015).

This paper aims to determine the role of individual sub-basins within a wider ecological network in a basin of the River Fiora and identify the potential conservation synergies with the current configuration of the surrounding N2K areas. More specifically, the research objectives are to 1) develop and apply a methodological framework to spatially link the WFD with HBDs 2) to account for structural and functional connectivity in the river basin level to identify areas which act complementary to the existing N2K sites in order to support river basin planning and management in the long term.

Materials and methods

Framework Development

Using landscape ecology principles, the framework links LULC manifestations and the composition of the matrix (river basin), as expressed in the WFD, to existing designations which receive statutory protection as part of the HBDs (Figure 1). The steps included:

a. the selection of indicators/criteria to assess LULC manifestations and the composition of the matrix within sub-basins (a proxy for functional connectivity);
b. evaluation of the position of every sub-basin with respect to every Natura 2000 site in the basin and surrounding area (structural connectivity);
c. evaluation of the overall connectivity as expressed by step a) and b);
d. identification of ecological network elements in the basin which can act in synergy with the existing N2K sites within and beyond the basin.

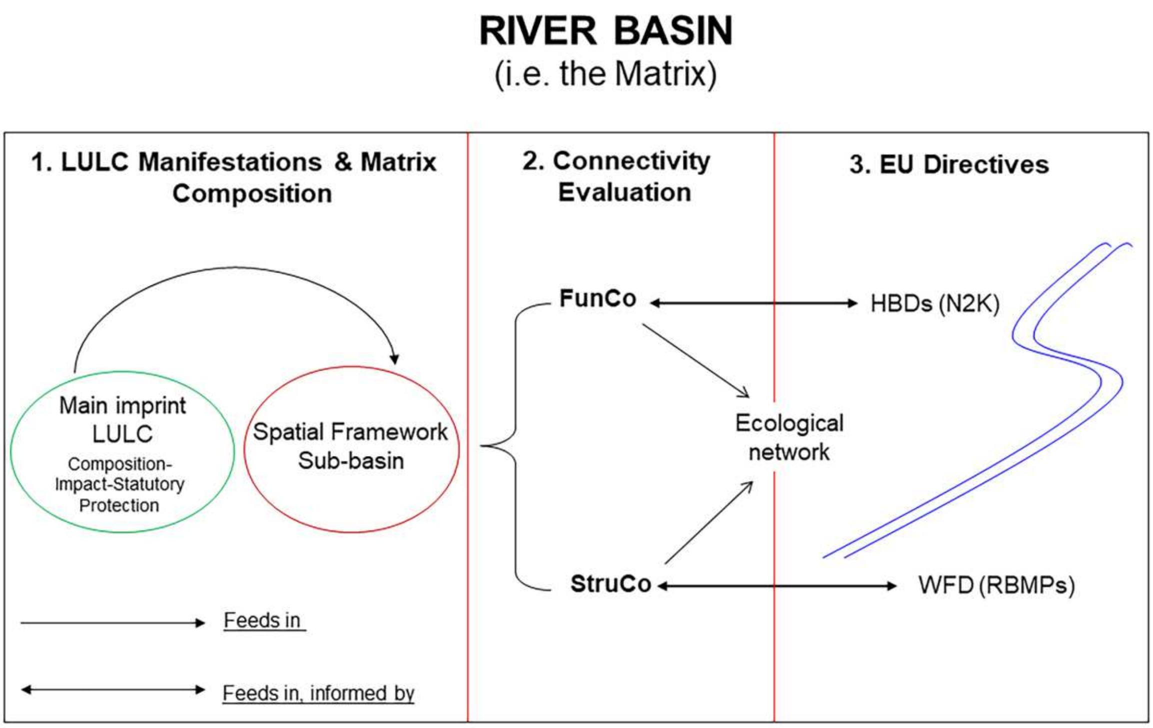

Figure 1. Schematic diagram of the integrated approach for river conservation assessment. LULC: Land Use/Land Cover changes; FunCo: Functional Connectivity; StrucCo: Structural Connectivity; HBDs: Habitats and Birds Directive; WFD: Water Framework Directive; N2K: Natura 2000 sites; RBMPs: River Basin Management Plans.

Study Area

The approach was tested in a Mediterranean river basin located in Southern Tuscany, the Fiora River basin (42°34' N, 11°34'E and 42°49'N, 11°35'E) (Figure 2). The study was carried out only in the Tuscan part of the Fiora river basin, since this area is protected under the HBDs through its designation as a SAC "Alto Corso del Fiume Fiora" (IT51A001). The area was selected because it features a wide range of land cover types, including small villages and two small towns (Santa Fiora and Pitigliano). The altitude range of the studied part of the river is from 687 m to 130 m above sea level, with a catchment area of c.423 Km^2.

The geology consists of Cretaceous and Miocene shales, sandstones and cobbles in the upper part, while of Quaternary effusive and pyroclastic rocks in the lower part. Within the valley floors, apart from the bedrock reaches, there are extensive drifts of fluvial alluvium (Carmignani et al., 2004). In the Tuscan part of the Fiora River it is possible to identify four fluvial types (Angiolini et al., 2011): the "headwater" in the upper part, the "low sinuosity", in the medium part, the "braided" in the lower part, the "incised" in the last stretch studied. The climate is humid Mediterranean with total annual rainfall ranging from 903 mm to 1003 mm and a mean temperature range of 11.2 to 14.5 °C. The river dries up almost totally during summer, while in autumn and spring it is affected by flood events. The catchment area is prevalently occupied by woodlands, including beech forests with *Abies alba*, deciduous forests dominated by *Quercus cerris* or mixed deciduous oak woods with a prevalence of *Q. cerris* and *Q. pubescens,* together with scattered pasture, crops (mostly cereal and fodder crops), and uncultivated areas (for a detailed description see Angiolini et al., 2011). Since low intensity agro-pastoral systems dominate, natural vegetation has been retained in the riparian zone with minimal human impact

Spatial unit delineation and indices derivation

Sub-basins within the larger Fiora River basin, were used to provide spatial units for conservation evaluation. Watershed layer was derived from a Digital Elevation Model (DEM, 75m × 75m resolution), using ArcGIS 9.2 (ESRI) and Spatial Analyst tools that allow for automated delineation of nested basins, applying a series of ''hydrologic enforcement'' algorithms that conditioned the elevation data to enable improved calculations of flow directions and river topology. Delineation resulted in 30 sub-basins (Figure 2). Delineation was not possible for a small part in the south of the basin. Here the river was subjected to considerable lateral migrations during the last 10 years, and the riverbed is located outside the boundary of the protected area while in the final narrowest part, the river flows inside a canyon (see Figure 2). The analyses were based on information derived from the DEM, the CORINE Land Cover (CLC) dataset at the 5th level (CPSG-CISIS, 2012). Roads network and urban settlements layers were also taken from CORINE (Bossard et al., 2000).

Evaluation of ecological integrity: the criteria

We used some of the most frequently applied criteria for ecological integrity assessment (Manolaki et al., 2020; Walston and Hartmann, 2018; McGarigal et al., 2018) evaluation including naturalness, diversity, richness, human interference and the percentage of protected areas within every sub-basin (Table 1). In addition, these criteria represent the manifestation of three major components of LULC imprints (composition, impact and statutory protection). Naturalness, diversity and richness are expressions of LULC composition within sub-basins, threat and fragmentation an expression of impact while statutory protection refers to the designation of N2K sites. LULC data were based on the 5th level of CLC available for Tuscany region at a scale of 1:250 000 and minimum mapping unit of 50ha (CPSG-CISIS, 2012). The criteria were assessed directly or by means of specific indicators, generating maps at the sub-basin level as follows:

1. <u>Naturalness</u>: was calculated based on the percentage (%) coverage of the semi-natural CLC classes. We used the CLC classes at the 5th level Natural woodlands (i.e. all classes under code 3, reforestation areas excluded), grasslands, pastures (codes 231, 321), garrigues (code 323) were given the highest naturalness values, while urban areas and crops were given the lowest. The final score of naturalness was then divided by the area of the sub-basin. The higher the naturalness value the more semi-natural land cover the sub-basin.
2. <u>Diversity</u>: With the diversity criterion we consider the number of different land cover classes found within a sub-basin, expressed with the Evenness index that quantifies how even the distribution of patch types in a land mosaic is. The index was calculated for each sub-basin with V-Late tool (Lang and Tiede, 2013).
3. <u>Richness</u>: Patch Richness Density (PRD) was used, since it standardizes richness to a per area basis that facilitates comparison among landscapes (McGarigal and Marks, 1995). PRD was calculated as the number of different patch types present within the landscape boundary divided by total landscape area in hectares. The higher was the value of this metric for a sub-basin, the higher the final score.
4. <u>Threat</u>: This criterion relates to the direct exposure to human disturbance caused by roads and settlements. The degree of human impact was evaluated as the percentage of cover of roads and urban settlements within the sub-basin. This was calculated taking into account the real area occupied by roads and settlements, plus a buffer zone of 100m, considered as "influence zone". The lower the percentage cover of roads and settlements, the higher the score assigned to the sub-basin.
5. <u>Fragmentation</u>: Landscape fragmentation due to infrastructure and urban sprawl has major environmental impacts (Forman et al., 2003; Girvetz et al., 2008). In the study area, road

network is the main responsible for fragmentation. For this reason, we used regional and provincial roads (two lane roads), to quantify fragmentation employing the Effective Mesh Size (Jaeger, 2000). The higher the value of the metric the more intact (not fragmented) is a landscape.

Statutory Protection: Due to the ecological importance of Fiora, a large part of the river basin is a SAC at the EU level (N2K site), therefore we calculated what percentage of each sub-basin was covered by the SAC, with higher values representing higher importance.

Calculation of scores

For each criterion a numerical value was assigned to each of the 30 mapped polygons to generate one map for every criterion employed within a GIS. We employed a common scale for every criterion (Vi) ranging from a minimum value of 0 to a maximum value of 1, using the following formula (Equation 1)

$$V_j=(Ci_x-Ci_{min})/(Ci_{max}-Ci_{min}) \quad \text{(Eq.1)}$$

where, Vi is the rescaled value for each criterion sub-basin j, Ci_x is the value X of criterion i calculated as described in Table Annex 1; Ci_{min} is the minimum value observed for criterion i along sub-basins; and Ci_{max} is the maximum value observed for criterion i along sub-basins.

The rescaled value (Vi) of each criterion was then multiplied by the specific weight of each criterion (Table 1) which represents their importance for conservation. The weight of each criterion was derived using experts' opinion. We asked 14 experts from 5 Mediterranean countries namely Italy, France, Greece, Portugal, Cyprus to evaluate the importance of each criterion chosen for this study, giving a numerical value from 1 to 5 (low to high) in a scale of increasing importance for the conservation significance evaluation. Experts were selected on the basis of track record and experience in Mediterranean river conservation, and included practitioners from relevant Government Agencies, academics and researchers. For each criterion we calculated mean and standard error of the values given by experts (responses for every criterion are presented in Table 1 with Mean, S.E., and weight). Weights were calculated on the basis of a standardization. For each criterion we calculated the sum of all the scores assigned by the experts and then divided by the sum of all the scores assigned by all the experts for all the criteria considered (weights values ranged from 0 to 1). We then multiplied every criterion map with the respective weight.

Table 1. Criteria employed and their weights

Criteria	Mean	S.E.	Weight
Naturalness	4.24	0.19	0.216
Diversity	4.03	0.30	0.163
Richness	3.36	0.26	0.154
Threat	3.41	0.39	0.157
Fragmentation	3.43	0.38	0.144
Statutory protection	3.86	0.31	0.166

Ecological Integrity index

Ecological Integrity index (S) for each sub-basin was calculated by summing up the weighed score of each criterion. For each sub-basin the final score was generated using the following equation (Eq. 2):

$$S = \sum Wi * Xi \qquad \text{(Eq. 2)}$$

Where S is the score, W_i is the weight factor for criterion i and X_i is the criterion score of factor i. We then used S index to generate a final map of conservation value for every sub-basin (Fig 3).

Ecological Network Design

We set to identify an ecological network at the basin scale as proposed by Bennett (2004) "*a coherent system of natural and/or semi/natural landscape elements that is configured and managed with the objective of maintaining or restoring ecological functions as a means to conserve biodiversity while also providing appropriate opportunities for the sustainable use of natural resources*".

In order to achieve this, ecological integrity index (S) was used herein as a proxy for functional connectivity split into three categories using *Jenks Natural breaks* in ArcGIS. In addition, the distance of every sub-basin from the surrounding N2K sites, those neighbouring the Fiora basin, was calculated in ArcGIS in order to measure structural connectivity. Three classes of structural connectivity were assigned as follows: *high* structural connectivity to basins connecting two or more N2K sites, *medium/moderate* structural connectivity to those traversing or adjacent to one N2K site and *none* to those with no obvious connections.

We used these two aspects of connectivity (structural and functional) to characterise sub-basins into one of the widely accepted constituent elements of ecological networks (Boitani et al., 2007) as shown below:

1) Core areas: these include all N2K sites which are either inside or bordering the basin (Figure 2);
2) Corridors: sub-basins of high functional and structural connectivity which connect two or more N2K sites;
3) Stepping Stones: smaller sub-basins of high functional connectivity that are intended to aid movement of individuals in the matrix;
4) Buffer areas: sub-basins of moderate to high functional connectivity which traverse the N2K sites;
5) Restoration areas: degraded sub-basins where management should focus on restoration actions and which can act as corridors or buffers in the long term.

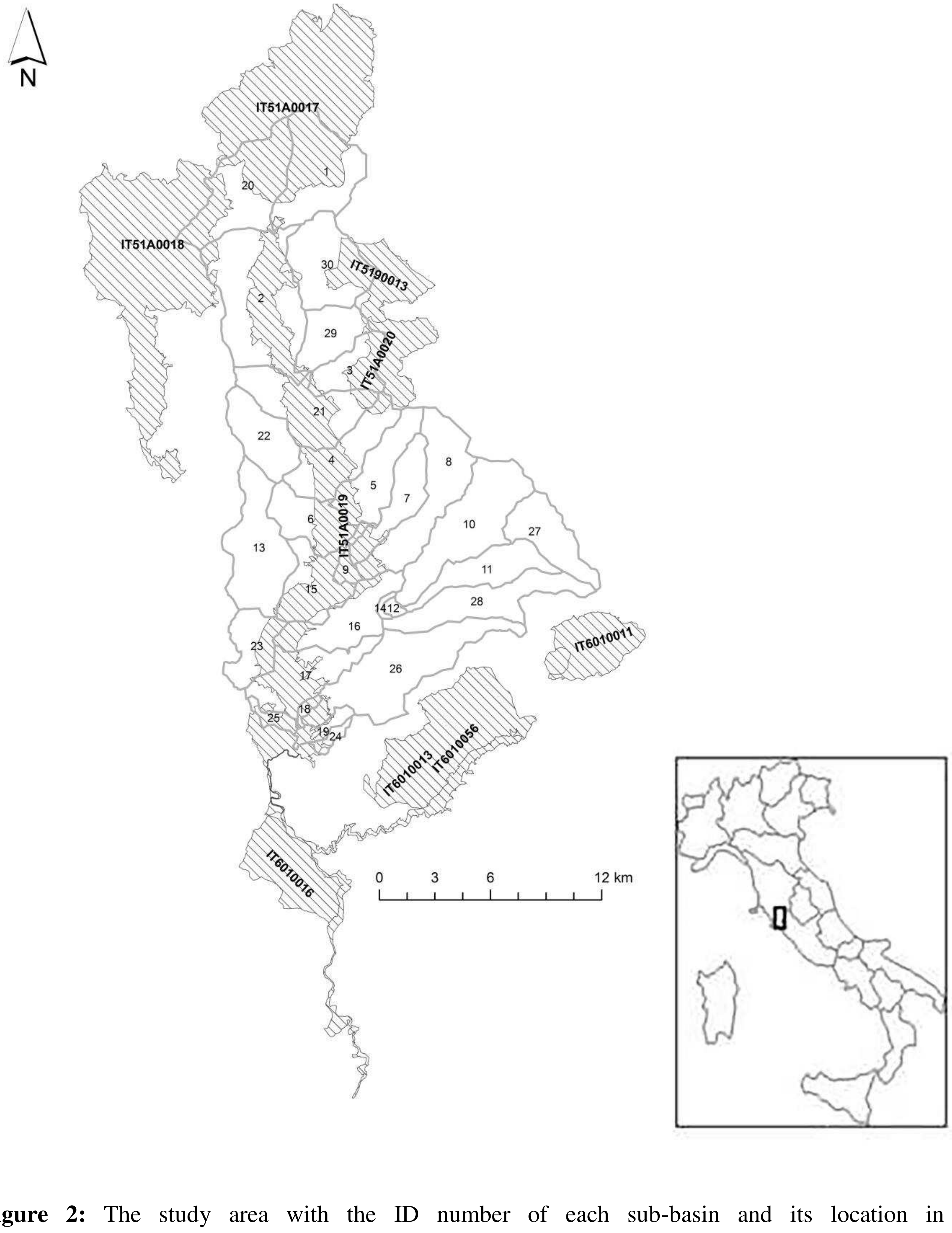

Figure 2: The study area with the ID number of each sub-basin and its location in Italy(inset)..Shaded polygons indicate N2K sites. IT51A0017: Cono vulcanico del Monte Amiata; IT51A0018: Monte Labbro e alta valle dell'Albegna; IT51A0019: Alto corso del Fiume Fiora IT51A0020: Monte Penna, Bosco della Fonte e Monte Civitella; IT5190013: Foreste del Siele e del

Pigelleto di Piancastagnaio; IT6010011: Caldera di Latera; IT6010013: Selva del Lamone; IT6010016: Monti di Castro.

Results

The contribution of each component (criterion × weight) to the ecological integrity of each sub-basin (S index) is illustrated in Figure 3. The results showed that low human presence (Threat), high naturalness and low road-induced fragmentation contributed significantly, i.e. higher than 20% to the ecological integrity of more than half of the river sub-basins (22, 21 and 16 out of 30 sub-basins) (Figure 3). This figure changes with the gradual decrease of the percentage contribution of Richness, Statutory protection and Patch diversity to the total ecological integrity of the sub-basins. Their contribution was higher that 20% only for sub-basins 4, 5 and 6 respectively (Figure 3).

Five out of six criteria contributed equally (≈ 20%) to the total ecological integrity of sub-basin 14, which has the highest S index (Figure 3). Despite its high integrity, the contribution of statutory protection to the total ecological integrity value of the site was zero, as sub-basin 14 does not include any SAC area. In contrast, in the case of sub-basin 18 (second highest ecological integrity), the contribution of statutory protection was the highest (>25%) while the contribution of diversity and naturalness were less than 10%.

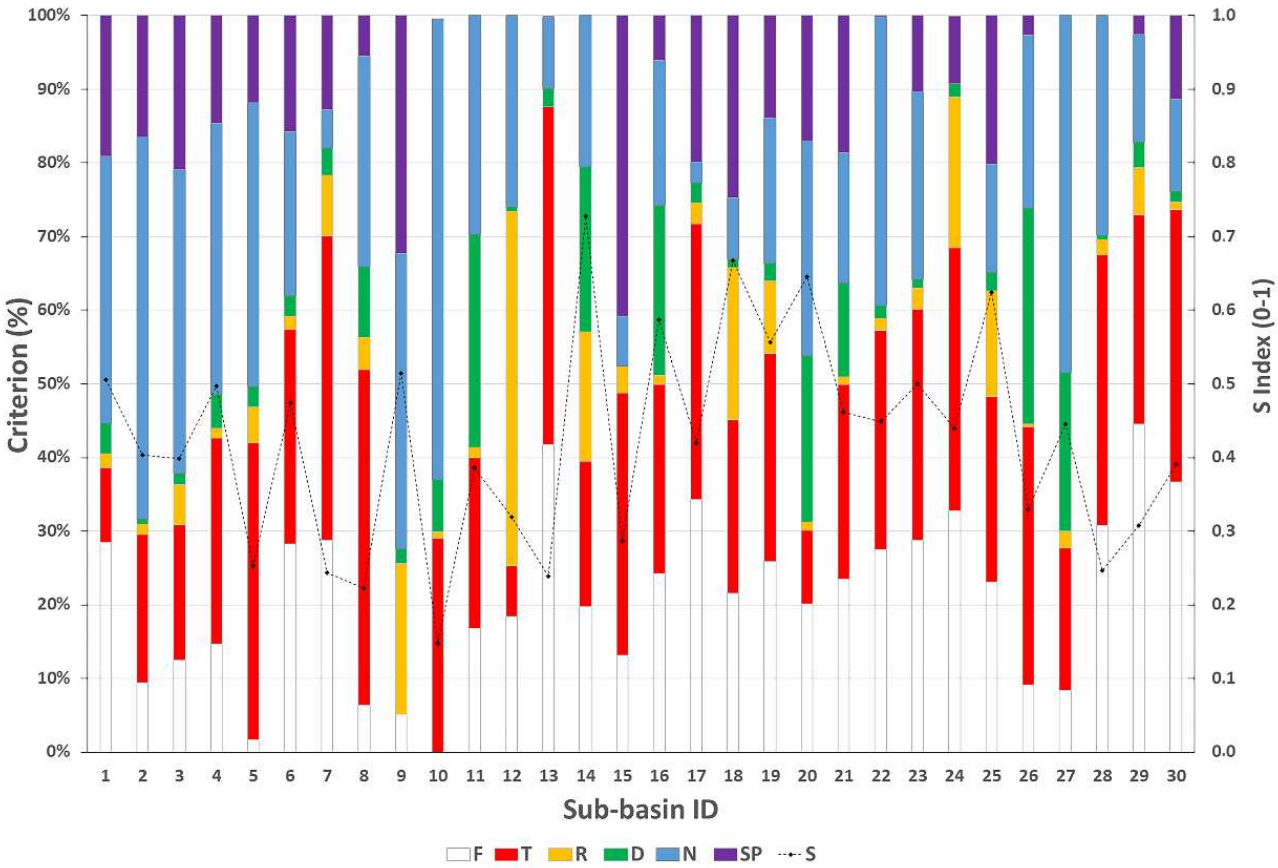

Figure 3. Percentage of each component (criterion * weight) contributed to S index calculation per sub-basin. Secondary axis showed S index per sub-basin. (F: Fragmentation; T: Threat; R: Richness; D: Diversity; N: Naturalness; SP: Statutory protection).

Ecological Network elements

The ecological integrity of each sub-basin (S index) was used as a proxy of the functional connectivity of each sub-basin and ranged from 0.15 (sub-basin 10) to 0.73 (sub-basin 14; secondary axis of Figure 3). Six of the sub-basins (20%) have high functional connectivity with S index ranging from 0.5 to 0.73. 14 sub-basins (47%) and 10 sub-basins (33%) showed lower functional connectivity with values ranging from 0.3-0.5 and 0.15 to 0.3 respectively (Figure 3 and 4A). None of the sub-basins have S value lower than 0.1 or higher than 0.75 (Figure 3, 4A).

Structural connectivity of the study area followed the same tendency as that of functional connectivity, with the majority of the sub-basins having medium connectivity (57%; 17 sub-basins), and 23% (7 sub-basins) and 20% (6 sub-basins) high and low structural connectivity respectively (Fig. 4B).

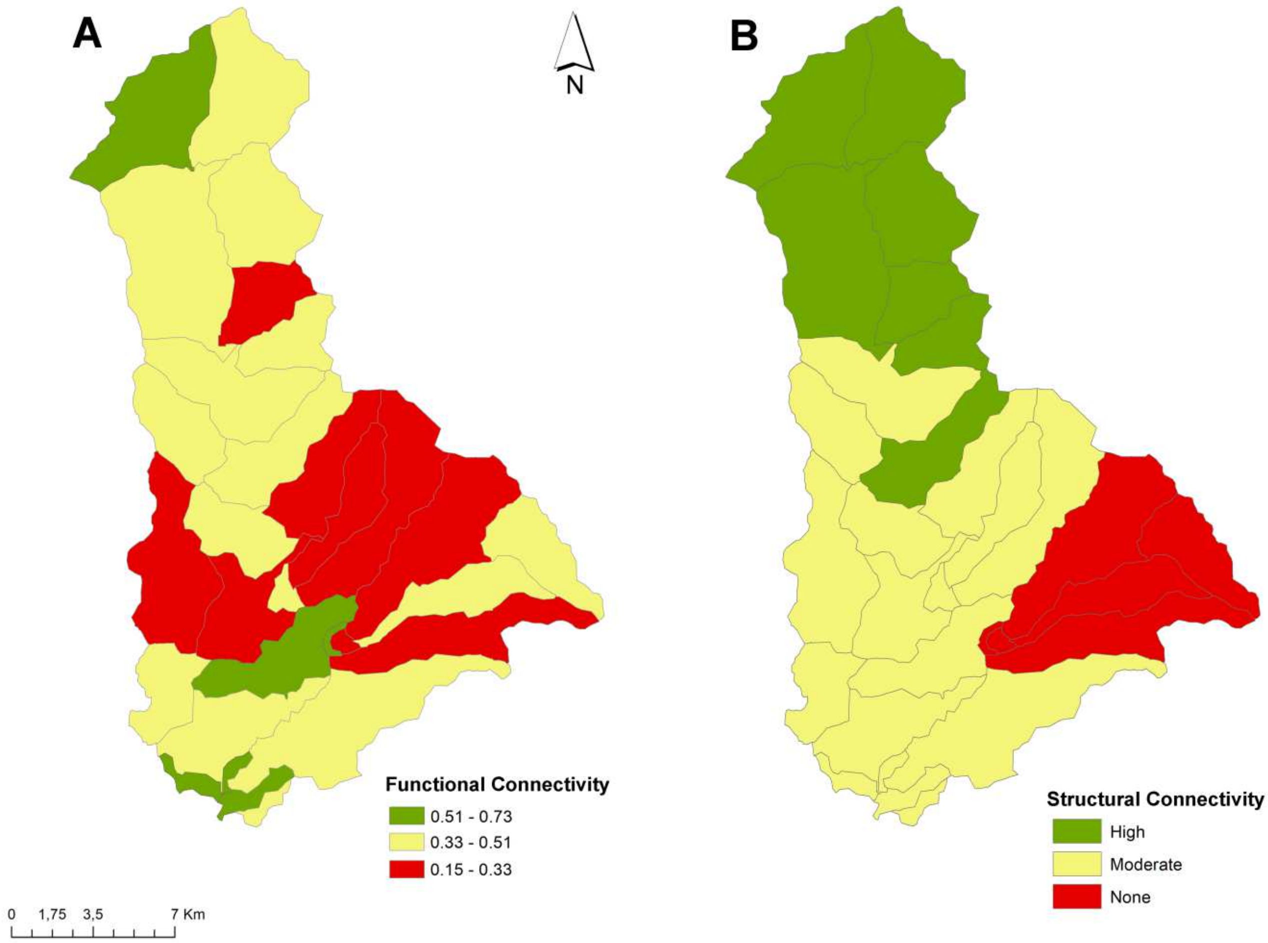

Figure 4: Functional and Structural Connectivity in the Fiora river catchment evaluated per sub-basin.

The overall connectivity of the study area, according to the conceptual framework set in the context of this work, is the combination of structural and functional connectivity (inset table in Fig. 5). Only one sub-basin (sub-basin 20) have *very high* and 10 sub-basins showed *high* overall connectivity accounting more than 37% of the study area (Figure 5). The next most abundant overall connectivity category is the *moderate* class (10 sub-basins) following by *low* class (20%). Three sub-basins have no connections with the N2K sites in the area (Figure 5).

Six of the sub-basins (no 1, 2, 3, 4, 20, and 30) were characterised as corridor areas i.e. the Fiora River basin N2K site (Figure 5). Sub-basin 29, apart from for the designation as a corridor, it has also been designated as an area to be considered for restoration activities, due to the low value of its functional connectivity. The majority of the sub-basins were identified as buffer areas (57%) of which, the 43% had also been designated as areas for restoration (Figure 5). Two sub-basins (no 11 and 27) were characterised solely as areas in which restoration activities should take place, while sub-basin 14 was characterised as stepping stone (SS) area.

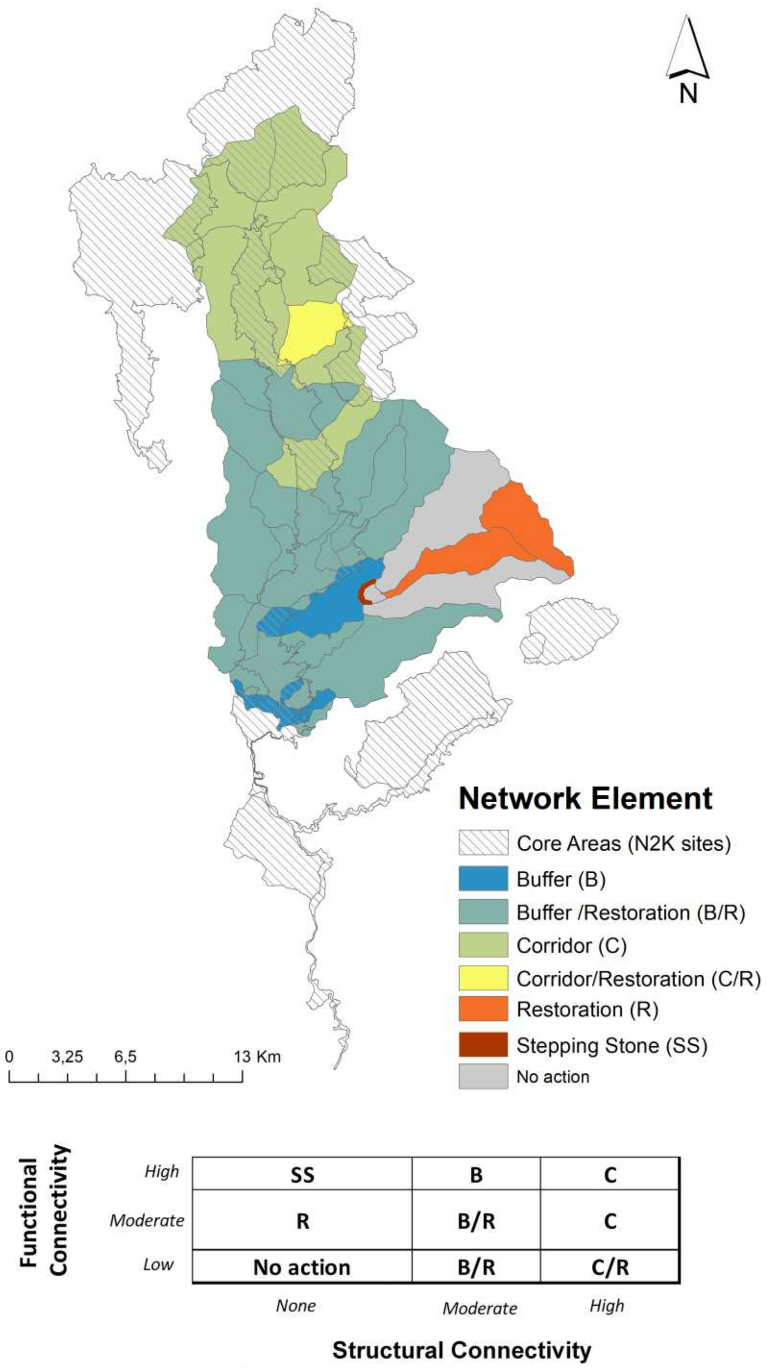

Figure 5. Ecological network elements in the Fiora basin.

Discussion

Land Use/Land Cover (LULC) is the main human imprint on landscapes worldwide whose spatiotemporal changes pose a threat to ecological processes and species distribution (Winkler et al., 2021). Assessing human–landscape interactions, or interactions within the landscape itself within a connectivity framework may inform or predict socio-economic and environmental (including climatic) futures (Brierley et al., 2006). In this paper, we have developed and applied a methodological framework to determine the role of individual sub-basins within a wider ecological network in the river Fiora River basin and identify the potential conservation synergies with the current configuration of the surrounding N2K areas.

There are many ways for designing and implementing an EN which may be species specific (Boitani et al., 2007) or as part of regional strategies to identify resilient areas for conservation (Samways and Pryke, 2015; Hermoso et al., 2020). Rivers are an integral part of ecological network theory (Jongman and Pungetti, 2004) and of the EU green infrastructure policy (European Commission 2013). The proposed network herein identifies (Figure 5) large corridor areas (4 sub-basins) in the north part of the basin while the middle and southern part of the basin comprises mainly areas which may act as buffers immediately to N2K or have the potential to play that role if appropriately restored. The presence of restoration and stepping stone areas is limited. This overall EN configuration can be explained by the fact that the north of the basin hosts areas with the highest naturalness and limited human impact while as we move towards the south there is lower diversity, highest fragmentation and human pressure values close to the rural lowland areas (Angiolini et al., 2017a).

The cornerstone of the EN concept is ecological connectivity which in this study was based methodologically on the evaluation of the overall connectivity (structural and functional) of the spatial units (in our case the sub-basins). Connectivity is a complex concept involving many interactions with many dimensions and spatiotemporal variations (Correa Ayram et al., 2016). While structural connectivity is straightforward to assess, functional connectivity, the response of organisms to the various elements of the landscape (Tischendorf and Fahrig, 2000) includes both a spatial and a temporal aspect. The assumption we make is that sub-basins with *high* ecological integrity are better placed to ensure ecological processes and therefore promote functional connectivity (at least) in the temporal sense (as defined by Auffret et al., 2015). It is in this context that the term functional connectivity is used herein. The study demonstrates that at the sub-basin level, *high* functional connectivity is limited to 5 sub-basins (16,18,19,20,25) with the remaining having either *moderate* or *low*. Overall, functional connectivity as intended herein was a combination of *high* or *moderate* naturalness, diversity and richness of land uses with lower intensity impacts (expressed by fragmentation and threat). However, human presence (Threat), naturalness and road-induced fragmentation were found to be the most determining factors in terms of functional connectivity of the Fiora River basin. In fact, these factors are dominant elements used in the assessment of ecological integrity at the landscape level (Manolaki et al., 2020; Walston and Hartmann, 2018; McGarigal et al., 2018).

Structural connectivity of the study area followed the same tendency with functional connectivity, with the majority of the sub-basins having medium connectivity (57%; 17 sub-basins), with 23% (7 sub-basins) and 20% (6 sub-basins) having *high* and *low* structural connectivity respectively (Fig. 4B). Sub-basins with *high* structural connectivity exhibit a distinct aggregation to the north part of the study area since there are more N2K surrounding that part of the basin. Usually, protected areas related to rivers are located upstream, due to topography and therefore land uses which are not in conflict with human activities, compared to downstream. This is why areas with increased connectivity (e.g. sub-basin 16) are more important in the lowlands along rivers.

Ecological integrity is explicitly included in HBDs. More specifically high conservation status, according to the directives, implies an excellent integrity of habitat structures, floristic composition or characteristic fauna of the habitat and the low intensity of negative impacts, while the functions of the habitat present excellent prospects (Council of the European Communities 1992). Even if the evaluation in this paper was not site-based but at the sub-basin level, ensuring landscape level integrity increases resilience in the long term (Pander et al., 2018; Fremier et al., 2015). In addition, site based protection is no longer enough since the pressures which taken place in the surrounding matrix influence habitat quality and species persistence within the sites (Vogiatzakis et al., 2015; Hermoso et al., 2015). In fact, HBDs support the creation of a network for nature conservation by promoting ecological connectivity rather than the protection of isolated areas. This is also the case with rivers and their catchments which are highly interconnected systems and are especially susceptible to human-induced habitat degradation, which impairs the overall basin quality (Tockner et al., 2010). In particular, LULC manifestations (i.e. fragmentation, nutrient loading, and channelization) affect river basin and stream ecological status, already assessed in the river basin level management programmes (senso WFD) but also assessed at the site level as part of the HBDs. The challenge is to ensure that the complementarity of the two directives moves from separate implementation into a common unified methodological framework.

Based on the explicit link between ecological integrity and ecological connectivity we provided a scheme introducing ecological integrity as a potential tool for an ecological network design in a river basin level, in an attempt to enhance conservation and management around N2K and to accommodate not only conservation but also ecological quality as proposed by the WFD. Therefore, the framework developed may be integrated into existing monitoring efforts or provide a reliable rapid appraisal alternative tool when resources are scarce. Conservation beyond protected areas should deal with enhancing resilience at the landscape level which can be achieved among other things with increased connectivity and improved management (Lawton et al., 2010). In that respect isolated assessments may underestimate threats and pressures and overlook issues of connectivity between protected areas and the matrix.

Rivers and wetlands in protected-area networks are underrepresented (Kingsford, 2011), while often conservation planning focus on river ecosystems neglecting their neighbouring elements (Linke et al., 2011; Angiolini et al., 2017b). Therefore, various studies have advocated a catchment approach for conservation of river ecosystems (Comino et al., 2016; Abellán et al., 2007) and the importance of evaluating their conservation status inside and outside protected areas (Nel et al., 2007) with emphasis on the effects of land-use changes (Meek et al., 2010; Wahl et al., 2013). In addition, most restoration projects occur at the reach level, with effects varying among taxonomic groups (Palmer et al., 2010; Lorenz et al., 2012). Whether restoration or conservation is the target in these cases building resilience at the river basin scale is the only way forward. Since conserving entire catchments in order to protect significant downstream habitats is not usually an option, lateral and longitudinal connectedness at the catchment level should be strengthened (Linke et al., 2011).

Enhancing connectivity at the basin level (riverscape) as in the case of Fiora, means enhancing connectivity within and between its constituent elements i.e. terrestrial and riparian. Constituent elements in such an ecological network have their own discrete management regime which of course leads to a common goal i.e. building resilience in an area. Sub-basins are part of, and should function as, the constituents' elements in an ecological network with N2K sites as core areas.

GIS-based multi-criteria decision making is widely employed in ecology/planning (Comino et al., 2016) while increasingly rapid ecological appraisal (e.g. Vogiatzakis et al., 2015; Manolaki et al., 2020) often relies on experts' opinion, in the absence of available information and/or fieldwork resources (Krueger et al., 2012) despite its critics (Stevenson-Holt et al., 2014). Among the criteria used in this study experts' level of agreement was higher for naturalness and richness and lower for threat and fragmentation. This can be probably attributed to the conceptual clarity and ease of assessment of the former. On the other hand, threat and fragmentation have various drivers and involve many processes which render their evaluation and a subsequent consensus more difficult to achieve.

In the past 20 years there has been a proliferation of methods and techniques on land use based ecological network analysis (Blasi et al., 2008; Modica et al., 2021) some relying on complex tools (Ribeiro et al., 2017). Therefore, the configuration of an ecological network remains dependant on the methodology used for its design. Our own approach is one of many plausible ecological networks as proposed by Bennett (2004), which although analytically simple, can be enriched with data on species and stakeholders' involvement (see review by Beier et al., 2011). It has the flexibility to include other factors for example land use dynamics, in particular land take, which could be incorporated under the criterion threat in the evaluation framework. However, as already discussed the overall human impact is relatively low in the area while land use changes at the basin level have been remarkably stable the past 20 years. In addition, it provides synergies with policies at the EU level and in particular with the Biodiversity Strategy for 2030, the Green Infrastructure as well as the Green Architecture of the new Common Agricultural Policy (CAP).

Since a large number of rivers and parts of the respective valleys are protected through the HBDs (Albrecht et al., 2012; Hofmann and Schmidt, 2012), WFD and HBDs together serve, in addition to nature conservation, the purposes of flood protection, climate change mitigation and adaptation (Janauer et al., 2015). However, and despite their alleged synergy each of these directives is currently implemented on its own, thereby potentially jeopardising the achievement of their respective goals (Beunen et al., 2009). Integrating river basin into all aspects of water quality, as the WFD suggests, is a direct recognition of the importance of land use as determinant of water quality and indirectly of the need for developing management and policies at the landscape level. The proposed methodology is a step towards that direction.

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Conflict of Interest

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRedit Author Statement

IV, CA: Conceptualization; AN, PM: Data Curation; AN, IV, PM: Methodology; AN, PM: Formal Analysis; AN, PM: Writing -original draft; CA, IV: Writing-review editing; CA: Supervision