



Riverine ecosystem in central Italy: an insight into EU Habitats of the Elsa River and a new bryophyte habitat for Italy*

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Abstract

Riverine habitats play a vital ecological role, offering key ecosystem services, regulating hydrology, and supporting high biodiversity, including species and habitats of conservation concern. This study aimed to identify Annex I habitats under the Habitats Directive (92/43/EEC) within a former protected area along the mid-upper Elsa River (Tuscany, central Italy). From 2021 to 2023, 85 vegetation relevés were collected to classify habitats, map their distribution, and assess major threats. Multivariate analyses revealed 15 Annex I habitats, including 32A0, reported here for the first time in Italy, and three priority habitats (7220*, 91E0*, 91AA*). Habitat 32A0, officially included in Annex I following the accession of Croatia to the EU, shows distinct ecological and structural features compared to habitat 7220*, despite sharing some bryophyte species. While the two often occur in close proximity or form mosaics, habitat 32A0 develops in active waterfalls with constant flow and low carbonate deposition, whereas habitat 7220* forms in slow-dripping areas with high tufa accumulation. The absence of habitat 32A0 from the Italian Habitat Manual underscores the need to revise national habitat classifications to improve identification accuracy and conservation strategies. Our study enhances knowledge of riverine habitats and stresses the importance of adaptive management to safeguard the Elsa River ecosystem. Key actions include continuous monitoring and control of invasive alien species. Incorporating the area into a nearby Special Area of Conservation would strengthen long-term protection in line with the EU Biodiversity Strategy for 2030.

Keywords

Biodiversity conservation, bryophyte-dominated vegetation, Natura 2000, tufa cascades, urban river, wetland habitats

Introduction

Rivers are hotspots of biodiversity, hosting a wide assemblage of species and provide irreplaceable services at both the ecosystem and societal levels (Albert et al. 2021; Faghihinia et al. 2021). Riverine habitats are characterised

by species that are specifically adapted to fluctuating environmental conditions, such as seasonal changes in water levels, variable nutrient influxes and temperatures. They are often characterized by high levels of biodiversity, including a significant proportion of threatened or community-interest species, which highlights the necessity

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of targeted conservation initiatives (Birk et al. 2020; Graziano et al. 2022). Despite their importance, riverine biodiversity in highly anthropized stretches of rivers is threatened from the direct and indirect effects of anthropogenic activities (Angiolini et al. 2023; de Simone et al. 2025). Urbanization, intensive agriculture, and the spread of both native and alien invasive species, degrade water quality, destabilize soils, and compromise the integrity of the habitats in these areas (Joy and Death 2013; Reid et al. 2019). Moreover, urban expansion often leads to physical changes in the riverine systems such as channelisation and alterations to the course of the riverbed, which in turn reduce soil water absorption, increase flood risk and accelerate erosion (Paul and Meyer 2001; Allan and Castillo 2007). Additionally, intensive agriculture produces nutrient and pesticide pollution, promoting water eutrophication with harmful effects for aquatic life (Bohus et al. 2023; Rivera-Pérez et al. 2023). Climate change further intensifies these pressures by causing more frequent and severe floods and droughts, which disrupt hydrological regimes and threaten specialised species (Schneider et al. 2013; Rivaes et al. 2022; Siddha and Sahu 2022).

In this context, the study and preservation of riverine and freshwater habitats is not only ecologically important for biodiversity conservation, but also for the wellbeing of human communities. These habitats, in fact, provide essential ecosystem services, including hydrological regulation, nutrient cycling, and bank stabilisation, as well as serving as refuges for a wide diversity of plant and animal species (Lind et al. 2019; Riis et al. 2020). The protection of these habitats is then essential for the conservation of threatened species, as well as for enhancing ecological resilience. This, in turn, enables adaptive responses to climate change and anthropogenic pressures that threaten ecosystem stability (Reid et al. 2019; Valentim et al. 2024).

Within the European Union, the Directive 92/43/EEC, more commonly referred to as the Habitats Directive (HD), demands that Member States identify and protect habitats of community interest and vulnerable ecosystems. The HD highlights the necessity of preserving areas with high ecological value through the Natura 2000 network (Ellwanger et al. 2018; Hödl 2018; Nucci et al. 2022; Spampinato et al. 2023). This regulatory framework recognizes the conservation value and the need for their protection and sustainable management of riverine habitats. Many of these habitats are therefore listed in the HD Annex I under three habitat macrotypes (e.g., macrotypes 3, 7, and 9). This is particularly important in the fragile context of freshwater connected habitats in the Mediterranean basin. Here, rivers are characterised by a high diversity of habitats and species (Stella et al. 2013; Fiaschi et al. 2023), being simultaneously exposed to strong anthropogenic pressures (Cramer et al. 2018; Fernández-Martínez et al. 2020, 2024). Consequently, a significant number of vegetation studies have been conducted in recent years, especially on rivers in central Italy (see Landi et al. 2009; Lastrucci et al. 2012; Angiolini et al. 2017). However, few authors have focused exclusively or even partially on habitats listed in

Annex I of the Habitats Directive, highlighting the high vegetation diversity (i.e. Landi et al. 2009; Mereu et al. 2012; Fanfarillo et al. 2023), vulnerability, and significant conservation value of HD habitats found within rivers and streams (Carli et al. 2016; Gigante et al. 2018). To protect biodiversity and designate protected areas, one of the most widely used methods is the assessment of habitats under HD, which is a mandatory action in many cases. Under these premises, this work focuses on a riverine area of high ecological value (Calattini et al. 2025), the mid-upper stretch of the Elsa River (Tuscany, central Italy). The stretch, which flows through the town of Colle Val d'Elsa, has been investigated in terms of its vegetation (Angiolini et al. 1999), while HD habitats remain largely understudied. The only data currently available consist of recent records regarding the presence of some habitats (Rivieccio et al. 2022; Tavilla et al. 2022). Thus, our study firstly aims to provide knowledge on the HD habitats within the boundaries of the Ex ANPIL (Area Naturale Protetta di Interesse Locale) “Parco Fluviale dell’Alta Val d’Elsa” (Siena, Italy), hereafter named Ex-ANPIL-Elsa. This urban stretch of the Elsa River is currently threatened by several anthropogenic pressures, including agriculture, tourism, urbanization, pollution, hydroelectric projects, and water abstraction (Calattini et al. 2025). Hence, providing the necessary tools for a proper assessment of the Ex-ANPIL-Elsa conservation value for its potential inclusion in the Natura 2000 Network is a priority.

The main focus of this research will be on riverine habitats, with a focus on evaluating their conservation status, distribution, and identifying the primary threats to their ecological stability. Secondly, the objective is to study the bryophyte-dominated vegetation occurring on the waterfalls along the Elsa River, in order to investigate its possible assignment to one or more habitats, given that these communities are poorly studied in Tuscany.

Material and methods

Study area

The River Elsa is a watercourse in Tuscany (central Italy) that flows through the Val d'Elsa from south to north. It originates in the Montagnola Senese, at an elevation of approximately 580 metres above sea level. It has a length of about 75 km, and its drainage basin covers an area of approximately 867 km². The Elsa flows through several municipalities, including Colle di Val d'Elsa, Poggibonsi, Certaldo, and Castelfiorentino, before merging into the Arno River. The study area (43°40.5793'N, 11°13.5088'E) is located in central Tuscany (Province of Siena, Italy; Fig. 1A) and is part of the “Parco Fluviale dell’Alta Val d’Elsa”, an ex-protected area designated by the Tuscany Region with Regional Council Resolution no. 256 of 16/07/1997 as an “Area Naturale Protetta di Interesse Locale” (ANPIL). It represents a key ecological corridor within the region, consisting of a riverine ecosystem (Fig. 1B, C) extending

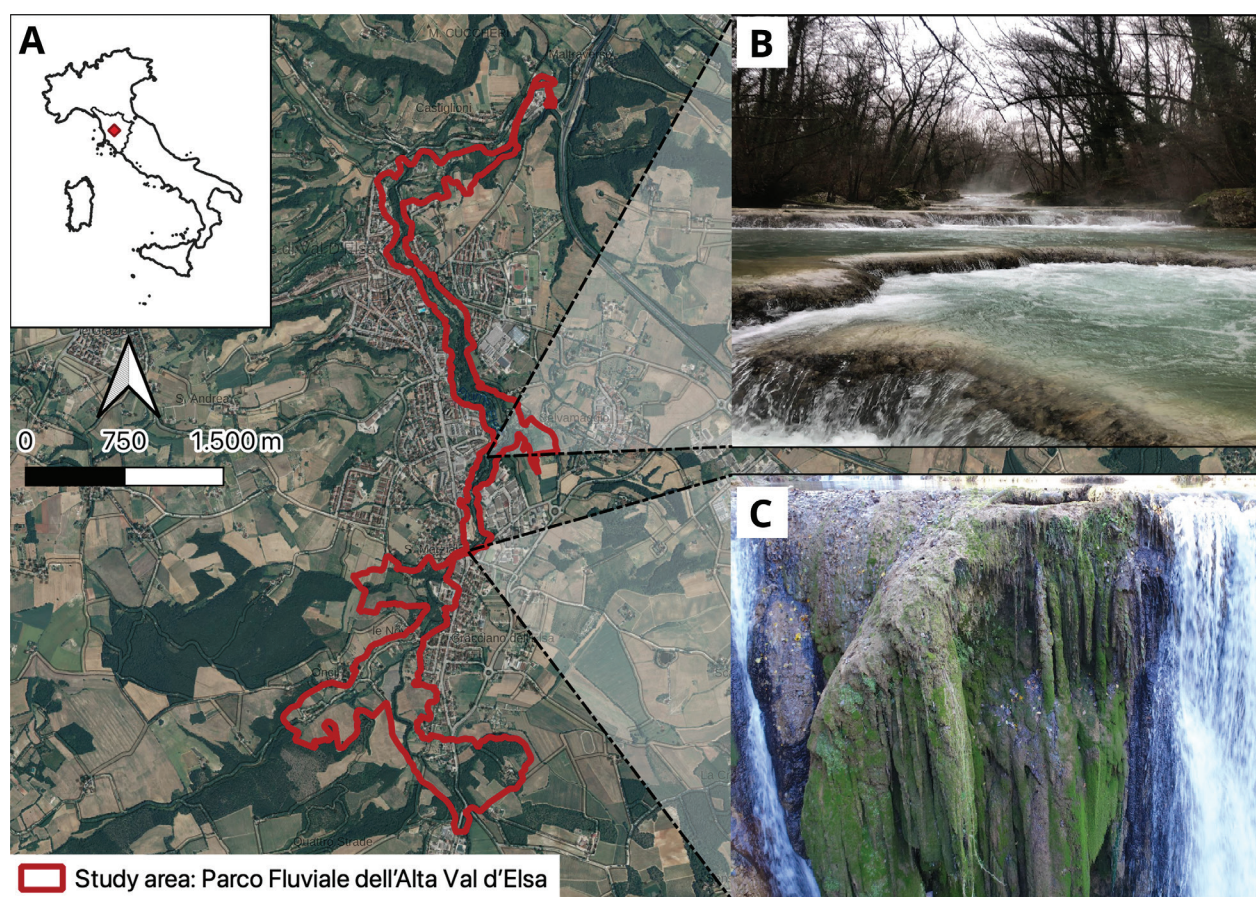


Figure 1. Study area. Location of the study area in Italy (A). Examples of riverine habitats encountered in the Elsa River (B, C).

along the course of the Elsa River for 7.7 km and covering approximately 203.6 hectares, in the municipalities of Colle di Val d'Elsa (Angiolini et al. 1999).

Since the 11th century, the Elsa River has undergone extensive human modifications. Key interventions include the “gore”, artificial channels to divert water toward the town centre for mills, paper mills, forges, and other industries (Gelli 2009; Ulivieri 2017). Expanding agricultural, residential, and industrial infrastructures have exerted pressure on ecosystems, contributing to erosion and habitat fragmentation (Muzzi 1998; Moretti and Soldani 2007).

The geology of the Elsa River reflects the interplay between water, tectonics, and erosion, with its deposits representing a significant natural and historical heritage (Barazzuoli et al. 2013). The study area is characterized by travertine deposits, formed by the precipitation of calcium carbonate from CO₂-rich waters. These deposits can be classified into thermal travertines, associated with hot springs, and calcareous tufa, typical of fluvial-palustrine environments (Capezzuoli 2013). In addition to travertines, the region features Mesozoic limestones, which form the geological substrate, and alluvial and fluvial deposits composed of sands, gravels, and clays. These sediments, transported and deposited along the active course of the Elsa River, contribute to the geomorphological complexity of the area (Del Zanna 1901; Barazzuoli et al. 2013). Tectonic activity has played a key role in shaping structural depressions and springs, while fluvial erosion

has sculpted terraces and steep escarpments. A notable geomorphological feature is the Diborrato waterfall, a 15-meter drop formed by differential erosion of travertine, where some important habitats were found during the field activities (Fig. 1C; Capezzuoli 2013).

From a climatic perspective, the macrobioclimate of the study area is Mediterranean, and the bioclimate can be classified as Pluviseasonal oceanic (Mediterranean). The thermotype is upper mesomediterranean (Mediterranean) and the ombrotype is lower humid. From the perspective of continentality, the bioclimate is weak semicontinental (Pesaresi et al. 2017). Meteorological data from a station in the Municipality of Colle di Val D'Elsa, near the study area, recorded an annual average temperature of 13.8 °C, an average temperature of 25.1 °C in the warmest month (August) and 6.9 °C in the coldest month (January) over the period 2000–2024. The mean annual precipitation is 861 mm distributed over 87 days, with rainfall concentrated in autumn and winter, while the summer months (July and August) experience a marked aridity period, despite occasional localized rainfall events (SIR Toscana 2025). Over the centuries, the extent of anthropogenic landscapes has increased, driven by agriculture, residential settlements, and industrial activities, leading to a progressive transformation of the natural environment (Moretti and Soldani 2007).

From a conservation point of view, the Ex-ANPIL-Elsa is currently undergoing evaluation for classification under

one of the categories outlined in Articles 2 and 6 of Regional Law 30/2015, while the only active protection currently in place is a municipal regulation (Comune di Colle di Val d'Elsa 2021).

Vegetation survey

Sampling was conducted between 2021 and 2023. The study primarily focused on aquatic and riparian habitats along the River Elsa, extending to adjacent areas including shores, forest fragments, and shrublands within the Ex-ANPIL-Elsa area. A total of 85 relevés were carried out across various HD habitats, using relevés sizes adapted to each habitat type based on the physiognomy, structure, ecology, and extent of the vegetation being studied. In line with the methodological guidelines of Biondi et al. (2009), Angelini et al. (2016), and Gigante et al. (2016), relevés sizes were selected according to the habitat macrotypes identified in the field: for macrotype 3 (standing and running water vegetation), relevés ranged from 2 to 4 m²; for macrotype 5 (shrub vegetation), relevés measured 25 m²; for macrotype 6 (grasslands), 16 m²; for macrotype 7 (spring habitats), relevés measured 0.49 m² (70 cm × 70 cm) were used; and for macrotype 9 (forest habitats), relevés sizes ranged from 90 to 200 m² (Chytrý and Otýpková 2003). An exception was made for the HD habitat of waterfalls (belonging to macrotype 3), for which the same relevés size as that used for macrotype 7 habitats was applied. The list of vascular plant and bryophyte species identified within each relevé was recorded, along with the estimated abundance of each species, expressed as visually assessed percentage cover. In addition, information on slope, aspect and water depth were recorded. More precisely, in the field, slope was measured using a digital clinometer, and aspect was measured using a field compass in °deg. For relevés in aquatic environments, water depth was estimated at the centre of each plot by inserting a rod to the bottom and subsequently measuring the wetted section with a measuring tape. Each relevé centre was geo-referenced using a phone GPS with an accuracy ranging from 5 and 10 m. For each relevé, data on elevation was subsequently extracted from the Digital Terrain Model of the study area (Regione Toscana 2025) using the open source software QGIS (QGIS.org 2021).

To identify the main environmental drivers of the plots assigned to habitats 32A0 and 7220* in the waterfalls along the Elsa River, we measured two additional variables: northness, and water availability. Northness is a variable related to relevés aspect and slope, calculated following Amatulli et al. (2018). Water availability was estimated visually as the percentage proportion of the plot surface covered by flowing or dripping water.

Vascular plants were identified according to Pignatti et al. (2017–2019), while mosses were identified according to Cortini Pedrotti (2001, 2005) and liverworts were identified according to Paton (1999). The taxonomic nomenclature follows Euro+Med PlantBase (2025) for vascular

plants and for bryophytes. Syntaxonomic nomenclature follows the original authors for associations and sub-associations, Biondi et al. (2014a) for alliances, and sub-alliances, and Mucina et al. (2016) for classes and orders.

Data analysis

As a first step, the species × relevés compositional matrix (283 species × 85 relevés) was Hellinger-transformed and used to compute a Hellinger distance dissimilarity matrix of relevés with the aim to reduce the importance of species with high cover values (Legendre and Legendre 2012). We applied hierarchical agglomerative clustering (UPGMA) on the Hellinger distance matrix to summarise the compositional structure. To interpret and compare the dendrogram of the resulting relevés clustering, we selected four cluster groups, a number that permitted the best ecological interpretation of sites. The cluster grouping results were subsequently analysed in terms of floristic composition to identify their typical species and HD habitat codes (*sensu* 92/43/EEC Habitats Directive; Gigante et al. 2016) using both the Italian and European Interpretation Manual of Habitats Directive (Biondi et al. 2009; European Commission 2013); to analyse relevés belonging to the habitat 32A0, given its absence in the Italian Manual, only the guidelines from the European Union's Habitats Directive (European Commission 2013) were used. Moreover, a Non-metric Multidimensional Scaling analysis (NMDS) based on the same Hellinger distance was performed to represent the relevés in a low-dimensional space by optimising the correspondence between original and in the ordination dissimilarities (Økland 1996). Finally, a Redundancy Analysis (RDA) was performed to measure the ecological differences in the constrained distribution of variance of sites pertaining to bryophyte-dominated HD habitats typical of flowing-dripping waters (habitat 32A0 and 7220*). For this purpose, we used the Hellinger distance dissimilarity matrix of relevés pertaining to habitat 32A0 and 7220*, accounting for the constraining variables slope, northness, water availability and total vegetation cover, to evaluate their role in shaping species composition, habitat structure, and the ecological differentiation between the two habitats. Slope and northness were selected because of their influence in driving riparian plant community composition (de Simone et al. 2025). Water availability was selected for its ability to disentangle habitat 32A0 from 7220* according to European Commission (2013). ANOVA-like permutations were performed to test the global significance of the RDA, its main axes of variation and the effect of each constraining variable (Legendre and Legendre 2012).

Results and discussion

The cluster analysis allows us to identify four distinct groups of relevés (Table 1), reflecting differences in their floristic composition and ecological characteristics (Fig. 2A). Overall, the clustering results confirm the

habitat subdivision carried out in the field based on expert ecological and phytosociological knowledge, although some habitats show strong floristic affinities reflecting their compositional and ecological similarity (Fig. 2A). These cluster groups corresponded to well-defined habitat types regulated by hydrological, structural, and environmental conditions. The first division separates Group 1, consisting of 17 relevés, from all the others. This group, defined as “Communities dominated by bryophytes”, includes relevés attributable to habitats of two different macrotypes (32 Running water, and 72 Calcareous fens), with plant communities pertaining to habitat 32A0 and 7220*, both characterized by a dominance of bryophytes in flowing-dripping water habitats. Their distinct clustering structure suggests that these habitats share a fraction of their species pools and have, to some level, similar specific abundances. Group 2 (27 relevés) includes relevés referable to habitats belonging to the macrogroup 3 (3140, 3150, 3260, and 3290) and named “Aquatic communities dominated by algae and hydrophytic species”. These habitats are all characterized by the presence and dominance of algae of the Characeae family or vascular hydrophytes. Their clustering structure confirms that these habitats are closely related, likely due to shared ecological factors such as standing or slow-moving waters, submerged conditions, and nutrient availability. Moreover, the distinction of aquatic communities dominated by algae and hydrophytic species (Group 2) from the communities dominated by bryophytes (Group 1) underscores their distinct ecological requirements, particularly in terms of water flow and substrate stability. Group 3 (19 relevés) encompasses habitats of macrohabitat 3 and 6 (3270, 3280, 6420, and 6430) and was named “Communities of tall-herb humid meadows”. This cluster group, well divided from the previous two, is characterised by the dominance of herbaceous species colonizing riverbanks and transitional zones between aquatic and terrestrial habitats. Floristic similarities of relevés belonging to this group reflect their shared influence by environmental conditions such as periodic flooding and moisture availability, which shape the composition of these river meadows. Moreover, their intermediate position between aquatic and terrestrial habitats highlights their role as ecotonal communities. Group 4 (22 relevés), named “Communities dominated by woody species,” includes forests and shrub-dominated habitats (macrohabitats 9 and 5; habitats 92A0, 91E0*, 91L0, 91AA*, 5130). These encompass both river woodlands and drier tree or shrub formations that are not directly influenced by the river. The clear separation of this cluster group from herbaceous (Group 3) and aquatic communities (Group 2) suggests a strong ecological differentiation, driven by factors such as substrate stability, soil depth, reduced hydric influence, and long-term successional processes. River woodland habitats (91E0*, 92A0, 91L0) cluster closely together, indicating their shared dependence on riverine conditions, whereas more detached formations (91AA*, 5130) are grouped separately, reflecting their adaptation to less humid and more stable habitats.

Table 1. Summary of the four main vegetation groups identified by the cluster analysis, with corresponding habitat codes (according to the EU Habitats Directive 92/43/EEC) and the number of relevés per Habitat.

Group	habitat	No. of relevés
1 Communities dominated by bryophytes	32A0	11
	7220*	6
	3140	6
2 Aquatic communities dominated by algae and hydrophytic species	3150	4
	3260	12
	3290	5
	3270	7
3 Communities of tall-herb humid meadows	3280	5
	6420	4
	6430	3
	5130	3
4 Communities dominated by woody species	91AA*	3
	91L0	3
	91E0*	8
	92A0	5

The NMDS ordination (Fig. 2B), with a stress value of 0.12, is a good representation of relevés dissimilarities. The solution was obtained after 20 random starts, with species scores projected from site scores. The NMDS was able to distinguish the different habitat macrotypes along the aquatic to terrestrial gradient, in accordance with the results of the cluster analysis. All four cluster groups (1–4) were clearly separated on the first and second axes. In NMDS, the stress value measures how well the ordination represents the observed dissimilarity data in a reduced number of dimensions. Lower stress values indicate a better fit. Along Axis 1, the relevés of Group 1, occurring in flowing or dripping water habitats and dominated by bryophytes, were grouped on the positive side, whereas those of Group 2, found in aquatic habitats, including both lentic and lotic environments, were positioned on the positive side. Along Axis 2, the relevés of Groups 1 and 2, associated with aquatic sites, were mainly grouped on the positive side, together with species such as *Hydrogonium bolleanum*, *Apopellia endiviifolia*, *Myriophyllum spicatum*, and *Potamogeton* spp.; by contrast, relevés from less humid sites (Group 4) were distributed on the negative side and were characterized by species such as *Bromus erectus*, *Carex flacca*, and *Juniperus communis*. Group 3, with transition habitats (3270, 3280, 6420, 6430) occupy an intermediate position between aquatic and terrestrial habitats, reflecting their ecotonal nature. Their placement in the ordination suggests a decreasing moisture gradient that influences floristic composition.

The distribution of groups in both analyses suggests the influence exerted by key riverine environmental factors, such as water depth, flood duration, and hydrodynamic disturbance. For example, habitat 7220* and 32A0, both characterized by a bryophyte-dominated vegetation, are clearly separated from all the others, confirming a peculiar assemblage adapted to high oxygenation and

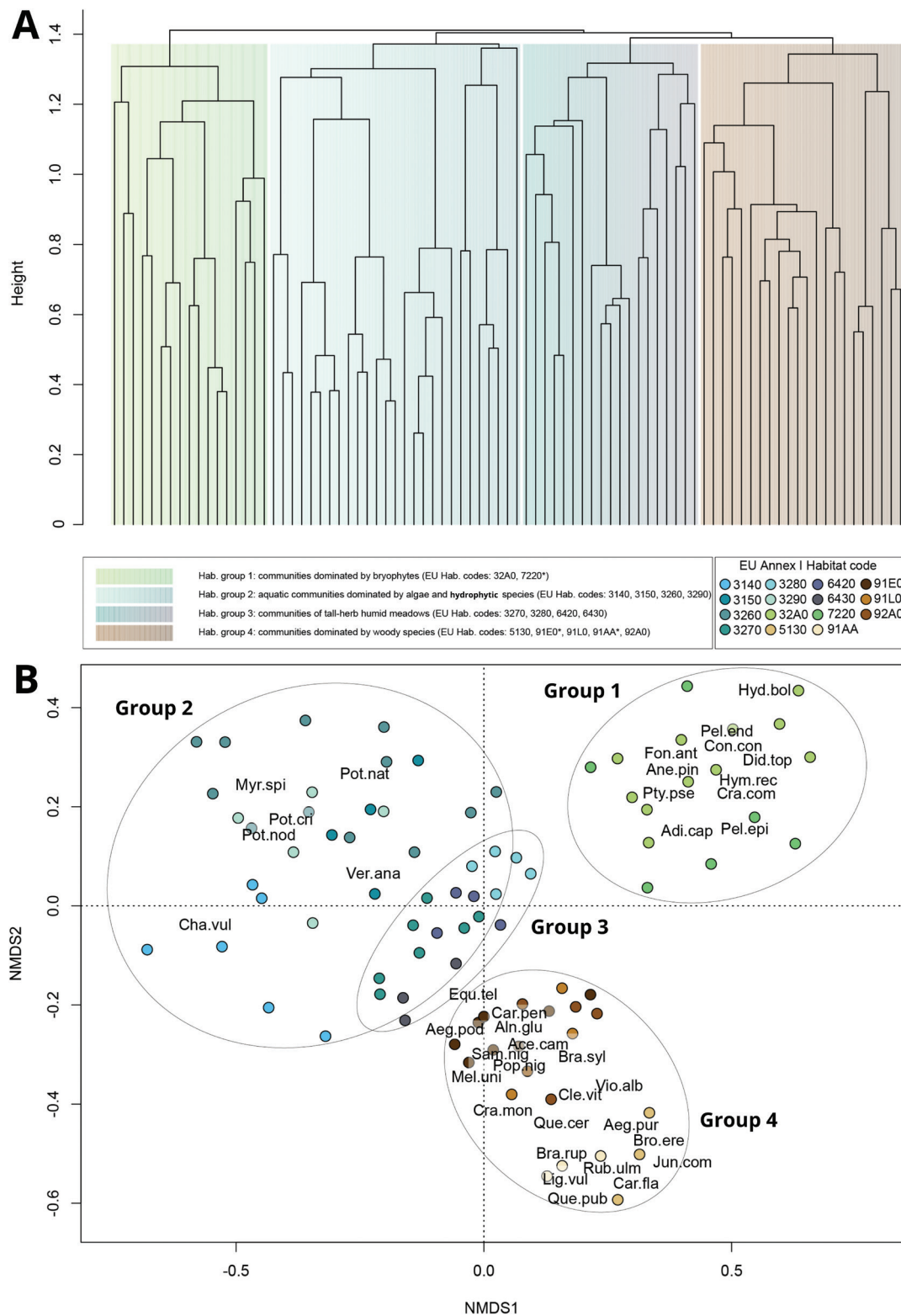


Figure 2. Results of the clustering and ordination of vegetation relevés. Results of the classification using UPGMA average agglomerative clustering (A). Results of the NMDS ordination (B). NMDS stress = 0.12. *Ace.cam* = *Acer campestre*, *Adi.cap* = *Adiantum capillus-veneris*, *Aeg.pod* = *Aegopodium podagraria*, *Aeg.pur* = *Aegonychon purpureocaeruleum*, *Aln.glu* = *Alnus glutinosa*, *Ane.pip* = *Aneura pinguis*, *Bra.rup* = *Brachypodium rupestre*, *Bra.syl* = *Brachypodium sylvaticum*, *Bro.ere* = *Bromopsis erecta*, *Car.flu* = *Carex flacca*, *Car.pen* = *Carex pendula*, *Cha.vul* = *Chara vulgaris*, *Cle.vit* = *Clematis vitalba*, *Con.con* = *Conocephalum conicum*, *Cra.mon* = *Crataegus monogyna*, *Did.top* = *Didymodon tophaceus*, *Equ.tel* = *Equisetum telmateja*, *Fon.ant* = *Fontinalis antipyretica*, *Hyd.bol* = *Hydrogonium bolleanum*, *Hym.rec* = *Hymenostylium recurvirostrum*, *Jun.com* = *Juniperus communis*, *Lig.vul* = *Ligustrum vulgare*, *Mel.uni* = *Melica uniflora*, *Myr.spi* = *Myriophyllum spicatum*, *Pel.end* = *Pellia endiviifolia*, *Pel.epi* = *Pellia epiphylla*, *Pop.nig* = *Populus nigra*, *Pot.cri* = *Potamogeton crispus*, *Pot.nat* = *Potamogeton natans*, *Pot.nod* = *Potamogeton nodosus*, *Pty.pse* = *Ptychostomum pseudotriquetrum*, *Que.cer* = *Quercus cerris*, *Rub.ulm* = *Rubus ulmifolius*, *Sam.nig* = *Sambucus nigra*, *Ver.ana* = *Veronica anagallis-aquatica*, *Vio.alb* = *Viola alba*.

specific hydrological dynamics. Their separation from aquatic habitats dominated by hydrophytes and Characeae (3140, 3150, 3260, 3290) underscores the importance of flow velocity and substrate stability in determining species distribution. Finally, woodland and shrub-dominated habitats relevant of cluster group 4 (91AA*, 91E0*, 91L0, 92A0, 5130) were in the lower right portion of the diagram, clearly separated from aquatic habitats. This distance indicates strong ecological differentiation, with the predominance of woody species adapted to more stable conditions, less directly influenced by fluvial dynamics. Overall, the NMDS confirms the ecological zonation of riparian and aquatic habitats, supporting the classification emerging from the cluster analysis.

In the following sections, the communities and HD habitats are described and commented on.

Group 1 – Communities dominated by bryophytes

32A0 Tufa cascades of karstic rivers of the Dinaric Alps (Suppl. material 1: table S1)

Habitat 32A0, recently added to the European manual following the accession of Croatia to the EU (European Commission 2013), is not yet officially included in the Italian manual, and the plant communities it supports are often misclassified as habitat 7220* – Petrifying springs with tufa formation (*Cratoneurion*). Nonetheless, Biondi et al. (2014b) hypothesised its presence at the Marmore Falls

and in several rivers of Italy. Specifically, these authors noted that the Habitat might be located in mountainous and hilly areas, and in karst zones of the Apennines and the Eastern Alps. This habitat falls under the broader category 32, “Running waters – stretches of watercourses with natural or semi-natural dynamics” and was originally described based on the waterfalls of Plitvice National Park (Topić and Vukelić 2009). Habitat 32A0 is typically found along stretches of rivers undergoing abrupt morphological changes that generate waterfalls, such as those in the Ex-ANPIL-Elsa, where it is quite frequent (Fig. 3). These environments are characterized by dripping and continuous water movement over most of the surface, which exerts physical and chemical stress on the vegetation. The habitat hosts plant communities dominated by bryophytes capable of withstanding strong water pressure, including both liverworts and mosses. Along the Elsa River, the most prevalent species include *Fontinalis antipyretica*, *Apopellia endiviifolia*, *Rhynchostegium riparioides*, and *Didymodon tophaceus*, all indicated as typical of habitat 32A0 (European Commission 2013). A frequent occurring species is *Palustriella commutata*, which is also found in habitat 7220*. Mosses display both pleurocarpous and acrocarpous growth forms; the pleurocarpous species, more sensitive to hydraulic force, often grow flattened against the rocky substrate (Glime 2021). The conservation status of this habitat appears generally good, but in a precarious balance, as is the case for many aquatic habitats. Since this is the first record in Italy and given the limited number of scientific studies available, it remains difficult to accurately assess its condition or make systematic comparisons.



Figure 3. Diborrato waterfall (Colle di Val d'Elsa, Siena, central Italy), where habitats 32A0 and 7220* are present. Habitat 32A0 occurs in the lateral parts of the image, where more water is present, while habitat 7220* is found in the central, drier area.

7220* Petrifying springs with tufa formation (*Cratoneurion*)

(Suppl. material 1: table S2)

This priority habitat, recognized under the Habitats Directive 92/43/EEC as 7220* develops near springs and on moist rock faces, where extreme environmental conditions support the establishment of distinctive communities dominated by non-vascular plants, particularly bryophytes (Onete et al. 2014; Couvreur et al. 2016). It can be found in a wide range of environments, including forests and open countryside, and is typically associated with the alliance *Cratoneurion commutati*. These formations are usually small in scale, either point-like or linear, and composed predominantly of hygrophilous and calcicolous mosses and liverworts. Through the absorption of carbon dioxide, these organisms facilitate the precipitation of calcium carbonate from water, leading to the formation of tufa or travertine, a biogenic rock (Couvreur et al. 2016; Denyer et al. 2023). In Italy, habitat 7220* is widespread across nearly all regions, although ecological and distributional studies remain limited. In Tuscany, as in other regions with active bryophyte specialists, it is relatively well documented but still generally underestimated (Rivieccio et al. 2024a). Due to the often small size of habitat patches and the need for specialist bryological knowledge, its identification is particularly challenging and requires both extensive fieldwork and subsequent laboratory analysis. In the Ex-ANPIL-Elsa area, it has been observed on humid rock faces and dripping surfaces, but not in sections with continuous water flow like those which are characteristic of habitat 32A0 (Fig. 3). These plant communities are physiognomically dominated by hygrophilous and calcicolous bryophytes such as *Cratoneuron filicinum*, *Palustriella commutata*, *Hymenostylium recurvirostrum*, *Oxyrrhynchium hians*, *Didymodon luridus*, *Pellia epiphylla*, and *Fissidens fontanus*, many of which are typical of habitat 7220* according to the European manual (European Commission 2013).

Comparison of habitats 32A0 and 7220* and proposal for Elsa River as a 32A0 Site

The only previous vegetation study on the Elsa River (Angiolini et al. 1999) focused exclusively on vascular plants and did not record any bryophyte-dominated communities. By contrast, our study identifies two distinct assemblages of non-vascular vegetation, attributable to two different Habitats Directive (HD) types: habitat 32A0 and habitat 7220*.

Distinguishing habitat 32A0 from 7220* is challenging both from the ecological and taxonomic standpoints. Through the ecological process of absorption of carbon dioxide from light to continuous dripping waters, which could be also intermittent according to seasonality, the presence of habitat 7220* is often correlated with the abundant formation of tufa or travertine, a biogenic rock. This characteristic is a key ecological element which distinguishes

habitat 7220* from habitat 32A0. Conversely, the latter occurs in areas affected by continuous dripping to flowing waters, less affected by seasonality, with a limited formation of biogenic rocks. Considering the taxonomic comparison, habitats 7220* and 32A0 can be confused when only species composition is examined. In fact, according to the Interpretation Manual of European Union Habitats (European Commission 2013), a group of species are characteristic of both habitat 7220* and 32A0, thereby allowing a partial comparative analysis. For this reason, analysing both the local ecological conditions and the composition of bryophytes within the community is mandatory to discriminate between these habitats. Habitat 32A0 includes bryophyte-dominated communities developing on waterfalls and in splash zones, where fast-flowing, aerated water imposes strong physical and chemical stress. These conditions select for specialized mosses and liverworts adapted to frequent submersion and high hydraulic energy like *Fontinalis antipyretica*, *Apopellia endiviifolia*, and *Rhynostegium riparioides*. By contrast, habitat 7220*, is a priority habitat occurring around springs and dripping calcareous rock faces where water rich in calcium carbonate leads to tufa (travertine) formation. Bryophytes in these communities, such as *Cratoneuron filicinum* and *Hymenostylium recurvirostrum*, not only tolerate humid and calcareous conditions but also actively promote carbonate precipitation, contributing to biogenic rock development.

The multivariate analysis of our dataset shows a clear separation of the two habitats based on their main environmental drivers (Fig. 4), confirming our interpretation. According to the permutation test, the Redundancy Analysis (RDA) was overall significant ($p < 0.01$), with only the first constrained axis significant ($p < 0.05$). The adjusted R^2 of the RDA was 0.16, with a cumulative proportion of constrained variance explained by the first two axes of 76.2%. Water availability was the only significant constraint ($p < 0.001$) with a contribution to the constrained variance of 41.9%. For this reason, our results show that Habitat 32A0 and 7220* have a distinct separation based on hydrological characteristics: while habitat 32A0 develops within active waterfalls with continuous water flow and availability and low carbonate deposition, habitat 7220* is correlated with slow-dripping water and high tufa accumulation. Although similarities in species composition occur (e.g. *Palustriella commutata* and *Eucladium verticillatum*), our results show that, at least in the context of the Elsa River, the frequent co-occurrence or mosaic distribution of these habitats makes hydrology and carbonate dynamics critical factors for correct habitat classification. Moreover, species like *Pellia epiphylla* and *Gymnostomum calcareum*, characteristic of habitat 7220* relevés in our study area according to the Italian manual, are not listed as indicators for this habitat in the European manual. This discrepancy highlights the critical need for better harmonization between national and EU-level reference documents.

Thus, based on our results, we propose recognizing Ex-ANPIL-Elsa as a site hosting habitat 32A0 “Waterfalls with bryophyte communities”, supporting its formal

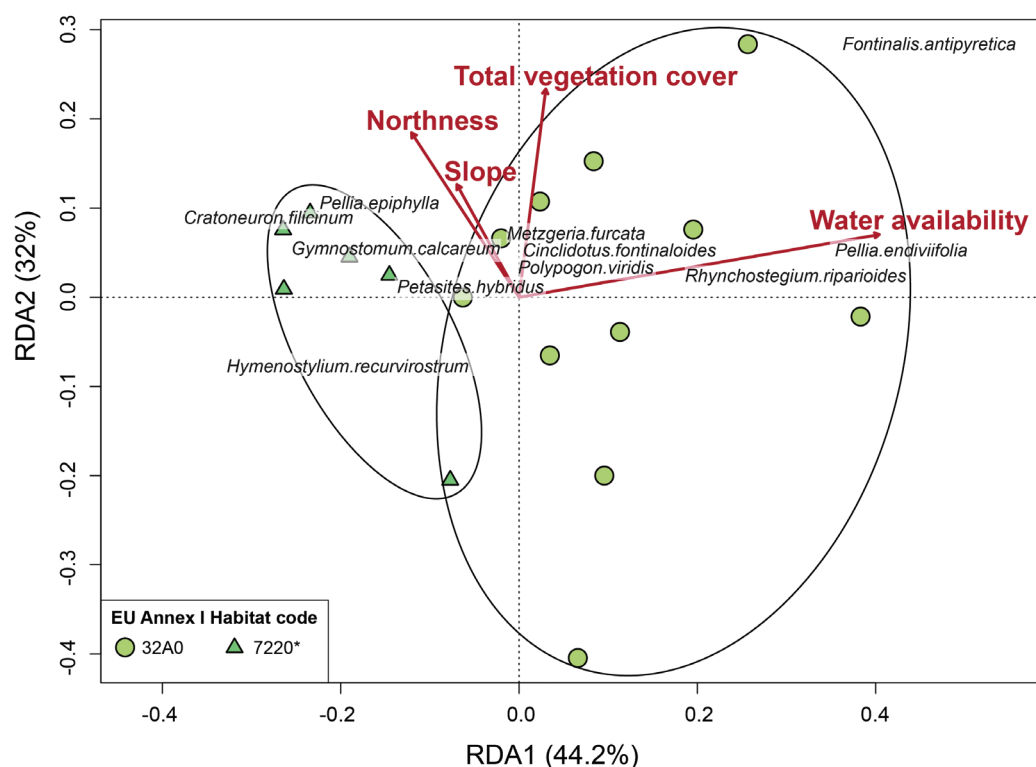


Figure 4. Redundancy analysis of the Hellinger-transformed Sites $\times$ Species matrix for habitat 32A0 and 7220*. Sites are plotted using their fitted site scores (lc scores). A scaling 1 was used for visualising the correlation triplot. Red arrows indicate the main environmental drivers of the two habitats.

inclusion in Annex I of the Habitats Directive for Italy, in line with the ecological and floristic evidence and the recommendations by Biondi et al. (2014b).

Group 2 – Aquatic communities dominated by algae and hydrophytic species

Habitat 3140 Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (Suppl. material 1: table S3)

This is one of the few freshwater habitats dominated by green algae belonging to the Characeae family, which form dense underwater meadows (Stefanidis et al. 2019). This habitat is quite common throughout Italy but has been little studied due to the difficulty in species identification (Pagana et al. 2024). According to recent reports from Tuscany, it appears to be present in many areas, both in large lakes and in small ponds (Rivieccio et al. 2024a, 2024b, 2025). This habitat is present within the study area in association with habitat 3150 – Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*-type vegetation, occurring in pools within the dry branches of the Elsa River, as well as in ponds and certain temporary water bodies. Here, some plant communities attributable to this habitat are dominated by *Chara vulgaris*, a widespread perennial species in freshwater ecosystems that forms submerged meadows, providing essential refuge and foraging areas for numerous aquatic organisms (Schneider et al.

2015). Conversely, other communities are characterized by the dominance of *Tolypella glomerata*, an annual species adapted to aquatic environments with temporary or limited water levels (Schubert et al. 2024), which is relatively rare within the study area. Notably, *Nitella tenuissima* was recorded in just one relevé, a species of conservation concern with only a few documented occurrences in Tuscany (Ravera et al. 2024). Overall, within the study area this habitat is highly localized and in poor conservation status, a condition mainly linked to the local morphology and hydrological characteristics rather than to significant anthropogenic pressures.

3150 Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*-type vegetation (Suppl. material 1: table S4)

Habitat 3150 includes species-poor plant communities growing in lakes and ponds, with azonal vegetation belonging to the *Lemnetea* or *Potametea* classes (Biondi et al. 2012). In Italy and Tuscany, it occurs in most water bodies and is commonly found in areas where it was previously unreported (Tavilla et al. 2022). Within the Ex-ANPIL-Elsa, azonal hydrophytic vegetation associated with this habitat develops in pools adjacent to the river, forming small and species-poor communities. The floating layer of these communities is dominated by *Lemna minor*, while the submerged vegetation consists of rooted hydrophytes, primarily *Potamogeton crispus* and *Zannichellia palustris*. This habitat, although present in the

study area, is spatially limited and often occurs in a mosaic with habitat 3140. The pools where this habitat occurs are small in size or characterized by low light availability, which restricts plant growth and diversity, resulting in generally low ecological quality and a conservation status that can be assessed as unfavourable.

3260 Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation

(Suppl. material 1: table S5)

Habitat dominated by aquatic macrophytes, with vegetation divided into two types: submerged vegetation exposed to strong currents (*Ranunculion fluitantis*) and floating vegetation found in areas with weaker currents (*Callitricho-Batrachion*; Biondi et al. 2009). This habitat is present throughout Italy and is among the most common along rivers and streams. In Tuscany, it is found in all the major rivers of the region (Rivieccio et al. 2021; Tavilla et al. 2022). One of the main challenges is distinguishing it from habitat 3290 – Intermittently flowing Mediterranean rivers of the *Paspalo-Agrostidion*, with which it shares some species (Lastrucci et al. 2010a). This habitat represents the most widespread aquatic vegetation type within the Ex-ANPIL-Elsa, occurring along most of its course, except in sections where strong currents, excessive water depth, or high shading levels prevent macrophyte colonization. Some aspects are dominated by *Potamogeton natans*, a species indicative of slow-flowing or stagnant waters; others, where the current is slightly stronger, are dominated by *Potamogeton nodosus*, often associated with *Myriophyllum spicatum* and *Zannichellia palustris*. A third community, identified within this habitat, is dominated by *Myriophyllum spicatum*, which has most of its biomass submerged. Overall, this habitat is well represented and generally in good conservation status within the study area, although localized pressures from bathing activities negatively affect some stretches of the river.

3290 Intermittently flowing Mediterranean rivers of the *Paspalo-Agrostidion*

(Suppl. material 1: table S6)

This habitat can be found in intermittent rivers where the riverbed remains dry for part of the year or where only isolated pools persist. Similar to habitat 3280, but characteristic of river stretches that undergo seasonal drying, it forms a continuum with habitat 3260, with which it shares some species (Lastrucci et al. 2010a; Biondi et al. 2012). In Italy, it is mainly found in Mediterranean rivers and is absent in the north. In Tuscany, it is primarily located in the central-southern areas, especially in medium-small rivers (Lastrucci et al. 2010a). The similarities with habitats 3280 and 3260 can make its classification challenging. In the study area the habitat consists of hygrophilous-nitrophilous herbaceous vegetation that develops in the

secondary branches of the Elsa River with intermittent flow. Here, this habitat is relatively rare since the main branch maintains a continuous water flow, while the secondary branches, which experience drying periods with only residual pools where the habitat establishes, are few and short in length. Characteristic species found in the study area include *Potamogeton natans*, *P. nodosus*, *Myriophyllum spicatum*, and *Paspalum distichum*. Overall, within the study area, this habitat is scarce and confined to a few short stretches, with an unfavourable conservation status due to river morphology and local conditions that are not optimal for its persistence.

Group 3 – Communities of tall-herb humid meadows

3270 Rivers with muddy banks with *Chenopodium rubri* p.p. and *Bidention* p.p. vegetation

(Suppl. material 1: table S7)

The habitat is characterized by pioneer hygrophilous-nitrophilous vegetation, dominated by annual species with a summer-autumn growth cycle. These communities develop on sandy, silty, clayey, and gravelly substrates that are only periodically flooded (Angiolini et al. 2005; Pellizzari 2020). They are often found in mosaics with other habitat types, from which a contingent of species not strictly typical of habitat 3270 may enter (Arrigoni and Papini 2003). In Italy and Tuscany, it is present in many rivers, especially in lowland areas where it has more ecological space. Compared to many riverine habitats, it reaches its optimal sampling period later in the season, and if surveyed too early, it may be undetected (Lastrucci et al. 2012). This habitat often forms a mosaic with other habitats that develop along the riverbank, but it is never very extensive due to the limited availability of suitable ecological space (Angiolini et al. 2005). According to these findings, the communities that characterize this habitat never reach large dimensions in the Ex-ANPIL-Elsa, as observed in major lowland rivers, also due to the lack of the necessary morphological characteristics in the stretch of the Elsa River (Assini 1998). This habitat can be rich in alien species, which play a significant role in determining its structure and function. In our study case the alien component of the habitat is represented by *Xanthium orientale* subsp. *italicum* and *Bidens frondosa*. Habitat 3270 is one of the rare cases where some alien species are characteristic and diagnostic (Lastrucci et al. 2012). Other typical species such as *Bidens tripartita*, *Mentha aquatica*, *Persicaria lapathifolia*, and *Cyperus fuscus* are frequent in our relevés. It is one of the least threatened habitats, at least in the study area, both due to the natural presence of alien species and its late phenology. Within the study area, this habitat shows a good conservation status, although it remains fragmented and confined to a few suitable sites compared to the broader development observed in other rivers.

3280 Constantly flowing Mediterranean rivers with *Paspalo-Agrostidion* species and hanging curtains of *Salix* and *Populus alba*

(Suppl. material 1: table S8)

This habitat colonizes fine-grained (silty) river deposits that are very moist, submerged for part of the year, and rich in organic material (Biondi et al. 2002). It is in contact on one side with forest habitats such as 92A0 – *Salix alba* and *Populus alba* galleries and 91E0* – Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*), and on the other with aquatic habitats such as 3260 and 3290 (Arrigoni and Papini 2003). In Italy and Tuscany, it is widespread along the banks of the intermediate and lower stretches of rivers (Mereu et al. 2012; Morabito et al. 2023). One of the main challenges is determining whether the community should be classified as habitat 3280 or 3290, especially in braided rivers (Baldoni and Biondi 1993; Angiolini et al. 2005). In the Ex-ANPIL-Elsa the hygrophilous meadows referable to habitat 3280 are dominated by rhizomatous grasses, particularly *Paspalum distichum*, which can form mono-specific grasslands, often associated with other grasses such as *Polypogon viridis* and creeping species like *Ranunculus repens*, all typical species of the habitat (Lastrucci et al. 2007). Along with habitat 3270, it is one of the rare cases where some alien species are characteristic and diagnostic; in our case, an example is *Paspalum distichum* (Assini 1998). It is common along the banks of the Elsa River, where it is found in contact with both habitat 3290 and the tall herb communities of habitats 6420 and 6430, which often replace it in areas further from the water or in more stable environments. Overall, in the study area this habitat shows a good conservation status, although it frequently occurs in a mosaic with adjacent habitats.

6420 Mediterranean tall humid herb grasslands of the *Molinio-Holoschoenion*

(Suppl. material 1: table S9)

The habitat is characterized by extensive hygrophilous herbaceous vegetation, primarily dominated by rush species that grow in moist environments but can tolerate periods of drought (Lastrucci et al. 2010b). This habitat is present throughout most of Italy, developing mainly in coastal wetland areas but also found along rivers and lakes in the interior. It is less frequent in northern Italy. In Tuscany, it occurs both along the coasts and in inland wetland areas (Lastrucci et al. 2010b; Landucci et al. 2013). This habitat has also been reported in other areas along the Elsa River, as noted by Riveccio et al. (2022). These communities are not particularly widespread within the Ex-ANPIL-Elsa, and where they occur, they cover relatively small surfaces. Here, the reference plant species of the habitat, that are also the dominant ones, are mainly graminoids, such as *Scirpoides holoschoenus*, *Agrostis stolonifera*, and *Calamagrostis epigejos*, accompanied by non-graminoid species like *Eupatorium cannabinum* and

Pulicaria dysenterica. Other frequent species are hygrophilous, such as *Lythrum salicaria* and *Mentha aquatica*, or alien as *Echinochloa crus-galli*, to indicate contacts with other habitats such as 3270 and 6430. The conservation of this habitat is closely tied to the alternation between flooding and water recession periods, without which the habitat tends to disappear (Lastrucci et al. 2010b). Overall, in the study area the habitat occurs in small patches but shows a good conservation status.

6430 Hygrophilous tall herb fringe communities of plains and of the montane to alpine levels

(Suppl. material 1: table S10)

The habitat is characterized by extensive hygrophilous and nitrophilous herbaceous vegetation, classified into two subtypes: lowland-hilly and montane-alpine (Biondi et al. 2009). By contrast to the communities constituting habitat 6420, this habitat is dominated by broad-leaved species, also named megaforbes (Morabito et al. 2024). It represents a widespread habitat type in Italy, particularly in Tuscany, where it commonly occurs along riverbanks and as an understory layer within humid or montane forests (Morabito et al. 2023). It develops along the banks of the Elsa River, especially in clearings within mesohygrophilous woodlands, as an ecotonal margin of *Alnus glutinosa* and *Populus* spp. stands, or along stabilized riverbanks. Within the Ex-ANPIL-Elsa, the characteristic flora consists mainly of mesophilic or hygrophilous species, which are relatively common; notable species include *Epilobium hirsutum*, *Petasites hybridus*, *Aegopodium podagraria*, *Lythrum salicaria*, and *Arctium lappa*, which are associated with the lowland-hilly subtype of 6430 (Fanfarillo et al. 2023). This habitat occurs extensively within the study area and shows an excellent conservation status, colonizing wide areas along the river.

Group 4 – Communities dominated by woody species

5130 *Juniperus communis* formations on heaths or calcareous grasslands

(Suppl. material 1: table S11)

This habitat, represented by shrubland pastures dominated by *Juniperus communis* and often featuring numerous shrubby species of the Rosaceae family and a high specific diversity, is a transitional habitat between pasture and forest (Evangelista et al. 2016). With the gradual abandonment of extensive pastoralism, this habitat initially expanded but later declined due to its rapid dynamism, quickly transitioning from pasture to shrubland pasture and eventually to forest. Although these shrublands are unstable communities in continuous evolution, they play a fundamental ecological role by serving as refuge for numerous animal species (Assini et al. 2014). Habitat 5130 is widely distributed in peninsular Italy, particularly in the

Apennines (Filibeck et al. 2025). In Tuscany, it is mainly found in the Apennine area and in other less anthropized regions such as the Metalliferous Hills and the Tuff hills of southern Tuscany. In more anthropized and industrial areas where pastoralism has long been absent, this habitat is rare. For this reason, in the study area, this habitat is rare due to pressures from urbanization and agriculture, making it a distinctive element that contributes to the phytocoenotic diversity. In particular, the communities present within the Ex-ANPIL-Elsa are represented by *Juniperus communis*, associated with *Prunus spinosa*, *Cornus sanguinea*, and *Crataegus monogyna*. The herbaceous layer hosts thermo-heliophilous species such as *Bromopsis erecta* and *Brachypodium rupestre*. However, the surfaces are extremely limited in size and in a highly dynamic state, resulting in a very poor conservation status and a likely disappearance in the coming years due to vegetation succession. Moreover, the study area is not particularly suitable for the development of this habitat, which further limits its occurrence and explains its unfavourable conservation condition.

91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae)
(Suppl. material 1: table S12)

This habitat develops in conditions with a shallow water table or in proximity to rivers, marshes, and, more rarely, lakes. It is a priority habitat primarily found in temperate areas as well as in Mediterranean hilly or montane regions (Angiolini et al. 2005; Landi et al. 2009), where it is often linked to a headwater section of river with high moisture level (Angiolini et al. 2011, 2017). In Italy, it is mostly present in the northern regions and along the Apennines, whereas in more Mediterranean contexts, it is replaced by poplar-willow woodlands (habitat 92A0) (Sciandrello et al. 2022). The same pattern is observed in Tuscany, where it is quite frequent (Morabito et al. 2023). There are no particular issues regarding its identification; however, in smaller rivers, it may be present with minimal extensions, making it difficult to sample. Along the Elsa River, this habitat is represented by riparian woodlands dominated by *Alnus glutinosa*, which are relatively common but restricted to the vegetation belt closest to the riverbank. Here, *Alnus glutinosa* is one of the most common trees within the hygrophilous vegetation zone, occurring on sandy-silty deposits. Numerous mesophilic and hygrophilous broadleaved species, such as *Acer campestre*, *Fraxinus excelsior*, and *Sambucus nigra*, contribute to both the dominant tree layer and the shrub layer, where *Rubus caesius* is also present with good coverage. The herbaceous layer is generally rich in megaforbs, forming habitat 6430 when the tree canopy becomes very sparse or is completely absent, and other hygrophilous, shade-tolerant, and nitrophilous species, such as *Petasites hybridus*, *Carex pendula*, and *Equisetum telmateia* become dominant. The conservation status of this habitat is generally good, in some areas even excellent, but it is negatively affected in

places subject to anthropogenic disturbance and in some patches where alien species, especially *Ailanthus altissima* and *Robinia pseudoacacia*, exert a strong impact.

91L0 Illyrian oak-hornbeam forests (*Erythronio-Carpinion*)

(Suppl. material 1: table S13)

Habitat 91L0 consists of various forest types, all characterized by mesophilous woodlands dominated by certain *Quercus* species and *Carpinus betulus*, which primarily develop in cool environments (Biondi et al. 2012; Jelinčić et al. 2024). In Italy, this habitat is mainly found in central and northeastern regions, while in Tuscany, it is primarily located in the Apennines and other hilly reliefs (Viciani et al. 2018). One of the main challenges is the field characterization of this habitat, as it frequently grades into beech forests, turkey oak, alder woods, and ash forests (Biondi et al. 2010). In the study area, the habitat is represented by mesophilous woodlands dominated by *Carpinus betulus*. These forest communities are characterized by significant vertical development and are found in the cooler areas of the Ex-ANPIL-Elsa. The tree layer is dominated by *Carpinus betulus*, accompanied by *Quercus cerris*, *Quercus robur*, *Corylus avellana*, and *Ulmus minor*. The herbaceous layer hosts an abundant nemoral flora typical of cool, humid woodlands. Among the most frequent species in the understory are *Hedera helix*, *Helleborus viridis* subsp. *bocconeii*, *Euphorbia amygdaloides*, *Aegopodium podagraria*, *Anemone apennina*, and *Primula vulgaris*. This is one of the rarest and most fragmented habitats in the area, existing in a dynamic relationship with shrublands of the *Prunetalia spinosae* order on one side and with the riparian woodlands of habitats 92A0 and 91E0* on the other hand. Overall, within the study area habitat 91L0 is limited in extent and shows a medium conservation status, due both to anthropogenic disturbance and to the morphological conditions of the site.

91AA* Eastern white oak woods

(Suppl. material 1: table S14)

The habitat consists of thermophilous Mediterranean woodlands dominated by species of the genus *Quercus* and other tree species such as *Fraxinus ornus*, *Carpinus* spp., and *Ostrya carpinifolia*, while the understory is more heterogeneous (De Chiro et al. 2014; Montecchiari et al. 2020). These forests are primarily found in edaphic-xerophilous positions and are mainly distributed in peninsular Italy and the islands, becoming rarer in the northern regions (Blasi et al. 2004; Proietti et al. 2021). In Tuscany, these woodlands are relatively common, especially in the hilly areas. Towards the coast, they are replaced by holm oak forests (*Quercus ilex*, habitat 9340), while in cooler areas, they may grade into beech forests or woodlands dominated by *Ostrya carpinifolia* and *Quercus cerris*, particularly in environments with more silicates or acidic soils (Biondi et al. 2003; Blasi et al. 2006). Identification and

sampling of this habitat do not present major challenges, except in transition zones where it comes into contact with other similar forest communities (Alberdi et al. 2019; Pesaresi et al. 2022). This habitat occurs in various parts of the Ex-ANPIL-Elsa, mainly in the initial section before reaching inhabited areas, where river-associated woodlands and urbanization-related habitat reduction led to its rarity and fragmentation. Within the study area, the habitat is composed of thermophilous woodlands dominated by *Quercus pubescens*, often associated with *Fraxinus ornus* and *Quercus cerris*. The shrub layer is dominated by *Ligustrum vulgare* and *Crataegus monogyna*, *Asparagus acutifolius* and *Rosa sempervirens* while in the herbaceous layer, *Hedera helix*, *Aegonychon purpureoeruleum*, and *Rubia peregrina* are predominant. Overall, its conservation status is not favourable, as the habitat is present but with limited extent, often invaded by species not fully typical of this forest type, and restricted to small areas frequently surrounded by anthropogenic structures or agricultural fields, particularly in zones further away from the river.

92A0 *Salix alba* and *Populus alba* galleries

(Suppl. material 1: table S15)

Among the most common woodlands along Mediterranean rivers are poplar and willow stands, which are often found together in a forest matrix forming habitat 92A0 (Ceschin and Salerno 2008; Gennai et al. 2021). These woodlands are communities that can occur both near the riverbed and at distances of several hundred meters, resulting in a heterogeneous herbaceous and shrub layer due to the numerous contacts with other vegetation types. They are widely distributed along the flat stretches of watercourses in peninsular Italy and Tuscany (Carli et al. 2016). However, moving northward and particularly in hilly and montane areas, these communities decrease in both frequency and extent (Foggi et al. 2017; Sciandrello et al. 2022). From an identification and sampling perspective, no significant challenges arise, except in transitional zones where the structure shifts from a shrub-dominated formation to an arboreal one (Foggi et al. 2017). Together with *Alnus glutinosa* woodlands (91E0*), with which they frequently occur in sequence, they represent one of the most common forest communities within the Ex-ANPIL-Elsa, although due to the morphology of the river valley, they form relatively limited extensions. In the tree layer of our relevés, species of *Salix alba*, *Populus alba* and sometimes *P. nigra* dominate, while in the shrub layer, the most frequent species are *Acer campestre*, *Cornus sanguinea* subsp. *hungarica*, and *Crataegus monogyna*. In the herbaceous layer, *Brachypodium sylvaticum*, *Hedera helix*, *Ranunculus repens*, and *Carex pendula* are commonly found. Overall, this habitat shows a medium conservation status in the study area: the incised morphology of the river, with riparian zones limited in width, allows its presence but only in reduced extensions. The main threat to its conservation is the spread of alien species, particularly *Ailanthus altissima* and *Robinia pseudoacacia*.

Threats and Conservation of Elsa River riverine habitats

The Ex-ANPIL-Elsa habitats are under significant threat from human activities, jeopardizing their delicate ecological balance and the diverse species and habitats they support. These pressures often intertwine, leading to a cascade of negative effects. One of the most pressing concerns is alterations to the hydrological regime. Habitats such as 3260, 3290, 32A0, 6420, and 7220*, all heavily reliant on consistent water availability, are particularly vulnerable. The diversion of water for agricultural and industrial uses, coupled with artificial flow regulation, can drastically plummet the river's discharge. This reduction in water availability leads to habitat fragmentation and the loss of sensitive species unable to adapt. For example, a significant drop in discharge could cause the complete drying up of lateral branches, which host habitat 3290. Habitat 6420 is also acutely affected by an altered hydrological regime; prolonged dry phases can lead to the colonization by hygrophilous shrubs like *Salix* spp. and *Populus* spp., leading to the entire replacement of the original habitat. Beyond water amount, also water quality is severely compromised, largely due to eutrophication and pollution. The inflow of chemicals from agricultural runoff and urban waste introduce an excess of nutrients into the river. This overload not only alters the plant composition, favouring some species over others, but also diminishes overall water quality. Habitats like 3140, 3150, 3260, 6420, and 7220* are directly impacted, experiencing significant shifts in their floristic composition and overall ecosystem degradation. In some cases, pollution also includes chemical or biological contamination, further disrupting the delicate balance of these aquatic environments. Physical disturbances also pose a significant threat to these fragile habitats. Intense recreational activities, particularly bathing in certain river sections, can cause a "trampling" effect. This is especially damaging to habitats 32A0 and 7220*, where the physical presence and movement of people directly harm and disrupt delicate structures and organisms. Habitat 3280 is particularly susceptible to this intense recreational bathing. Furthermore, physical modifications to riverbanks, including channelization, urbanization, and artificialization, lead to direct habitat loss and reduced ecological connectivity. These modifications are a primary threat for habitat 3270, reducing its colonizable area, and also significantly impact 3280 and 6430. Habitat 6430 also faces threats from the use of heavy machinery during riverbank alterations. Another widespread and pervasive threat is the invasion of alien species, which disrupts the structure and dynamics of ecosystems, compromising local biodiversity. Invasive species such as *Robinia pseudoacacia* and *Ailanthus altissima* rapidly colonize riparian forest formations (such as 91E0* and 92A0), altering their structure and ecological functions. Similarly, alien plants change the composition of herbaceous communities of habitat 6430. On the contrary, according to Bolpagni (2013), habitat 3270 and 3280 are dominated by alien plants but can be considered of conservation interests, performing important ecosystem roles

or service as refuges for animal species. In forested areas, deforestation and timber exploitation contribute to ecosystem fragmentation, favouring the spread of invasive species and jeopardizing habitats of high conservation value such as 91AA*, 91E0*, 91L0, and 92A0. Habitat 91AA*, predominantly located in agricultural areas, faces significant pressure from agricultural expansion and forest logging for timber production. For abandoned pastures like 5130, while currently without active pressures, there is a potential threat of landowners undertaking cutting operations to restore pastureland, leading to habitat alteration.

Beyond land-use pressures, recreational activities, especially river-related tourism, which has grown exponentially in the last 10 years, represent an additional disturbance. Trampling and bathing in areas with travertine formations can alter moss and algae communities, threatening the ecological stability of habitats 32A0 and 7220*. Finally, agricultural expansion poses an ongoing pressure on Elsa River ecosystems, as the progressive conversion of natural environments into cultivated land leads to the contraction of habitats such as 5130, 91AA*, 91L0, and 92A0.

The interplay of these disturbances highlights the urgent need for targeted conservation and management strategies to preserve the biodiversity of the Elsa River and ensure the long-term ecological functionality of these riverine ecosystems.

Conclusions

This study provides a comprehensive assessment of the distribution and conservation status of riverine habitats within the Ex-ANPIL-Elsa (Siena, central Italy), highlighting their exceptional richness and ecological diversity. The area hosts 15 community-interest habitats, including one new HD habitat for Italy and two priority habitats of high conservation value. Our assessment confirms that Elsa River habitats are under strong anthropogenic pressures: unchecked urbanization, alterations of the hydrological regime, and the spread of invasive alien species such as *Robinia pseudoacacia* and *Ailanthus altissima*. These factors drive ecosystem fragmentation and degradation, threatening ecological stability and biodiversity. Among them, hydrological alterations are the most critical, directly affecting priority habitats such as 7220* and 91E0*. A key outcome is the urgent need to update the national habitat classification system of Italy to include new habitats like 3180 and 32A0. Despite being recognized in the 2013 EU manual, 32A0 is absent from the outdated 2009 Italian manual, often misclassified as 7220*. Our findings confirm its presence and its clear ecological and floristic distinction from 7220*: the former associated with waterfalls and rapids shaped by strong hydrodynamics, the latter with dripping rock faces and travertine formation. Correct classification is essential for effective preservation. From a conservation perspective, this study strongly advocates for strengthening protection measures for the Ex-ANPIL-Elsa. Its high biodiversity and key role in ecological connectivity make enhanced

protection crucial. Currently, the regulation of the area is limited to the municipal level, lacking broader regional or EU-level protection. However, the potential inclusion of the study area within the Nature 2000 network could represent a significant stride towards the protection and sustainable management of its riverine habitats. This would directly contribute to the ambitious goals of the EU Biodiversity Strategy 2030, specifically the target of protecting 30% of European land and sea areas (Spiliopoulou et al. 2023). In this perspective, the Municipality of Colle di Val d'Elsa has already undertaken an initiative to include the area within the SAC of the "Montagnola Senese" (IT5190003), a crucial step towards securing its future. Inclusion within the Natura 2000 network would enable the conservation of riverine HD habitats through several concrete actions such as: i) continuous monitoring of habitat conservation status to track changes and inform adaptive management; ii) stronger land-use regulations to effectively limit the negative impact of urbanization on water quality and overall ecosystem stability; iii) targeted control of invasive alien species through strategic interventions to curb the spread of species that disrupt habitat structure and functionality.

In conclusion, the Ex-Anpil-Elsa is an area of remarkable ecological and conservation value. Preserving it demands integrated management strategies based on updated scientific data and supported by a more robust regulatory framework. Only through effective and targeted protection measures will we be able to safeguard its unique biodiversity and ensure its long-term ecological functionality. These findings underscore the critical need for dedicated conservation efforts to maintain the ecological and functional diversity of fluvial and riparian ecosystems.

Competing interests

The authors have declared that no competing interests exist.

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Data availability

Data are available in Suppl. material 1.

Author contributions

Relevés with a dominance of vascular plants were collected by Tiberio Fiaschi and Silvia Cannucci. Relevés with a dominance of algae were collected by Tiberio Fiaschi. Relevés with a dominance of bryophytes were collected by Ilaria Bonini, Giulio Pandeli, and Tiberio Fiaschi.

Tiberio Fiaschi: Conceptualization, Methodology, Investigation, Data Curation, Visualization, Writing – Original Draft. Leopoldo de Simone: Conceptualization, Methodology, Investigation, Data Curation, Visualization, Formal analysis, Writing – Original Draft. Francesco Mascia: Writing – Review & Editing, Validation. Ilaria Bonini: Writing – Review & Editing, Validation. Bruno Foggi: Writing – Review & Editing, Validation. Matilde Gennai: Writing – Review & Editing, Validation. Giulio Pandeli: Writing – Review & Editing, Validation. Simona Maccherini: Writing – Review & Editing, Validation. Emanuele Fanfarillo: Writing – Review & Editing, Validation. Claudia Angiolini: Conceptualization, Methodology, Validation, Writing – Original Draft, Project administration.

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Supplementary material 1

Original vegetation relevés

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Data type: xlsx

Explanation note: These are the original vegetation relevés used in the manuscript.

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