METHODOLOGICAL GUIDE FOR THE CONSERVATION OF AQUATIC DIVERSITY IN THE PICOS DE EUROPA NATIONAL PARK

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1. Background

This document is part of the DIVAQUA project - *Improving aquatic diversity in the Picos de Europa* - cofounded by the European Commission's LIFE+ program under the Nature and Biodiversity sub-program.

The main objective of DIVAQUA is to improve the conservation status of habitats and species of community interest (those described in Annexes I, II, and IV of the Habitat Directive; 92/43/EEC) in the aquatic ecosystems of the Picos de Europa National Park and the surrounding area. To achieve this, the project includes the development of 3 preparatory actions (Action A) and 4 conservation actions (Action C), in addition to others related to the monitoring of these (Action D), as well as the dissemination of the project and its most relevant results (Action E).

In this context, the present proposal for a Methodological Guide has been developed as part of Action A3: *Development of guidelines and a methodological guide for the conservation of aquatic diversity in mountain areas*, resulting directly from sub-action A3.4: *Proposal for a methodological guide for the conservation of aquatic diversity*.

2. Introduction

2.1. Mountain aquatic ecosystems facing global change.

Continental aquatic ecosystems (e.g., rivers, lakes, etc.) are highly sensitive to global change, defined as large-scale environmental transformations caused by the intensification of human activities that can affect the atmosphere, water resources, soil, biodiversity patterns, etc. Among the different continental aquatic ecosystems, those associated with mountain areas are particularly sensitive to these changes, due to their small size (i.e., volume, flow, or depth), which makes them very vulnerable to variations in the natural hydrological and thermal regime (Schmeller et al., 2018). Additionally, higher altitude at which these ecosystems are found create unique environmental conditions, such as steep terrain and sharp temperature gradients, which support organisms and biological communities that are highly adapted to these conditions. These conditions, in turn, can be considered adverse for other organisms and communities that thrive more successfully in lower parts of the watershed (Körner, 2007).

Due to their high sensitivity and the dominance of altitude-dependent environmental conditions, mountain aquatic ecosystems respond particularly strong to changes in climatic conditions. These changes severely affect mountain aquatic biological communities, such as invertebrates (Cereghino & Lavandier, 1998) or fish (Jonsson & Jonsson, 2011), as well as several metabolic processes occurring in these ecosystems (e.g., primary production or respiration; Estévez et al., 2017). As result, mountain aquatic ecosystems are especially impacted by climate change.

In the last decades, significant changes in land-uses and vegetation cover in mountain areas around the world have been observed. The replacement of forested areas by agricultural, livestock, or recreational uses, such as ski resorts, can cause runoff problems, as these changes in vegetation cover tend to increase erosion and hillside transport processes, as well as the sediment load to aquatic ecosystems (i.e., rivers, lakes, and wetlands). Higher entry of such materials into watercourses, favoured by the steep mountainous terrain and the degradation of vegetation cover, leads to greater sedimentation of the aquatic bed, producing significant changes in the aquatic communities of primary producers (Niedermayr & Schagerl, 2010), as well as a subsequent cascade of trophic and metabolic effects on other communities and processes within these ecosystems.

Another activity that particularly affects mountain areas, due to their specific topography that facilitates the presence of gorges and steep slopes, is the artificial storage of water for hydroelectric power generation, irrigation, or water supply. This activity, and the associated hydraulic infrastructures (i.e., dams and reservoirs), produces significant changes in the hydrological regime and hydraulic conditions of the affected rivers, producing major changes in both, the abiotic (e.g., composition of the riverbed) and biotic elements (e.g., structure and composition of river communities; Cereghino & Lavandier, 1998). Additionally, the environmental changes brought about by these infrastructures often facilitate the expansion of invasive species, which take advantage of these changes over native organisms. This has been documented in mountain rivers with hydroelectric uses affected by the invasive diatom *Didymosphenia geminata* (Ladrera et al., 2015).

Another significant environmental problem described in mountain aquatic environments in recent decades is related to the expansion of new pathogens and diseases. This phenomenon may be originally linked to changes in natural environmental conditions, as well as to the tourism activities that take place in some mountain systems, where the numerous worldwide visitors may act as vectors of new pathogens. For example, the Picos de Europa National Park (in northern Spain) had around 2 million visitors in 2016. In this mountain area, the presence of a new *Ranavirus* from the Iridoviridae family has been detected. This pathogen, not recorded in this area until the 21st century, is causing the collapse of many amphibian populations native to this National Park, and it is believed that its arrival and spread may be related to the massive presence of visitors in certain parts of this area (Price et al., 2014).

In addition to the uniqueness and fragility that mountain aquatic ecosystems show in response to environmental changes induced by human activity, these ecosystems must be conserved and protected for the large number of benefits they provide to human societies. Mountain aquatic ecosystems provide resources to human society and are also capable to regulate different environmental processes, as well as to improve the quality of life for people who visit them during their leisure time. The resources and services provided by natural or semi-natural ecosystems are referred to as ecosystem resources, and they are particularly relevant in mountain aquatic ecosystems. For example, according to Viviroli and collaborators (2007), mountains can be considered "Water Towers," as they typically receive higher precipitation, which they can store as lakes, snow, ice, wetlands, or groundwater, and later distribute during drier and warmer periods to lower areas of the watershed.

In order to protect mountain ecosystems and the ecosystem services (EESS) they provide to human societies, approximately 19% of the world's mountain surface, excluding Antarctica, currently has some form of protection (e.g., National Park, LIC/ZEC of the Natura 2000 Network, etc.; Jacobs et al., 2021).

2.2. Objectives

Despite the importance and fragility described, there is currently no common and global proposal to monitor and assess the conservation status of mountain aquatic ecosystems and their long-term response to global environmental changes (i.e., global change; Peñas et al., 2023). However, advancements made by projects like UNESCO GLOCHAMORE on a global scale, or by The Eastern Rivers and Mountains Network on a regional scale, in the USA, should be considered. This means that, in many cases, these monitoring systems are not based on appropriate sampling designs, leading to the following errors and weaknesses:

1. Low frequency in data/sample collection.
2. The use of different methodologies that are not comparable for achieving a common goal of characterization/evaluation.
3. The lack of definition of key variables/parameters needed to identify and quantify the effects that global change has on these ecosystems and the ecosystem services they provide to society.

In the specific case of Spain, 6 out of 10 of the country's mainland National Parks (NPs) are located in mountain and high mountain areas (Fig. 1). Despite being in the same country and have relatively similar environmental conditions, the monitoring programs of these NPs do not follow a common methodological framework or guide. Therefore, each NP establishes its own criteria, methods, and monitoring protocols.

Thus, comparing results between different areas is challenging, making it difficult to identify environmental trends and patterns on broad spatial and temporal scales (Bonache et al., 2016). In this regard, it is necessary to establish common protocols and apply them continuously over long periods of time (i.e., >10 years), as well as to harmonize and standardize the collection of field data and samples, defining an appropriate frequency to allow the characterization of different environmental processes and changes associated with the intensification or abandonment of human activities (daily, monthly, seasonal, annual data, etc. Haase et al., 2018).

Figure 1. Location of the 6 mountain National Parks of peninsular Spain. 1- Picos de Europa, 2- Ordesa y Monte Perdido, 3- Aigüestortes i Estany de Sant Maurici, 4- Sierra de Guadarrama, 5- Sierra de Las Nieves y 6- Sierra Nevada.

Following the deficits described above, as well as the experience from over a decade of monitoring the aquatic ecosystems of the Picos de Europa National Park (NP), partly thanks to the implementation of the LIFE DIVAQUA project, this Methodological Guide is presented to establish a proposal for a common framework to monitor and maintain the good condition of the mountain aquatic ecosystems of the Picos de Europa National Park, which can also be used as a reference for other mountain NNPP in Spain and the rest of Europe. To achieve this main objective, we also focus on different partial goals:

- Determine the most relevant environmental patterns (e.g., climatic, hydrological, etc.) for the conservation of mountain aquatic ecosystems and predict their future evolution under different scenarios.
- Identify the most important abiotic variables (e.g., water quantity and quality) to determine the conservation status of the different mountain aquatic ecosystems, and define the frequency of data/sample collection for their adequate characterization and evaluation.
- Identify the most significant biotic variables (i.e., metabolic processes and biological communities/populations) to assess the conservation status of the different mountain aquatic ecosystems, and define the frequency of data/sample collection for their proper characterization and evaluation.
- Determine the socioeconomic dynamics surrounding these ecosystems, as well as the relationships established between the natural and human environments (e.g., livestock farming, tourism, etc.).

3. Study area

A fundamental aspect for the development of a guide like the one presented in this document is the appropriate knowledge of the study área and its main elements. Below, the most relevant information needed to characterize the physical and biological environment of the Picos de Europa is summarized. Since almost all of the river ecosystems that drain the Picos de Europa are grouped into two different river basins, the Sella basin and the Deva-Cares basin, the area referred to in this section extends to the entire surface occupied by both basins, from their headwaters to their mouths (Fig. 2).

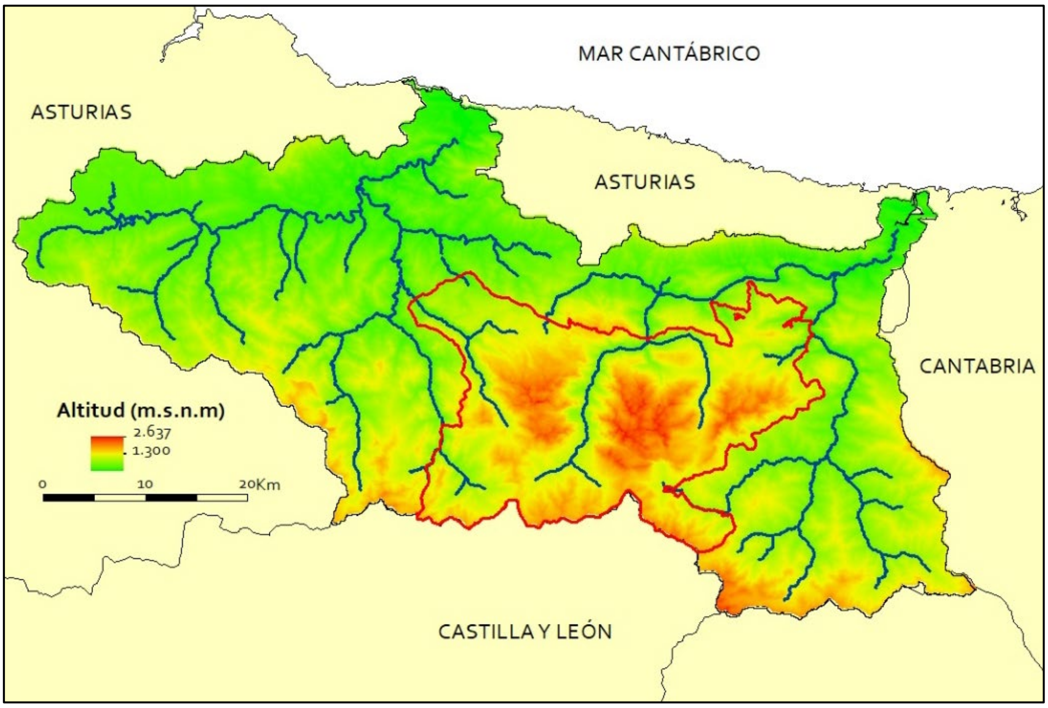


Figure 2 Limits and main hydrographic network of the Sella and Deva-Cares basin. The Picos de Europa National Park is delimited by a red line.

3.1. Physical environment

3.1.1. Geology and geomorphology

Sella and Deva-Cares basins are located in northern Spain and drain part of the Cantabrian Mountain Range, flowing into the Cantabrian Sea. The Cantabrian Mountain Range, in whose heart the Picos de Europa NP is located, is a mountain chain 400 km long that extends from west to east, parallel to the northern coastal line of Spain, between the autonomous communities of Galicia and Basque Country. Picos de Europa NP covers an area of 66,030 ha, extending across the provinces of Asturias (West; 27,477 ha), Cantabria (East; 14,973 ha), and León (South; 23,580 ha). The Cantabrian Mountain Range was originated during the Alpine orogeny and is flanked to the south by the sinorogenic basins of the Duero and Ebro rivers, and to the north by the Cantabrian Sea (Atlantic Ocean). The Cantabrian Mountain Range can be divided into three sectors:

1. Vasco-Cantabrian Sector, located in the eastern area. This sector features robust Mesozoic series and landforms that generally do not exceed 1,500 m.a.s.l.
2. Asturian Massif, located in the central area of this Mountain Range. Its maximum altitude reaches 2,648 meters at the summit of Torrecerredo Peak. In this sector, the Mesozoic cover has undergone significant erosion, exposing a Paleozoic basement uplifted during the Alpine deformation. The Picos de Europa NP is located in this massif.
3. Western Sector, which has lower elevations and does not present Mesozoic sediments. The Alpine deformation is recorded by some tertiary basins limited by faults

3.1.2. Climatology

Throughout the Cantabrian Mountain Range, the climate is predominantly temperate Atlantic, although it varies from temperate oceanic to sub-Mediterranean oceanic and hyper-oceanic sub-Mediterranean climates (Rivas-Martínez et al., 2004). Additionally, there are seven thermal-climatic belts distributed over just 150 km (termotemplated, mesotemplated, supratemplated, orotemplated, mesosubmediterranean, orosubmediterranean, and supramediterranean).

The average annual temperature ranges from 6ºC in the highest mountain áreas, to 15ºC in the more coastal locations. The average annual precipitation in the Picos de Europa NP varies between 800 mm in the Liébana Valley (Cantabria) and 3,000 mm in the wettest areas of Asturias. The highest precipitation occurs in winter and spring, although storms can appear at any time of the year. In the more mountainous areas, snowfall is common from late autumn until well into spring.

3.1.3. Hydrology

Within the Asturian Massif are the basins of the Sella and Deva-Cares rivers, draining the Picos de Europa NP at their headwaters. These rivers originate in the upper regions of the Cantabrian Mountain Range and, after a short course, flow into the Cantabrian Sea, making them rivers with a very steep gradient.

The Sella River basin has a drainage area of 1,284 km^2. The Piloña River, whith a drainage area of 509 km^2, is the most important tributary. The Sella River, with a length of 66 km, has an average annual flow at its mouth of about 30 m^3/s. According to the National Hydrological Plan (RD 1/2016), the Sella River basin is formed by 20 natural surface water bodies (river type), of which only the water body known as Río Piloña I (code ES143MAR000761) has an ecological status lower than good, thus failing to meet the quality requirements established by the Water Framework Directive (WFD; 2000/60/EC).

On the other hand, the Cares River flows into the Deva River in the lower part of the basin with an average annual flow of 22 m^3/s. The Cares River has a length of 55 km and a drainage area of 534 km^2. The Deva River, 72 km long and with a drainage area of 644 km^2, has an average annual flow of 18 m^3/s upstream of the confluence with the Cares

River, making the average annual flow of the Deva-Cares basin, before flowing into the Cantabrian Sea, reach 40 m^3/s. The Deva-Cares basin is divided into 15 natural surface river type water bodies. In this basin all water bodies meet the quality requirements set by the WFD, except for the water bodies Río Duje II (ES129MAR000570) and Río Casaño (ES130MAR000600), which have an ecological status lower than good (RD 1/2016).

3.2. Biotic environment

The climatic and topographical conditions described above produce the development of very diverse plant communities. The potential vegetation in both basins corresponds to a mixed deciduous forest. The most common tree species are those characteristics of the Atlantic climate, such as beeches (*Fagus sylvatica*), oaks (*Quercus robur*), chestnuts (*Castanea sativa*), elms (*Ulmus* spp.), hazelnuts (*Corylus avellana*) and ashes (*Fraxinus excelsior*). All of these species are characteristic of the Cantabrian-Atlantic phytogeographic province, which is part of the Eurosiberian region. The plant formations exhibit a staggered distribution in bioclimatic layers depending on climatic conditions, slope orientation, and soil characteristics.

The main bioclimatic belts in this area are:

- The hilly belt, which extends from the coastal line up to 500 m.a.s.l. Here, crops and eucalyptus plantations (*Eucalyptus globulus*) are abundant, while natural forests have reduced in area due to human activity. In the countryside associated with this altitude level, it is common to find mammals such as the fox (*Vulpes vulpes*), the mole (*Talpa europaea*), the rabbit (*Oryctolagus cuniculus*), and the hare (*Lepus capensis*), as well as several rodents and shrews. Additionally, many passerine birds can be found, such as the goldfinch (*Carduelis carduelis*) or the wren (*Troglodytes troglodytes*), along with raptors like the common buzzard (*Buteo buteo*). Certain species of reptiles (e.g., slow worm, *Anguis fragilis*) and forest invertebrates, such as the deer beetle (*Lucanus cervus*), are also common.
- The montane belt, 500 - 1,100 m.a.s.l., is the home to native oak (*Q. robur*) and beech (*F. sylvatica*) forests. Oak forests are predominantly found on sunny southern slopes. These forests are very diverse due to strong sunlight penetration. In contrast, beech forests are found on shady slopes, forming a monospecific forest due to the shaded orientation of the forest and the density of the foliage, which prevents light from reaching the forest floor. Alongside these forest formations, there are abundant successional stages, such as grasslands, scrublands and heaths. Montane forests are notable for the presence of species like the brown bear (*Ursus arctos*), the wolf (*Canis lupus*), as well as various ungulates (e.g., *Cervus elaphus*), rodents (e.g., *Apodemus silvaticus*), mustelids (e.g., *Martes foina*), and bats (e.g., *Barbastella barbastellus*). Among the birds, forest species such as the goshawk (*Accipiter gentilis*), woodpeckers (e.g., *Dryocopus martius*), or the nearly extinct capercaillie (*Tetrao urogallus*) are prominent. The most characteristic invertebrates include forest

beetles (e.g., *Rosalia alpina* or *L. cervus*) and some molluscs of community interest, such as the Quimper snail (*Elona quimperiana*).

- Above the montane belt, at the subalpine belt, the birch (*Betula alba*) appears, which structures the highest tree formations in the area. Above 1,600 meters, the majority of the vegetation cover consists of low-growing plant elements, with legumes and grasses dominating, forming the mountain grasslands. Among the most characteristic animal species in high mountain areas are mammals like the chamois (*Rupricapra pirenaica parva*) or the snow vole (*Chionomys nivalis*). The notable birds include the alpine chough (*Pyrrhocorax graculus*), the wallcreeper (*Tichodroma muraria*), and the gray partridge (*Perdix perdix*).

The river basins of the Sella and Deva-Cares have over two-thirds of their area protected under some form of environmental designation, primarily under the Natura 2000 Network (see Habitats Directive; 92/43/EEC; Fig. 3), including 5 Special Protection Areas for Birds (SPA; codes: ES0000198, ES0000248, ES0000003, ES1200009, and ES1200001) and 9 Special Areas of Conservation (SAC) of both fluvial (ES1200032, ES1200035, and ES130008) and terrestrial nature (ES0000003, ES1200009, ES1200008, ES1200001, ES1200043, and ES1300001). Overall, the Natura 2000 spaces located in this territory contain, according to the official forms submitted by the Government of Spain to the European Union (updated in 2015), 40 habitats and 80 species of community interest (those described in Annexes I and II, respectively, of the Habitats Directive). The species of community interest include 13 species of invertebrates, 5 of fish, 2 of amphibians, 2 of reptiles, 12 of mammals, 6 of plants, and 40 of birds.

Figure 3. Special Areas of Conservation (SAC) of the Natura 2000 Network in the Sella and Deva-Cares basin.

In addition to the areas included in the Natura 2000 Network, in this area stands out the Picos de Europa NP, wich was the first National Park in Spain, upon being declared a National Park of the Covadonga Mountain in 1918.

4. Climate characterization and temporal historical and future variation.

Climate observation and monitoring is essential for understanding how its variation affects the conservation of the natural environment and the development of the different socioeconomic activities. Initiatives like Copernicus aim to coordinate different monitoring efforts at European scale, complementing the work done by National Meteorological Services, as well as developing climate services tailored to the current needs of their users. In Spain, this monitoring task primarily falls to the Spanish Meteorological Agency (AEMET) through different observation networks (automated, secondary network, etc.). However, the spatial coverage of these networks is very heterogeneous, with areas poorly represented, such as mountain regions. Additionally, this work presents mistakes and/or temporal data gaps, meaning periods when a particular station does not record observations, which complicates the use of the databases generated by these systems in studies requiring high-resolution climatic information.

The need to address this problem has led to the emergence of several methodologies to generate climate information in high-resolution grids (e.g., 1 km mesh) on a global or national scale (Herrera, 2011). Nonetheless, the quality of the resulting products has proven to be limited for different regions, particularly for the eastern Cantabrian area (Bedia et al., 2013). Therefore, several regional projects have developed specific climate atlases, taking into account the geographical peculiarities and available historical records. Cantabria was one of the pioneering regions in this type of study, developing a methodology that has been subsequently refined by Predictia Intelligent Data Solutions SL (hereinafter, Predictia) and applied to other Autonomous Communities, such as the Asturias and the Basque Country. These climate atlases need to be periodically updated, incorporating new available information and thus extending the considered time period. In this context, to characterize the climate of the Sella and Deva-Cares basins, an update of the Climate Atlas of Cantabria, the Basque Country, Asturias, and northern Castilla y León was carried out up to the year 2021.

4.1. Methodological development and establishment of scenarios.

To characterize the climate of the Sella and Deva-Cares basins, climate projections of precipitation and temperature (maximum, minimum and average) have been used for two time periods generated by Predictia through the application of a set of bias adjustment methods, commonly called bias adjustment. These are:

- Historical, covering the period from January 1, 1971, to December 31, 2015.

- RCP8.5 scenario, defined by the Intergovernmental Panel on Climate Change (IPCC), covering the period from January 1, 2007, to December 31, 2097.

The projections used were obtained by processing the output data from Climadjus with observations from grids in the geographical area of interest at a spatial resolution of 1 km. For the development of the baseline climatology, Predictia used data provided by the EURO-CORDEX initiative. The main method of bias adjustment used is EQM (Empirical Quantile Mapping method for bias correction), which was selected and configured independently for each target variable. For subsequent validation, the projections from the historical period were employed, following the validation scheme defined in "VALUE Action COST." This scheme considers different aspects of the statistical distribution of the projections. The data were received in GeoTiff and NetCDF formats for both time periods.

According to the IPCC, the climate is a highly complex system resulting from the interaction between five major components: 1- atmosphere, 2- hydrosphere, 3- cryosphere, 4- terrestrial surface, and 5- biosphere. Therefore, to determine what the future climate will be, it is necessary to understand the climate system and its response to external disturbances that can alter the system's radiative balance. A measure of the net change in the energy balance in response to these disturbances is radiative forcing. As a consequence of human activity, the climate system may exhibit disturbances in the composition of the atmosphere and on the terrestrial surface. To understand this evolution, the so-called emission scenarios have been defined, which are plausible representations of the future evolution of emissions of substances that could be radiatively active (e.g., greenhouse gases, aerosols, etc.), based on a coherent set of assumptions about the driving factors (e.g., demographic and socio-economic development, fossil fuel use, etc.) and the main relationships established between them.

The RCPs (Representative Concentration Pathways) are the most widely used scenarios and encompass time series of emissions and concentrations of the complete range of greenhouse gases, aerosols, and chemically active gases, as well as land use and land cover. Each concentration pathway offers one of the many possible scenarios that would lead to specific radiative forcing characteristics (Fig. 4). Although only long-term concentration levels are of interest, they also show the trajectory followed over time to reach the relevant outcome. The RCPs, originally RCP 2.6, RCP 4.5, RCP 6, and RCP 8.5, are labelled according to a possible range of radiative forcing values in the year 2100 (2.6, 4.5, 6, and 8.5 W/m^2, respectively).

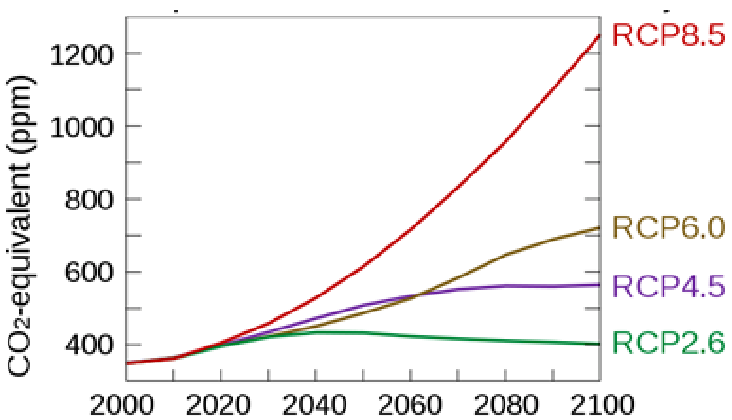


Figure 4. CO_2 concentrations in parts per million - equivalents for the different emission scenarios according to the four RCPs used by the fifth IPCC assessment report to make predictive models.

In the study conducted for the Sella and Deva-Cares basins, changes related to climate dynamics observed over recent decades (historical period) and simulated for the future RCP8.5 scenario were analyzed. The RCP8.5 scenario is a Business As Usual (BAU) scenario, where emissions continue to increase throughout the 21st century.

Although the obtained values are on an annual scale and from observation points or grid points, both spatial and temporal groupings were performed for the presentation and analysis of the generated results. The temporal grouping aimed to extract information on an annual scale at specific stations in the basins, located in upper, middle and lower sections, while the spatial groupings were done taking into account both the territory's extent and its elevation.

Based on the set of projections generated using the aforementioned techniques, the future evolution of the mean values of the described variables was analyzed, along with their values during the historical period. This information is presented in two types of charts: projection maps and space-time evolution charts. Projection maps are spatial representations of a variable's value at each observation or grid point, shown through a color scale. This type of representation provides a spatial image of how the future climate might change in the Picos de Europa National Park. Thus, projection maps have been made for both the historical period (1971-2015) and the simulated RCP8.5 scenario (2016-2097).

4.2. Temperature regime

Seasonal differences in precipitation are largely explained by atmospheric circulation and its interaction with the complex orography of the area, while temperature is strongly influenced by the Earth's tilt. In general, the results show an increase in average, maximum, and minimum temperatures for the future period compared to the historical period.

4.2.1. Average temperature

Average temperature can be grouped into two clearly distinct zones (Fig. 5a and 5b). A strong thermal gradient is observed from north to south, corresponding to the coastal and mountainous areas, respectively. The warmest temperatures occur at low altitudes, along the coast, while the lowest temperatures are found at high altitudes, such as in the Picos de Europa. The greatest temperature difference is observed along the Sella axis and in the southwestern area of the Deva-Cares basin (Fig. 5c).

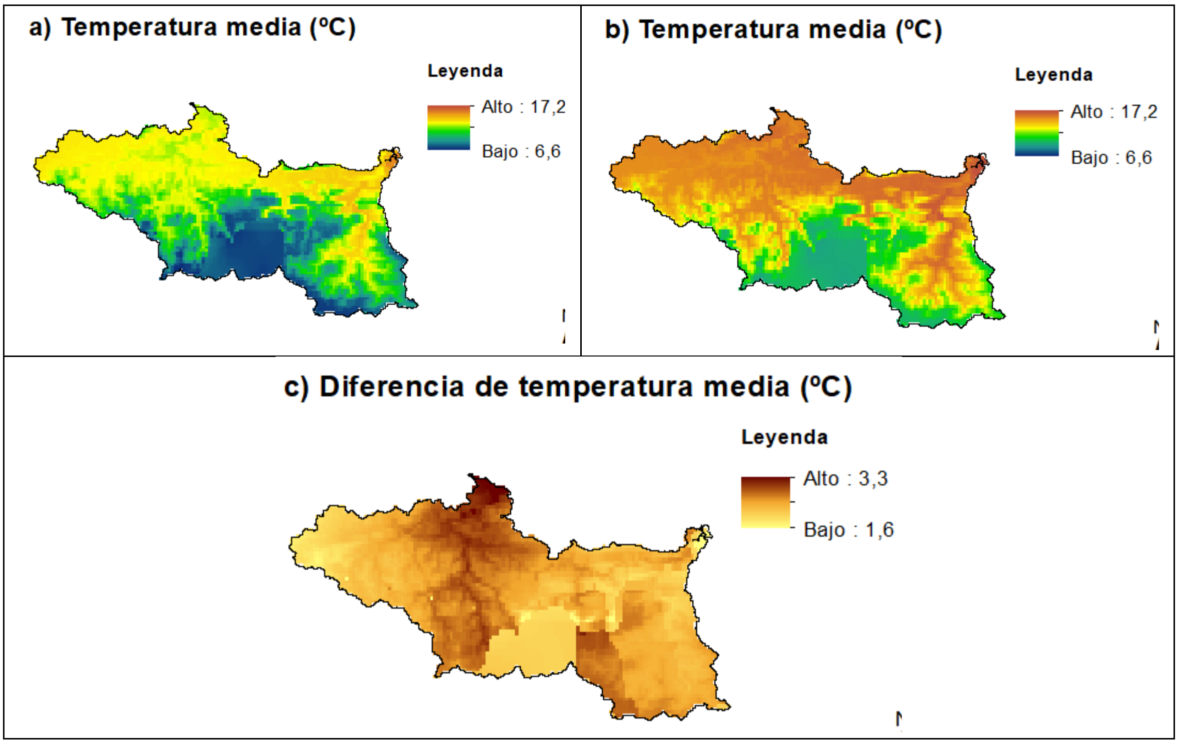


Figure 5. Maps of annual average temperature in the Sella, Deva and Cares basins during the historical period (a) and the future (b) and the difference between both (c).

The spatial distribution of the mean temperature shows a wide range of values. In the historical series, half of the territory has a temperature between 12°C and 14°C, while in the future it will rise to 14°C/16°C (Fig. 6). In 80% of the territory, the temperature will increase by an average of 2.5°C by the end of the century under the RCP8.5 scenario compared to the current situation.

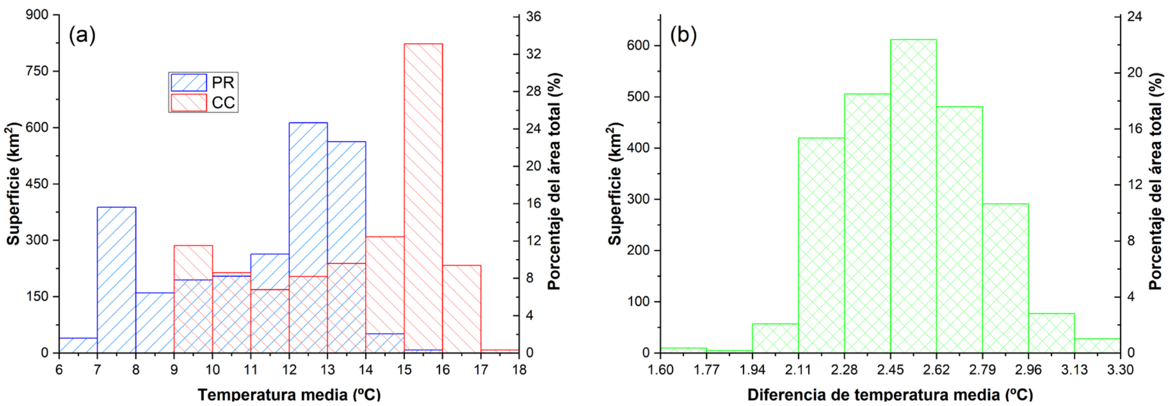


Figure 6. Distribution of the mean surface temperature of the Sella and Deva-Cares basins during the historical, PR and future series, CC (a) and the difference between the two (b).

The adjustment made to the mean temperature data for the historical period shows that over the past 40 years, it has increased by approximately 0.8°C at the selected stations (0.02°C/year). The predictions indicate a more pronounced upward trend than the current one (0.05°C/year). It is worth noting that no significant differences are observed between the river sections in high areas (Casaño, Duje at the headwaters, and Bullón), middle areas (Quiviesa, Deva in Beares, and Cares), and lower sections of the Cares and Deva rivers (Fig. 7).

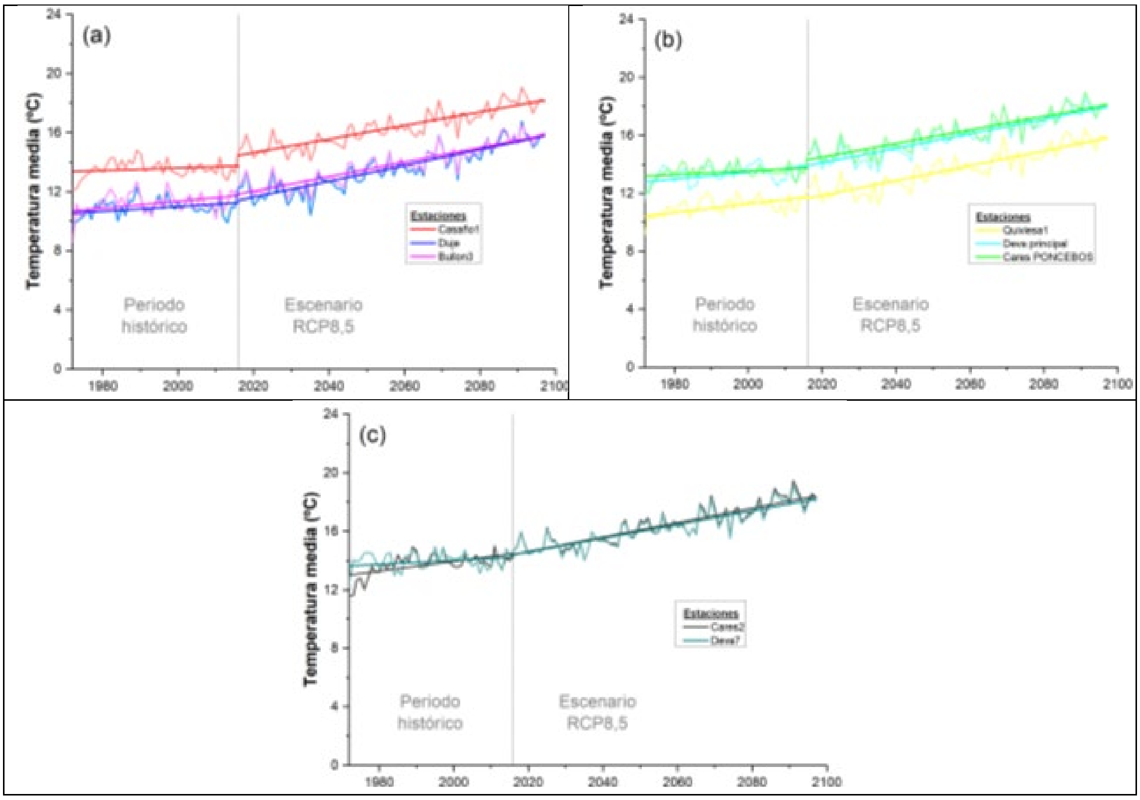
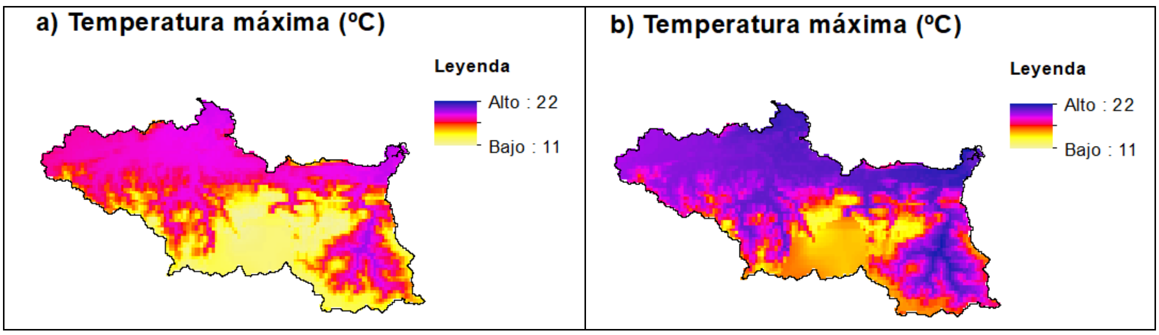


Figure 7. Time series of average temperature in different stations of the upper (a), middle (b) and lower (c) reaches of the Deva-Cares basin.

4.2.2. Maximum temperature

The maps of the spatial distribution of the maximum temperature (Fig. 8) follow the same pattern described above for the average one. The hottest areas are located at low altitudes and close to the coast, while the coldest areas are found at higher elevations and in the central-southern part of the described territory.



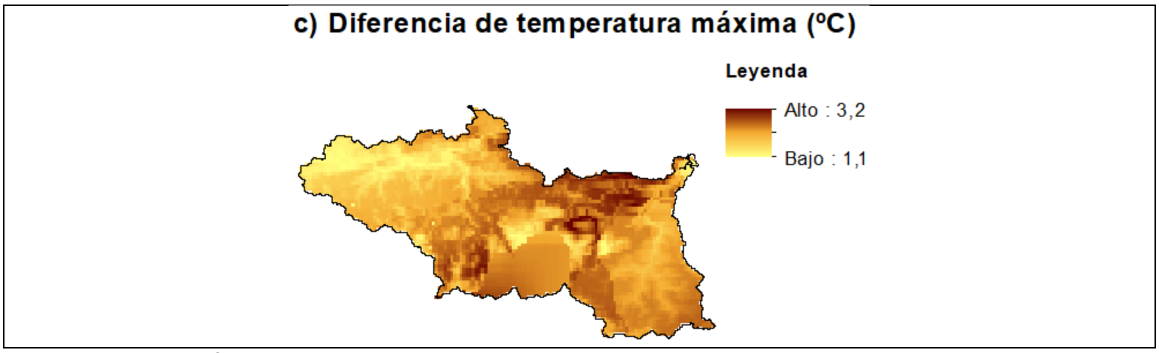


Figure 8. Maps of maximum annual temperature in the Sella, Deva, and Cares basins during the historical period (a) and future (b) and the difference between the two (c).

The increase in maximum temperatures is more uniform, with the lower area of the Sella basin showing the least variation (approx. 1°C) and the area to the west of the Cares basin exhibiting the highest one (approx. 3°C). The analysis based on the surface area studied shows that, while in 50% of the territory the maximum temperature is between 17°C and 19°C for the historical period, in the future, the maximum temperature will range between 19°C and 21°C (Fig. 9).

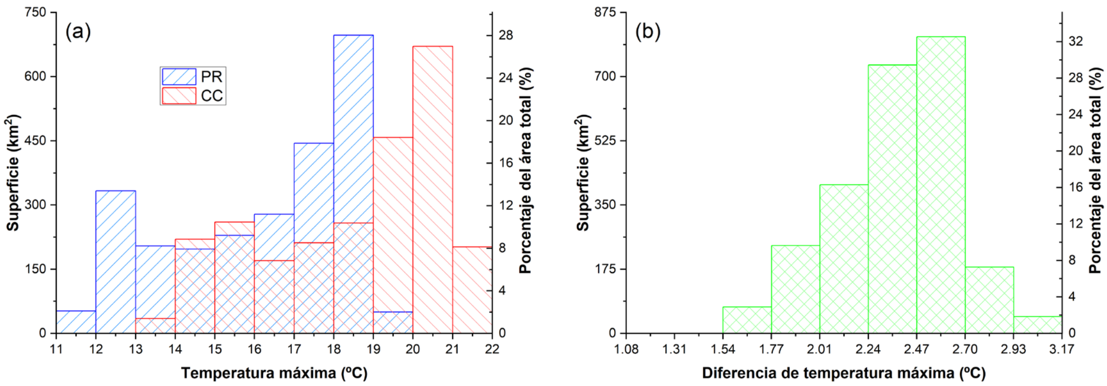
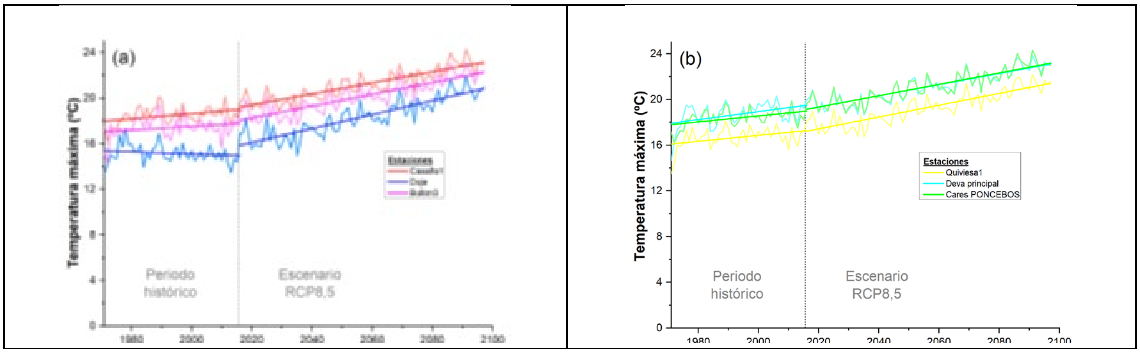


Figure 9. Distribution of the maximum temperature in the Sella, Deva, and Cares basins during the historical, PR and future series, CC (a) and the difference between both in the same (b).

The adjustment made for the maximum temperature data in the historical period shows that over the past 40 years it has increased by approx. 0.8°C at the selected stations (0.02°C/year), similar to the average temperature. Predictions also indicate a more pronounced upward trend than the current one (0.05°C/year). No significant differences are observed between the three types studied (high, medium, and low; Fig. 10).



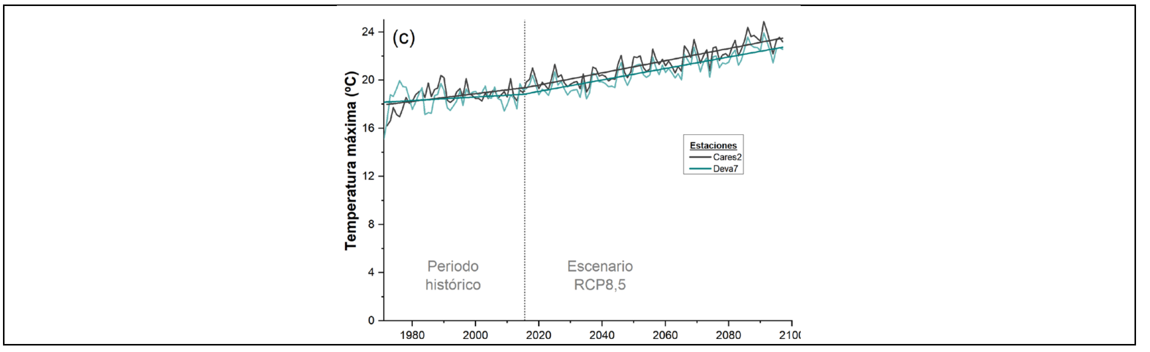


Figure 10. Time series of maximum temperature in different stations of the upper (a), middle (b) and lower (c) reaches of the Deva-Cares basin.

4.2.3. Minimum temperature

The maps of the distribution of minimum temperatures (Fig. 11) are similar to the previous ones. However, in this case, the opposite occurs compared to maximum temperatures, where the variations are more pronounced and the locations are inverted. This results in a difference of nearly 4.5°C in the coastal area of the Sella, near Ribadesella.

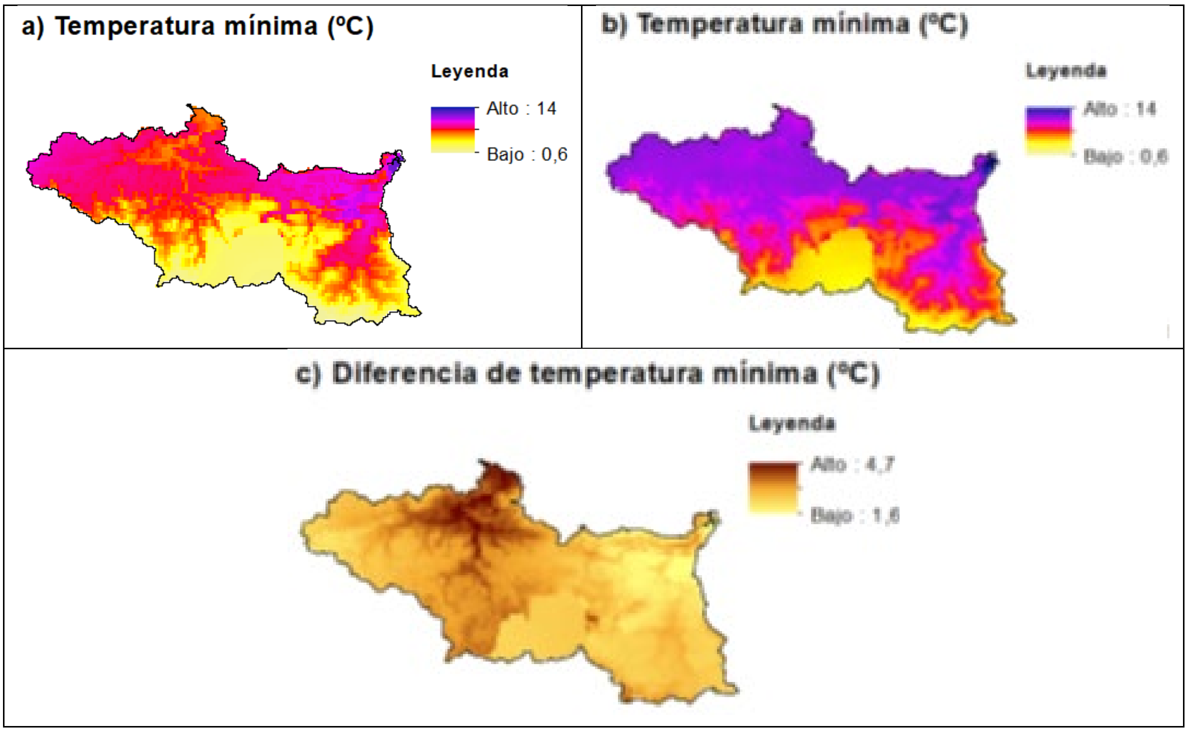


Figure 11. Maps of minimum annual temperature in the Sella, Deva, and Cares basins during the historical period (a) and future (b) and the difference between the two (c).

In the last decades, 50% of the territory covered by these basins had an average minimum temperature between 6°C and 9°C, which is expected to rise to 10°C-12°C in the future scenario, as in more than 70% of this area the temperature will increase between 2.2°C and 4.0°C according to the proposed models (Fig. 12).

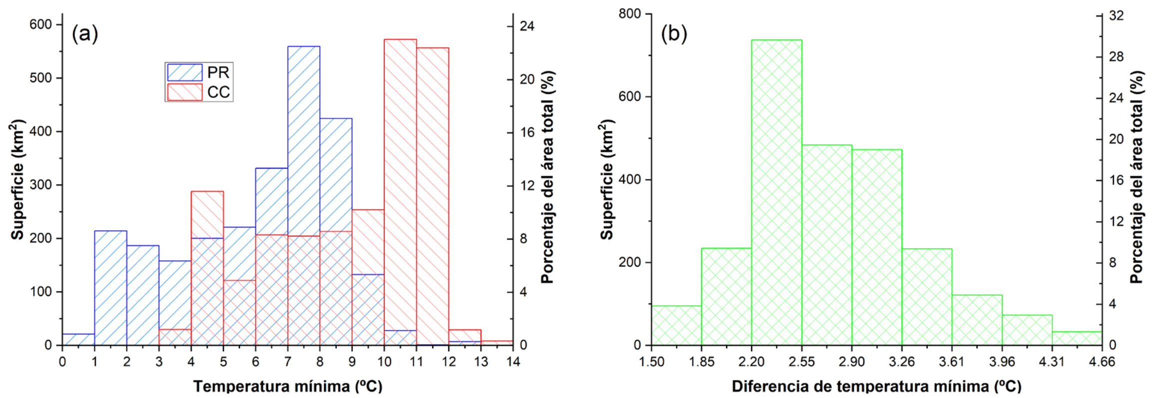


Figure 12. Distribution of the minimum temperature in the Sella, Deva, and Cares basins during the historical, PR and future series, CC (a) and the difference between both in the same (b).

The adjustment done to the minimum temperature data for the historical period shows that over the past 40 years the temperature has increased by approximately 0.8°C at the selected stations (0.02°C/year). Predictions indicate a more pronounced upward trend (0.05°C/year). As in the previous cases, no significant differences are observed between the three types of sections studied (Fig. 13).

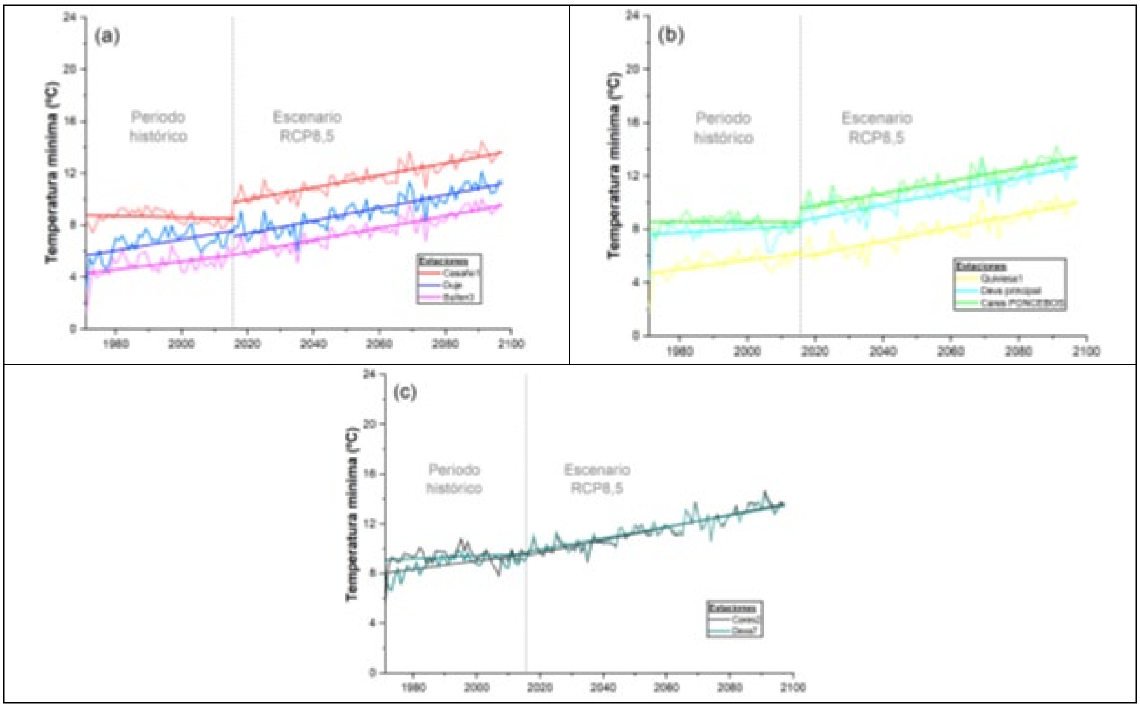


Figure 13. Time series of minimum temperature in different stations of the upper (a), middle (b) and lower (c) reaches of the Deva-Cares basin.

As expected, a relationship between temperature and altitude is observed, regardless of the period considered. Thus, a relatively constant temperature is noted between 0 and 400 meters of altitude, and a decrease in the temperature/altitude relationship is observed between approximately 500 and 1,600 meters (Fig. 14). In the historical period (Fig. 14a), a thermal decrease with altitude of about 0.44°C/100 meters is observed, a trend that will continue in the future (Fig. 14b).

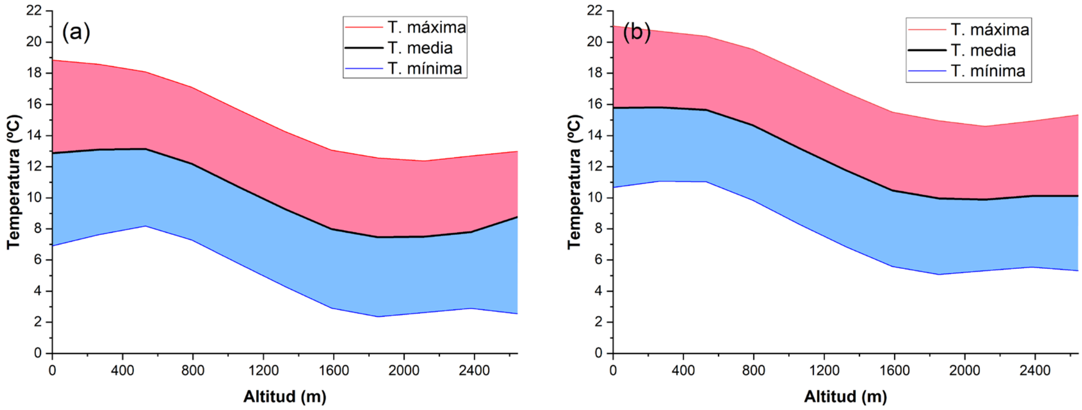


Figure 14. Mean temperature, max. and min. according to the height in the historical series, PR (a) and future, CC (b).

For the 3 temperatures (average, max. and min.) the difference between both periods remains around 2.5ºC between 800 – 2,500 m.a.s.l. However, at sea level the minimum temperature will experience an increase of up to 3.7ºC (Fig. 15).

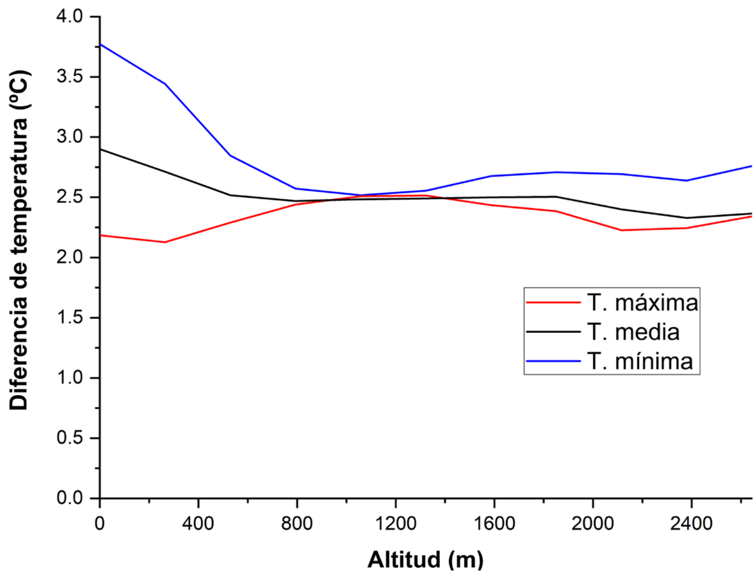


Figure 15. Mean, max and min temperature difference between the time series according to height.

4.3. Precipitation regime

Mean precipitation data show a general decrease for the future period compared to the historical one (Fig. 16). It is observed that most of the rainfall is concentrated in high mountain areas, while the lowest amount of precipitation occurs in low-altitude areas. Precipitation exceeds 1,000 mm/year in 90% of the territory (except for the lower elevations of the Liébana Valley and some areas near the town of Arriondas), with marked peaks in the Picos de Europa. A reduction in the average precipitation in the area can be seen during the future RCP8.5 scenario compared to the historical period.

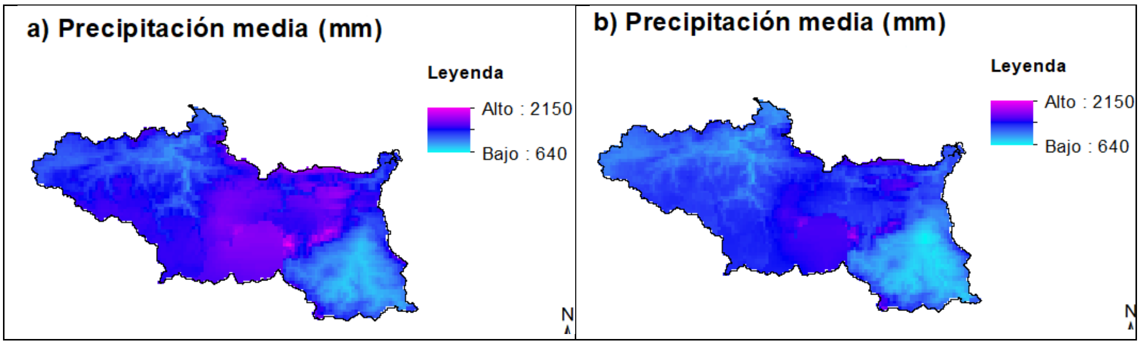


Figure 16. Maps of average annual precipitation in the Sella, Deva and Cares basins, during the historical period (a) and future (b).

While in the historical period 20% of the territory showed an average annual rainfall of 1400-1500 mm, in the future this average will be 1200-1400 mm. In Figure 17, it is shown that approximately 70% of the area will experience a reduction of the annual precipitation around 100 - 220 mm. In general, in both cases, historical and future, the average precipitation increases with altitude, with a variation of approximately 650 mm between the lowest (0 m) and the highest altitude (2,644 m) in the historical period, and 560 mm in the future scenario.

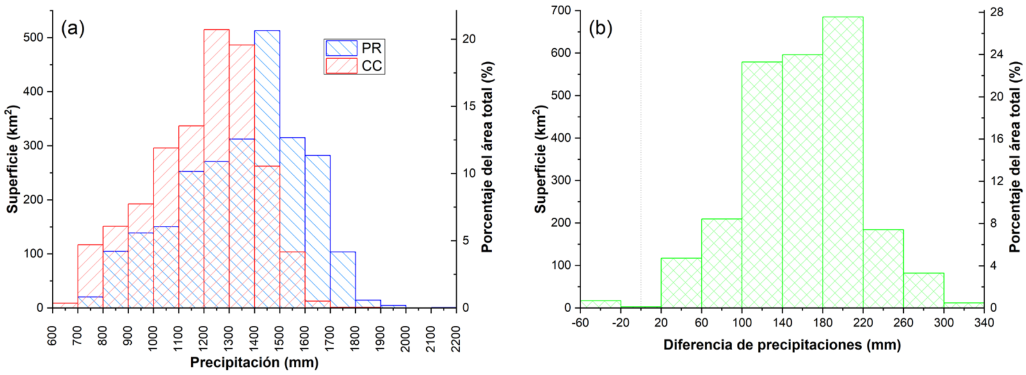


Figure 17. Distribution of the amount of rain collected in the Sella, Deva, and Cares basins during the historical, PR and future series, CC (a) and the difference between both (b).

In the current case, precipitation increases by 23 mm for every 100 meters, whereas in the future is expected to decrease to 20 mm. Average precipitation will be lower in the future compared to the historical period for any altitude range (Fig. 18).

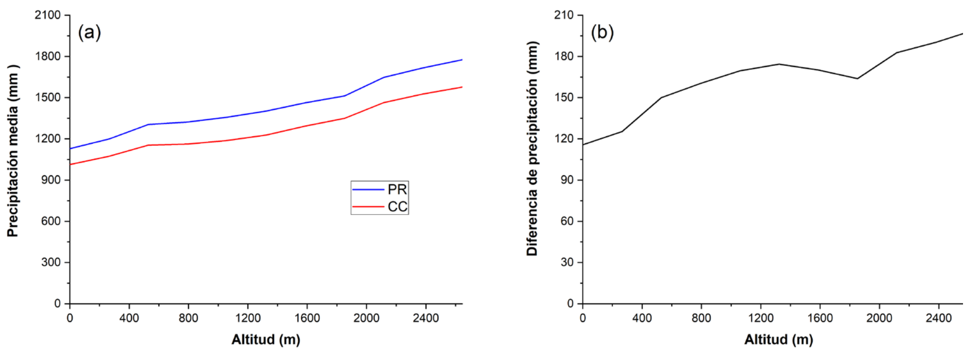


Figure 18. Average precipitation in mm in relation to altitude in the historical, PR and future series, CC (a) and difference between both (b).

Below are the temporal distributions of precipitation at different points in the Deva-Cares basin, grouped into upper (Casaño, Duje at the headwaters and Bullón), middle (Quiviesa, Deva at Beares and Cares), and lower sections of the Cares and Deva rivers. In these 3 cases (upper, middle, and lower sections), historical data shows an upward trend in precipitation. However, the predictions made for the RCP8.5 scenario indicate that this will not happen in the future, and precipitation at each station will decrease over time (Fig. 19). No notable differences are observed in the segregation by sections in these cases. Precipitation is expected to decline by an average of 2.6 mm/year.

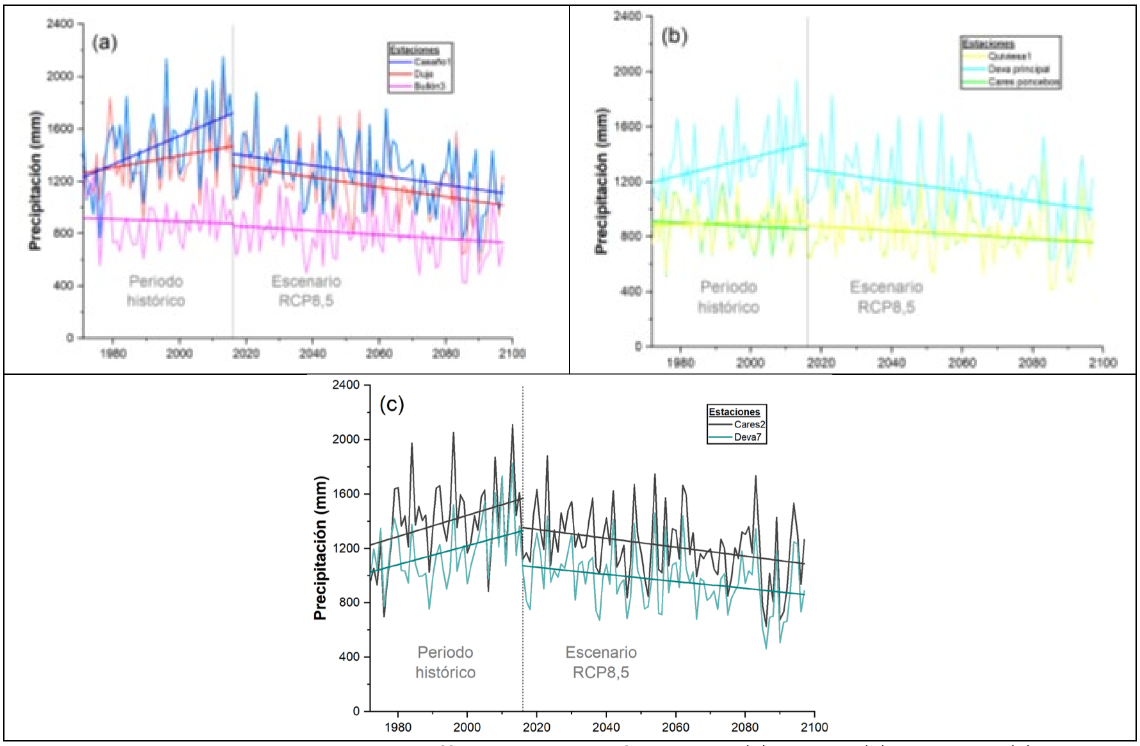


Figure 19. Precipitation time series in different stations of the upper (a), middle (b) and lower (c) reaches of the Deva-Cares basin.

5. Characterization of vegetation and riparian forests

5.1. Characterization of land uses and coverage. Terrestrial habitats.

Of all the processes driving global change, changes in land use and land occupation are considered one of the main causes of natural habitat degradation and associated biodiversity loss (Lambin & Geist, 2006; Fig. 20). These changes represent the most important factor in relation to the impacts on natural resources (Hunsaker & Levine, 1995), interacting intensely with other elements (Vitousek et al., 1997) and generating different impacts, such as alterations in the spatial structure and functioning of landscapes at local, regional, and global scales (Valladares, 2004b). The study of the magnitude of these changes over time, as well as the evaluation and understanding of the factors that regulate them, will largely determine our future ability of adaptation. In this context, any alteration of vegetation cover or water regimes—due, for example, to forest fires, deforestation, or land occupation for residential or industrial purposes—can lead to irreversible erosion processes, soil fertility loss, or overexploitation of water resources.

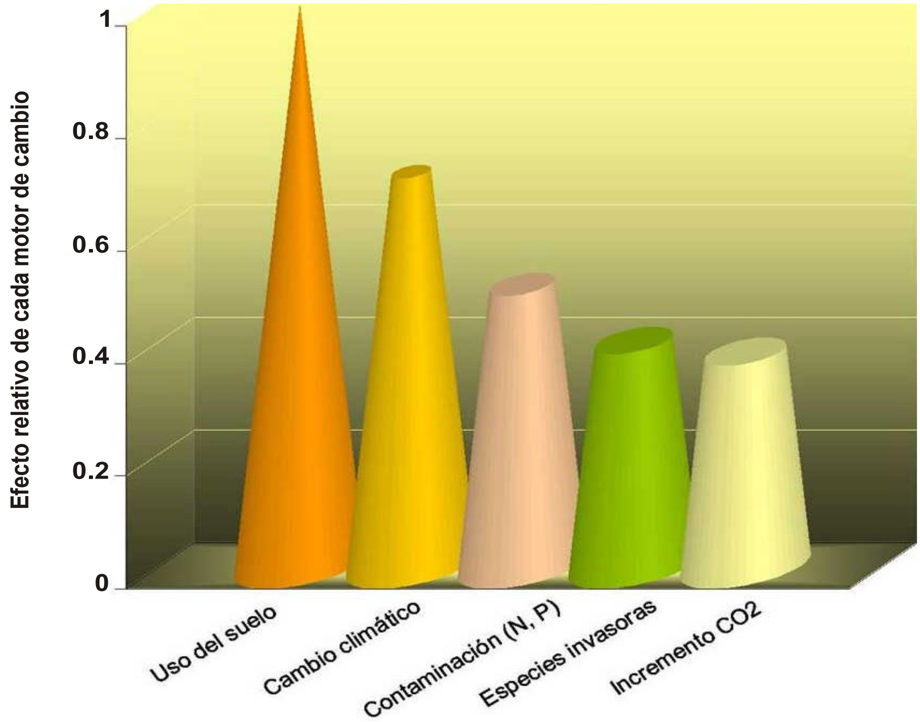


Figure 20. Changes in land use are the main cause of biodiversity loss in Mediterranean ecosystems. From Sala et al. (2000) and Valladares (2004b).

In Spain, large-scale production-focused forest repopulation programs were carried out during the XX century, continuing to a lesser extent under the Common Agricultural Policy (CAP) framework. To restore the balance between production and market capacity while ensuring equitable income levels for farmers and respect for the environment, the CAP reform set key objectives of greater efficiency and competitiveness in farms (Regulation EEC 2328/91), as well as a gradual reduction of surplus sectors. The abandonment of poorer and marginal lands, far from intensive production centers, the decrease in livestock density and the stop of extractive activities have contributed to forest expansion (Pueyo & Beguería, 2007; Fig. 21). As a result, there has been a notable increase in biodiversity associated with these ecosystems, improved soil quality and the hydrological cycle and a significant reduction in surface erosion (Suárez-Seoane et al., 2002). This has been the case in the less arid sub-Mediterranean marginal areas, while in more arid areas, overgrazing and widespread environmental degradation, combined with unfavourable climate cycles, have considerably hindered the spontaneous recovery of vegetation (Valladares, 2004). In this context, European regulations require the development of appropriate management plans to facilitate decision-making aimed at achieving sustainability goals. The processes governing landscape dynamics, as well as the factors driving land use changes, must be analyzed and related to the quantity and quality of water resources in order to manage them properly under future ecological and socioeconomic scenarios of uncertain nature.

Figure 21. Changes in land occupation are due to natural and anthropogenic forces that cause divergent pressures on the territory. Doctoral Thesis University of León (Álvarez-Martínez et al., 2014).

Therefore, the reasons justifying the study of land use and occupation changes are multiple and diverse. Until a few decades ago, these analyses were carried out by the government through surveys or in-situ sampling. Today, remote sensing image processing and the application of GIS techniques and spatial modeling allow for the visual and analytical evaluation of temporal changes in the territory in a semi-automatic and much more precise manner (Álvarez-Martínez et al., 2014), thanks to their ability to track dynamic processes (Roughgarden et al., 1991). Due to the large amount of available information and techniques, it is possible to adapt the analyses to specific temporal and spatial scales (e.g., annual scale for monitoring post-fire vegetation recovery, or detailed spatial scale for monitoring forest successional dynamics; Chuvieco & Congalton, 1989). In this context, an analysis of land occupation and the changes that have occurred over the last 3 decades has been developed, based on environmental variables and remote sensors, using satellite images and LiDAR data for the area described in this document. Additionally, vegetation diversity patterns have been explored using the methodology proposed by Álvarez-Martínez et al. (2018a). The aim of this approach is to provide detailed ecosystem maps, which are later integrated into landscape or functional units corresponding to small hydrological basins covering the entire study area, in order to provide a framework for evaluating ecological functions and services. This methodology involves integrating in-situ data with predictor variables related to abiotic limiting factors and remote sensing, through data mining techniques. In this case, the results have been statistically validated for the entire work area, also using field information and expert judgment.

5.1.1. Datos in-situ

As a source of in-situ information, all available habitat data were collected, following the Ecological Bases for the Description of Spanish Habitats (Hidalgo, 2009). The cross-references between vegetation units and habitat types were adapted for the study area using the European Nature Information System (EUNIS 2017). Of the 41 habitats described, 24 were selected for modelling (see Table 1). Some habitat types were discarded due to a lack of data and unsatisfactory results in previous analyses. In most cases, these were related to scattered categories and microhabitat conditions that were difficult to assess at the scale of the analyses performed. Presence data were obtained from detailed vegetation maps (1:25,000 or higher) distributed across the study area and adjacent regions. In addition, these data were supplemented by specific field campaigns to achieve an adequate level of geographic and environmental sampling for the habitat types. In total, more than 5,000 points were collected to train the land occupation and habitat distribution models. To meet statistical requirements, the spatial autocorrelation of the dataset was evaluated using Moran's Global I statistic, implemented in ArcGIS 10.8 software, selecting, for each habitat type, training points that were at least 500 m apart from each other. Ultimately, more than 2,000 data points were retained for the modeling exercises.

Hábitat	Código	Nombre hábitat *sensu* Directiva Hábitats
Bushes	4030	European dry heathlands
	4060	Alpine and boreal heathlands
	4090	Endemic Oro-Mediterranean heaths with gorse
	5120	*Cytisus purgans* montane formations
Pastures	6160	Mediterranean orophilous grasses of *Festuca indigesta*
	6170	Alpine and subalpine calcareous grasslands
	6210	Semi-natural dry grasslands and calcareous scrub facies.
	6230*	Grassy formations with *Nardus*, with numerous species, on siliceous mountainous substrates.
	6510	Lowland mowing meadows.
Rocky outcrops	8130	Western Mediterranean and thermophilic scree
	8210	Calcareous rocky slopes with chasmophytic vegetation
	8220	Siliceous rocky slopes with chasmophytic vegetation
	8230	Siliceous rocks with pioneer vegetation of *Sedo Scleranthion* or *Sedo albi-Veronicion dilleniid.*

Hábitat	Código	Nombre hábitat *sensu* Directiva Hábitats
Forest	9120	Atlantic acidophilic beech forests with *Ilex* and *Taxus*.
	9150	Middle European calcicolous beech forests.
	9160	Pyrenean-Cantabrian oak and ash forests.
	91E0*	Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior*.
	9230	Galician-Portuguese forests with *Quercus robur* and *Q. Pirenaica*
	9240	Marcescent Mediterranean oak forests
	9260	*Castanea sativa* forests
	92A0	Gallery forests with *Salix alba* and *Populus alba*
	9330	cork oak forests of *Q. suber*
	9340	*Q. ilex* and *Q. rotundifolia* forests
	9380	*Ilex aquifolium* forests

Table 1. List of habitat types modelled for the Sella and Deva-Cares basins and their code and name according to the Habitats Directive (92/43/EEC)

5.1.2. Predictive variables

As sources of abiotic information, biophysical data describing the climatic and topographic variability of the study area were selected. A climate database for the Iberian Peninsula (200 m resolution; Ninyerola et al., 2007) and a digital elevation model (DEM) obtained from LiDAR data with a 5 m. pixel size (CNIG, 2020) were used for this purpose. The Euclidean distance to the nearest river and other open water bodies, obtained from the BTN25 database (CNIG, 2020), was also calculated to account for water accumulation in the soil at the pixel scale. All layers were resampled to 30 m to match the spatial resolution of the satellite images (see below). For climate data, natural neighbor interpolation (Sibson, 1981), a weighted averaging method that uses neighborhood functions around each original data point, was applied. Since multicollinearity can lead to variance inflation and parameter bias, Spearman bivariate correlations were run between all predictors. Each variable in a highly correlated pair ($r > 0.7$) was retained or discarded based on univariate results, expert knowledge, and related literature.

At the satellite data level, all Landsat 8 *Operational Land Imager* scenes (OLI; path 202, row 30) from 2013-2016 were obtained from the United States Geological Survey (USGS) servers as standard Level 1T data products. Additionally, all Sentinel 2 images from the ESA were collected through the Hub https://scihub.copernicus.eu/. Three scenes per selected period were necessary to cover the entire study area. Those with the highest solar elevation, the least cloud cover, and taken near the peak greenness (April to October) were retained. Finally, they were corrected using standard methods for Landsat 8 and the sen2cor algorithm for Sentinel 2, and combined to produce a single cloud-free and spatially continuous image using a maximum value composite method (Álvarez-Martínez et al., 2010, 2018). Different products for subsequent modelling analysis were developed from mosaics and combined into a multiband raster stack composed by:

- The corrected image bands, allowing any combination of surface reflectance values that could aid in classifying each target cover/habitat type.
- Two spectral or vegetation indices: the normalized difference vegetation index (NDVI) and Tasselled Cap (TC) transformation products, commonly used in species and community distribution models. NDVI values are obtained from visible and near-infrared reflectance measurements of vegetation and represent a measure of total photosynthesis and net primary production (Pettorelli et al., 2005). TC integrates all spectral channels, providing ecologically meaningful information on vegetation greenness, land brightness, and soil moisture (Lozano et al., 2007).

LiDAR data for the study area were downloaded from the PNOA database (CNIG, 2020), with a resolution of 0.5 points/m². Native files were processed with the LAStools processing toolbox, obtaining variables that provide information on vegetation height and canopy structure, which also enhance the predictive capabilities of the multispectral images. Based on the local variability of the LiDAR responses, two different layers were developed:

- ✓ 90th percentile of the maximum vegetation height within 5-meter pixels.
- ✓ Average vegetation height in moving windows of 3 pixels to assess canopy structure.

The original surface elevation models (SEM) were developed with a spatial resolution of 5 m. and were resampled to match the 30 m pixel size of Landsat, along with the other predictor variables, in order to achieve a spatial resolution consistent with the analysis of temporal changes.

5.1.3. Predicting modelling

Based on the available information, models were trained to develop land use and cover maps for 3 different dates (1985, 2000 and present) using supervised classification methods (Álvarez-Martínez et al., 2018). The accuracy for the present was evaluated using part of the in-situ data collected, which differed from the training data, while the accuracy for the earlier periods was derived from historical aerial photographs or external data (e.g., Corine Land Cover maps). Some misclassifications were detected due to specific phenological states, which depend on particular annual climatic conditions that have affected the signal observed from the satellite. Three maps consistent with the observed trends for the entire period were chosen. The classification process at the level of landscape structure or physiognomic vegetation units also allows for the creation of a cartographic base upon which individual habitat distribution modelling will be established (e.g., forests, scrublands, grasslands, rocky areas, and wetlands).

The habitat distribution modelling was developed using MaxEnt to predict the Area of Occupancy (AOO) for each habitat type (Álvarez-Martínez et al., 2017). MaxEnt was prioritized over other alternatives because it only uses presence data as a response variable, efficiently resolving complex classifications. Multiple classifiers, such as maximum likelihood, were avoided since not all existing habitat types in the study area were adequately sampled, lacking suitable absence data. Pseudo-absence data for MaxEnt were randomly sampled throughout the study area while maintaining a minimum distance of 500 m between points to avoid spatial autocorrelation effects. To determine the local AOO for each habitat type, the predictor layers described above were used. The modeling generated a raster map of presence probability with continuous values from 0 to 1 for each of the 24 described habitats (Table 1), with 1 representing the maximum probability of a particular habitat occurring at any point in space. Thresholds of probability were selected to define habitat patches that minimize the omission/commission error ratio in the predictions. Values below this specific threshold are unlikely for a particular habitat, and are labelled with a value of 0 on the final maps. Comparing the individual probabilities of different models is not straightforward since the probability values depend on the sampling schemes and the bias of the available data (Merow et al., 2013). This effect was minimized by consistently using the same predictor variable set and the same pool of pseudo-absence data (Elith et al., 2009). Thus, each pixel was assigned to the class with the highest suitability, resulting in a combined map in which each pixel was associated with one of the 24 habitats or none if two (or more) showed a similar probability of occurrence. These transitional areas, or ecotones, nonetheless have great ecological importance for conservation, and can be spatially identified for different management purposes (Tapia et al., 2005).

Once the models were executed, an independent validation of the obtained predictions was carried out through field campaigns, where expert botanists visited over 2,000 vegetation patches of more than 1 ha following a stratified random sampling design to ensure a comprehensive study of the dominant environmental gradients in the landscape (Guisan & Zimmermann, 2000), avoiding inaccessible points and ecotones. These training points were overlaid with the maps of landscape units and habitat distribution, obtaining confusion matrices and overall metrics of accuracy and precision.

5.1.4. Landscape classification results

Current land cover shows a fragmented pattern in the study area, with forest masses mainly distributed in headwaters and a matrix of scrubland dominated by ecological succession processes (Fig. 22). Human interference has led to a reduction of the original forest cover, primarily due to logging that occurred between 1940 and 1980, which has been confirmed by the analysis of changes and trends observed in the study area over the past decades.

Rural depopulation and the reduction of the primary sector have allowed for the recovery of early forest stages in abandoned fields. The valley bottoms are occupied by meadows with hedges and residual farmland, while patches of bare soil and rocky outcrops appear throughout the area, interspersed with heaths in the higher zones. The anthropogenic effect has caused a significant change in the functionality of the ecosystem and the services it provides to society.

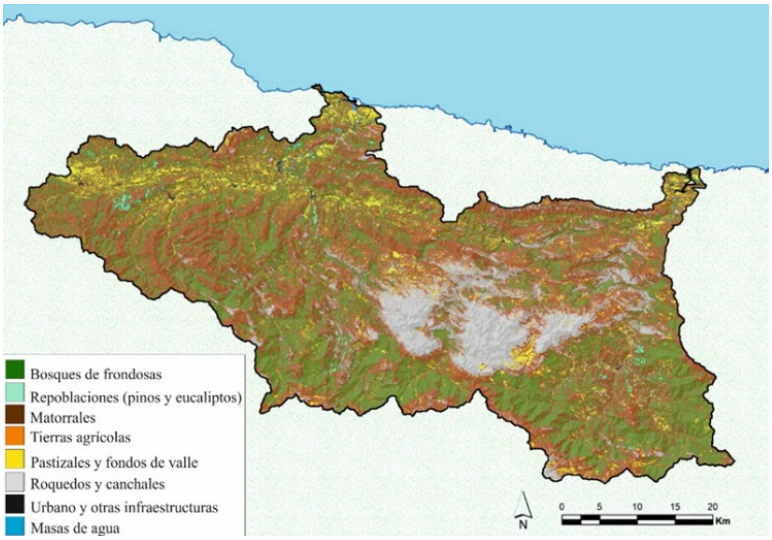


Figure 22. Current land uses and covers in the Sella and Deva-Cares basins.

5.1.5. *Maps of habitats or vegetation classes*

Once the current land use was analyzed, habitat models demonstrated a high capacity to estimate the distribution of the 24 typologies at the regional scale within each of the landscape categories. Figure 23 shows, as an example, the result for the habitat 4030 - European dry heaths and 9120 - Atlantic acidophilus beech forests with *Ilex* and, sometimes, also *Taxus* in the shrub layer

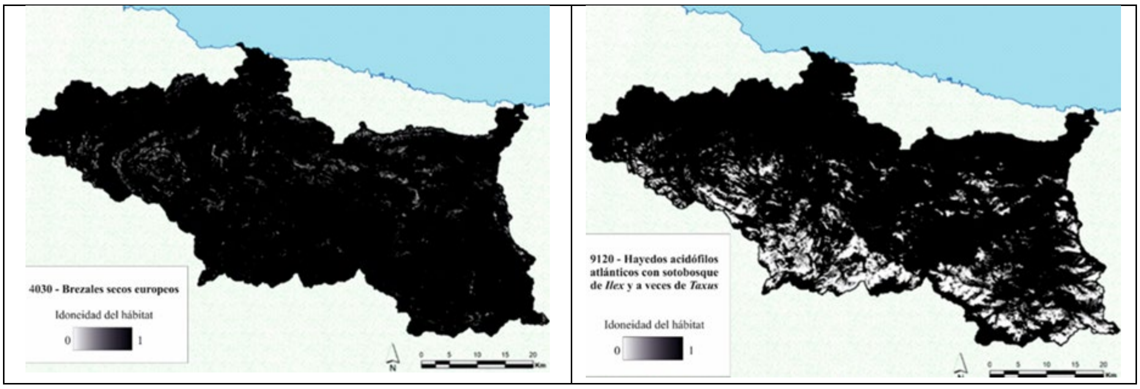


Figure 23. Distribution maps of the Potential Area of Occupation (AOO) for habitats 4030 and 9120.

Models showed consistently high predictive capacity values, with mean AUC values of 98.6 ± 1.2% for the distribution of all analysed habitat types. The AUC area, or area under the ROC curve (Receiver Operating Characteristic), is a statistical tool used to measure the accuracy of predicting binary events (whether they occur or not). The external validation, conducted with independent points, showed an overall accuracy of 67.59%, reaching over 80% for some forest habitats. Highest errors were observed in habitat types with a low number of training points. Notably, the system's ability to discriminate between forested and non-forested areas was almost flawless, with negligible classification errors. The combination of individual maps provides a representation of the spatial patterns of habitat types across the landscape (Fig. 24).

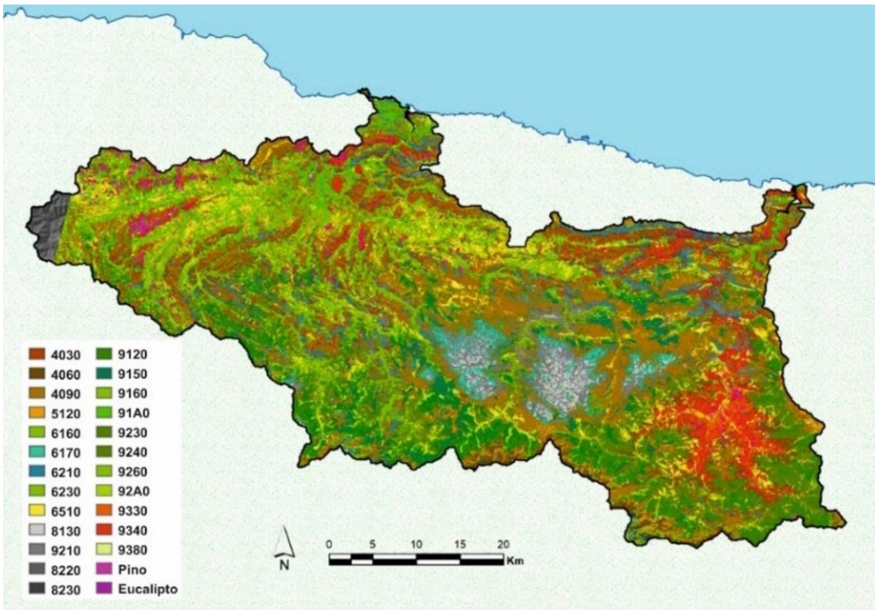


Figure 24. Vegetation map obtained by combining individual predictions (AOO).

These vegetation patterns can subsequently be merged with integral landscape units to reduce omission/commission errors produced by the random dispersion of typologies across contiguous pixels for the probabilistic assignment typical of satellite image classifications. This merging also allows for further analysis related to ecosystem functioning and services (Fig. 25).

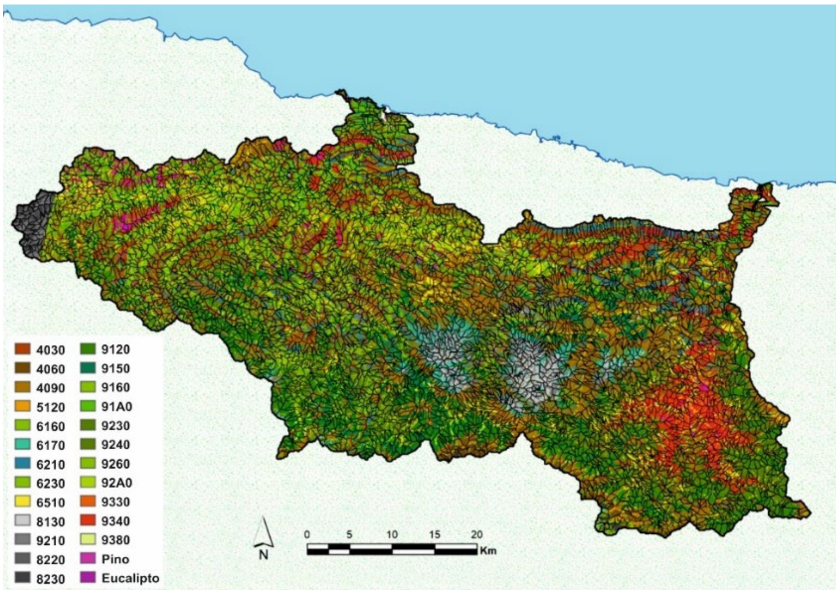


Figure 25. Vegetation map combined with landscape units (watersheds) for subsequent analyses related to ecosystem functioning and services.

5.2. Riparian vegetation

Riparian area plays a fundamental role in aquatic ecosystems, contributing to landscape biodiversity by providing habitats for several species and acting as an ecological corridor. Additionally, it performs essential functions, such as sediment and pollutant retention, water temperature regulation and water storage in floodplains, making it an important provider of provisioning and regulatory ecosystem services (Naiman et al., 2010). However, riparian forests have traditionally been subjected to anthropic disturbances, leading to significant alterations in their composition, structure and functioning (Logan & Furse, 2002). For example, in mountain ecosystems, the regulation of natural flow regimes due to dam and weir construction has affected sediment transport and the frequency, magnitude and duration of floods.

To characterize the riparian vegetation in the watersheds of the Sella and Deva-Cares rivers and to assess its conservation status, models of riparian forest habitats have been developed. Different indices were calculated for the assessment of their conservation status by using remote sensing techniques, allowing for landscape-scale analysis. This exercise has been carried out for the following habitats:

- Priority habitat 91E0*: Dominated by species such as the common alder (*A. glutinosa*), the ash (*F. excelsior*), and several tree-like willows (mainly the white willow, *S. alba*). These forests develop on soils rich in alluvial deposits and are periodically flooded by river floods, although they are well-drained and aerated during dry seasons. Alders are plant formations located along riverbanks, forming the first band of vegetation that withstands river flows. However, the natural characteristics of the alder, which cannot tolerate significant changes in the water table and requires a constant level of moisture, lead to the replacement of alder groves with willow stands in riverbeds of higher torrentiality and gravel beds.
- Habitat 92A0: These are riparian forests of the Mediterranean plains dominated by *S. alba*, *S. fragilis*, and other willows. It also includes Mediterranean riparian forests and those of Central Eurasia with several strata where, in addition to willows, appear species from the genera *Populus*, *Ulmus*, *Alnus*, *Acer*, *Tamarix*, *Fraxinus*, or *Quercus*. The tall poplars or aspens, *P. alba*, *P. caspica*, *P. euphratica*, are the dominant species in height, although their presence can be null or scarce in some communities dominated by elms, ashes, or willows

5.2.1. Evaluation of the conservation status of riparian vegetation

Given the need to develop measures aimed at improving the management and conservation of riparian environments, the Ministry for Ecological Transition and the Demographic Challenge of the Spanish Government has developed a methodology to inventory, characterize and assess the habitats associated with riverbanks (Lara et al., 2019). To evaluate the conservation status of the riparian forest in the watersheds of the Sella and Deva-Cares rivers, we combined the results generated according to the methodology described in the previous section, along with the proposal defined by the Spanish Ministry (Lara et al., 2019).

In this context, to assess the conservation status of habitats 91E0 and 92A0, pixels from the territory that did not reach an Area of Occupancy (AOO) of 0.37 for habitat 91E0 and

0.28 for habitat 92A0 were eliminated, optimizing the current distribution of each habitat based on the 10th percentile of the training dataset (Pérez-Silos, 2016). Next, the mean NDVI value was calculated for the riparian forest formations of each of these two habitats that met the "favorable" height criterion in the MITERD report (height > 2.5m based on PNOA LiDAR data), assuming they correspond to forest masses in their best physiological condition (i.e., vigor represented by NDVI). Finally, an *ad hoc* conservation index was calculated that combines the thresholds determined in the MITERD report (obtained from the LiDAR database) with the NDVI threshold established for forest masses with "favorable" height (obtained from spectral databases) at the functional unit level (Table 2)

Conservation status	LiDAR*	NDVI - 91E0*	NDVI - 92A0
Favourable	>2,5	>0,68	>0,76
Unfavourable-inadequate	1,5-2,5	<0,68	<0,76
Unfavourable -poor	<1,5	<0,68	<0,76

Table 2. Thresholds of average LiDAR and NDVI values of the riparian forest used to classify functional units in conservation states (*Height thresholds according to MITERD report).

5.2.2. *Evaluation of riparian transversal connectivity*

To evaluate the transversal connectivity of the riparian environment, the percentage of riparian forest coverage at the functional unit level was used as indicator. This approach combines the following criteria (see Table 3):

1- Space occupied by the type of vegetation in a standard strip along the banks.

2- Extent of contact between the woody riparian vegetation and the natural vegetation of the slopes.

	Space occupied by vegetation in a standard band next to banks	Extension of contact between riparian woody vegetation and natural hillside vegetation
Favourable	> 80%	> 75% contact with natural climatophilous vegetation
Unfavourable-inadequate	30% - 80%	Among 25%-75% contact with natural climatophilous vegetation
Unfavourable-poor	<30%	≤ 25% contact with natural climatophilous vegetation

Table 3. Thresholds of space occupied by vegetation along the banks and extent of transverse contact adapted from the MITERD report.

In this case, it has been assumed that the percentage of riparian forest coverage is an indicator of the space occupied by it at the functional unit level, and that, with a higher percentage of forest coverage, there is a greater probability of contact between the woody riparian vegetation and the natural vegetation of the slopes, if present. To calculate the percentage of forest coverage at the functional unit level, the number of pixels with the presence of forest was divided by the total number of pixels occupied by each river unit. This percentage has been calculated separately for each type of habitat, considering three degrees of transversal connectivity:

1. Favourable connectivity degree: Forest coverage >80%.
2. Unfavourable-inadequate connectivity degree: Forest coverage 30-80%.
3. Unfavourable-poor connectivity degree: Forest coverage <30%.

5.2.3. Results of the evaluation of riparian habitats conservation status

To improve the understanding of factors that could determine the characteristics of the riparian environment in the Sella and Deva-Cares basins, changes in the variables considered in the former sections (probability of occurrence, conservation status, and degree of connectivity) have been evaluated in relation to the basin size and the altitude of each functional unit, two key factors in the regulation of water flow to the river, water availability, and accessibility to the stretch (Lara et al., 2004; Table 4).

Catchment size			Altitudinal belt		
	Area	N		Altitude	N
Small	<10 km^2	434-1290	Low	<600 m	56-254
Medium	10-100 km^2	81-223	Medium	600-1200 m	283-580
Large	>100 km^2	28-103	High	>1200 m	56-254

Tabla 4. Classification values of functional units by basin size and altitude. N= Number of functional units for each basin and size class for habitat 91E0* and 92A0, respectively.

<u>91E0 y 92A0 habitat distribution</u>*

The distribution area of habitats 91E0* and 92A0 is segregated by bioclimatic regions (Fig. 26). Specifically, habitat 91E0* predominates in the Atlantic region (northern area of described basins), while habitat 92A0 is dominant in the Mediterranean region (southern area). It is noteworthy that there are areas where these habitats coexist, mainly in the southern zone of the area defined by the Sella and Deva-Cares basins (Fig. 26).

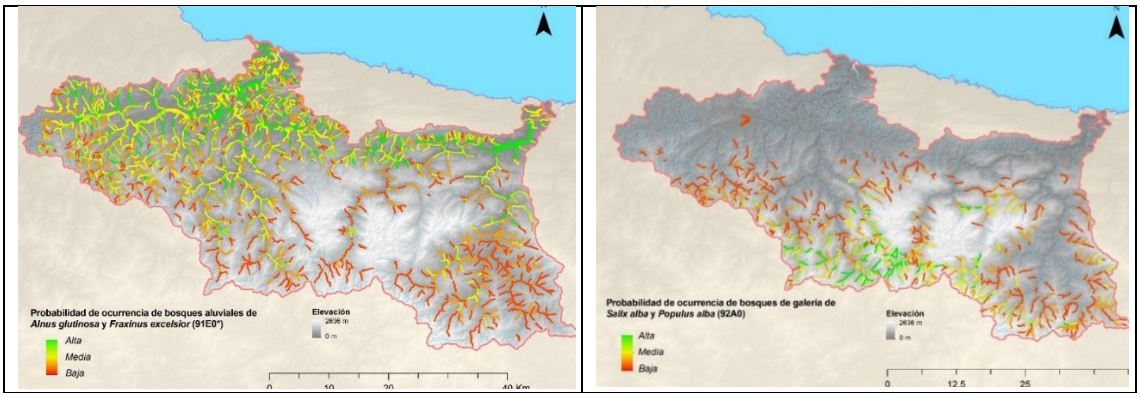


Figure 26. Result of the habitat 91E0* (left) and 92A0 (right) distribution models.

Regarding the probability of occurrence based on basin size and the altitudinal belt of the functional unit, it has been observed that for habitat 91E0*, this probability increases in the lower belt and decreases with altitude (Fig. 27). Additionally, the maximum values of probability of occurrence are found in the lower belt of large basins. This pattern can be explained by greater water availability and better environmental conditions for the development of this habitat in large basins, as well as higher precipitation and lower occupation of climax forests at lower altitudes (compared to medium altitudes; Lara et al., 2004).In contrast, the probability of occurrence of habitat 92A0 is similar across different altitudinal belts (Fig. 27). This may be due to the fact that the habitat dominated by willows and poplars is more resistant to changes in water availability than the habitat dominated by alders and ashes or, alternatively, that the selected basin size classes and altitudinal belts do not capture the natural variability inherent to this habitat

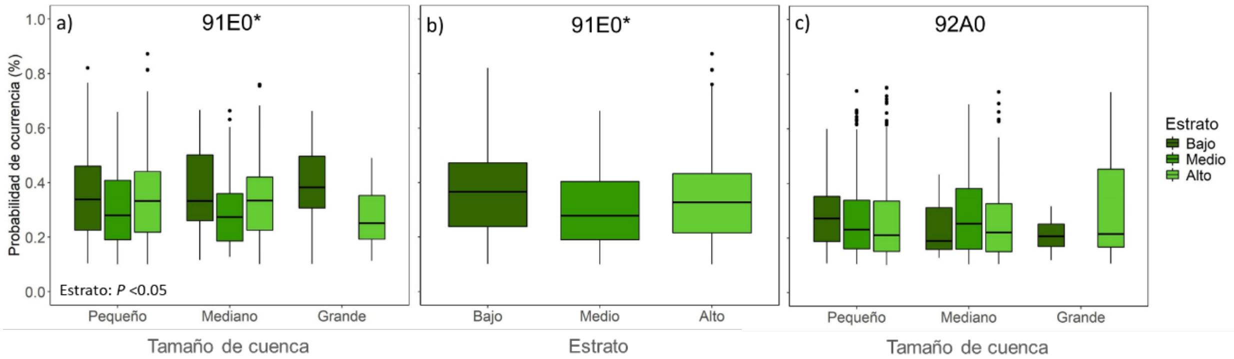


Figure 27. Changes in the probability of occurrence of 91E0* by basin size and altitudinal belt (a) and grouped by altitudinal belt (b), and of habitat 92A0 by basin size and altitudinal belt (c). Boxes = median and 25th and 75th percentiles. Lines = minimum and maximum values at 1.5 times the height of the box. Points = extreme values.

Riparian forest conservation status

Regarding the conservation status of habitat 91E0, most of the studied functional units show, equally, a favourable and unfavourable-poor conservation status (41% in both cases), while the remaining 17% presents an unfavourable-inadequate status (Fig. 28). In contrast, habitat 92A0 is predominantly favourable in most units (67%), while being in an unfavourable-inadequate and poor state to a lesser extent (26% and 6%, respectively).

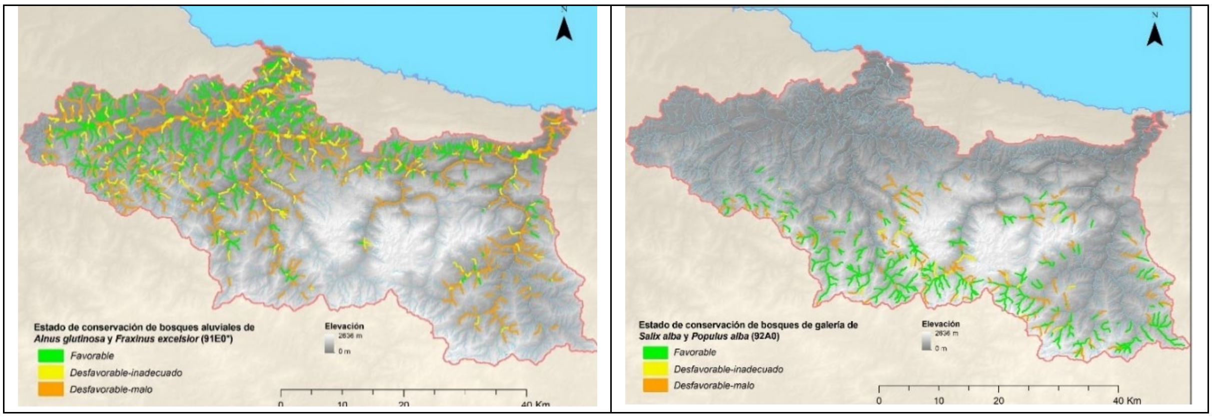


Figure 28. Result of the evaluation of the conservation status of habitat 91E0* (left) and 92A0 (right).

For 91E0* a higher proportion of functional units with a favourable conservation status is observed in small and medium-sized basins (Fig. 29), which typically corresponds to less anthropized headwater areas. In contrast, in large basins, which likely have a greater area of exploitation, an unfavourable conservation status predominates (Álvarez-Martínez et al., 2018b). For habitat 92A0, a favourable conservation status predominates across all three basin sizes, with similar proportions among them, while the proportion of favourable status increases in higher altitudes, particularly in large basins (Fig. 29). This may be because habitat 92A0 more efficiently utilizes the greater availability of water associated with large basins, leading to greater height and physiological condition, as measured by NDVI.

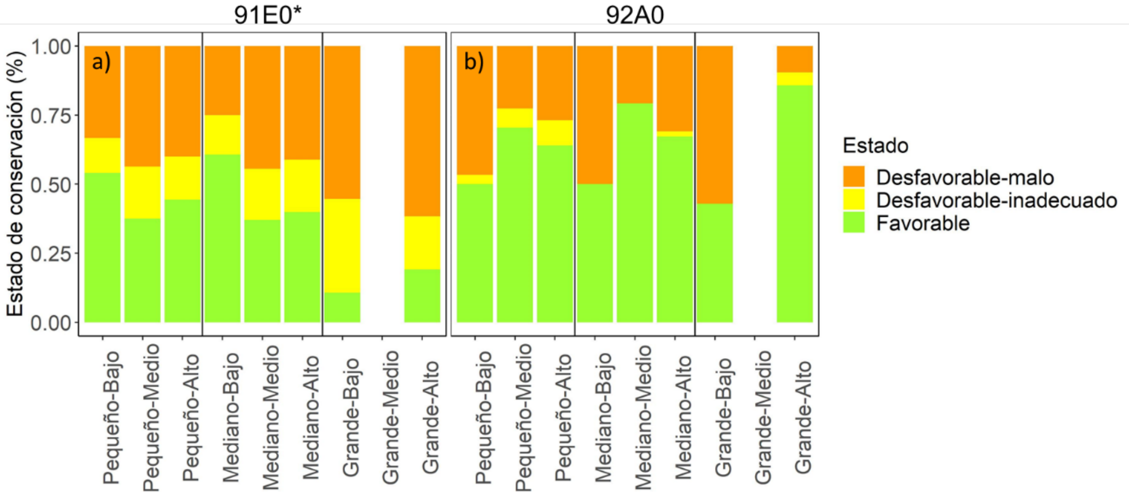


Figure 29. Proportion of functional units with the different conservation states of the habitat 91E0* (a) and 92A0 (b) with the basin size and altitudinal stratum.

Riparian conectivity degree

Riparian forest in the Sella and Deva-Cares basins shows a medium degree of transversal connectivity (37% 91E0*and 34% 92A0; Fig. 30).

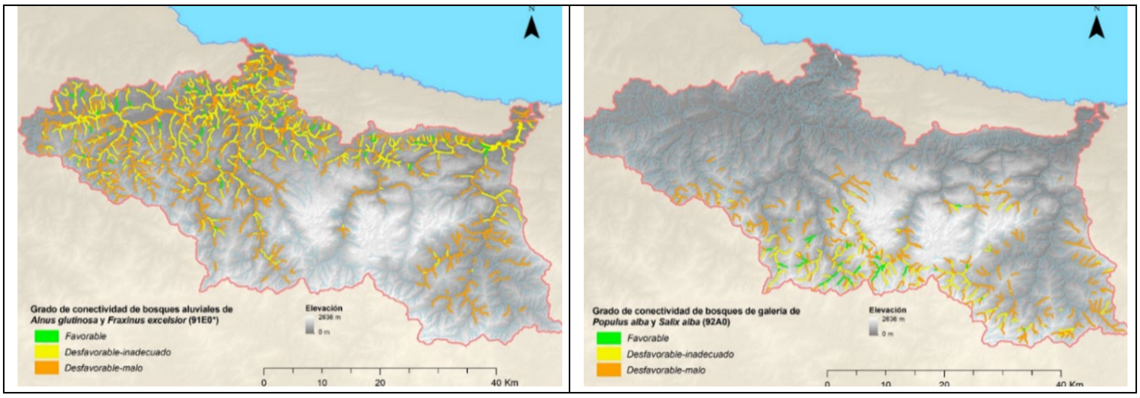


Figure 30. Degree of transverse connectivity of habitat 91E0* (left) and 92A0 (right).

In particular, greater cross-connectivity is observed in small basins and in the low and high altitudes for habitat 91E0* (Fig. 31), whereas habitat 92A0 showed no differences in connectivity related to basin size or altitude (Fig. 31). Similar to what was previously described, the results for 91E0* may be due to the fact that small basins and the high altitude in headwater areas show fewer anthropogenic alterations and greater forest cover (Álvarez-Martínez et al., 2018b). However, one would expect the riparian forest to have higher coverage values in the middle and low altitudes compared to the high one, which has greater torrentiality and shallower soils. This supports the previous idea that the most accessible sections, which are more exploited for anthropogenic activities, experience a more pronounced deterioration in riparian forest connectivity. Alternatively, these results suggest that there are strong floods and erosion processes in the middle sections that hinder the natural development of the riparian forest, along with greater accessibility and anthropization due to the historical land use associated with productive activities.

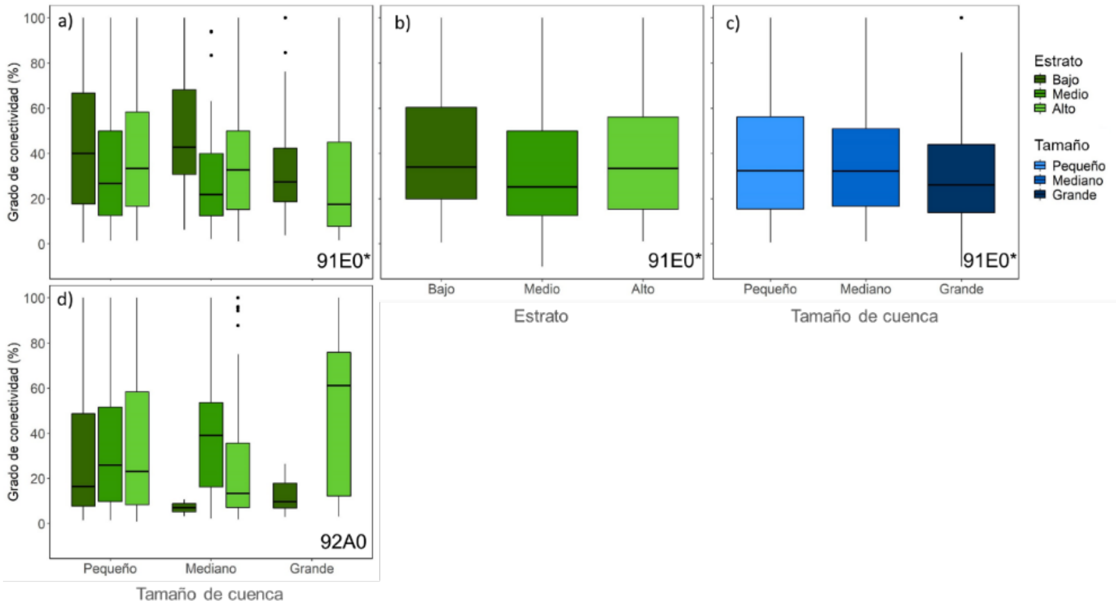


Figure 31. Changes in the transverse connectivity of 91E0* by basin size and altitude (a) and grouped by stratum (b) and basin size (c), and of 92A0 by basin size and altitudinal belt (d). Boxes = median and 25th and 75th percentiles. Lines = minimum and maximum values at 1.5 times the height of the box.

After evaluating the conservation status of 91E0* and 92A0 habitats, was observed that 92A0 has a better conservation status in the study area than 91E0*. Furthermore, each one of these habitats exhibits a different pattern in terms of conservation status. Habitat 91E0* tends to present more unfavourable conditions in large basins, as a result of greater anthropic pressure, while 92A0 shows no significant differences between different basin sizes. Regarding the influence of altitude, habitat 92A0 presents, compared to 91E0*, a greater proportion of favourable status in higher strata, which could be explained by the higher tolerance of willows to temporary hydrological regimes and more unstable environments.

On the other hand, both habitats show a relatively low degree of cross-connectivity (<40%) in the described basins, with unfavourable conditions being the most common for both. The degree of cross-connectivity for habitat 91E0* also clearly responds to basin size and altitude stratum, decreasing in medium strata and medium to large basins. This may indicate anthropization of land use or the existence of erosive processes that increase the instability of the riparian zone. In terms of connectivity, habitat 92A0 did not show significant differences based on altitude or basin size, again displaying lower sensitivity to these factors compared to 91E0*.

Protecting and restoring the cross-connectivity of the riparian environment with adjacent ecosystems is key to maintaining processes such as regulating flow velocity, sedimentation processes, energy and material exchange with the terrestrial system, and filtering nutrients and contaminants. In the case of the Sella and Deva-Cares basins, habitats 91E0* and 92A0 may be experiencing a degradation process associated with human activity, particularly notable in the case of habitat 91E0*.

6. Socioeconomical and water uses characterization

Sella and Deva-Cares river basins include a total of 25 municipalities (14 in Asturias, 8 in Cantabria, and 3 in Castilla y León), of which 11 are located within the boundaries of the Picos de Europa National Park (6 in Asturias, 3 in Cantabria, and 2 in Castilla y León, Table 5)

6.1.1. Demografía

The demographic evolution shows a slight population decline in the last 20 years in almost all the municipalities of this area (Table 5). As a whole, and according to data from the National Institute of Statistics (INE), the total census population went from 50,710 inhabitants in 2000, to 50,036 in 2010 and 45,425 in 2020.

	2000	2010	2020	PNPE	CCAA
Cabezón de Liébana	679	690	604	NO	Cantabria
Camaleño	1,101	1,050	930	YES	Cantabria
Cillórigo de Liébana	1,103	1,345	1,299	YES	Cantabria
Peñarrubia	284	366	319	NO	Cantabria
Pesaguero	402	345	295	NO	Cantabria
Potes	1,573	1,511	1,332	NO	Cantabria
Tresviso	60	78	59	YES	Cantabria
Vega de Liébana	999	846	719	NO	Cantabria
Oseja de Sajambre	360	275	224	YES	CyL
Posada de Valdeón	490	487	415	YES	CyL
La Pernía	479	392	316	NO	CyL
Amieva	905	805	628	YES	Asturias
Bimenes	2,174	1,894	1,679	NO	Asturias
Cabrales	2,371	2,253	1,941	YES	Asturias
Cangas de Onís	6,343	6,756	6,209	YES	Asturias
Caso	1,99	1,848	1,457	NO	Asturias

	2000	2010	2020	PNPE	CCAA
Nava	5,656	5,635	5,276	NO	Asturias
Onís	893	797	734	YES	Asturias
Parres	5,563	5,804	5,315	NO	Asturias
Peñamellera Alta	740	607	498	YES	Asturias
Peñamellera Baja	1,621	1,326	1,227	YES	Asturias
Piloña	8,892	7,994	6,875	NO	Asturias
Ponga	760	678	572	NO	Asturias
Ribadesella	6,245	6,301	5,688	NO	Asturias
Sobrescobio	818	898	814	NO	Asturias

Tabla 5. Population of the municipalities of the Sella and Deva-Cares basins.

These data show that the population decline has intensified since 2010. From 2010 to 2020 there was a relatively constant annualized decrease in the population, affecting both men and women similarly, although it seems that the trend stabilizes in the last three years of the series (2020-2022; Fig. 32).

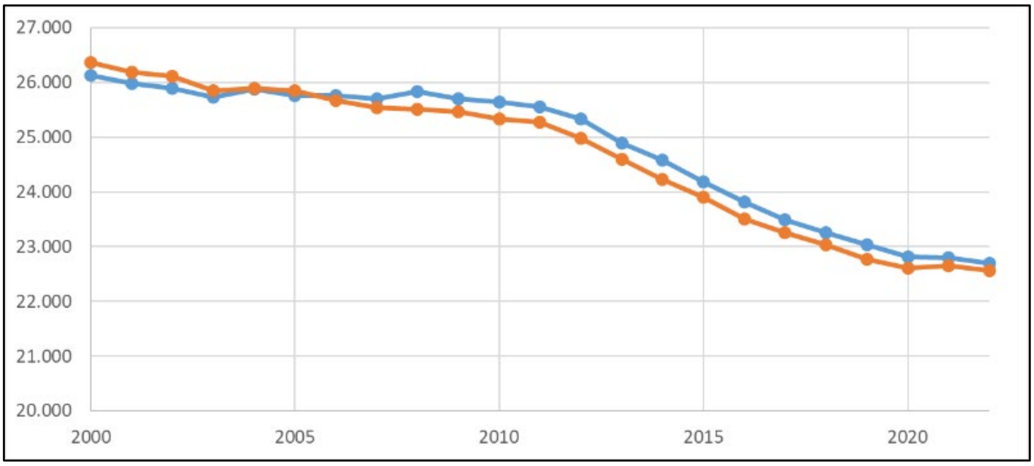


Figure 32. Evolution in the number of inhabitants registered in the 25 municipalities of Sella and Deva-Cares by sex between 2000 and 2022 (Blue: men; Red: women). Source, INE.

Population from foreign countries was around 2,000 inhabitants, which accounts for 4.5% of the total population, with their origin being primarily European (55%) and American (40%). In relation to the age distribution, of the 45,256 inhabitants recorded in 2022, 16% correspond to the child population (<16 years), 57% to the working-age population (16-64 years), and the remaining 24% to the population over 64 years. As shown in Figure 33, 2022 population census groups most of the described population (45,256 inhabitants) in the municipalities of the lower and more western area of Asturias, with Piloña and Cangas de Onís being the municipalities with the highest number of inhabitants (6,862 and 6,260, respectively). In contrast, the least populated municipalities are those located in the interior at higher altitudes, with Tresviso in Cantabria being the municipality with the lowest number of inhabitants, with only 54 people recorded in this municipality.

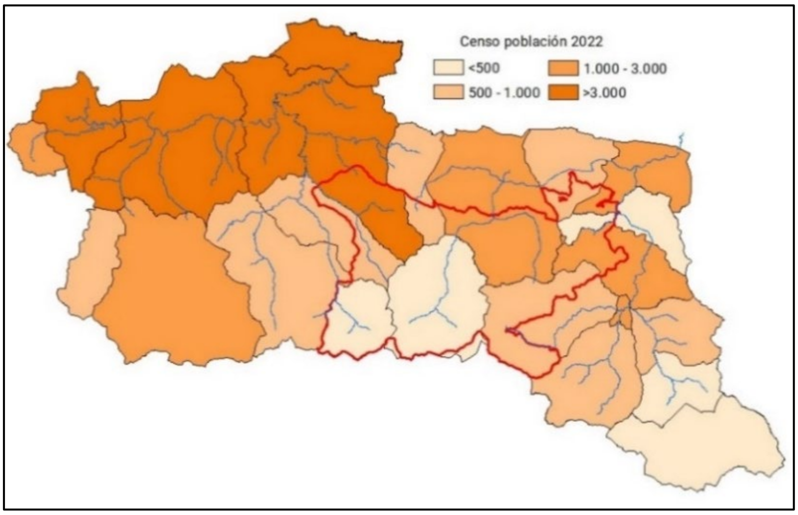


Figure 33. Population census by municipality for the year 2022 (Source: INE).

6.2. Sectorial economic activity

Primary sector. Extensive livestock farming and the related industry, such as cheese production, are important activities in the area. A clear example is the municipality of Cabrales and Onís, which has 38 and 11 companies dedicated to cheese production, respectively. In 2017, this area had a total of 122,152 livestock (17,200 goats, 21,453 sheep, and 83,499 cattle; equine data is not included), having a decrease compared to previous years (125,380 in 2015 and 123,811 in 2016). Municipalities with the largest livestock populations are in Asturias, especially Piloña (14,728), Cangas de Onís (13,460), and Cabrales (12,056; Fig. 34). In contrast, the municipalities of Potes (114) and Tresviso (435), both in Cantabria, have the lowest number of livestock.

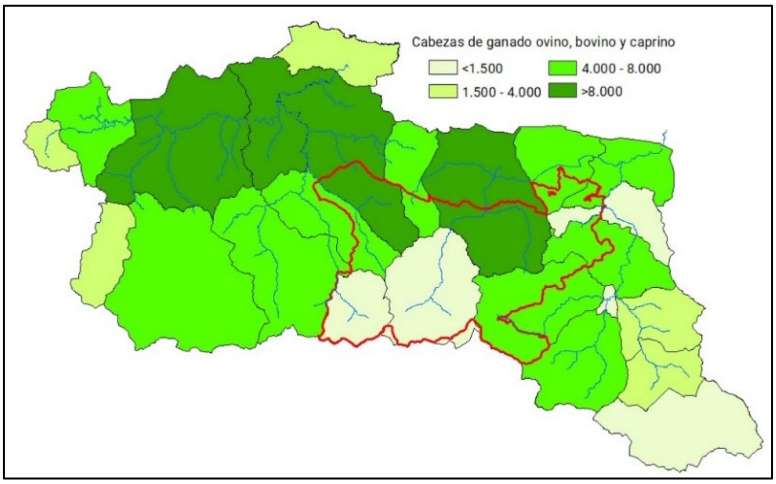


Figure 34. Census of goats, sheeps and cattle heads by municipality in 2017 (Source: INE).

Secondary sector. The municipalities of Asturias have the highest number of companies engaged in industrial and construction activities (Fig. 35). Piloña is the municipality with the largest number of companies in this sector (168), while the National Statistics Institute (INE) does not report any activity in this sector for 15 of these 25 municipalities:

La Pernía, Oseja de Sajambre, Posada de Valdeón, Amieva, Caso, Onís, Peñamellera Alta, Ponga, Sobrescobio, Cabezón de Liébana, Camaleño, Peñarrubia, Pesaguero, Tresviso, and Vega de Liébana.

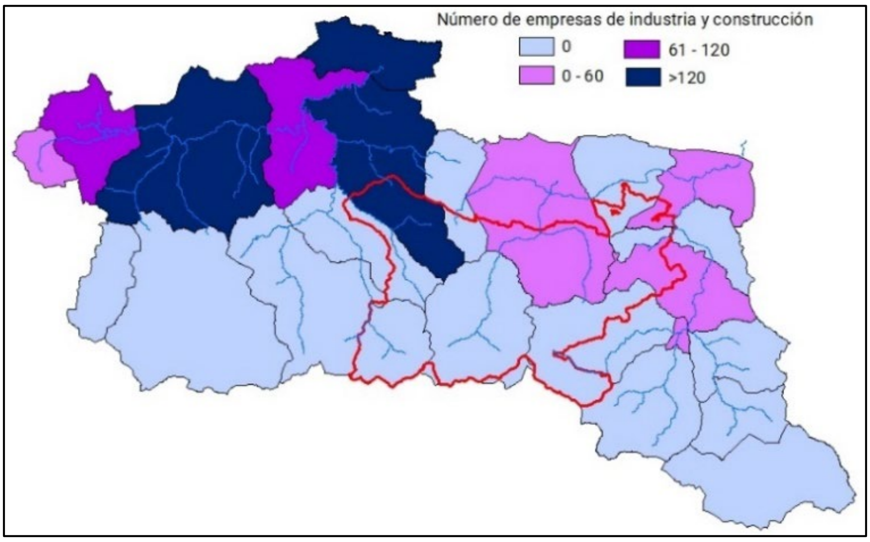


Figure 35. Companies dedicated to industrial activity and construction in 2020 (Source: INE).

Tertiary sector. Tourism is an important activity for the area's economy, being the leading economic sector by revenue in some municipalities. The main driver is the National Park, which reached 2.1 million visitors in 2016. Within the park's boundaries, the most visited sites are the Lakes of Covadonga, in Asturias, and the Fuente Dé cable car area in Cantabria, while in León, Posada de Valdeón is the population center that concentrates most of the tourist activity. Cangas de Onís has the highest number of service sector companies (353; Fig. 36). As in the previous case, the INE does not report any business activity linked to the tertiary sector in 15 of these 25 municipalities (La Pernía, Oseja de Sajambre, Posada de Valdeón, Amieva, Caso, Onís, Peñamellera Alta, Ponga, Sobrescobio, Cabezón de Liébana, Camaleño, Peñarrubia, Pesaguero, Tresviso, and Vega de Liébana).

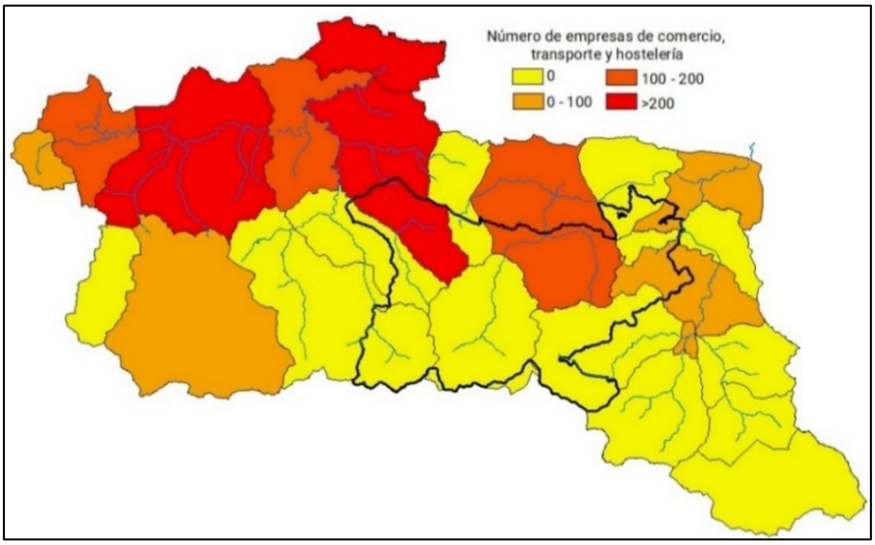


Figure 36. Companies dedicated to commerce, transportation and hospitality in 2017 (Source: INE).

6.3. Water uses in Picos de Europa National Park and surrounding area

Water is one of the most important natural resources for all these economic sectors. In the case of the primary sector, water is essential for meeting the needs of livestock, both in stables and when they are in semi-freedom conditions. For the secondary sector, water is the driving element that enables the generation of hydroelectric energy, which is a very relevant activity in these watersheds. Finally, for the tertiary sector, water plays a fundamental role, as many tourism and leisure activities are linked to this element, such as sport fishing, kayaking on the Sella and Deva rivers, and snowshoe mountain routes, among others. Additionally, water bodies are some of the most relevant and iconic spaces in the national park, such as the Lakes of Covadonga (Enol and Ercina lakes).

6.3.1. Primary sector water uses

According to data published by the Picos de Europa NP (Llaneza & García, 2016), around 60,473 livestock heads were recorded in 2015 in the 11 municipalities that are part of the Park (14,793 sheeps, 11,590 goats, 31,285 cattle, and 2,805 horses). According to Hernández Bedeni (1984), it is estimated that cattle consume about 50-70 l/day, which can reach 100 l in the case of lactating cows. This consumption is 6-12 l/day for goats and sheeps and 25-75 l/day for horses. These estimates of water consumption are made for adult animals and can vary depending on the type of feed, time of year, temperature, etc. Taking average values, we would obtain that the livestock in the Picos de Europa NP consumes the following amount of water:

- Cattle: 31,285 head * 60 liters * 365 days/year = 685,141,500 liters/year.
- Horses: 2,805 head * 50 liters * 365 days/year = 51,191,250 liters/year.
- Sheep: 14,793 head * 9 liters * 365 days/year = 48,595,005 liters/year.
- Goats: 11,590 head * 9 liters * 365 days/year = 38,073,150 liters/year.

Based on this data, it can be estimated that the livestock sector established in the Picos de Europa NP consumes about 823 million l/year (0.82 Hm^3), which, for reference, duplicates the maximum volume of La Jocica reservoir, on the Dobra River (0.4 Hm^3). When livestock is in extensive farming conditions during the summer, the herd obtains water from various sources, mainly from watering points, springs, lakes, and wetlands, or from the riverbeds themselves. Within the boundaries of the NP, a total of 235 watering points, 177 springs, and 285 lakes, lagoons, and wetlands have been inventoried (Fig. 37), where livestock can often be seen obtaining water during the periods when they are in the pastures under semi-freedom conditions.

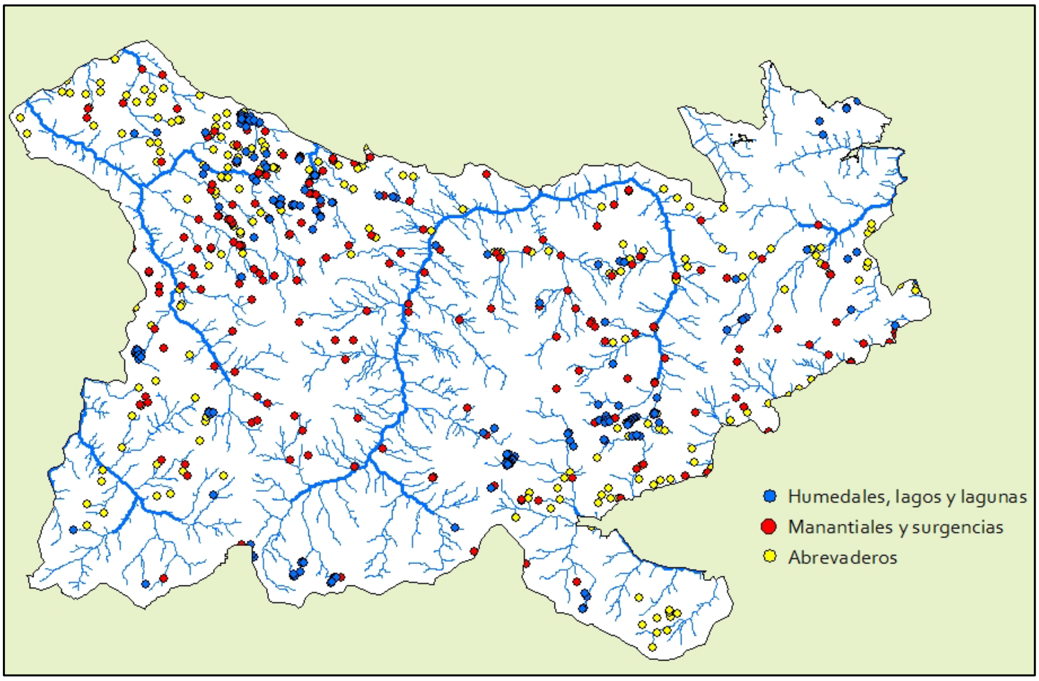


Figure 37. Location of watering holes, fountains and wetlands in the Picos de Europa National Park.

However, in some cases, watering points are in poor conditions or overexploited by livestock, leading them to dry up for extended periods. This forces the livestock to approach natural water environments to obtain water (e.g., springs, wetlands, etc.), which compromises their conservation status. This situation is also affected by specific water extractions to meet other irrigation needs related to the maintenance of gardens, fruit trees, etc. (Fig. 38).

Figure 38. Pump for extracting water from a spring and concentrating livestock on a water trough. Both photos are taken inside the Picos de Europa National Park.

Regarding primary sector, in addition to the water consumption associated with the livestock, the Cantabrian Hydrographic Confederation estimates a total of 2.99 Hm^3/year to meet the agricultural demand of the municipalities in the Picos de Europa NP (CHC, 2015). This consumption is distributed as follows:

- Sella Basin: 0.42 Hm^3/year: Oseja de Sajambre 0.04 Hm^3/year, Amieva 0.20 Hm^3/year, and Onís 0.18 Hm^3/year.

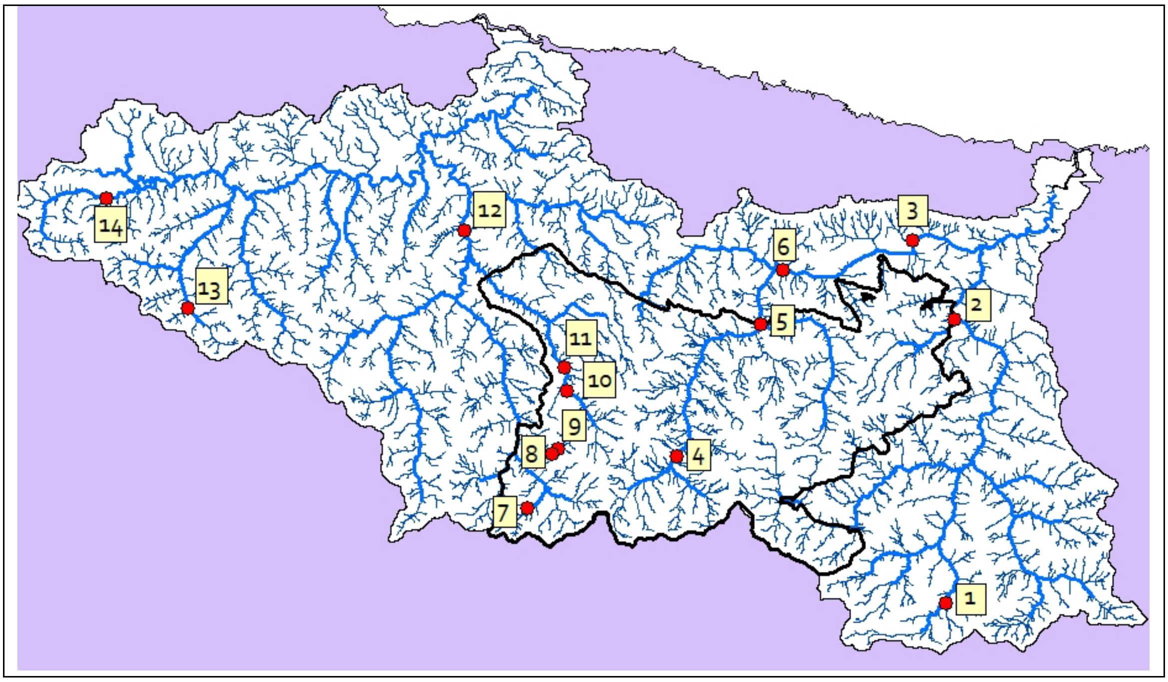
- Deva-Cares Basin: 2.57 Hm³/year: Cabrales 0.26 Hm³/year, Peñamellera Alta 0.10 Hm³/year, Peñamellera Baja 0.24 Hm³/year, Camaleño 1.02 Hm³/year, and Cillórigo de Liébana 0.95 Hm³/year.Cuenca del Sella; 0,42 Hm^3/año: Oseja de Sajambre 0,04 Hm^3/año, Amieva 0,20 Hm^3/año y Onís 0,18 Hm^3/año.

Therefore, taking all these consumption estimates together, it can be concluded that the activities of the primary sector carried out in the municipalities of the Picos de Europa NP amount to 3.81 Hm³/year.

6.3.2. Sencondary sector water uses

Within the secondary sector, the most relevant use of water in the PN and its surroundings is for the generation of hydroelectric energy. In the basins of the Sella and Deva-Cares rivers, there are 14 facilities for hydroelectric use, most of which have infrastructures (reservoirs, canals, etc.) within or near the limits of the PN (Fig. 39).

Additionally, it should be noted that in the Sella basin, industrial activities have their own water supply of 1.85 Hm³/year (Nestlé España 1.73 Hm³/year and Fuensanta 0.12 Hm³/year; CHC, 2015). However, these industries are located in the sub-basin of the Piloña River, so their supply is not closely linked to the flows generated at the headwaters of the Sella. On the other hand, in the Deva-Cares basin, there are no industries with their own supply according to the information provided by the Cantabrian Hydrographic Confederation (CHC).



Nº	Nombre	Concesionaria	Río	Caudal máx turbinado (l/s)	Salto bruto (m)	Potencia (kw/h)
1	Cucayo	Sociedad Eléctrica Río Frío	Frío	2.000	209	4.994
2	Urdón	REPSOL	Urdón	3.000	372	5.962
3	Niserias	Centrales eléctricas del Principado	Cares	6.000	4	161
4	Cordiñanes	ALTANO	Cares	6.000	195	9.800
5	Camarmeña	REPSOL	Cares	8.000	220	13.600
6	Arenas de Cabrales	REPSOL	Cares	14.000	78	8.877
7	Güeyu de Zarambal	Salto de Sajambre	Zalambral	260	175	312
8	Ribota	PREMISA	Zalambral	1.200	330	2.055
9	San Pedro	PREMISA	Agüera	800	307	1.833
10	Restaño	ALTANO	Dobra	8.800	217	14.400
11	Camporriondi	ALTANO	Dobra	4.000	510	15.200
12	Caño	EDP España	Sella	9.000	11	971
13	El Retorno	Estudios y Explotación Recursos	Ranera	1.500	108	1.360
14	Perancho	Ayto. de Gijón	Fuente Santa	300	522	910

Figure 39. Location of hydroelectric projects in the Sella and Deva-Cares basins and main characteristics (CHC, 2015).

6.3.3. Tertiary sector water uses

It is difficult to quantify water uses by the tertiary sector, which is increasingly relevant in the PN and its surroundings, as described above. As a reference to assess the needs of the resident and visitor population, the Urban Demand Units (UDU) defined by the CHC (2015) have been considered. In the case of the UDUs of the Deva System, which includes the municipalities of Cabezón de Liébana, Cabrales, Camaleño, Cillórigo de Liébana, Peñamellera Alta and Baja, Peñarrubia, Pesaguero, Posada de Valdeón, Potes, Ribadedeva, Tresviso, and Vega de Liébana, they amount to 1.94 Hm^3/year. On the other hand, the UDUs of the SE Sella amount to 7.06 Hm^3/year, although this volume of water is reduced to 1.71 Hm^3/year if the consumption of the municipalities located in the middle/lower part of the basin, such as Piloña or Ribadesella, is not considered.

7. River, wetlands, lakes, springs and water-trough cartography.

Environmental management of any area, especially if it is a protected area, such as the NNPP (highest figure of environmental protection in Spain; Law 30/2014), requires the generation of a comprehensive database that allows the characterization of the environmental (physical and biological) and socio-economic components of the area to be managed. These databases serve as the basic support for the subsequent development of tools that enable the determination of the biophysical interactions that occur between the different elements constituting the territory. The use of such tools is essential for identifying the causes and effects of various environmental changes that may arise from direct human actions (i.e., anthropogenic pressures), as well as those processes occurring on broader temporal and spatial scales, such as those caused by global change (Monk et al. 2007).

Therefore, the information these databases must collect is very heterogeneous, including data on variables and elements of different natures (physical, biological, socio-economic, etc.) and data from various sources, representative of different spatial and temporal scales (in situ samples, satellite information, predictions from environmental modeling, etc.). This variability in the origin and nature of the information used for the environmental management of protected areas requires the development of digital platforms that facilitate both the management of the data itself and its use for characterization, evaluation, and modelling/prediction of the different elements that make up the area to be managed. In the case of aquatic ecosystems, it is essential to have good digital cartographic information that allows for their localization, metadata documentation, and the ability to associate environmental and biological information with each inventoried and mapped water body.

7.1. Fluvial cartography. Development of a spatial framework for data management.

During the last decade, significant progress has been made in the development of digital platforms applied to the management of aquatic ecosystems, especially river ecosystems, through the use of virtual basins and synthetic river networks. These digital tools provide an appropriate spatial framework for organizing and managing information at different hierarchical levels, ranging from river stretches to basin/sub-basin, allowing interrelationships to be established between the aquatic environment, the terrestrial environment and different climatic processes (i.e., atmospheric). Additionally, their use also addresses data gaps, allowing for continuous predictions across the entire river network based on available predictive variables for each case. These tools have been successfully applied to continuously determine water quality (Álvarez-Cabria et al., 2016), hydrological regimes (Peñas et al., 2016), or the assessment bioindicator communities of river ecosystem integrity, along with their associated quality indices (e.g., IBMWP for invertebrates or percentage of salmonids; Álvarez-Cabria et al., 2017). For the development of these synthetic river networks and the subsequent definition of virtual basins, the use of specific software programs is required to delineate these networks from digital terrain models (DTM). Among these, NETMAP (Benda et al., 2007), developed by the Earth System Institute, has been successfully employed in different scenarios, allowing, among other things, to:

1. Define the basins into which a given territory is divided (virtual basins) and the river systems that drain each of them (synthetic river network).
2. Segment the generated synthetic river network by applying different criteria: presence of hydraulic infrastructure, of tributary axes, changes in ecological typology or water body status (see Spanish National Hydrological Plan), etc.
3. Link information from any pixel of the territory (virtual basin) to the different segments defined for the synthetic river network.
4. Delineate riparian and floodplain areas.

5. Map the fluvial and riparian landscape at different spatial scales (basin, sub-basin, or segment level).

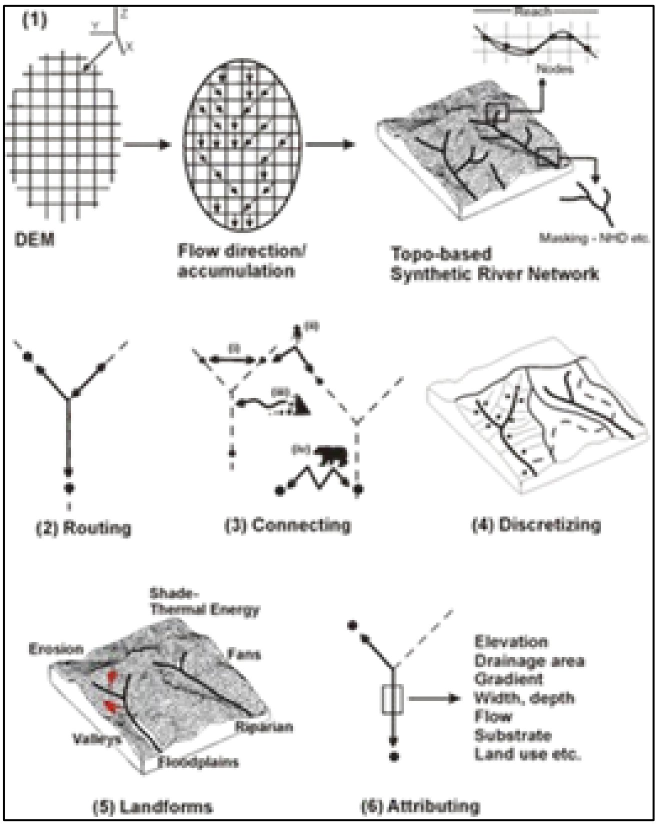


Figure 40. Synthetic river network and NETMAP properties to attribute information to each stretch, together with the delineation of other characteristics of the river landscape. From Benda et al. (2016).

After the development of these digital tools as a support for the management of aquatic ecosystems, it is necessary to create a database of the most relevant environmental variables, allowing each section of the synthetic river network to be provided with different information (i.e., metadata): climatic, topographic, lithological, edaphic, hydrological, biological, demographic, land use, etc. These networks also enable the digital location of several elements on the own network, such as the different anthropic alterations, water discharge, dams, weirs, bridges, fords, water intakes, etc. and the inclusion of the most relevant attributes to characterize each of these pressures (e.g., dam height, channelization length, conservation status, etc.).

The use of these tools not only allows linking environmental information with each river stretch of the synthetic river network, but also enables different calculations using common Geographic Information System (GIS) tools (e.g., ArcMap, QGIS), as well as NETMAP. Some examples include:

- Mean or cumulative value of a particular environmental variable for the entire watershed draining into a river segment.
- Mean or cumulative value of a particular environmental variable for the sub-basin where a specific river segment is located.

- Mean or cumulative value of a particular environmental variable in the riparian zone.
- Number of pressures upstream (point-type pressures).
- Length of pressures upstream (linear-type pressures).
- Distance to the nearest pressure upstream or downstream.

Finally, digital basins and synthetic river networks coupled with environmental databases, along with the use of appropriate statistical techniques (e.g., regression techniques and data mining), not only allow the generation of continuous data across the entire spatial framework (Fig. 41) but also open the door to modelling the dynamics of biotic and abiotic elements under different future scenarios. This provides the ideal framework for land managers, researchers, or other stakeholders with interests in this environment to make informed decisions in anticipation of predicted changes, as well as the ability to assess different scenarios and options that may arise in the future.

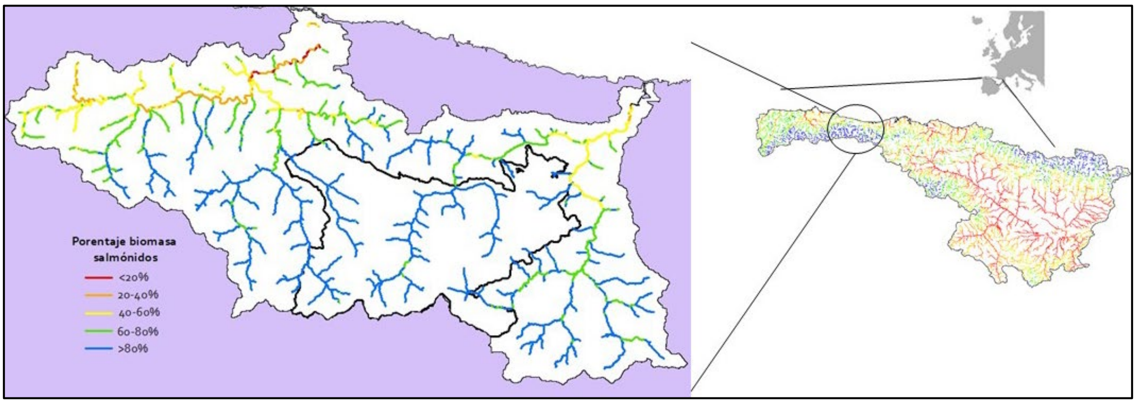


Figure 41. Virtual basin and synthetic river network of the Ebro and Cantabrian demarcations with the percentage of salmonid biomass modelled by each river section (sections of 0.5-1 km). The same expanded results are shown for Sella and Deva-Cares (see Álvarez-Cabria et al.; 2017).

7.2. Cartography of lentic water bodies

7.2.1. Lakes and lagoons

The distribution and typology of lentic water bodies, corresponding to lakes and lagoons in the study area, were developed sequentially, first using official data sources and then verification and result update methodologies.

Initially, the information collected in the MTN25, part of the basic cartography of the Spanish state, was used. This is a periodically updated digital series that includes data from the National Topographic Base at a scale of 1:25,000 (BTN25) and the Land Occupation System of Spain (SIOSE), with particular attention to wetland typologies and unique aquatic elements. The sheets corresponding to the study area, 46 in total, are freely available at the download center of the National Geographic Information Center (CNIG) in raster format (ECW; Enhanced Compression Wavelet or improved wavelet compression, and GeoTiff) and the ETRS 89 Geodetic Reference System for the peninsula, in UTM Zone 30 North projection. ECW files can be quickly compressed and decompressed, allowing efficient processing on conventional computers.

The procedure used takes advantage of the fact that the ECW sheets consist of 3 bands for the red, green and blue channels (RGB) and can differentiate various elements by colour tones across the 3 channels, treating them like multispectral images. By applying a classification using the maximum likelihood algorithm, a file is generated where the categories of interest are differentiated. Using GIS analysis, the raster information is transformed into vector format by selecting only the elements belonging to the water body category. Repeating this process iteratively for the 46 sheets generates the vector database for lentic and lotic water bodies, comprising 3,860 elements. This semi-automatically generated database was then used to complement other existing databases (Picos de Europa NP and IHCantabria). The information was analysed in detail by experts through photointerpretation, fieldwork, and in-situ data collection, resulting in the final database of lakes and lagoons (Fig. 42), with a total of 70 water bodies distributed across the entire study area.

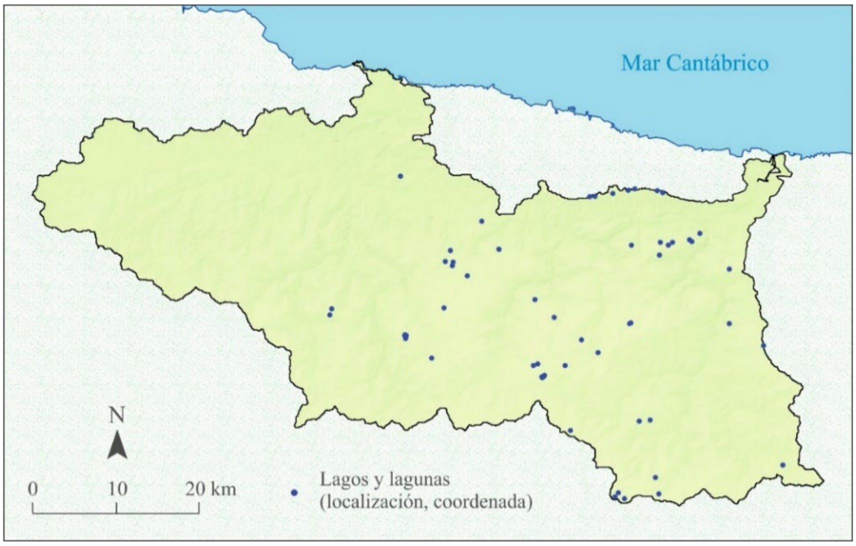


Figure 42. Lentic water bodies, lakes and lagoons of the Sella and Deva-Cares basins.

7.2.2. *Peatlands and wetlands*

The mapping of peatlands/wetlands, which is not available in the previous MTN25 database, has been developed using supervised ecological modelling techniques. This combines aerial photography analysis and satellite image classification from Sentinel 2 with the combined development of spectral indices and multitemporal monitoring. Photointerpretation is a valuable tool for mapping complex systems such as mountain wetlands. This high-spatial-resolution image analysis method provides a detailed and accurate view of the topography and terrain characteristics, offering a panoramic perspective of the landscape. It enables the efficient identification and mapping of land occupation, even in areas that are difficult to access, by expert operators with field knowledge of the systems of interest and their responses in this type of optical information source. Additionally, aerial photographs provide highly useful visual information for obtaining quantitative data on the characteristics of water bodies. By observing specific tones and textures in the images, one can deduce the depth and transparency of the water, the aquatic vegetation and other key elements of these ecosystems, as well as their spatial-temporal variability. The comparison of aerial photographs taken at different times (inter- and intra-annually) makes it possible to identify fluctuations in size, shape and location, which provides a valuable information on their temporal dynamics. In this case, photointerpretation of aerial photographs was particularly useful due to the complexity of the mountainous area mapped, as well as its large extent, providing an overview that allows the collection of quantitative data and indicators of its structure and functioning.

Based on the in-situ information provided by visual analysis, the wetland mapping was developed using time series of satellite images from the Sentinel 2 program. These images were selected for 3 different phenological periods in 2019 (April-May, June-July, and August-September) with the lowest cloud cover possible and a high solar elevation angle to avoid topographic shadows, which mostly occur during the summer period. The mission provides free images for any user and consists of two identical satellites, Sentinel 2A and 2B, which orbit simultaneously 180º apart, with a temporal resolution of 5 days. In this context, two types of classified products are available: L1C level, which corresponds to radiometrically and geometrically corrected data with reflectance above the atmosphere (top of atmosphere, TOA), including ortho-rectification and spatial registration in a global reference system with UTM/WGS84 projection; and L2A level reflectance with values at the bottom of the atmosphere (BOA). The latter is obtained using the Sen2Cor plugin in the Sentinel-2 SNAP tool, which is also used for topographic correction of the images using a Digital Elevation Model (DEM), resampling the bands that require it to a 10-meter pixel size.

Subsequently, with the images at the desired radiance level, a detailed cloud presence analysis was performed for the study region to develop Maximum Value Composites (MVC). Cloud gaps in the images were filled with pixels from other cloud-free images as close as possible in time, with similar reflectance values after applying the same correction process. Once the gaps were patched and the MVC created for each band of each image, an extraction by mask was performed for the entire study area for each of the image bands and derived spectral indices.

From the Sentinel 2 images downloaded, corrected to BOA level, and composed into MVC products for each defined annual period, three spectral indices were generated, selected after analyzing the most commonly used indices in classification and soil moisture location models. These indices were calculated with the Sentinel 2 image bands. They are:

- NDVI Index (Normalized Difference Vegetation Index): Described in previous chapters of this document, this index allows estimating the amount, quality, and development of vegetation by measuring the intensity of radiation from certain bands of the electromagnetic spectrum that vegetation emits or reflects. Its value ranges from -1 to +1, with higher values corresponding to better vegetation health and lower values to poor health or absence of vegetation. The relationship of this index with soil water presence is determined by the fact that NDVI variations imply changes in vegetation condition, which in this study is extrapolated to mean variations in soil water presence. High NDVI values indicate water absence, and low values indicate its presence.

- NDWI Index (Normalized Difference Water Index): This index identifies areas of high moisture saturation, serving as a measure to determine water stress in vegetation, soil moisture saturation, or direct delineations of water bodies such as lakes and reservoirs. NDWI values range from -1 to +1, with negative values indicating dry surfaces with high vegetation water stress, values near zero indicating areas with vegetation cover and non-saturated soil, and higher values indicating saturated hydration zones.
- SAVI Index (Soil Adjusted Vegetation Index): A variant of NDVI to avoid distortions in exposed soils, where variables like temperature or humidity can influence image bands and the results of the indicator. In this case, SAVI reduces this influence by adding an additional factor L= 0.5 to the NDVI equation, allowing it to work in scenarios with incipient vegetation development. The values and their interpretation are similar to those described for NDVI.

The goal of incorporating phenological information is to maximize the predictive capacity of the wetlands models. Figure 43 shows the results of these indices calculated for the study area during the indicated periods.

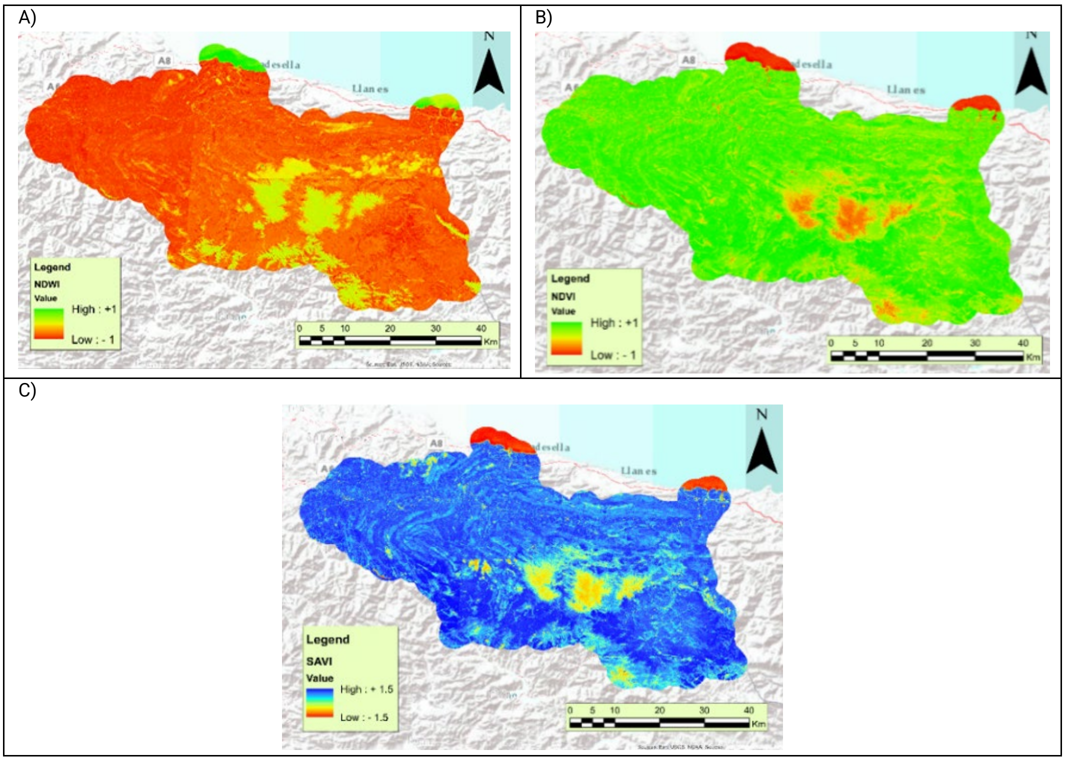


Figure 43. NDWI values for April-May (A), NDVI for June-July (B) and SAVI for August-September (C).

As a detail, Figure 44 shows the spatial variations of the SAVI index in photointerpreted wetland areas in Vegacomeya.

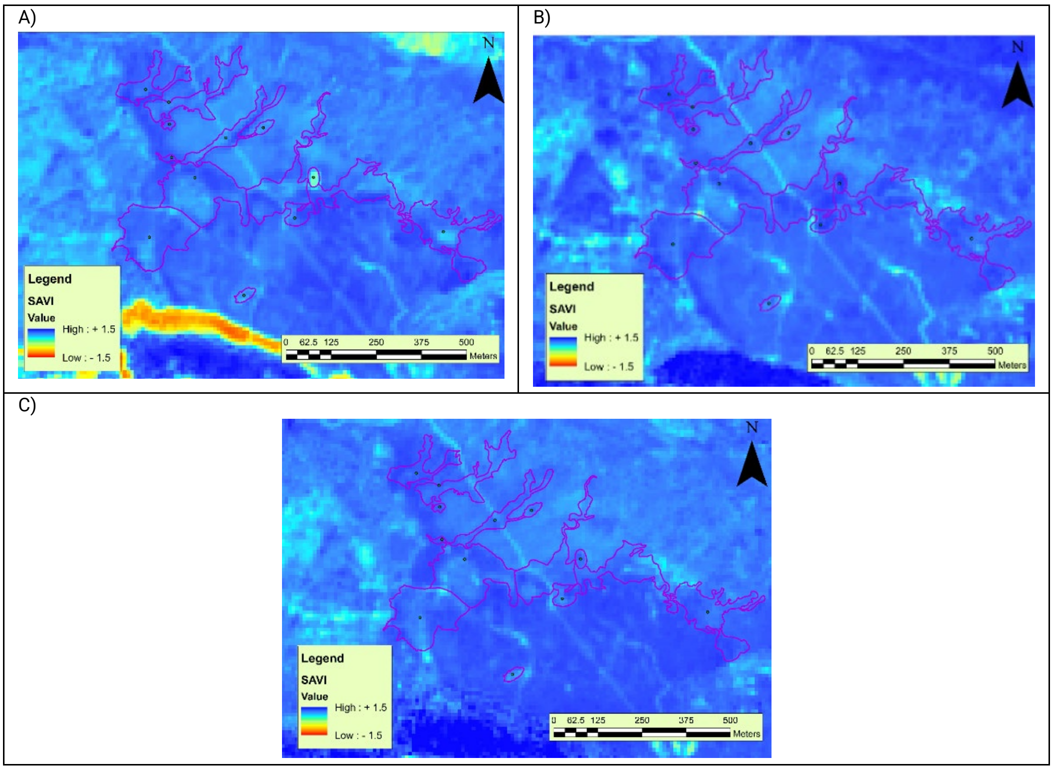


Figure 44. AVI values in Vegacomeya for April-May (A), June-July (B) and August-September (C).

As seen in the previous Figures, wetland mapping in mountainous areas presents significant challenges due to the ecological complexity of these environments, with numerous environmental gradients operating in the same location, as well as the spectral similarity between areas interpreted by experts as wetlands and adjacent areas. This makes it difficult for medium-resolution satellite sensors to achieve precise and accurate results when classifying these types of aquatic habitats. However, the combined use of photo-interpretation and image-based models proves to be an effective approach to tackle this challenge and achieve an accurate representation of these complex systems. As previously explained, experts in photo-interpretation and vegetation can use their experience and knowledge to distinguish wetlands within the landscape continuum, as well as to locate and map their occurrence in specific and representative locations of the dominant abiotic gradients. On the other hand, satellite image-based models offer a robust tool for the quantitative analysis and automated classification of aquatic and purely terrestrial features. These models, as explained earlier, use algorithms and image processing techniques to map ecosystems across large areas, revealing patterns and distinctive features that are difficult to detect with the naked eye. In this way, experts in photo-interpretation and fieldwork can use the results of satellite image-based models as a guide to identify and delineate new areas of interest, validating previous classification results and feeding new models with more records and greater geographic and environmental representativeness. This process requires visual verification and validation of areas of interest, statistically defining the accuracy and error rate using high spatial resolution images or conducting new field visits in areas identified as probable occurrences. This feedback provided by photo-interpreters can help refine and adjust the automated classification algorithms, leading to more reliable and precise results in wetland mapping. Thus, it can be concluded that the combined use of photo-interpretation and satellite image-based models is a suitable strategy for large-scale wetland mapping in mountainous areas, as developed in the area described here. This approach significantly improves the quality of conventional maps and contributes to the management and conservation of aquatic resources in mountainous regions.

As a result of the cartographic developments described here, a total of 215 wetlands were inventoried (Figure 45). The lakes and lagoons described in the previous section, as well as the wetlands, have been represented in a GIS database as point locations associated with X and Y coordinates, corresponding to the centroid of the actual patches in the landscape. Meanwhile, wetlands were photo-interpreted as polygons, allowing for the analysis of the spatiotemporal variability of the spectral indicator values used in the study, thereby increasing the predictive capacity of the models when reclassifying probabilistic outputs into dichotomous maps of presence/absence of the habitat of interest (Álvarez-Martínez et al., 2018).

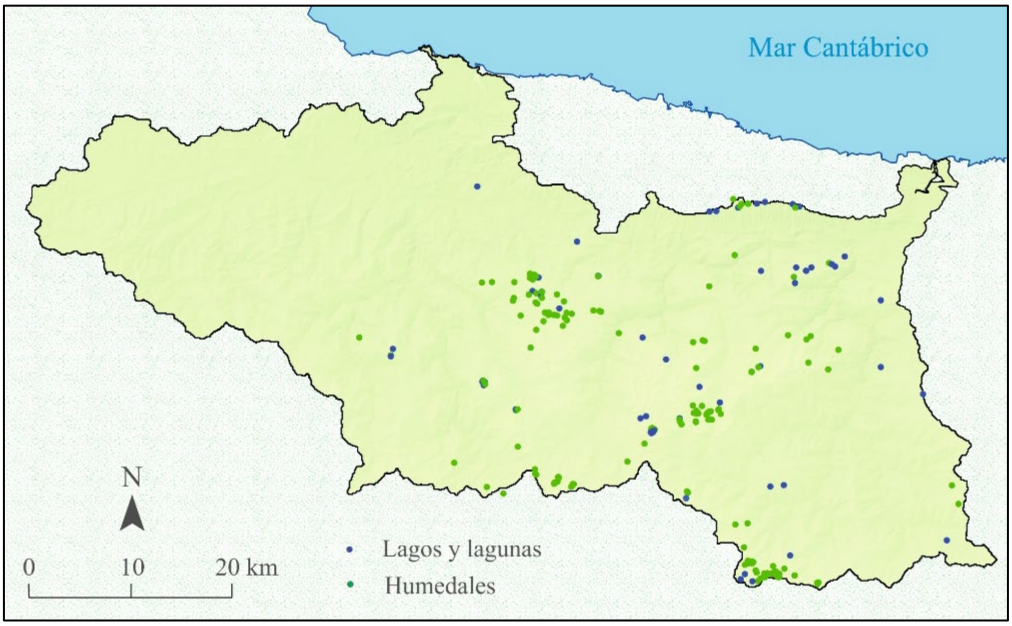


Figure 45. Distribution of lakes and lagoons, and wetlands in the Sella and Deva-Cares basins.

7.2.3. *Springs and watering troughs*

Watering troughs are small infrastructures used to supply water to livestock. In Picos de Europa they are mainly used when the livestock is in the mountain pastures in semi-wild conditions. As previously mentioned, watering troughs are of vital importance to the primary sector in this area, where livestock graze extensively for much of the year (May-November). During this period, the watering troughs, along with the natural springs and water sources in the park, are sometimes the only places where livestock can access water.

In addition to their importance for livestock supply, a space like the Picos de Europa NP requires proper management of these water points because, under certain conditions, watering troughs can act as reservoirs for diseases. These diseases can spread from wild animal populations, which also use the troughs, mainly ungulates, to the livestock. Thus, it is common to find good practice guides, workshops, and publications issued by various Spanish authorities warning about the risk of spreading certain epizootic diseases, such as tuberculosis or African swine fever. These documents point out that watering troughs, irrigation ponds, and other water points are particularly problematic areas for disease transmission between wild species populations and livestock.

Another reason why proper management of these water points is recommended is that the watering troughs are also of vital importance for certain wild amphibian populations to complete their life cycles. These points attract various species of frogs, toads, and newts for egg-laying and subsequent larval development until they reach adulthood. For these reasons, having a complete map of the watering troughs and natural springs distributed throughout an area like the Picos de Europa NP and its surroundings is essential. In this case, a comprehensive database created by the park's management body was used, which inventories 235 watering troughs and 25 springs (i.e., natural springs and seeps), in addition to 7 other water points classified mostly as reservoirs. Based on this complete database, which includes the coordinates of each watering trough and other metadata, a final and corrected digital map was created in shp format, where each trough was geolocated using photointerpretation with orthophotos (see the digital map of the watering troughs in the Picos de Europa NP in Figure 37 of this document).

For the springs, which include natural springs and seeps, the same database provided by the park’s management body was used, although this is a very incomplete database for mapping these water bodies, as it only inventories 25 springs. To complement this information, cartographic maps at a 1:25,000 scale (MTN25) of the park and its surroundings were used. From these maps, the springs were located and later incorporated into a digitized map in shp format, through photointerpretation with orthophotos, along with the springs described in the park’s database. After this process, 231 springs were mapped in the Sella and Deva-Cares basins, of which 177 are located within the Picos de Europa NP (see Fig. 37 of this document). Within the boundaries of the Picos de Europa NP, the mapped springs and watering troughs are distributed as follows:

- Asturias: 102 springs and 141 watering troughs.
- Cantabria: 35 springs and 75 watering troughs.
- Castile and León: 40 springs and 19 watering troughs.

8. Hydrologic characterization and hydro-period

8.1. Snow variability

The monitoring and analysis of snow cover were carried out using remote sensing techniques based on a time series of satellite data, processed and integrated at basin scale.

To monitor the variation of snow cover in the Sella and Deva-Cares basins, daily images derived from the MODIS/Terra satellite (MOD10A1F) were used, produced by the National Snow and Ice Data Center (NSIDC). This global level 3 dataset provides daily cloud-free snow cover derived from the MODIS/Terra Snow Cover Daily L3 Global 500m SIN Grid (MOD10A1). The grid cells of MOD10A1 obscured by cloud cover are filled by retaining clear-sky views from previous days. For identifying the snow cover from MOD10A1, the Normalized Difference Snow Index (NDSI) was used. Snow-covered land typically has very high reflectance in visible bands and very low reflectance in shortwave infrared bands. The NDSI reveals the magnitude of this difference. Additionally, each data file includes three scientific datasets (CGF_NDSI_Snow_Cover, Cloud Persistence, and MOD10A1_NDSI_Snow_Cover) and two quality data fields (Basic_QA and Algorithm_Flags_QA). Pixels identified as snow undergo several filters specifically developed to mitigate commission errors (i.e., detecting snow where there is none) and to flag uncertain snow detections. Moreover, snow-free pixels are screened for very low illumination to avoid possible snow omission errors. The screening results, as well as the location of inland waters, are stored as quality flags in the 'Algorithm_Flags_QA' SDS. This time series of images, obtained between 2000 and 2021, and corresponding to the northern half of the Iberian Peninsula (located in the grid h17v04), were downloaded from the existing NSIDC repository (https://nsidc.org/data/MOD10A1F/versions/61) in an extension format known as HDF-EOS (Hierarchical Data Format - Earth Observing System). A total of 7,998 images were used for this study.

The first step in processing the data was converting the format, transforming it from HDF-EOS to rasterized files projected in geographic coordinates (WGS84). For this, the SNAP software, designed by the European Space Agency, was used. Once this step was completed, pixels with CGF_NDSI_Snow_Cover values ≤100, corresponding to the percentage of snow cover, were selected. Furthermore, values were filtered using the "Basic_QA" quality indicator, selecting only those considered "Excellent" or "Good." The rest of the pixels were masked. Next, each of the areas of interest, corresponding to the Sella and Deva-Cares basins, was cropped from the set of 7,998 images comprising the entire historical MODIS dataset for this area. This resulted in the generation of over 150,000 processed files in ArcGIS, which were then exported to obtain the daily snow cover data. After cropping the images, the accumulated snow cover area for each basin was estimated, along with the percentage relative to the maximum extent, and the standard deviation over the time series of daily data from 2000-2021. Additionally, the average percentage of snow cover per pixel was estimated to identify areas with greater snow coverage (see Fig. 46).

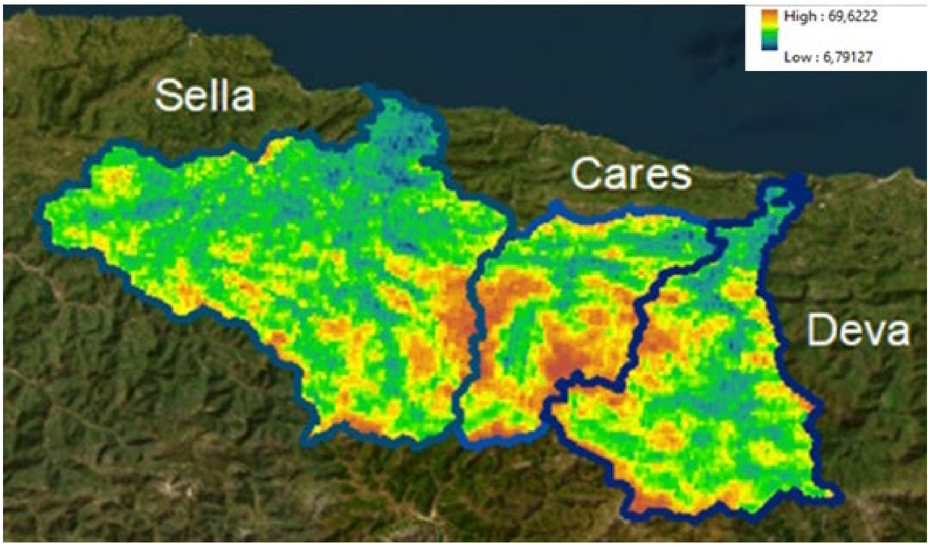


Figure 46. Average percentage of snow cover per pixel in the Sella and Deva-Cares basins.

Below are the preliminary results obtained from the time series of snow cover images collected between February 2000 and December 2021 (Fig. 47). Of the three basins, the Sella basin shows the highest values during extreme events, although the general trend is common across all three basins, with a progressive loss of snow-covered area.

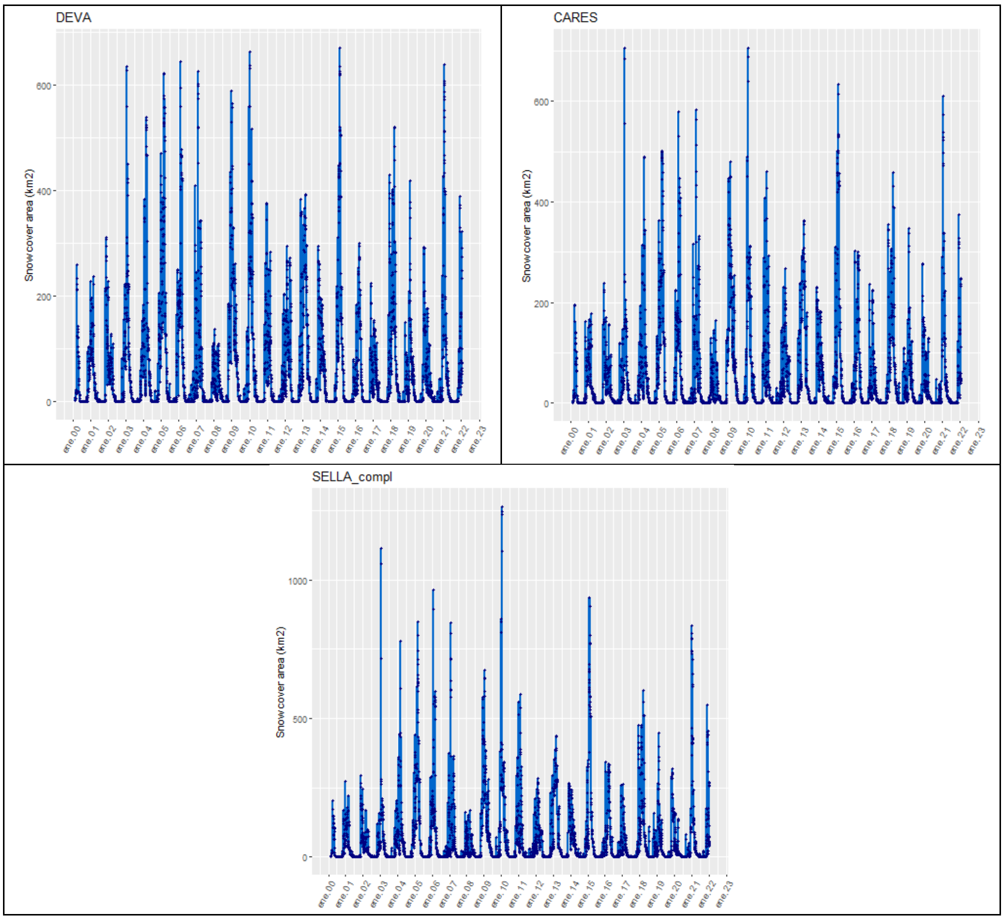


Figure 47. Temporal evolution of snow cover in the Sella and Deva-Cares basin.

8.2. Temporal variation of lentic water bodies

The lakes, lagoons, and wetlands previously described exhibit a highly seasonal variability, depending on their nature, size, and morphology in response to the hydrological cycle (Keddy, 2010, Batzer & Sharitz, 2007). Additionally, human activities (e.g., water extraction) and climate change (i.e., changes in precipitation and temperature regimes) can produce important variations in the surface area they occupy (Pan et al., 2023; Woolway et al., 2020). However, the ability to monitor these changes at a regional scale, considering their diversity in types and morphologies, remains limited. Systematically and effectively characterizing the temporal regime of lentic water bodies is essential for better understanding their response to various environmental change factors and the consequences these changes may have on their functions. This information is key in areas like the Picos de Europa National Park, where environmental conservation must balance with anthropogenic uses within the park and its surroundings.

In this context, data obtained through remote sensing techniques can provide detailed information about the characteristics and properties of the land surface at local, regional, and even global scales, and equally important, over time. Based on data obtained through remote sensing, various indices can be calculated to synthesize the information gathered. The characterization of seasonal variation in the lakes, lagoons, and wetlands within the Sella and Deva-Cares river basins was conducted by calculating the following remote sensing-based indices:

- AWEInhs (Automated Water Extraction Index no shadows): This was designed to differentiate between water and other surfaces with similar reflectance (e.g., clouds, snow) when ground shadows are not a potential source of error (Feyisa et al., 2014). Positive AWEInhs values indicate the presence of water.

  $$AWEInhs = 4 \times (Green - SWIR) - (0.25 \times NIR + 2.75 \times SWIR)$$

- NDVI, previously described in this document.

These indices were calculated using 51 cloud-free images from the Sentinel-2 L1 satellite (obtained from the USGS EarthExplorer repository and ESA's Copernicus Open Access Hub), corresponding to 4 scenes covering the entire area, acquired on 17 dates between April and October in the period from 2017 to 2020. The image series were atmospherically corrected using iCOR (De Keukelaere et al., 2018) to obtain surface reflectance measurements necessary to homogenize the data regardless of the acquisition date (or atmospheric conditions). Once corrected, the previously described spectral indices were calculated to evaluate both changes in water surface area and wetland extent for 213 previously mapped lentic water bodies (see Section 7.2 "Mapping of Lentic Water Bodies" in this document).

The selected lentic bodies have a minimum area of 100 m² and belong to two major groups:

- Lentic bodies generally dominated by water surfaces: Lakes (n=12) and lagoons (n=36). The lakes were classified into two types based on their area: large (area > 10,000 m²) and small (area < 10,000 m²).
- Lentic bodies generally dominated by vegetation: These correspond solely to wetlands (n=165).

To identify possible types within the lagoon and wetland subgroups, a cluster analysis was performed using two variables that determine the structure and functioning of lentic water bodies: i) area and ii) the Topographic Wetness Index (TWI), which represents the potential for moisture concentration or water accumulation (Figure 48). Surface area and TWI were calculated using tools in the ArcGIS software (v.10.7.1).

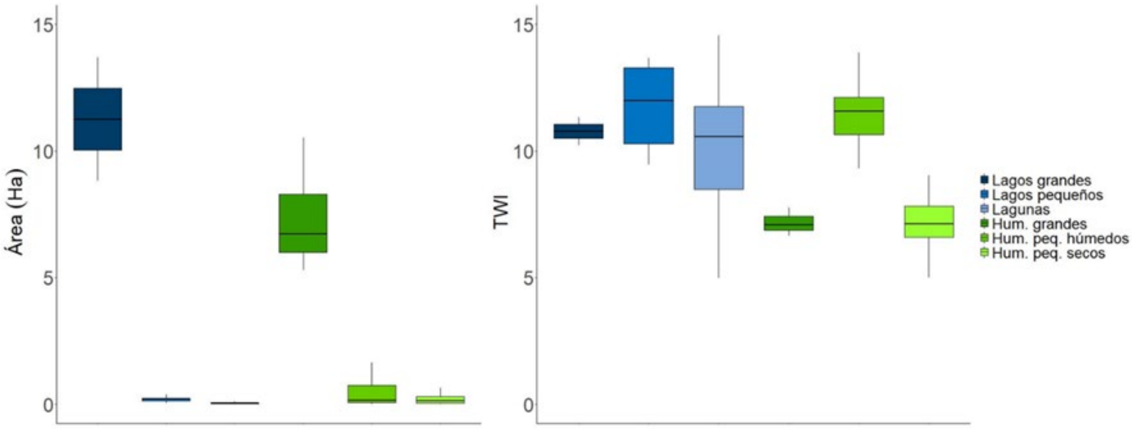


Figure 48. Area and Topographic Wetness Index (TWI) for each type of lentic water body. Boxes show the median and the 25th and 75th percentiles; vertical lines show minimum and maximum values that fall within 1.5 times the height of the box.

Thus, the following types were obtained for each group: 1) Lakes and lagoons (Large lakes, 2; Small lakes, 10; and Lagoons, 34), and 2) Wetlands (Large wetlands, 8; Wet small wetlands, 60; and Dry small wetlands, 97).

For each group and type, the median and the median absolute deviation (MAD) of the AWEInhs and NDVI indices were calculated for each month. As shown in Figure 49, the different groups and types of lentic water bodies displayed characteristic median values and ranges, with large lakes showing the most distinct values compared to other lentic bodies. Additionally, a change in the median values of each type is observed depending on the month of the year. This indicates that the selected indices are appropriate for evaluating the temporal variation of the different lentic bodies present in the Picos de Europa National Park.

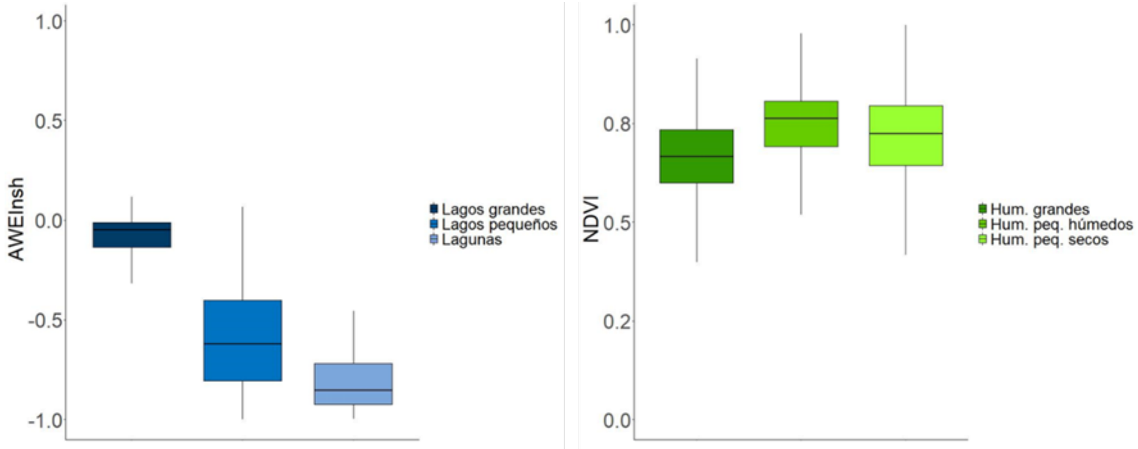


Figure 49. Variation of the AWEInhs and NDVI indices for each type of lentic water body throughout the year at the pixel level. Boxes show the median and the 25th and 75th percentiles; vertical lines show minimum and maximum values that fall within 1.5 times the height of the box.

Subsequently, the median value ±1DAM was used to determine the range of characteristic values of each type with respect to which the Sentinel-2 scenes were reclassified to information layers 0-1 (0: pixels with index values outside the characteristic range of each type, 1: pixels with index values within the characteristic range of each type) in a buffer of 20 m with respect to the perimeter of the lentic body mapped in low flow (Fig. 50).

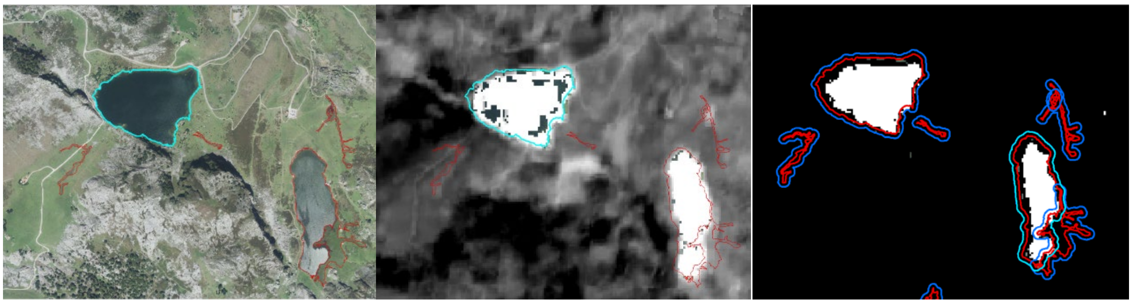


Figure 50. Sentinel-2 scene processing. Left: satellite image of lakes Enol and Ercina. Center: Sentinel-2 scene corresponding to the AWEInsh index (May 2020). Right: reclassified image (0-1) according to the median value ±1MAD calculated for the "great lakes" lentic body type. The perimeter of the lentic body is shown in red and the 20 m buffer is shown in blue.

Reclassified images for each type of lentic water body were combined according to the season: 1) Early spring: April, 2) Late spring: May and June, 3) Summer: July, August, and the first half of September, and 4) Autumn: the second half of September and October. In this way, relative extent (*extensionr*) of each selected lentic water body was estimated, as the percentage of pixels with values of 1 for each index and type relative to the total pixels of each lentic body obtained from the previous mapping (i.e., perimeter plus a 20 m buffer). To characterize the variation in the extent of the lentic bodies between seasons, the change in extent in a given season relative to the previous one (Δ) was calculated using the following formula: $\Delta = (extensionr(i) - extensionr(i-1)) / extensionr(i-1)$, where *i* represents each season of the year (i.e., early spring, late spring, summer, and autumn). To assess whether the Δ for each season differed from the previous one, 95%

confidence intervals (CI) were calculated using the bootstrapping approach with the R package boot (Canty & Ripley, 2019). This method allowed the seasonal dynamics of each type of lentic body in the study area to be characterized.

Results showed that the seasonal variation pattern of surface area observed for large and small lakes was similar, while lagoons exhibited more stable behaviour throughout the year. Specifically, and as expected, both, large and small lakes, showed a relative increase in their surface area in late spring compared to early spring, and a decrease in summer compared to late spring (Fig. 51). The increase in the surface area during late spring seems to be due to the effect of rainfall and snowmelt, while the summer decrease is likely related to reduced water input combined with higher evaporation rates (Carlson et al., 2020). Furthermore, small lakes increased again in extent in autumn compared to summer, probably due to rainfall during this season. However, this effect was not observed in large lakes, suggesting that the latter are less dependent on precipitation input than small lakes, or that the relative change caused by rainfall is much smaller in these cases. In fact, although the delta values between seasons are significantly different for large lakes, the change (Δ) ranges between 0.05% and -0.10%, which aligns with the idea that the extent of these types of lentic water bodies is stable (Horne & Goldman, 1994). On the other hand, lagoons exhibited high variability in relative extent for each season, and no significant variation was observed between seasons (Fig. 51). This suggests that small water bodies are highly sensitive to local climatic and environmental conditions (e.g., soil type; Dimitriou et al., 2009).

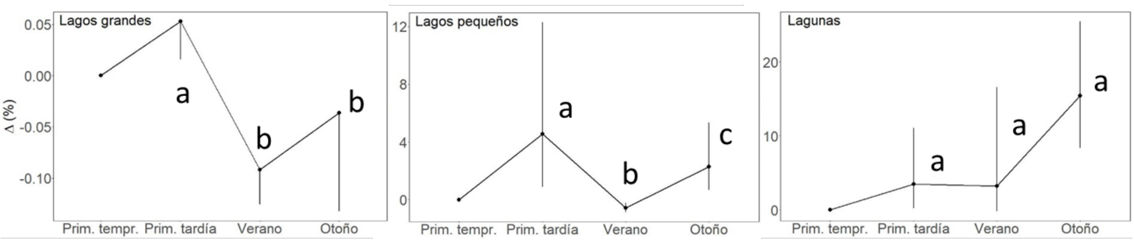


Figure 51. Seasonal pattern of extension Δ for large lakes (a), small lakes (b) and lagoons (c). Mean values ± CI. Significant differences are indicated with different letters.

The relative extent of large wetlands showed an increase during late spring compared to early spring, followed by a decrease in summer, and remained stable from summer to autumn (Fig. 52). This change pattern seems to be explained by the rainfall and snowmelt in spring, along with reduced water input and increased evaporation during summer, similar to other water bodies (Keddy, 2010). For small, wet wetlands, which have a higher potential for water retention, a more pronounced seasonal variation was observed. Their extent increases due to both spring precipitation and snowmelt, as well as autumn rains, while decreasing during the drier, warmer summer months (Fig. 52), consistent with studies such as Carlson et al. (2020). In contrast, small, dry wetlands, which have a lower potential for water accumulation, did not show a significant pattern of change in extent throughout the year (Fig. 52). This could be explained by the high variability in the relative extent of these wetlands according to the season, likely influenced by local-scale environmental factors such as vegetation cover, water retention capacity (Nhamo et al., 2017), or even human interventions like water extraction.

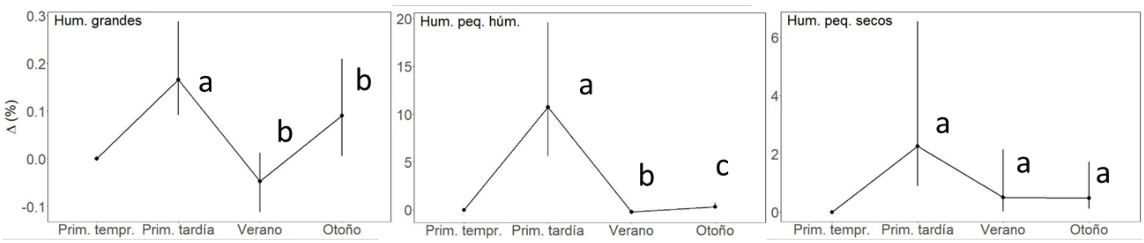


Figure 52. Seasonal pattern of extent Δ for large (a), small wet (b) and small dry (c) wetlands. Mean values ± CI. Significant differences are indicated with different letters.

The observed change patterns in lentic water bodies within the Picos de Europa NP highlight the critical importance of water inputs, not only from precipitation, but also from snowmelt in late spring. This is especially relevant for large lakes and wetlands, increaing their vulnerability to climate change, as the reduction of spring snow cover is one of its main effects (Notarnicola, 2022). Additionally, the significant variability in the changes in extent observed for lentic water bodies, particularly in the case of small-sized ones, such as lagoons and small, dry wetlands, suggests the possible existence of subtypes. The behavior of these subtypes could be influenced by other environmental factors, such as water consumption or extraction.

8.3. Hydrological model

It is often difficult to get the necessary information to determine the quantity and quality of water in a river basin at a specific moment and for a particular river stretch. This information is especially relevant in the context of global change, where alterations in thermal and precipitation regimes can lead to a decrease in water contributions and an increase in the demand for different uses, such as irrigation or livestock. The past, present, and future challenges related to the quantity and quality of water, and their spatial-temporal variations, have prompted hydrologists to develop modelling tools that allow for the analysis and understanding of the spatial-temporal representation of material and energy flows within a basin (Terink et al., 2015). These modelling tools have led to the development of hydrological models, which are defined as input-output models based on the water balance, simulating the evolution of water storage, its flows, and the potentially associated physical and chemical properties of the land surface and subsurface (Horton et al., 2022). Currently, hydrological models are essential tools for effective water resource management, as they provide results with high spatial and temporal resolution, enabling the exploration of different future scenarios.

There are multiple hydrological models, including, among others, the SPHY model (Terink et al., 2015), SWAT (Neitsch et al., 2009), VIC (Liang et al., 1996), TETISv9 (IIAMA, 2021), and WiMMed (Polo et al., 2010). The main differences between models developed so far lie in the number and level of detail of the hydrological processes they incorporate, the field and scale of application, and the method of implementation (Terink et al., 2015). Therefore, the choice of one model over another depends on the objectives and the purpose of the study being conducted.

For the Sella and Deva-Cares basins, a spatially distributed model has been used. A distributed model is characterized by breaking down the study area into a regular grid of cells with a known resolution. Thus, unlike aggregated or semi-distributed models, where the calculation unit is the watershed or sub-basin, distributed models calculate the water balance over each cell in the grid (Fig. 53). Therefore, although they usually require greater computational effort, this type of model allows for obtaining results at any point in the basin, as well as providing a better representation of the spatial variability of the hydrological processes involved (Diputación Foral de Gipuzkoa & IDOM, 2018)

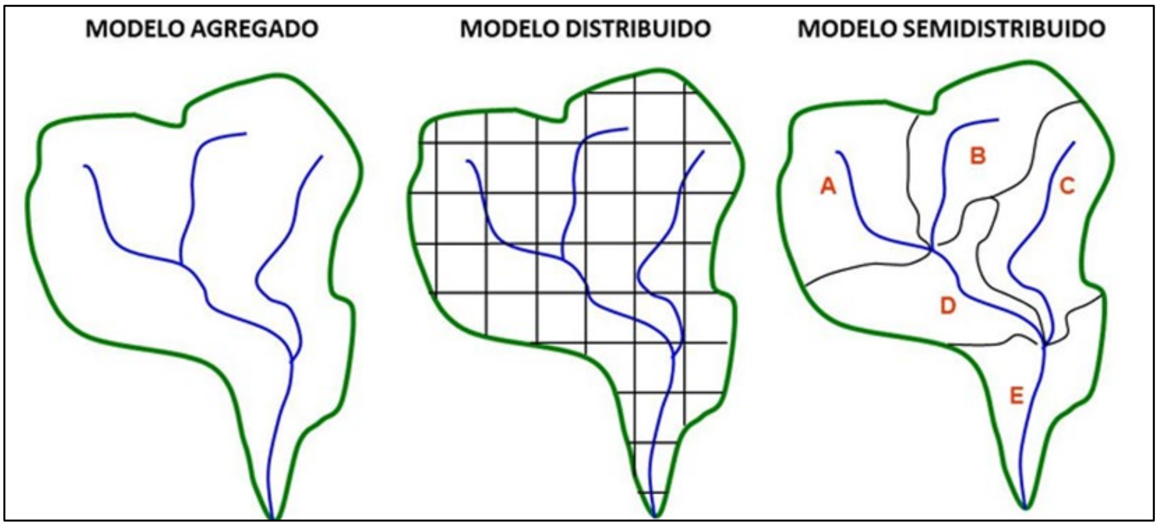


Figure 53. Graphic representation of different hydrological models according to their aggregation. Adaptation from Regional Council of Gipuzkoa & IDOM (2018).

Specifically, the distributed model selected for the Sella and Deva-Cares basins is the Open Source SPHY (Spatial Process in Hydrology) model, which has been successfully applied in previous studies, mainly related to agriculture (Biemans et al., 2019), climate change (Lutz et al., 2016), land cover (Eekhout et al., 2020) and hydrological regimes. Its selection is primarily due to its ability to integrate most hydrological processes, its relative ease of implementation, its flexibility for use in a wide range of hydrological applications, and its potential for exploring different scenarios at various scales. This capability enables it to identify the possible changes that climate regimes and land use may exert on the water cycle (Fig. 54).

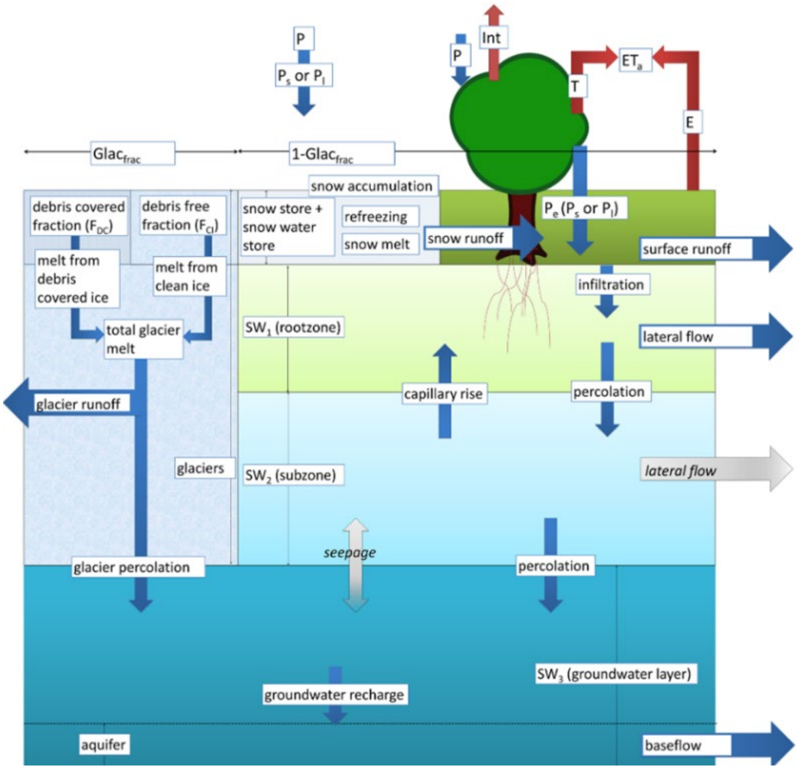


Figure 54: SPHY model structure (Terink *et al.*, 2015).

SPHY is a spatially distributed model of the "bucket with leaks" type that is applied cell by cell in raster format, allowing the evaluation of changes in storage and flows over space and time, where mass conservation is the main underlying concept. Each cell may be free, partially, or fully covered by glaciers, with cells without glaciers being characterized by a different type of land cover. The soil column structure is divided into two upper storage areas and one lower groundwater storage area. Drainage from these storages occurs through surface runoff, lateral flow, and base flow, respectively. The sum of these components, along with snow and glacier melt (if present), represents the specific runoff for each cell, which in turn constitutes the volume of water available for routing. Precipitation falling in a cell can be classified as rain or snow, depending on temperature. Part of the precipitation will be intercepted by vegetation and evaporated, while another part of the liquid precipitation will either turn into surface runoff or infiltrate the soil. The remaining non-evaporated water will contribute to river discharge through lateral flow from the top soil layer or through base flow from the groundwater layers. Snow precipitation contributes to snow storage, the balance of which is updated based on the simulation of accumulated or melted snow amounts. Glacier melt contributes to river discharge through a slow component and a rapid component, with the former being percolation to the groundwater layer that eventually becomes base flow, and the latter being direct runoff. Therefore, SPHY integrates different hydrological processes such as: rain-runoff, lake/reservoir outflow, cryospheric processes, evapotranspiration, and soil hydrological processes. Some of these processes are contained in modules that can be activated or deactivated based on their relevance to the study and the characteristics of the basin. This allows for a reduction in model run time and a decrease in the number of necessary input data. The modules present are: glacier, snow, groundwater, dynamic vegetation, simple routing, and lake/reservoir routing (for a more detailed description of SPHY, see Terink et al., 2015).

Based on the available data and the characteristics of the Sella and Deva-Cares basins, as well as their main processes, the selected modules for using SPHY in these basins were the groundwater, simple routing, and snow modules. In both basins, the results of the hydrological modelling were obtained at a spatial resolution of 100 m and a temporal resolution of daily scale. SPHY requires dynamic input data (climatic data on precipitation and temperature) and static data (DEM, land cover, and soil characteristics). Additionally, in these two basins, two scenarios have been considered in the modelling (historical-PR and future-CC) covering the periods 1971-2015 (historical-PR) and 2016-2097 (future-CC), with different climate and land cover data for each period. Model calibration was performed using the location corresponding to a gauge station in each basin (Puentellés for Deva-Cares and Villamayor for Sella) through an optimization algorithm to calibrate the model parameters against the observed flow data from the gauging stations over a period of three years. The most sensitive parameters were selected for calibration based on previous experience with SPHY. The objective function used to evaluate the model fit during the calibration phase was the Nash-Sutcliffe efficiency coefficient "log NSE" (Nash & Sutcliffe, 1970). Log NSE values <0.5 are considered unacceptable, while values between 0.6 and 0.8 are considered good, and values >0.8 are considered excellent. For the Puentellés gauging station, a LogNSE value of 0.72 was obtained for the period 2007-2009, and a value of 0.68 was obtained for the Villamayor gauging station for the period 2002-2004. Figure 55 shows the comparison between the modelled and observed hydrographs at both gauging stations (Puentellés and Villamayor). It can be seen that the simulation adequately represents the shape and character of the observed hydrograph, although it underestimates the maximum flows of some occasional events

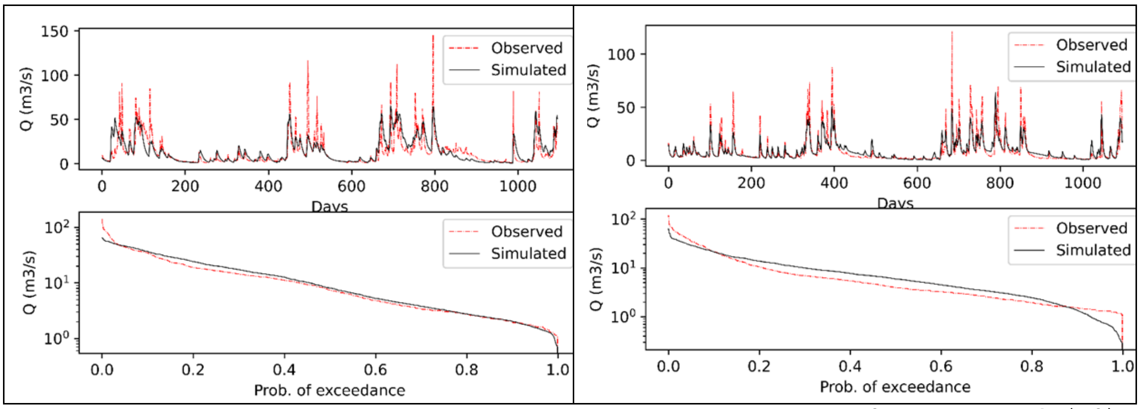


Figure 55. Model calibration. Comparison between the observed and simulated flow at Puentellés (left) and Villamayor (right) gauge station for the calibration period.

Model validation was carried out for the complete historical period with observed data from each gauge station available for that period. In this case, two criteria were used:

the log NSE coefficient and the Percentage Bias (PBIAS), where absolute PBIAS values less than 15% are considered acceptable. Ultimately, both basins achieved Log NSE values >0.6 and PBIAS values <15%. Figure 56 shows the comparison between the modelled and observed hydrographs at both gauge stations for the validation period, where a similar trend to that represented in the calibration period can be observed.

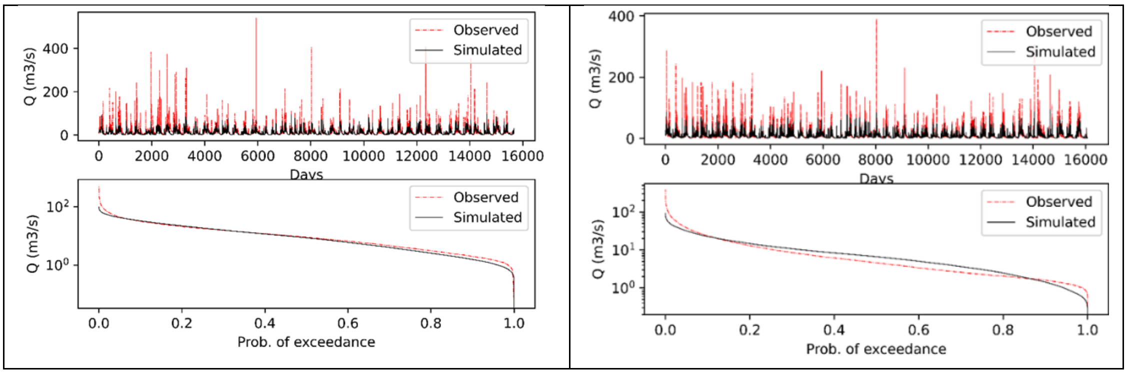


Figure 56. Model validation. Comparison between the observed and simulated flow in Puentellés (left) and Villamayor (right) gauge stations for the validation period.

Finally, based on the calibrated parameters, the model simulation was launched for the historical (1971-2015) and future period (2016-2097) for each of the two basins, obtaining the following variables at a daily level: 1) total runoff, 2) routed total runoff, 3) snow runoff, 4) baseflow runoff, and 5) rain runoff. Using data from the SPHY model simulation, it is possible to extract daily flow values at specific locations to analyse the hydrological change between scenarios through the generation of flow duration curves and the calculation of various hydrological indices. Furthermore, to eliminate the effect of river size in the classifications, the flow series must be normalized beforehand. In both basins, daily flow values were extracted at 28 specific locations, distinguishing the type of location between upper, middle, and lower reaches. These data were used to obtain the flow duration curves and calculate all the hydrological indices shown in Table 6 for the development of the LIFE DIVAQUA project.

Group	Name	Description
1) Magnitude of annual and monthly flows	L1	Linear moment that represents the average of the annual flow duration curve
	l2	Linear moment that represents the variance of the annual flow duration curve
	lcv	Linear moment that represents the asymmetry of the annual flow duration curve
	lca	Linear moment that represents the coeff. variation of the annual flow duration curve
	lkur	Linear moment that represents the kurtosis of the annual flow duration curve
	M1-M12	Caudal medio mensual de cada mes
2) Magnitude and duration of annual extremes	XLF	Magnitude of the minimum flow of X days duration
	XHF	Magnitude of the maximum flow of X days duration
	BFI	Base flow index= 7LF/l1

Group	Name	Description
3) Temporality of extreme events	Jmin	Julian day of maximum annual flow
	Jmax	Julian day of minimum annual flow
	Pred	Predictability
4) Frequency and duration of flow pulses	FRE2-10	Mean number of flow events greater than 2, 3…10 times the annual median flow of 7 days duration
	nPHigh	Mean number of events of flow exceeding the flow with probability of exceedance of 25%
	dPHigh	Mean duration of flow events greater than the flow with probability of exceedance of 25%
	nPLow	Mean number of events of flow exceeding the flow with probability of exceedance of 75%
	dPLow	Mean duration of flow events greater than the flow with probability of exceedance of 75%
5) Change rate	Pos	Mean value of the magnitude of all positive flow changes
	nPos	Mean number of days/year with increasing flow
	Neg	Mean of the magnitude of all negative flow changes
	nNeg	Mean number of days/year with decreasing flow
	Rev	Number of hydrological reserves

Tabla 6. Definition of the calculated hydrological indices.

Results reveal a projected decrease in the mean annual flow in the future scenario of 18% and 21% in the Sella and Deva-Cares basins, respectively (Fig. 57). The index I2, which accounts for this variance, similarly decreases in the future, with values of 12% and 19%, respectively. Meanwhile, indices Icv, Ika, and Ikur are expected to increase in the future scenario, indicating that there will be an increase in variability in flows and anomalous events.

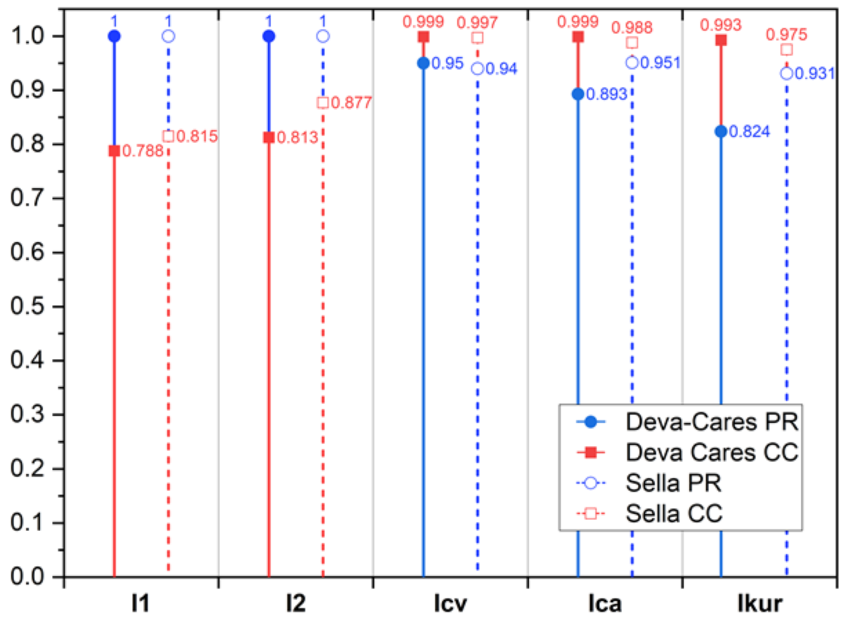


Figure 57. Normalized hydrological indices of the average values of I1, I2, Icv, Ika and Ikur for each time period (PR and CC) in the Sella and Deva-Cares basins.

Regarding mean monthly flow for both periods, there is a widespread reduction in flow (Fig. 58), with greater differences observed from October to January, where the model predicts an average decrease of 25%. It is also noteworthy that the peak in April (M4) in the historical series will shift to March (M3) in the future. This temporal advancement of the flow peak is primarily attributed to rising temperatures and decreasing precipitation, which would lead to several predictable effects on the snowpack. These effects are significant in basins with a rainy-snowy regime, such as the Sella and Deva-Cares basins. Among these effects are the delay in snow appearance, an increase in the rain-to-snow ratio, a reduction in the snow season, and the advancement and shortening of the melting period (Barranco et al., 2018).

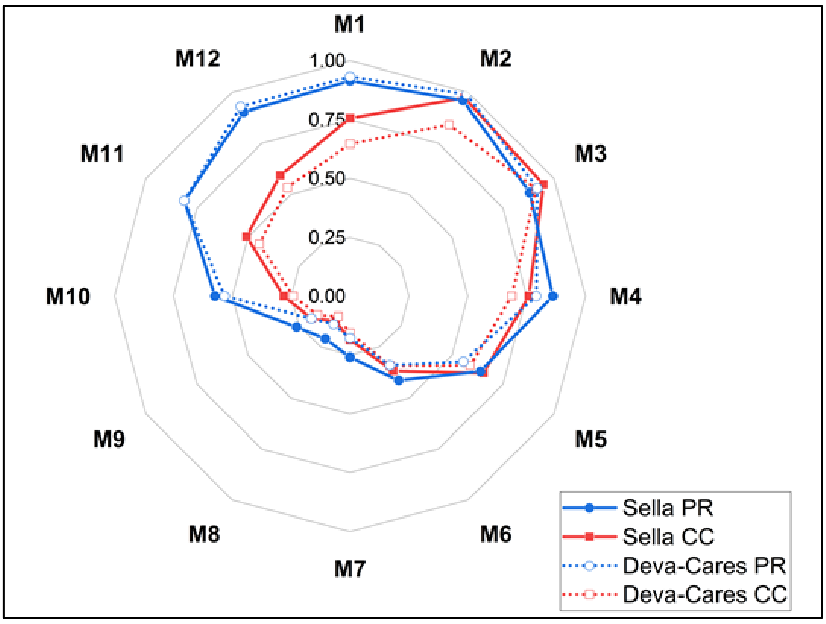


Figure 58. Normalized average values of the monthly flow in both time periods (PR and CC) and for the Sella and Deva-Cares basins.

The widespread increase in temperature and the decrease in precipitation in the future scenario, along with changes in land cover, will lead to a general flow reduction in both basins, as previously discussed. The decrease in the magnitude of max. and min. flows could also affect water quality, as its dilution capacity would be reduced. The reduction in flow and the change in temporal patterns, along with the increase in the variability of anomalous events, may negatively impact biodiversity and the river ecosystem functioning, as well as their interaction with marine and/or terrestrial ecosystems (Sanz & Galán, 2020). In this context, it is important to highlight that the population dynamics of certain species inhabiting these basins, such as salmon (*S. salar*), could be severely altered (Thorstad et al., 2021).

The rise in temperatures and the variability in precipitation may also increase water demand for supply and irrigation. In this sense, the potential impact on the livestock sector, which, as previously described, is one of the pillars of the region's economy, is particularly relevant. The tourism sector could also be affected in relation to different activities that take place in the aquatic environment, (e.g., canoeing) impacting the economy derived from tourism. Furthermore, changes in the hydrological regime could have significant implications for the management of the reservoirs in these basins (see Fig. 39), as these changes could alter their operation and management in relation to the future-established hydrological space-time patterns.

Therefore, it can be concluded that the distributed SPHY model has yielded satisfactory results for modelling the hydrology of the Sella and Deva-Cares basins, where the alteration of flow regimes and the decrease in water resources in a future scenario could have multiple consequences, both economic, social, and environmental. Thus, the results obtained through hydrological modelling are of great importance and utility for understanding the effects of global change and for conducting space-time analyses that enable the design of efficient and responsible use of water resources, in order to implement adequate strategies for their conservation and management.

8.4. Water level sensors

Beyond the use of hydrological models, or as a source of data for their development, calibration, and validation, it is essential to have hydrological data measured in the field. To characterize in situ the hydrological cycle of a given water body it is necessary to have gauge stations that can continuously measure the amount of water flowing through a river stretch or stored in a lake or lagoon. Traditionally, water agencies have operated gauge stations which requires civil engineering work for their installation (Fig. 59). This results in high installation and maintenance costs, in addition to generating a certain impact on the aquatic and riparian environment.

Figure 59. Example of a traditional gauging station installed on the Júcar River.

Due to their high cost and impact, traditional gauge stations are often scarce and ineffective in mountainous areas, especially in those that have protection figure, due to the limited size of headwater rivers and the impact they cause on them. To address these issues, the market currently offers more reasonable solutions, both in terms of economic cost and intrusion into the adjacent aquatic and terrestrial environments. One such solution has been implemented in the Sella and Deva-Cares basins, where a network of 8 gauge stations has been implemented (7 of which are within the boundaries of the National Park; Fig. 60). These gauge stations continuously measure the water column height and temperature, every 30 minutes, thereby characterizing the hydrological and thermal cycles of these rivers. This data is transmitted in real-time and can be accessed openly through the web portal http://picoseuropa.ihcantabria.com/.

- Picos 1: Installed at the headwaters of the Sella River, near the town of Vierdes de Sajambre.
- Picos 2: Installed in the lower part of the Bulnes River, before it joins the Cares River.
- Picos 3: Installed at Fuente Dé, the source of the Deva River.
- Picos 4: Installed in the lower part of the Duje River, before it joins the Cares River.
- Picos 5: Installed in the lower part of the Urdón River, before it joins the Deva River.
- Picos 6: Installed at the headwaters of the Cares River, near the town of Posada de Valdeón.
- Picos 7: Installed on the Cares River, downstream of Caín.
- CCant 1: Installed on the Deva River, near the town of Beares. This is the only one of these 8 gauging stations located outside the boundaries of the National Park.

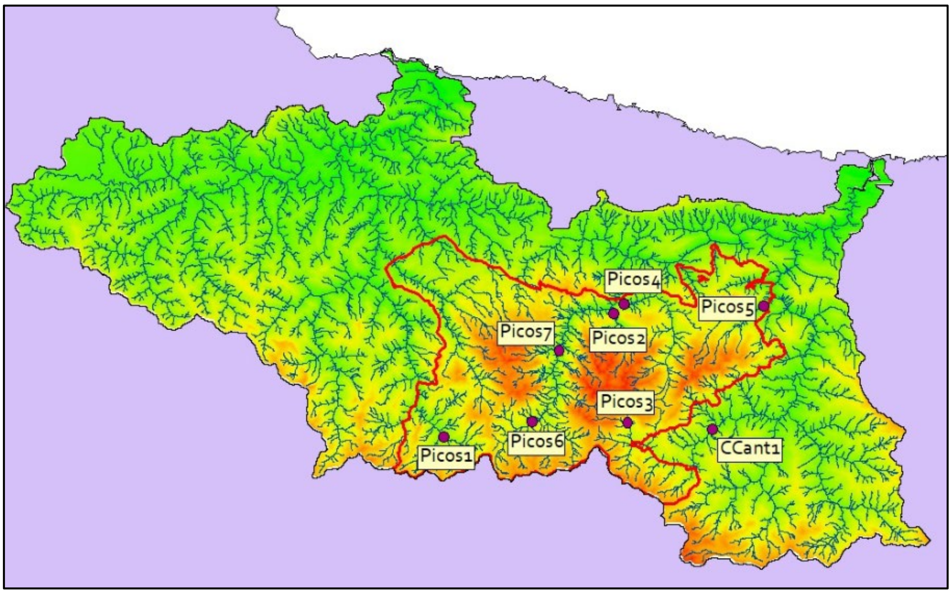


Figure 60. Location of the 8 gauges installed in the Sella and Deva-Cares basins.

Each of these 8 stations consists of a high-precision pressure and temperature level transmitter, the Keller Series 36XW, which is anchored to the riverbed. This transmitter connects to a remote, autonomous data transmitter and data-logger, either the GSM-2 or ARC-1, which is installed outside the river channel, typically buried or fixed to some infrastructure (e.g., a bridge). The latter sends the level and temperature data collected by the Keller Series 36XW transmitter via email to the data download server, eventually incorporating the information into the indicated web portal (Fig. 61). Additionally, the data transmitters (GSM-2 or ARC-1) are equipped with a pressure gauge to compensate for the pressure measured under the water column with atmospheric pressure, thereby obtaining the real water level measurement (i.e. water column height).

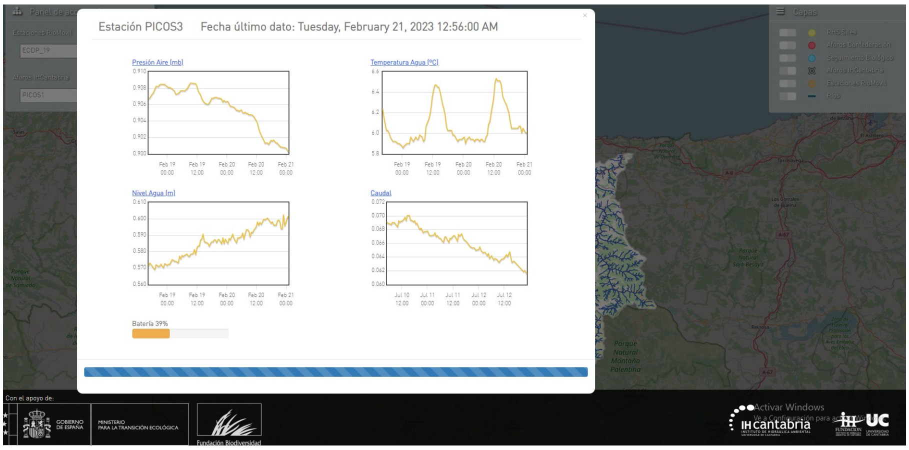


Figure 61. Screenshot of the picoseuropa.ihcantabria.com portal with the Picos3 capacity information.

As shown in Figure 62, these gauge stations are minimally intrusive, as they do not generate any impact on the riverine and riparian environment once installed, unlike traditional gauge stations. All technical specifications of the instruments that make up the described gauging stations can be consulted on the Keller website (http://www.keller-druck.com/home_e/paprod_e/gsm2_e.asp).

Figure 62. Gauge stations in Picos4 (Duje river) and Picos6 (Cares river; Posada de Valdeón).

To transform water level data (m) into the value of the flowing discharge (m^3/s), it is necessary to develop and validate a hydraulic model for the sections where the gauge stations are installed. Of the 8 gauge stations installed in the Sella and Deva-Cares basins, Picos1, Picos2, and Picos3 have such hydraulic models. The following steps were carried out to achieve this process (see Fig. 63):

1. Characterization of the dry channel: A Faro Focus3D Laser Scanner was used (Technical specifications http://www.faro.com/en-us/products/3d-surveying/faro-focus3d/overview) . This device allows for collecting more than 900 million points measured by infrared laser in a single scan, covering an area of 360º around the scanner and 310º in vertical section. The Faro Focus3D Laser Scanner can measure points with millimetre precision, reaching distances of up to 80 meters. This laser can perform each scan at medium resolution in less than 3 minutes. (See technical specifications at Faro).
2. Topographic and hydraulic characterization of the wet channel: This was conducted at various transects of the section to be characterized using a FlowTracker-ADV current velocity meter from SonTek. (Technical specifications at http://www.sontek.com/productsdetail.php?FlowTracker2-Handheld-ADV-1).
3. Generation of models: with the collected data, a digital terrain model was generated for each section, along with a detailed hydraulic model that allowed for the adjustment of discharge curves. From these curves, the flowing discharge is calculated using the water column height data sent by the GSM-2/ARC-1 sensors.

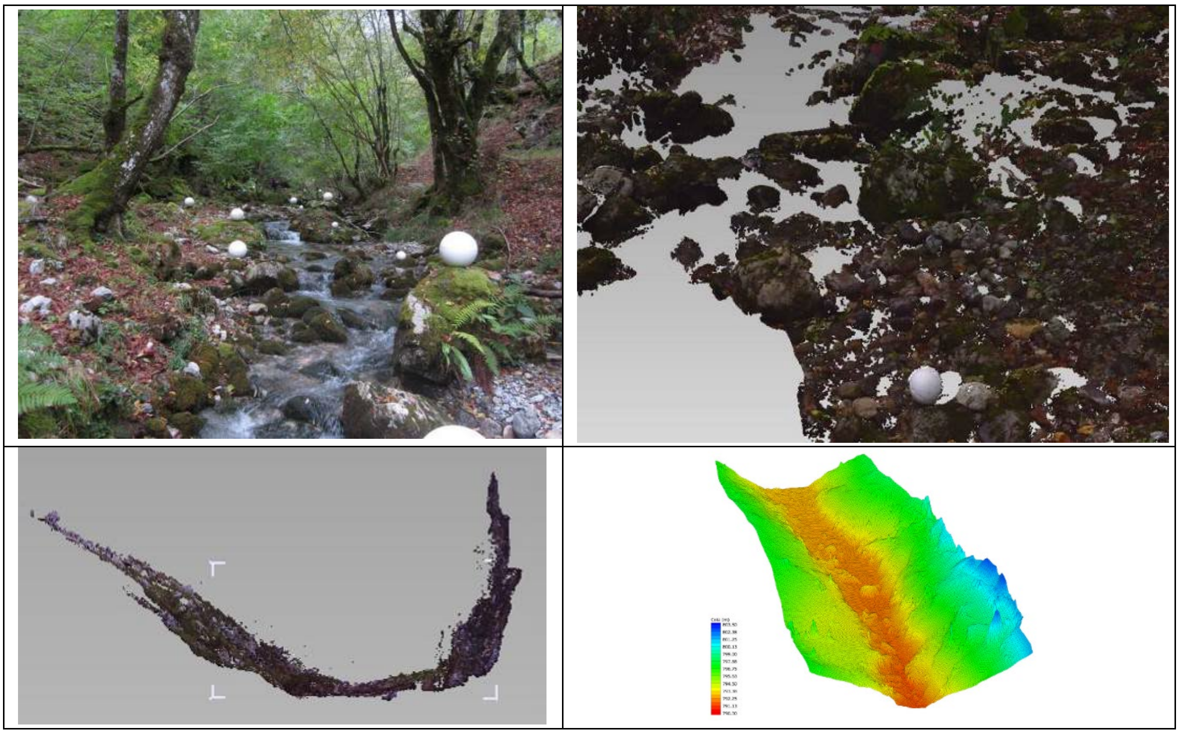


Figure 63. Topographic characterization of the section: preparation of the section with spherical references for laser scanning, 3D image after laser scanning, cross section of the river and digital model.

9. Water quality

9.1. Water quality in river ecosystems

Any planning guide or instrument promoting the conservation of biological diversity in mountain aquatic environments and the integrity of these ecosystems, in general, must include the proposal of a monitoring system that provides the necessary information to characterize and evaluate its physical and biological components, as well as the different processes occurring in these ecosystems. The most relevant physical component of an aquatic ecosystem is, as its name suggests, the water. Therefore, as described in the previous sections of this document, it is essential to know the amount of water circulating through a river ecosystem (i.e., flow) or the volume of water contained in a lentic water body (e.g., lake) and determine its temporal variability (i.e., hydrological cycle). Similarly, it is necessary to determine water quality, as it depends on the structure and composition of the biological communities that settle in these ecosystems, as well as the dynamics of the various processes that characterize them (e.g., river metabolism; see below).

This section presents a proposal for monitoring the aquatic ecosystems of the Picos de Europa NP, established from the experience gained over more than a decade of work in this area. In this case, the design and establishment of a long-term monitoring network is proposed, from which it will be possible to understand the potential spatial and temporal changes, whether punctual or large-scale, that may occur in the characterized rivers. Understanding this change dynamic is the most appropriate way to differentiate natural changes inherent to the aquatic environment from those generated as a consequence of impacts produced by human activity. This acquired knowledge allows for characterization, diagnosis and planning, allowing the design of tools that enable us to anticipate what will happen in a future scenario with different change scenarios. Following these criteria and needs, monitoring networks should be designed so that the results derived from their use are suitable to avoid two types of errors commonly described in certain natural resource monitoring programs (see Downes et al., 2022):

- Type I Error: Failing to identify a relevant impact/change that is affecting the monitored environment.
- Type II Error: Identifying a relevant impact/change that does not actually exist and is not affecting the monitored environment.

To achieve this, the monitoring network must have a enough number of study/sampling points that are affected by the most relevant impacts to be monitored, as well as other points that, while having similar environmental conditions, do not have any significant anthropogenic impacts, showing their natural environmental conditions remain unaltered (i.e., control or reference points). Under this premise, there are two different approaches that can be applied to the design of a monitoring network.

- **Reference Condition Approach**: Following this approach, the monitored environment (e.g., the river network) should be compartmentalized into ecological types (i.e. typologies), to minimize environmental variability among study/sampling points. This allows the comparability among points of the same typology, avoiding the comparison of points located in environments that differ significantly from each other (e.g. different geology, or catchment location headwater Vs lower areas). Once the different typologies are defined, minimally impacted points must be identified, from which the reference conditions specific to their type can be established. In this case, the observed deviation between a sampling point and its reference conditions is associated with the degree of impact, which can be quantified based on the deviation of an index, variable, or parameter from its reference value. This is referred to as the Ecological Quality Ratio (EQR). This approach is used, for example, by the Water Framework Directive (WFD) to assess the ecological status of water bodies in the various Member States of the European Union. For the definition of ecological typologies, the WFD itself establishes two options that include different environmental variables to consider in each case.

  Although the use of reference points/conditions is a proposed method for monitoring large territories, this approach may not be suitable for achieving the objectives pursued by a guide like the one presented in this document, as reference conditions are not dynamic, meaning they do not capture the inherent interannual natural variability of the river environment. For example, the reference value and the EQR of a particular quality index derived from a specific bioindicator community (e.g., IPS for the diatom community or IBMWP for invertebrates) do not vary, thus failing to account for how interannual natural variability in hydrological regime or temperature can affect the structure and composition of these biological communities and, consequently, the value of the indices/metrics used for their assessment. For this reason, monitoring networks established under this approach may incur Type I and II errors when comparing different years in which environmental conditions have varied significantly due to natural factors (e.g., dry years versus wet years).

- **Control-Impact (CI) Approach**: This approach is more suitable for designing a monitoring network aligned with the objectives described in this guide. In this case, a series of sampling points must be specifically located to characterize the impacts/alterations that are desired to be monitored, whether punctual (e.g., effluent) or non-punctual (e.g., diffuse runoff from slopes). Once these sampling points are established, control points must be selected that show environmental characteristics as similar as possible to the affected points (i.e., same typology) but do not have the alterations affecting the latter. Therefore, in this case, the typological classification occurs after the location of points affected by impacts. Once the typological differentiation is established, including the sampling points affected by the pressures/impacts to be monitored, controls must be sought in locations belonging to the corresponding type.

The advantage of this approach is that sampling the points that make up these networks allows for pairwise comparison of results obtained between an affected point and its control(s), determining whether the observed variation in an affected point is due to the effect of the impact itself or, conversely, if it is a change generated by the natural variability inherent to the river environment (e.g., variability between different dry or wet years). This fact makes it less likely to incur in the previously indicated Type I and II errors under this approach

Following CI approach, a monitoring river network of the Picos de Europa NP was designed and operated since 2010 (Barquín et al., 2014). Below, the methodological process used to design this network is described, particularly proposed to assess the effects produced by:

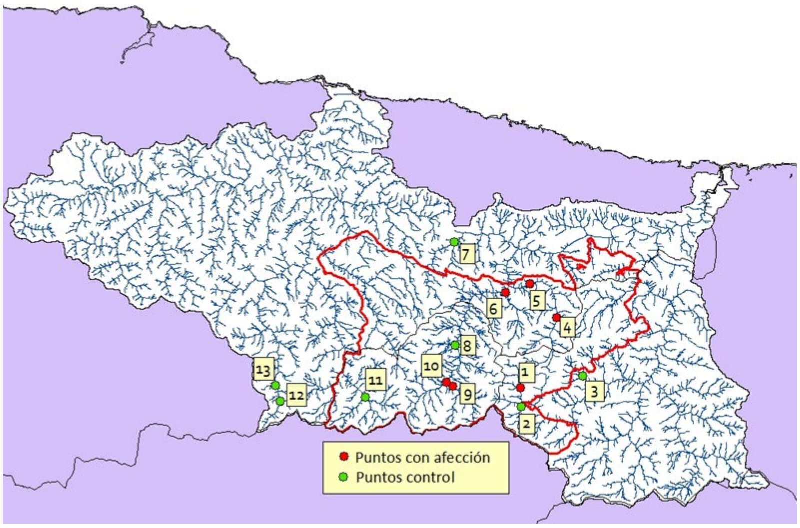
1. **Urban point effluents** on the river ecosystems of the Picos de Europa NP. Three river sections affected by urban effluents in the localities of Posada de Valdeón (Cares River), Tielve (Duje River), and Bulnes (Bulnes River) were selected. The Fuente Dé spring, source of the Deva River, was also included, which receives effluent from various establishments in the area.
2. **Runoff processes** on the river ecosystems of the Picos de Europa NP. In this case, two sub-basins that generate high loads of material linked to runoff processes were selected. This was based on a virtual basin and a synthetic river network (see above) where 261 different sub-basins were identified. Subsequently, the runoff generated by each of these sub-basins was modelled, considering their topographic, geological characteristics and land uses. The model was developed using the SWAT software (Soil and Water Assessment Tool) and applied to 2 variables: 1) sediment concentration and 2) nitrates transported to the watercourses of each sub-basin from the contributing basin, considering both input and output data for each sub-basin. It should be noted that this model does not consider the inputs produced by point effluents, only modelling the diffuse entry of both compounds into the river courses. For this reason, other chemical compounds, such as phosphate, whose origin is typically more associated with urban point effluents, were not considered in this case (Álvarez-Cabria et al., 2016).

   To identify the sub-basins that generate the most runoff, data from the time series covering the years 1990-2010 were used to avoid errors in the results due to changes in land use that may have occurred since the 1990s when incorporating data prior to that date. Once the average monthly results generated by the model for each of the 261 defined sub-basins during the period 1990-2010 were obtained, the average for each of these sub-basins was calculated, meaning that each sub-basin is represented by a single data point for each of the two modelled variables. From these loads and using the average annual flow, also averaged through SWAT, the concentrations of the indicated variables were calculated, ultimately obtaining an input and output concentration for both sediment and nitrates in each of the 261 sub-basins in the study area. Taking all 261 sub-basins together, an average sediment concentration of 1.393 mg/l was obtained, while the median value is much lower at 30 mg/l. In the case of nitrates, the modelled average concentration in the sub-basins forming the studied river network reaches 0.98 mg/l, with the median value being very similar at 0.94 mg/l. For nitrates, the model did not yield concentrations higher than 2 mg/l, so it is considered that, according to the results of the SWAT model, no section of the studied river network is significantly affected by the diffuse entry of this compound. However, after applying this process, 29 sub-basins were identified that receive sediment concentrations exceeding 1000 mg/l. Based on these values, two sections affected by runoff processes were selected: one located in the upper part of the Duje River and another belonging to the basin of the Arenal River.

Once these 6 points with different impacts were selected (4 affected by urban effluentss: Posada de Valdeón, Tielve, Bulnes, and Fuente Dé; and 2 affected by runoff processes: Duje and Arenal), their corresponding control points were sought. To define types and select controls that show environmental conditions as similar as possible to these six affected sections, the following environmental variables were considered:

1) The area of the contributing basin, calculated with tools available in commonly used GIS programs (e.g., ArcMap).
2) The order of the river stretch (Strahler Number).
3) The slope of the river stretch, calculated with tools available in GIS programs (e.g., ArcMap).
4) Forest cover on the banks, derived from tools available in GIS programs, based on data available from SIOSE (Land Occupation Information System in Spain).
5) Forest cover in the contributing basin, derived from tools available in GIS programs, based on data available from SIOSE.
6) Insolation calculated using the GIS tool called Solar Radiation Area. This tool calculates the total radiation received by each pixel during august under simulated clear sky conditions, considering both terrain topography and the geographic location of the section. Once this information was obtained, the average radiation for each section was calculated based on the radiation received by each pixel present in the section itself.
7) Hydrological characteristics according to a previously developed classification (see detailed description in Peñas et al., 2014).
8) Physical habitat characteristics according to a previously developed classification (see detailed description in Belmar et al., 2019).

After this process, a network of points was established for the monitoring of the river ecosystems of the Picos de Europa NP. This network has been characterized annually through in situ sampling during the summer period, from 2012 to the current year, 2024 (water quality, biological communities, metabolism, etc.; this section only reports on water quality; the other parameters, processes, and communities are described in the following sections of this document). This network is composed by 13 study/sampling points (Fig. 64). All affected points are located within the NP. However, to find control points that meet all the expectations/assumptions, part of the river network that drains outside the Park had to be considered, with four controls located in its vicinity (Fig. 64)



Tipology	Nº Figure	Sampling point	Treatment
T1	11	Sella river	Control
	10	Cares river	Urban effluent
	9	Arenal river	Runoff
	13	Ponga river	Control
T2	7	Casaño river	Control
	4	Duje river headwater	Runoff
	6	Bulnes river	Urban effluent
	2	Salvorón river	Control
	3	Seco river	Control
	5	Duje river Tielve	Urban effluent
T3	8	Farfada spring	Control
	12	Ponga spring	Control
	1	Fuente Dé spring	Urban effluent

Figure 64. Location, typology and impact of the 13 sampling points that make up the monitoring network of the river ecosystems of the Picos de Europa National Park.

In the annual monitoring conducted at these 13 points to assess water quality, among other ecosystem components, several physicochemical variables are measured. From the database generated by the operation of this monitoring network, long-term changes in water quality can be determined, as well as whether these changes are due to natural variability inherent to the fluvial environment or, conversely, induced by human action/global change. The variables measured annually during in summer (August) are:

- Variables determined in situ using multiparameter probes: pH, electrical conductivity, water temperature, and concentration and percentage of dissolved oxygen saturation.
- Variables determined in the laboratory through water sampling: concentration of ammonium, nitrate, nitrite, phosphate, silicates, and suspended solids. Nitrate concentration is a good indicator of the impact that certain land uses, such as agriculture, have on water quality through runoff (Álvarez-Cabria et al., 2016). Ammonium and nitrite are other ions associated with the nitrogen cycle. The stability and persistence of these ions in water are lower than that of nitrates, but their toxicity is greater for aquatic organisms. On the other hand, phosphate concentration is usually more linked to the presence of urban pollution, which is generally introduced into fluvial ecosystems through point sources, such as those associated with wastewater treatment plant effluents (Álvarez-Cabria et al., 2016).

In addition to this type of monitoring network to generally assess water quality, it is advisable to include other points and variables in cases where the area to be characterized has specific issues. For example, in the case of the Picos de Europa NP, mining activities, primarily associated with the extraction of sphalerite, manganese, mercury, cinnabar and iron, were highly relevant until the early 20th century. Currently, some of these mines, such as the abandoned Las Mánforas mine located at the source of the Duje River, may introduce certain amount of heavy metals into groundwater and surface waters due to infiltration and runoff from the abandoned tailings ponds resulting from mining activity. In these cases, an ad hoc characterization of the variables that best determine the degree of impact caused by the use/alteration to be characterized and assessed is recommended. In this case, to determine the possible impact that the tailings ponds deposited at the Las Mánforas mine may have on the Duje River, water samples were taken at the spring located immediately downstream of these ponds, which corresponds to the source of this river (Fig. 65). Water samples were also taken at the two points on the Duje River included in the previously described 13-point network (Duje River at the head and Duje River at Tielve; see Figure 64) and at the controls identified for this typology (Casaño River and Seco; Fig. 64). At these 5 points, the concentrations of various heavy metals were analysed through water sampling in September 2016. A sample was taken at the mines, while 2 samples were taken at the other 4 points as replicates. The metals analysed in this case were arsenic, cadmium, iron, mercury, manganese, lead, and zinc.

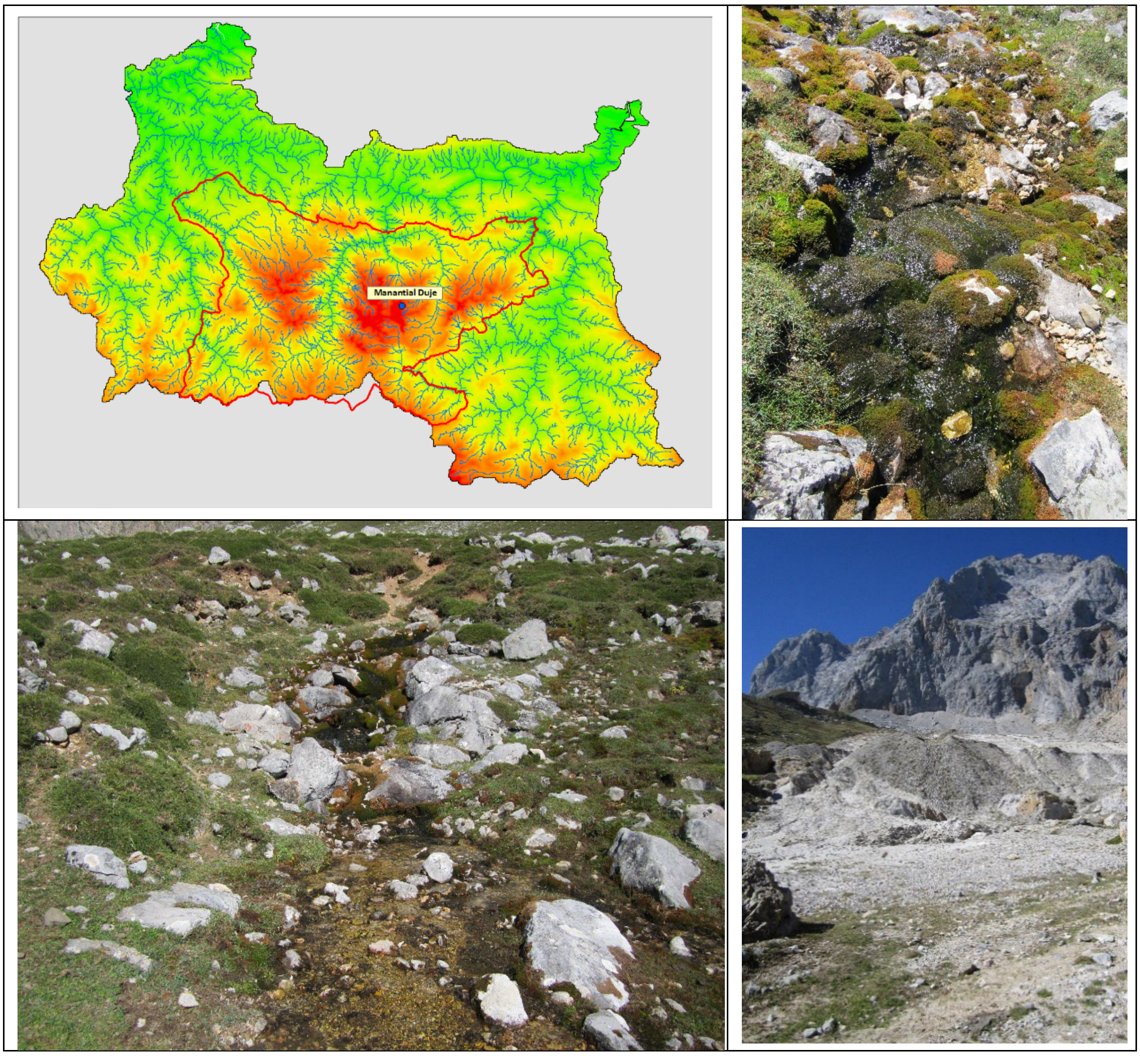


Figure 65. Spring where water samples were taken to assess the effect of the abandoned Las Mánforas mine on the quality of the water of the Duje River and image of the fines pond of the mine.

The results obtained indicate that, according to Royal Decree 817/2015, of September 11, which establishes the criteria for monitoring and evaluating the status of surface waters and environmental quality standards, the concentrations obtained (Table 7) comply, a priori, with the environmental quality standards (EQS) for continental surface waters. However, it should be noted that the detection limits for cadmium and lead in the analyses performed are higher than the limits for the annual average and the maximum allowable concentration established in this Royal Decree.

	Arsenic (µg/l)	Cadmium (µg/l)	Iron (µg/l)	Mercury (µg/l)	Manganese (µg/l)	Lead (µg/l)	Zinc (µg/l)
Casaño R1	< 1	< 0.5	< 20.7	< 0.2	< 4.7	< 1	< 20
Casaño R2	< 1	< 0.5	< 20.7	< 0.2	< 4.7	< 1	< 20
Seco R1	< 1	< 0.5	< 20.7	< 0.2	< 4.7	< 1	< 20
Seco R2	< 1	< 0.5	< 20.7	< 0.2	< 4.7	< 1	< 20
Duje Tielve R1	< 1	< 0.5	< 20.7	< 0.2	< 4.7	< 1	< 20
Duje Tielve R1	< 1	< 0.5	< 20.7	< 0.2	< 4.7	< 1	< 20
Duje cabecera R1	< 1	< 0.5	< 20.7	< 0.2	< 4.7	2.1	48.9
Duje cabecera R2	< 1	< 0.5	31.7	< 0.2	7.0	3.4	62.6
Mánforas spring	< 1	< 0.5	< 20.7	< 0.2	< 4.7	2.5	156.3

Table 7. Concentration of the metals analysed in the Duje River and its control sites (09/23/2016).

On the other hand, taking into account the values proposed by the US-Environment Protection Agency (US-EPA) to assess the effects that metals can produce on freshwater biological communities (Table 8), it is observed that the lead concentration reached values that can produce chronic effects in the communities of the Duje River (Duje1) and in the characterized Spring (source of the Duje River). On the other hand, the concentration of zinc described in the spring also exceeded the limit proposed by the US-EPA as a reference from which this metal can cause chronic and acute effects on river biological communities (Tables 7 and 8). It should be noted that the concentrations proposed by the US-EPA depend on the hardness values of the water, being an established and normalized proposal for waters with an average hardness of 100 mg/l of CaCO3, a value similar to that described in the characterized area.

	Arsenic (µg/l)	Cadmium (µg/l)	Iron (µg/l)	Mercury (µg/l)	Manganese (µg/l)	Lead (µg/l)
Chronical effects	150	0.72	1,000	0.77	2.5	120
Chronical effects	340	1.80	-	1.40	35.0	120

Tabla 8. Concentraciones propuestas por la *US-EPA* como límite para que el arsénico, cadmio, hierro, mercurio, plomo y zinc causen afectos crónicos y agudos en las comunidades biológicas de agua dulce.

Metal concentration in these points correspond to the summer season, a period in which there was no relevant precipitation and the river flow showed basal values. Therefore, the concentrations described could come mainly from the own groundwater (especially in the case of the spring), and do not seem to be related to basin washing and runoff processes

9.2. Water quality in lakes and lagoons

Other very important aquatic ecosystems in mountain areas are lakes and lagoons, which are especially relevant in the case of the Picos de Europa NP. This Park prominently features the Covadonga Lakes (Lago Ercina and Lago Enol), which see a high number of visitors, particularly from June to September. To determine the water quality of the lakes and lagoons in Picos de Europa NP, six lakes and lagoons were selected for long term monitoring, where water samples are taken annually to measure the same variables previously indicated for river monitoring. The lakes and lagoons that are annually monitored since 2018, always during the summer, are: the lakes of Lloroza, Ercina, Enol, Bajero, Cimero, and the lagoon and stream of Liordes (Fig. 66).

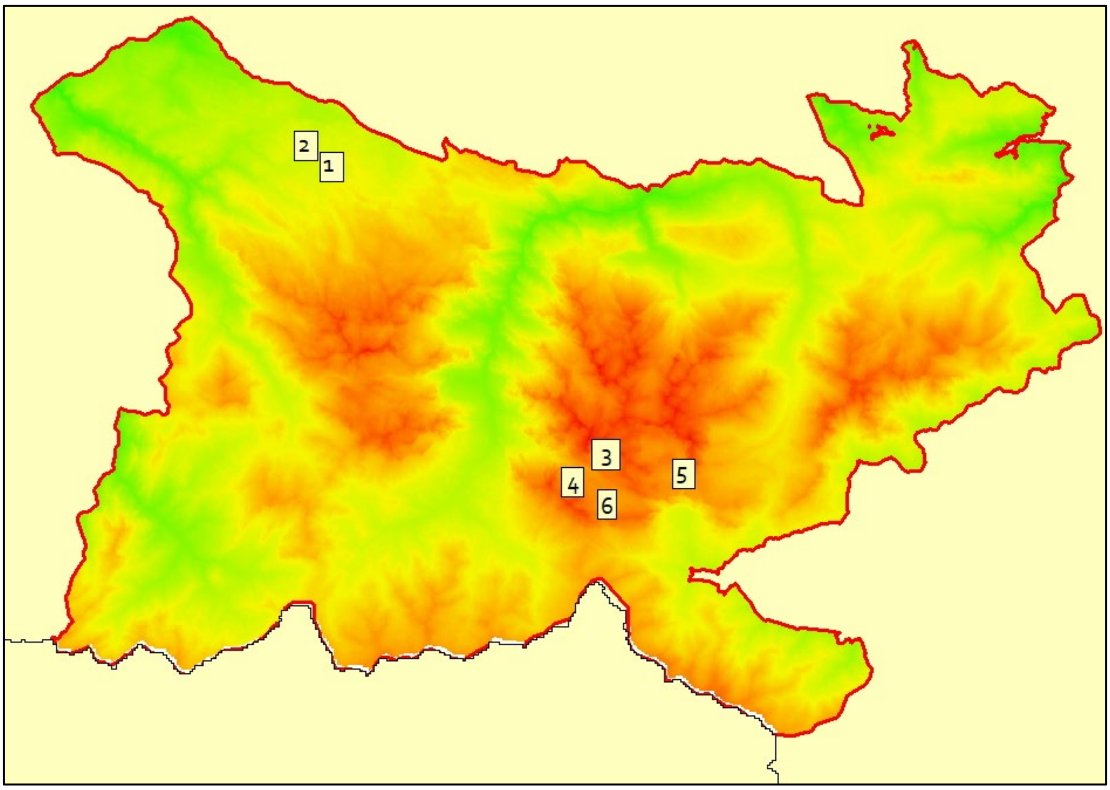


Figure 66. Location of the 6 lakes and lagoons where annual monitoring of water quality is carried: 1- Lake Ercina, 2- Lake Enol, 3- Lake Cimero, 4- Lake Bajero, 5- Lake Lloroza and 6- Regato and Liordes lagoon.

9.3. Seasonal variability of water quality

In addition to the monitoring networks previously described for the annual and long-term tracking of lacustrine and fluvial ecosystems in the Picos de Europa NP, 3 field campaigns were carried out in 2020 and 2021 to determine how the physicochemical characteristics of water vary seasonally. To achieve this, a network of 127 study points was designed under the framework of the LIFE DIVAQUA project (Fig. 67), distributed across different types of aquatic habitats (rivers, springs, watering places, lakes, and wetlands) where water quality was monitored in spring and autumn. At these 127 points, the same physicochemical variables were measured as those described earlier for the monitoring network established at the 13 points in the fluvial network of the Picos de Europa NP and the surrounding area (Fig. 64). The implementation of this extensive sampling network has enhanced the knowledge of how the water characteristics of mountain aquatic ecosystems vary seasonally. Furthermore, the location of study points in aquatic ecosystems of different typologies has also enabled the determination of the suitability of each for the development of various aquatic species, such as amphibians, which can thrive in different aquatic niches.

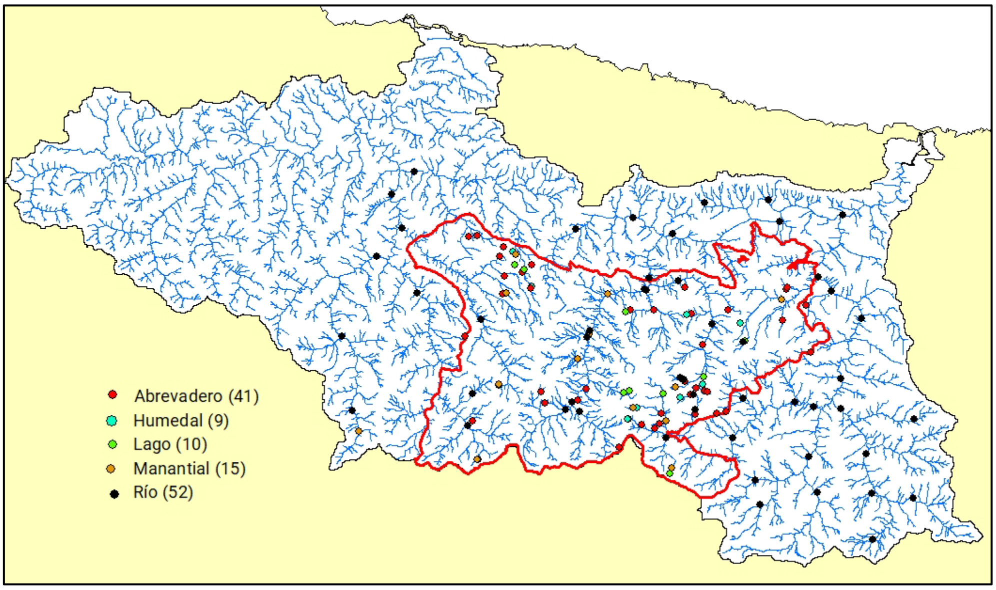


Figure 67. Location of the 127 study sites where water characteristics of different aquatic environments (water troughs, wetlands, lakes, springs and rivers) were seasonally characterized in 2020 and 2021.

10. River connectivity

Connectivity is a fundamental property of river ecosystems, influencing different patterns and processes inherent to these ecosystems (González-Ferreras et al., 2019). From a hydrological perspective, connectivity is defined as the transfer of matter, energy, and/or organisms through water (Pringle, 2001). Although connectivity encompasses interactions across the four riverine dimensions (longitudinal, lateral, vertical, and temporal), all of which are important for aquatic biodiversity, longitudinal connectivity—understood as the connection between different longitudinal segments of a river network— is especially important for fishes due to their movements and migrations undertake to complete their life cycles (Segurado et al., 2015).

While river networks are naturally fragmented by the presence of waterfalls or rapids, anthropic activities have further fragmented these ecosystems through the introduction of physical (e.g., dams, weirs), biological (e.g., pathogens) and physicochemical barriers (e.g., thermal or chemical pollution). This alteration of the river longitudinal connectivity can lead to multiple environmental changes and impacts within river networks, mainly affecting the quality, quantity and accessibility of habitat patches and patterns of fish population dispersal due to the barrier effect (Larinier, 2000). Consequently, fragmentation and habitat loss can result in several adverse effects on fish populations, such as the extinction of isolated populations (Morita & Yamamoto, 2002), genetic divergence (Hansen et al., 2014), or asymmetric dispersal (Junker et al., 2012), among others.

Given that both, the structure of the river network and the life history and/or dispersal traits of species, can affect connectivity among aquatic populations, it is essential to assess the longitudinal connectivity at the river basin level. Several procedures exist for evaluating the level of fragmentation of a river basin, and the choice of method depends on the objectives and area to be analysed (Kemp & O'Hanley, 2010). The most commonly used methods, due to their versatility and simplicity, are Connectivity Indices, which have been applied to the Sella and Deva-Cares basins. These indices calculate the percentage of fragmentation of a given basin, taking into account the position of each obstacle and its cumulative effect on the river network. The study of river connectivity fragmentation using Connectivity Indices involves three steps: 1) Inventory of obstacles and collection of information regarding each obstacle, 2) Calculation of the passability degree of each obstacle, and 3) Calculation of the Connectivity Index. Following these steps, the first task in the Sella and Deva-Cares basins was to generate an inventory of all obstacles present in both basins, both natural and artificial, as well as to collect all available information about them (location, height, width, presence of fish passages, use, obsolescence, etc.), creating a digital database with all this information. The inventory was generated by compiling all the information available from different databases. These include:

1) Inventory of pressures in surface waters conducted by the Hydrographic Confederations during the first cycle of implementation of the Water Framework Directive (DATAGUA, 2008).
2) Inventory of transverse barriers from the European AMBER project (Adaptive Management of Barriers in European Rivers).
3) Inventory of pressures IMPRESS 2, developed and maintained by the Hydrographic Confederation of the Cantabrian Sea.
4) Inventory of natural and artificial obstacles in the Principality of Asturias carried out by SIGMA Servicios de Gestión Medioambiental S.L.
5) Inventory of natural and artificial obstacles in the Deva-Cares river basin prepared by González-Ferreras et al. (2019).
6) Information provided by the park ranger service personnel.

In addition, these sources of digital information were supplemented by verifying or eliminating barriers that currently do not exist through aerial imagery and field visits. Finally, a total inventory of 126 obstacles was obtained (65 in the Sella basin and 61 in the Deva-Cares basin; Fig. 68). Subsequently, a passability index was calculated for each of these obstacles. The passability of an obstacle differs for each fish species and depends on many factors, such as the height of the barrier, the presence of pools at its base, or the flowing water volume, among others. Therefore, there is no standardized method for evaluating passability applicable to all cases. In this case, a river connectivity assessment methodology developed for Cantabria was applied, which uses the maximum jumping ability of the fish species, the height of the barrier, and the presence/absence of fish passage (ICFC, IHCantabria – Government of Cantabria, 2011). The species used to assess the connectivity of the Sella and Deva-Cares basins was the salmon, as it is a priority species of community interest for the Habitats Directive, considering a maximum jumping height of 0.75 m for this species. To apply the ICFC to these basins, the synthetic river network described earlier in section 7.1 of this document was used, which is segmented into sections of 500-1000 m, where each of the obstacles inventoried in this study was georeferenced. Finally, the Dendritic Connectivity Index (DCI; Cote et al., 2009) was applied to the salmon, as its calculation differs for diadromous (migrating between rivers and the sea) or potamodromous (restricted to freshwater) species. The equation developed to calculate river connectivity for diadromous fish (DCID), in the case of salmon, is as follows:

$$DCI_D = \sum_{i=1}^{n} \frac{l_i}{L} \left(\prod_{m=i}^{M} c \right) x\ 100$$

Where l_i is the segment length i, L the total length of the catchment, n the number of segmentes in the catchment and c is the ICFC value of every assessed barrier m (m = 1, …, M) among the mouth and the segment i. The result of this index represents a percentage that results from the accumulation of barriers between the mouth and each segment and length. The value of this index increases from the mouth upstream towards the headwaters, depending on the number of obstacles found (Fig. 68). The result obtained in the study area shows a connectivity of 17% for the Sella basin, while in the Deva-Cares basin, it only reaches 10%. These percentages reflect the probability that a salmon will reach each segment of the basin from the mouth, and this probability decreases upstream with the presence of each new obstacle. For this reason, a large number of headwater sections are identified as inaccessible for salmon, especially in the Deva basin. This supposes a significant problem for the survival of populations of this species, as headwater rivers present better environmental conditions for spawning and the development of salmon fry (Rodeles et al., 2019). The reduction in the distribution area of salmon, associated with the presence of obstacles, increases the vulnerability of their populations, reducing their long-term survival capacity (Junge et al., 2014). On the other hand, we must not overlook the passability of these same obstacles downstream, which can affect the migration of fry and juvenile salmon. Moreover, hydropower dams can also cause significant mortality due to the entry of individuals into the diversion channels and the turbine zone (García de Leaniz, 2008).

It should be noted that the results presented, along with other social and economic criteria, can be applied to prioritize the obstacles on which action should be taken to increase the area occupied by salmon in these two basins. In this case, priority should be given to the permeability of the main rivers (Sella, Deva, and Cares) to facilitate the reconnection between the upper and lower parts of the river networks.

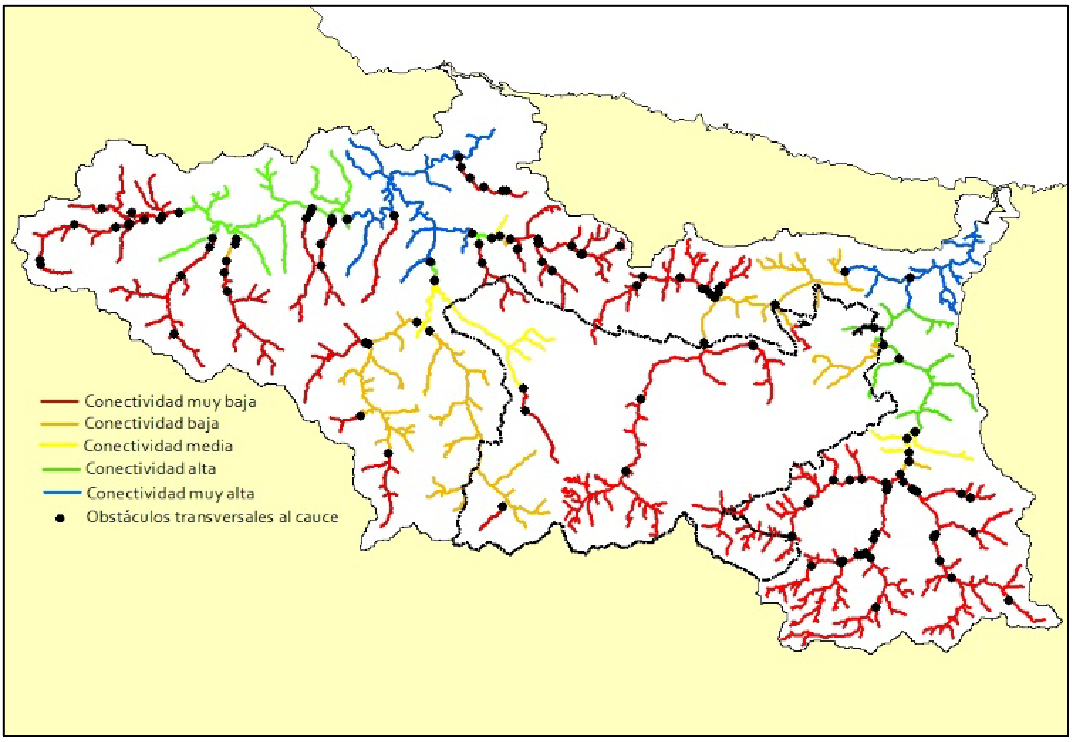


Figure 68. Result of the Dendritic Connectivity Index for Atlantic salmon in the Deva-Cares and Sella river basins and location of the obstacles identified in both basins.

11. Biological communities

The set of organisms that require an aquatic environment to successfully complete some phase, or the entire, their life cycle make up the biodiversity of an aquatic ecosystem. In the case of mountainous areas, as indicated above, the main natural aquatic ecosystems to consider when characterizing their biodiversity can be grouped into: 1) springs, 2) rivers and streams (fluvial ecosystems), 3) lakes and ponds (lacustrine ecosystems) and 4) peatlands/wetlands, although groundwater and other smaller water bodies can also be considered. Additionally, some organisms also find certain artificial water points to be of great importance for completing their life cycle. This is the case of amphibians, which often lay their eggs in livestock watering troughs or irrigation ponds, where they also undergo their subsequent larval development. Each of these types of aquatic ecosystems presents different environmental conditions, forming distinct niches capable of harbouring different taxonomic groups that form characteristic biological communities in each of these aquatic habitats.

A second aspect to consider when characterizing the biological component of the aquatic environment is related to the purpose for which this characterization is being conducted. Among these possible purposes, the following stand out:

1. Establishing global biodiversity patterns and their spatial and temporal trends, for which as much information as possible must be obtained about all the organisms inhabiting the characterized aquatic environment. This allows for the

determination of spatial and temporal patterns of taxonomic richness, α, β, or γ biological diversity, etc.

2. Monitoring and evaluating catalogued species. These are species listed in regional, national, or european catalogues for the protection/conservation of wild species, such as species of community interest described in Annex II of the Habitats Directive. In this case, it is necessary to obtain the required information to determine the distribution area of the species, the size and structure of its population or populations, as well as the temporal dynamics shown by these descriptors of its conservation status (trend of the distribution area, and the size and structure of its population). Following the diagnostic methodology proposed by the Habitats Directive, this is the information needed to report the conservation status of species of community interest, which can also be applied to other species listed under different protection frameworks.
3. Monitoring to determine the integrity of the aquatic environment. In this case, biological communities able to reflect changes in the integrity of aquatic environment (i.e., bioindicator communities) must be characterized, such as invertebrate, fish, or diatom communities. European environmental water legislation (WFD; 2000/60/EC) sets the bioindicator communities that must be monitored to determine the ecological status of different water bodies in European Union member states (i.e., rivers, lakes, reservoirs, transitional waters, etc.). In this case, the methodology used by each State to characterize and assess these bioindicator communities (e.g., sampling methods, quality indices, etc.) may vary, although the different proposals must follow the methodological guide described by the Directive and undergo a process of intercalibration. The goal is to obtain consistent results that allow comparability between the different methods applied by each member state and, most importantly, between the results obtained after implementing this process.
4. Monitoring to detect the presence of alien species: These species are one of the main drivers of biodiversity loss worldwide, which is why Target 6 of the Kunming-Montreal Global Biodiversity Framework highlights the need to "*reduce invasive alien species or mitigate their impacts on biodiversity and ecosystem services*". To achieve effective conservation of a natural area, it is necessary to detect the presence and distribution of alien species that pose a serious threat to native species, their habitats and ecosystems.

Traditionally, the characterization of aquatic biological communities has been carried out through sampling and subsequent taxonomic identification of the organisms collected based on their anatomical characteristics. As a result, the composition of the sampled community is obtained, meaning the different taxa that comprise it, and its structure, referring to the absolute or relative abundance/density of each taxon present in the sample relative to the total number of organisms quantified in it. This approach can yield suitable results for addressing objectives 1 and 3 mentioned earlier, as long as the appropriate protocol is applied to each characterized community. However, for catalogued species monitoring, more targeted and specific protocols for each species are generally applied, without considering the rest of the community to which it belongs.

As an evolution of these "traditional" sampling and determination methods, a new approach has been developed over the past decade, based on water sampling and the identification of organisms whose DNA is present in the water by using metagenomic techniques. This innovative technique is known as environmental DNA or eDNA. eDNA relies on the identification of taxa through the cells they leave in the environment. This technique has been widely used in recent years to assess and monitor biological diversity as a whole (eDNA metabarcoding), as well as for the study of specific species (eDNA barcoding). Its applications are diverse, allowing for the achievement of several of the aforementioned objectives.

In the Picos de Europa NP, both approaches are applied to characterize the most relevant aquatic biological communities. Below is a detailed account of the work carried out in this area to characterize aquatic communities through traditional sampling and the application of the eDNA technique. In all cases, the samples used to characterize these communities, following both approaches, were obtained from the network of 13 study points described earlier in section 9 of this document (see Fig. 64), which, as previously mentioned, is sampled annually during the summer season. In the case of eDNA sampling, the same 13 points were sampled, as well as additional new ones (127 localities: river sections, lakes, springs, wetlands, and watering troughs; see below) across 3 different campaigns (spring and summer 2020 and spring 2021; Fig. 67).

11.1. Traditional sampling

The following describes the work carried out in the Picos de Europa NP, and its surroundings, to characterize different aquatic biological communities through the application of traditional sampling methods. This work has been conducted annually, without interruption, from 2012 to the present (2024).

11.1.1. River invertebrate communities

These communities include any non-vertebrate animal larger than 500 µm which maintains a direct relationship with the river environment, at least during some stage of the life cycle.

Since invertebrate communities are composed by a large number of different taxonomic groups with highly heterogeneous trophic requirements, their activity has a significant influence on the river ecosystems functioning. They are a fundamental link between the different energy sources of these ecosystems and the secondary consumers (e.g., fish, amphibians, birds, etc.). Additionally, invertebrate communities have been extensively studied due to their great biological diversity, allowing for the development, creation, and testing of different ecological models concerning the interactions between biological communities and the environmental conditions dominating both the aquatic and adjacent terrestrial environments.

The great diversity of organisms included in these communities also makes them respond gradually to the degradation of river ecosystems, which is why they are commonly used as bioindicator communities to assess the integrity of river ecosystems specifically and aquatic ecosystems in general. More specifically, these communities are excellent bioindicators of river ecosystem integrity for the following reasons (see Bonada et al., 2006):

- Sensitivity: Invertebrate communities provide a quantifiable response to different specific disturbances in the environment, such as water pollution and hydromorphological alterations.
- Selectivity: Depending on the indices used, the analysis of invertebrate communities yields specific results for different disturbances.
- Scientific validity: The responses of invertebrate communities to several anthropogenic disturbances have been widely described from a scientific perspective. Likewise, these responses have also been statistically validated.
- Reliability: The analysis of invertebrate communities provides known and acceptable levels of uncertainty.
- Cost-effectiveness: The use of invertebrate communities as bioindicators provides a large amount of information in relation to the invested effort (economic, time, personnel, etc.).
- Simplicity: The methodologies designed to work with invertebrate communities generate results that are easily applicable and interpretable, allowing for their generalized and standardized use.
- Comparability: These results can also be compared across wide geographic areas, allowing for interterritorial comparisons.
- Predictive capacity: The use of invertebrates as bioindicators allows for the establishment of models capable of predicting the effect of different anthropogenic pressures on these communities

For all these reasons, the long-term monitoring system proposed for the aquatic ecosystems of the Picos de Europa NP incorporates the characterization and evaluation of invertebrate communities. The following protocols were applied:

1. To characterize the structure and composition of the invertebrate community at each one of the 13 points described above (see Fig. 64), samples are taken from two different hydraulic habitats: pools and runs. A surber-type net is used to conduct 3 samplings in pools and 3 in runs, which are then grouped to obtain a single invertebrate sample for pool and another one for run in each site (each composed of three surber samples). The use of the surber net allows for the quantification of the sampled area (Fig. 69), so after the treatment and identification of the samples, it is possible to determine the density of individuals for each identified taxon in each sample (i.e., individuals/m²).

2. In addition to the two samples taken with the Surber net(pool and run), another sample is taken at the same study site using a kicker net in other microhabitats present in the section (e.g., riparian roots, leaf litter accumulations, etc.). This sample is taken following the protocol established by the Ministry of the Environment of the Government of Spain for monitoring the ecological status of freshwater water bodies (ML-Rv-I-2013), commonly known as the 20-kick method. The purpose of this protocol is to distribute the sampling effort across the different microhabitats present in the study site to characterize the full diversity of invertebrates present, as many taxa tend to preferentially select certain habitats over others (Álvarez-Cabria et al., 2011). Since the monitoring in the Picos de Europa NP already includes two samples associated with pools and rapids, the kicker net sample is done with less effort. Instead of 20 kicks, the sample consists of just 10.

Figure 69. Sampling of invertebrates with a surber net at the study site located in the Bulnes river and part of a sample on a plastic tray.

Once in the laboratory, the samples from rapids and pools are identified to the lowest possible taxonomic level, generally to genus and species for almost all groups, obtaining data on the density of each taxon in both, pools and runs. On the other hand, the sample taken with the kicker net, also called the multi-habitat sample, is identified only at the family taxonomic level, without counting abundances or densities, recording only the presence of the identified families. Based on these data, analyses of the structure and composition of the invertebrate community associated with pools, runs, or both habitat types can be conducted by summing both samples.

With this data, it is possible to perform, for example, long-term community similarity analyses considering the Control/Impact design of the sampling network, as well as other environmental variables characterized throughout this document (e.g., hydrology, food resources, water quality, etc.). This allows for determining whether a change in these communities is influenced by a natural environmental variable or by an anthropogenic impact.

Moreover, to assess the integrity of invertebrate communities through the calculation of quality indices/metrics, as indicated by current sectoral legislation (WFD), is necessary to understand the structure and composition of these communities at the family taxonomic level. To calculate these indices from the described samples (one pool sample, one run sample, and one multi-habitat sample at each of the 13 points/year), the community identified at the lowest possible taxonomic level in pools and rapids is translated to the family level. Then, the presence of families identified in the multi-habitat sample, which were not found in the pool and rapid samples, is added.

After this process, the calculation of several commonly used indices to evaluate the integrity of river ecosystems during the study period has been obtained. For example, Figure 70 shows the evolution of the Iberian Biomonitoring Working Party (IBMWP) index and the number of families of Ephemeroptera, Plecoptera, and Trichoptera (EPT) for the 6 study points classified under typology 2 throughout the entire data series (2012–2022). Considering that a decrease in the value of these indices reflects the loss of integrity of the invertebrate communities, it can be observed that the control points of this typology (Seco, Salvorón, and Casaño) generally maintain higher values for both indices compared to the points affected by urban effluents (Duje at Tielve and Bulnes) and runoff (Duje at the headwaters).

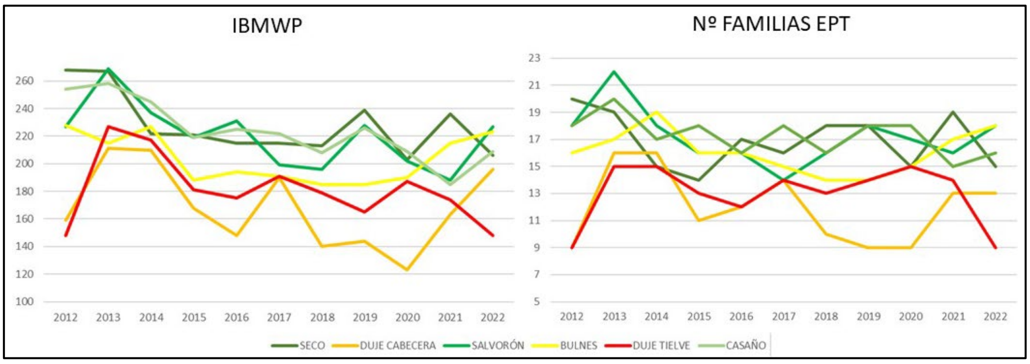


Figure 70. Variation of IBMWP and the number of EPT families in the 6 study sites of typology 2 (2012-2022). Green: control points and warm tones: affected points.

This simple analysis also allows us to observe, among other things, how these indices show less interannual variability in control sites than in the impacted ones. This pattern seems to reflect that river sections in better conservation status allow for the development of more stable communities in the long term, despite environmental changes. In contrast, communities in poorly conserved river stretches are more affected by the interannual environmental variability of the river environment (e.g., hydrological and temperature regimes). Therefore, it is expected that the effects of global change will have less impact on the invertebrate communities that develop in well-preserved river sections.

As already indicated, the generated database also allows for ecological studies to determine how these communities interact with the adjacent aquatic and terrestrial environment. For example, the results obtained up to 2022, presented at the first edition of the Research Conference of the Picos de Europa NP, held in Posada de Valdeón

(16/10/2023), showed through a non-metric multidimensional scaling analysis (NMDS) how the diversity of invertebrates and other indices that assess the integrity of these communities (METI and IASPT) positively correlate with the same axis as most of the communities described at the control points. In contrast, the communities affected by effluents showed a similar arrangement in this two-dimensional space to that obtained by various parameters indicating poor water quality, such as nitrate and phosphate concentrations and electrical conductivity (Fig. 71).

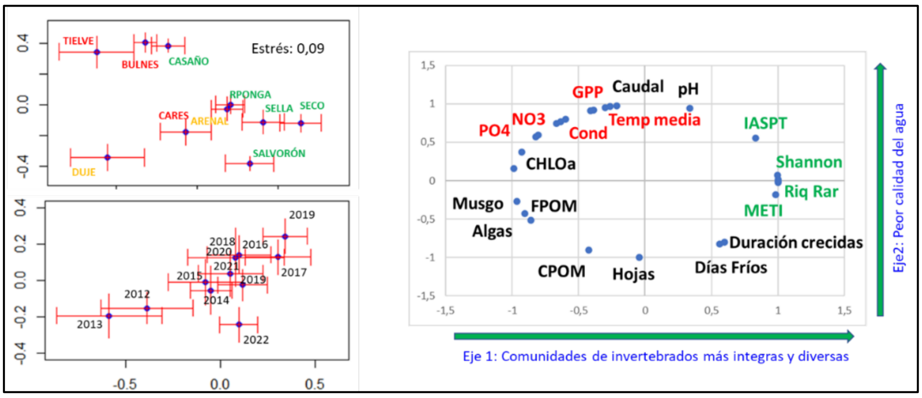


Figure 71. Non-metric multiscale analysis of the 110 river invertebrate communities described in the period 2012-2022 (does not include springs). Communities from the 5 control sites are represented in green, in red in the 3 sites affected by urban effluents and in orange the 2 affected by runoff processes.

11.1.2. *Diatom communities*

Another aquatic organism community commonly used to assess the integrity of river ecosystems is the one formed by phytobenthic algae, specifically diatoms. Like invertebrates, diatoms possess several characteristics that make them ideal organisms for use as aquatic bioindicators (Ciutti, 2005).

Diatom communities was annually characterized and evaluated in the same 13 sites network during the same campaign in which water and invertebrate samples were collected, although the analysis of diatom communities began in 2017. Two diatom samples were taken at each one of these sites in pools and runs. Three stones were collected from the riverbed in runs and another three from pools, selecting 10 cm x 10 cm approximately. These stones must be from the central part of the river channel and completely submerged, avoiding isolated branches or areas located under infrastructures (e.g., bridges). The stones are brushed, and the scraped biofilm is collected in plastic trays. From this, 30 ml of biofilm is extracted for the diatom sample, preserved with diluted alcohol until further laboratory analysis. Once in the laboratory, a specific preparation is made for each sample (permanent preparation) using a mounting medium with a high refractive index (e.g., 1.65) to allow diatom visualization under microscope (Fig. 72). The preparation method used for these samples is oxidation with hydrogen peroxide (UNE-EN 13946:2014). Then, 400 individuals (valves) are identified in each sample down to the species taxonomic level, whenever possible, following the methodology described in the UNE-EN 14407 standard. For diatom identification, a phase-contrast microscope equipped with a micrometric grid to measure the diatoms and a 100x magnification is used.

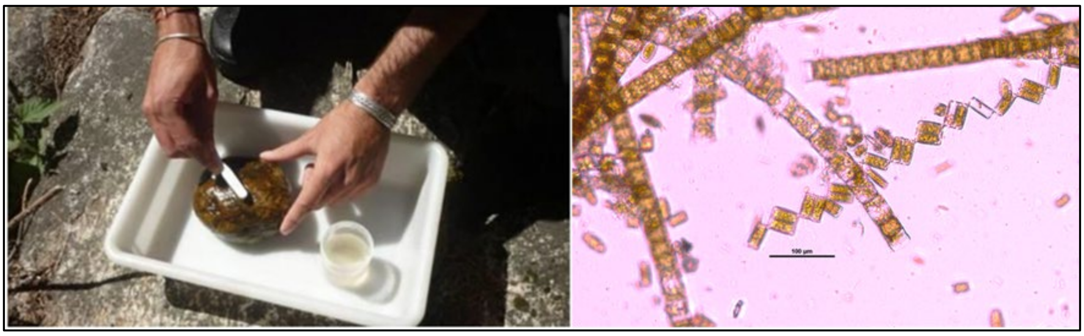


Figure 72. Sampling of benthic diatoms and *Melosira* spp. and *Diatoma* spp. in microscope.

Once the taxonomic identification and valve counting have been completed, a description of the structure (relative abundance of each species) and composition (species richness) of the diatom community for each sample is obtained. These data allow for the calculation of indices that assess the integrity of the river. The most commonly used index is IPS (Cemagref, 1982), which allows for the analysis of the interannual variability of these communities (Fig. 73). For example, when analyzing the variability of the IPS and the taxonomic richness of diatom species in Type 2 sites, it is observed that this index generally shows higher values at control sites (Seco, Salvorón, and Casaño) compared to affected by effluents (Duje at Tielve and Bulnes). However, no clear differences are observed between control sites and the affected by runoff processes (Headwater Duje). In contrast, a higher species richness is observed at sites affected by effluents compared to the control sites. This is because, in many cases, intermediate levels of disturbance increase the species richness of diatoms compared to oligotrophic environments or areas with significant anthropogenic impacts (Townsend et al., 1997). However, it is important to note that higher alpha diversity values (e.g., number of species in a sample) often correspond to lower beta diversity (rate of change between adjacent communities). As a result, diatom communities in disturbed regions tend to show greater homogeneity (i.e., lower beta diversity; Goldenberg Vilar et al., 2014).

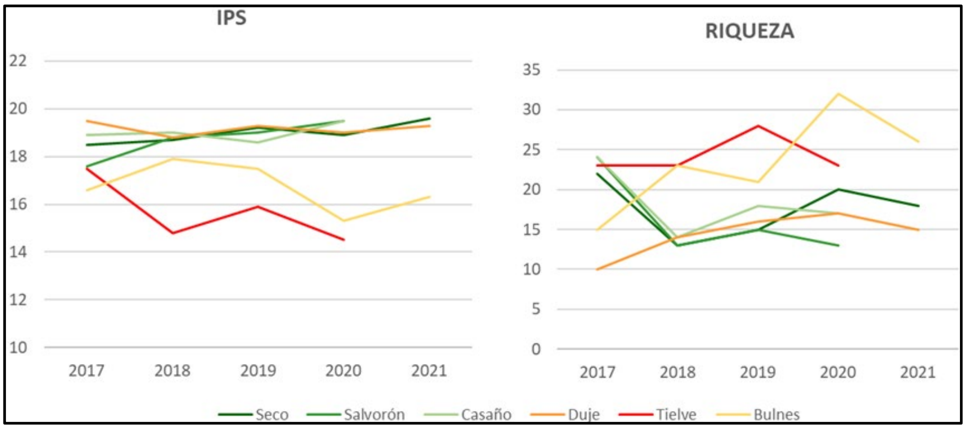


Figure 73. IPS index and richness of diatom species in the 6 type 2 study sites (2017-2021). Green: control sites. Warm tones: affected sites.

Based on the structure and composition of these communities, statistical analyses can be conducted to determine their variability in response to impacts or environmental variability inherent to the aquatic environment (e.g., temperature, hydrological regime, etc.). In the NMDS analysis shown below, the similarity of diatom communities is observed after applying the Bray-Curtis index. Samples from control points are mainly arranged in the negative zone of axis 1 of the NMDS (Fig. 74), while samples affected by effluents appear in the central part of the ordination. Samples affected by runoff (Arenal and Duje) do not show more similarity to each other than to the control sites. However, it is noted that the diatom community of the Duje River is particularly different from the communities at the other points (Fig. 74). Along axis 2 of the NMDS, there is some variation in community composition in relation to the sampling year. The communities from 2021 are associated with lower values on axis 2, while the samples from 2018 and 2019 are found in the lower area and lower-right quadrant, with higher values along axis 2 of the NMDS (Fig. 74).

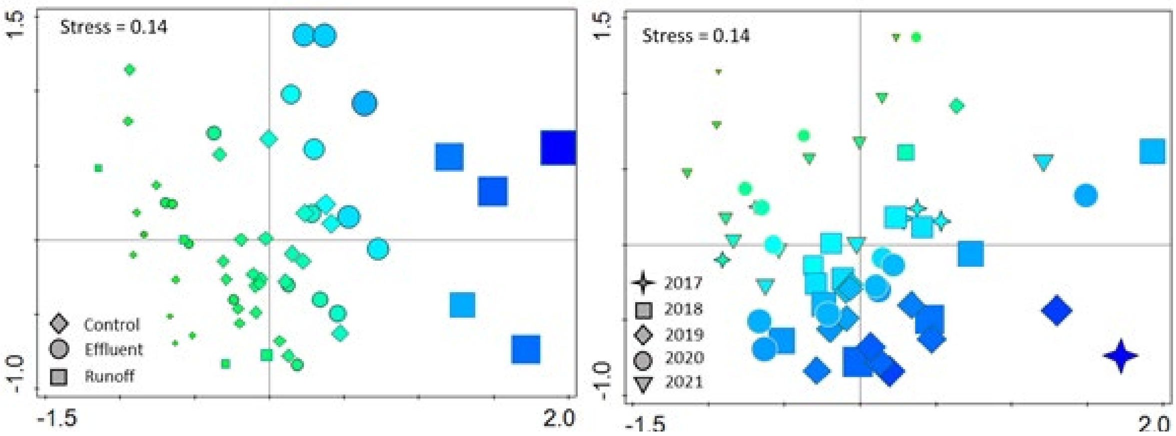


Figure 74. NMDS for diatom communities of the Picos de Europa NP. Colour and size scales are shown for axis 1 (left) and axis 2 (right) values.

11.1.3. Macrophyte communities

Aquatic macrophytes correspond to a very heterogeneous group of plants from a systematic and evolutionary point of view. Macrophytes are considered a key element in the food chains of aquatic ecosystems. They are autotrophic organisms that are visible to the naked eye, including macroalgae, bryophytes, and cormophytes. Among cormophytes, two major groups stand out:

1. Hydrophytes: aquatic plants that complete their biological cycle with all parts submerged or floating on the water's surface.
2. Helophytes: amphibious plants with the lower part submerged and the upper part emerged.

These communities are good indicators of the integrity of the aquatic environment, as they are highly sensitive to the following changes:

- Water quality: reduction in water transparency, eutrophication, changes in conductivity, and salinity.
- Hydromorphological conditions: variations in flow regime, hydraulic conditions, river continuity, or bed characteristics.
- Land use and coverage: changes in land use within the drainage basin can also lead to variations in the composition and structure of macrophyte communities (Kuhar et al., 2007).

Additionally, the use of macrophytes as bioindicators has several advantages, such as the relative ease of taxonomic identification, as they are visible to the naked eye and fixed to the riverbed. Therefore, most of the taxonomy can be performed in situ, on the own river stretch, without the need for collection and subsequent identification in the laboratory. Moreover, they have a relatively long lifespan, allowing them to reflect seasonal and interannual disturbances in their structure and composition. To characterize macrophyte communities at the Picos de Europa network (13 sampling sites), a 100 m. stretch of the river is studied, where the coverage of different macrophyte species is estimated by using the "Sampling and Laboratory Protocol for Macrophytes in Rivers" (ML-R-M-2015; Fig. 75). Taxa that cannot be identified in the field are collected and preserved in Kew liquid until they can be identified in the laboratory. The species relative coverage data allow for ecological analyses and the estimation of quality indices such as the IBMR (Haury et al., 2006).

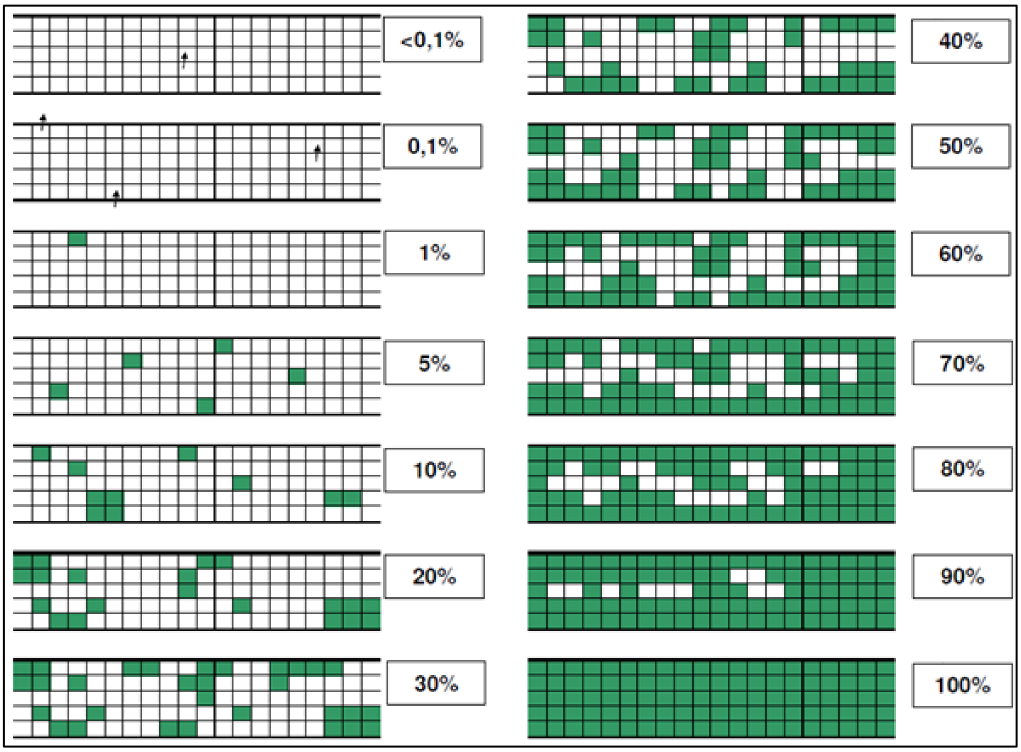


Figure 75. Example of macrophyte coverage scale. Figure taken from Sampling Protocol and Macrophyte Laboratory in Rivers: ML-R-M-2015.

Macrophytes began to be characterized in 2018 within the monitoring network established for the rivers in Picos de Europa NP. Figure 76 shows the variation in the IBMR index in type 2 study sites. Data series indicates that the control sites (Seco, Salvorón, and Casaño) had higher IBMR values than sites impacted by effluents (Duje in Tielve and Bulnes). As previous cases, the upper Duje, which is affected by runoff, did not show significant differences in IBMR values when compared to the control points. Regarding macrophyte richness, no clear distinction is observed between control sites and those impacted by anthropic pressures (Figure 76). This suggests that while the IBMR index effectively captures the impact of point-source pollution, macrophyte richness alone might not be a strong indicator of environmental integrity in this context.

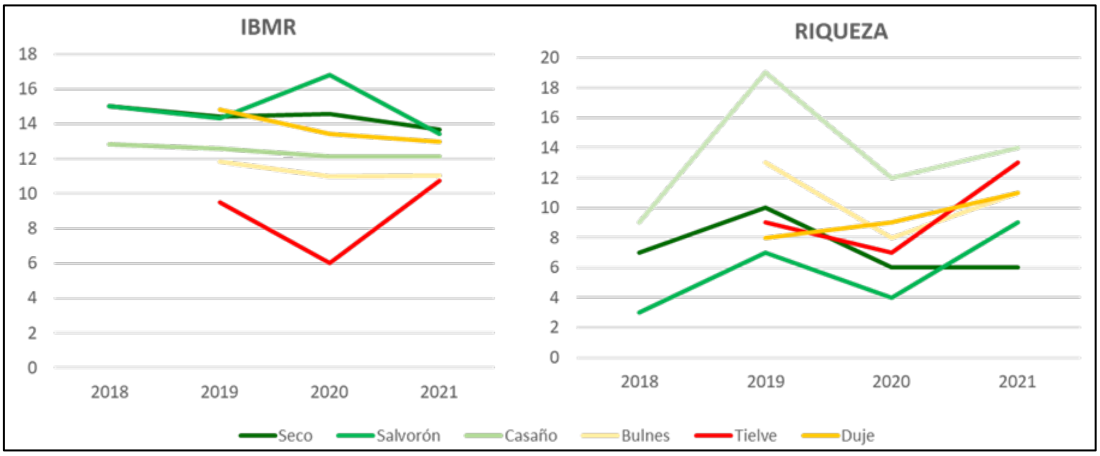


Figure 76. Variation of the IBMR index and macrophyte richness in typology 2 sites (2018-2021). Green: control sites. Warm tones: affected sites.

The Bray-Curtis similarity analysis represented through a NMDS does not reveal a greater similarity between the control samples in relation to the affected by runoff or effluents (Fig. 77). Contrary, a slight variation is observed in relation to the year, where we see that the samples from 2018 have, in general, lower values regarding axis 2 of the NMDS, except for the point of the Duje River (Fig. 77) and 2020 and 2021, which generally present higher values, although not for all sampled points.

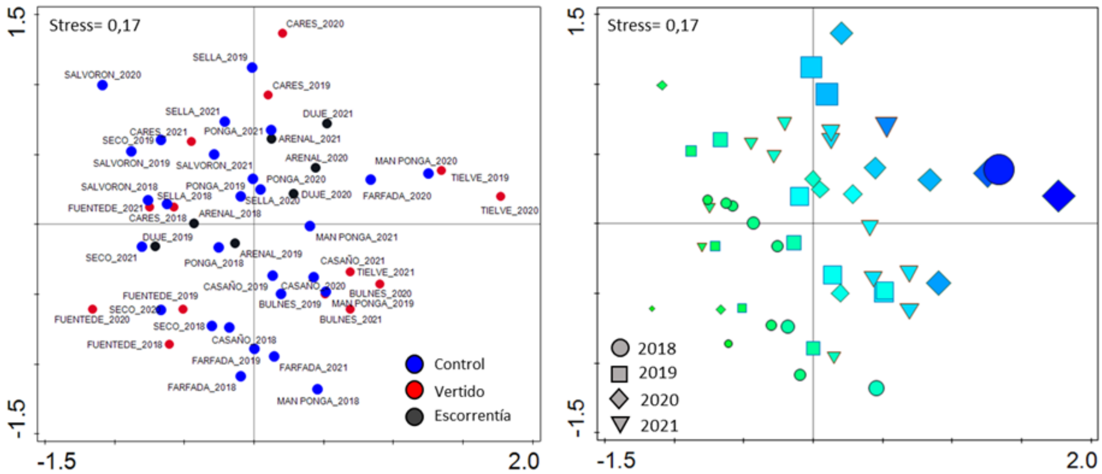


Figure 77. Non-metric multiscale analysis for the 52 macrophyte communities described in the Picos de Europa NP monitoring network (13 points * 4 years).

11.1.4. Fish communities

Ichthyofauna is considered a good indicator of the integrity of aquatic ecosystems due to the sensitivity that fishes show to different physico-chemical and, especially, hydromorphological alterations, being a biological quality element required by the WFD.

Fish occupy different trophic levels, situated near the top of the river trophic pyramid. Therefore, they can reflect the conservation status of aquatic ecosystems through the analysis of the structure and composition of the communities they form. Additionally, their greater longevity, size, and mobility, compared to other bioindicator communities, give this group an indicator value at the meso-habitat or river segment level (Durán & Pardo, 2007).

The objective of the fish sampling is to obtain data on the structure and composition of these communities. In the 13 sites monitoring network, annual fish sampling has been carried out during the dry season (august) from 2012 to the present (2024). Sampling is based on the standard "EN ISO 14011:2003. Water quality. Sampling of fish using electricity" and on the electrofishing sampling protocols used by national water agencies. The sampling technique used is electrofishing (Figure 78), based on creating an electric field that alters the behaviour of fishes, facilitating their capture. The fishing has been carried out with a portable device with a power of 1.3 kW, capable of generating direct current at 300 or 500 V, or pulsating up to 940 V with a variable frequency of 25-100 Hz. Before starting the electrofishing, a measurement of the water conductivity is taken to adjust the intensity of the current converter, as the intensity of the current needed for electrofishing decreases as conductivity increases; therefore, in low conductivity waters, a higher voltage is necessary. Other physicochemical parameters such as pH or temperature are also measured during the sampling

Figure 78. Technicians carrying out fish sampling with electric fishing at Picos de Europa.

The 13 sampling points of the monitoring network are wadable. The sampling length depends on the river's width, always considering that the sampled area (length x width) contains different types of mesohabitats to sample the greatest possible habitat heterogeneity. At all sampling sites, natural obstacles have been used as closures for the sampled area, both upstream and downstream.

The method of successive captures and the Carle & Strub method (Carle & Strub, 1978) have been applied to estimate fish population abundance. Captured fishes are identified at the species level, the number of individuals is counted, and their weight and fork length (or total length, depending on the species) are recorded. To avoid generating stress in the captured specimens, eugenol is used for sedation. After recording the data, the fish are placed in recovery boxes situated in the riverbed, which are equipped with small holes to facilitate water entry and exit and oxygenation (Fig. 79). Subsequently, the fish are returned to the river near the area where they were captured. Data obtained during the field sampling allow for the subsequent estimation of fish community densities and biomasses at each sampling point, with the aim of enabling long-term monitoring. Other data, such as age structure, can be obtained through visual analysis based on the frequency distribution of fork lengths or through age determination by collecting scale samples.

Figure 79 Recovery boxes used in field sampling.

In the fish sampling conducted at the 13 study sites, from 2012 to 2024, only common trout (*Salmo trutta*) specimens were captured, except for a few eel (*Anguilla anguilla*) specimens caught in the Casaño, Salvorón, and Seco rivers in some years, with no other fish species being found, although we fished salmon (*S. salar*) in 2023 and 2024 in other study site belonging to the DIVAQUA monitoring network. Trout populations at Arenal, Cares, Ponga, and Sella have shown a similar pattern throughout the sampled time serie, with density declines from 2012 to 2015 (Figure 80A). Cares River, affected by a urban effluent, has consistently shown a density higher than that of the other sites (Fig. 80A).

Regarding Bulnes, Casaño and Tielve, it is noteworthy that the density of the Tielve River increased from 2012 to 2016, due to actions taken at this site to eliminate/reduce the livestock effluents (Fig. 80B). In the case of Duje, Salvorón, and Seco, they have shown a trend of increases and decreases in population density over the years (Fig. 80C). Finally, the group that includes the springs has a lower density of trout than the other ones (Figure 80D). It is important to indicate that no fish were captured in the spring of Fuente Dé (the source of the Deva River) during the years 2012 to 2019 (Figure 80D).

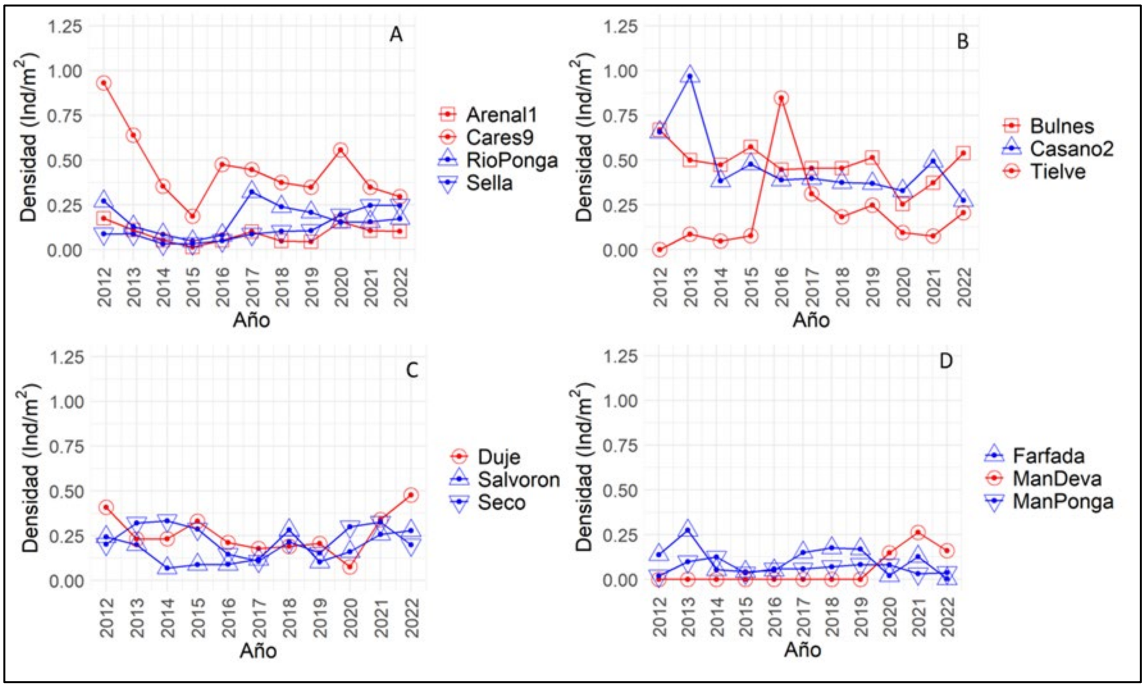


Figure 80: Trout density (Ind/m^2) captured in the first pass (2012-2022) for the points that make up the monitoring network (Arenal, Cares, Ponga and Sella, A; Bulnes, Casano2 and Tielve, B; Duje, Salvoron and Seco, C and Farfada, Deva and Ponga springs, D).

11.2. Environmental DNA and key elements

The technique known as environmental DNA (eDNA) has a wide range of potential applications, depending on both, the type of sample being analyzed (the nature of the substrate from which the genetic material is collected) and the group of organisms being studied. In the Picos de Europa NP, this technique has been applied to detect key species (catalogued, pathogenic, and invasive) using specific primers. General primers have also been employed to analyse aquatic biodiversity as a whole, with a particular focus on bioindicator communities of the integrity of the aquatic environment (e.g., invertebrates or diatoms).

11.2.1. Sampling

Samples were collected from water, sediment and biofilm in the rivers/streams, lakes/ponds, and watering places of the Picos de Europa NP and its surroundings, as the genetic material present in the water appears to have a shorter retention time than in biofilm and sediment (Joseph et al. 2022). Furthermore, the availability of samples taken from different media facilitates the detection of various taxa, allowing for a more comprehensive representation of the aquatic communities in this area.

The followed protocol depends on the nature of the substrate. However, for all of them, the genetic material has been collected by filtering a specific volume of water using a peristaltic pump that creates a vacuum through a flask in filtering columns, employing filters with a pore size of 0.45 µm. In all cases, sterilized materials with sodium hypochlorite (3%) were used. Additionally, to prevent cross-contamination with biological material external to the sampling site, all filtering was conducted in the field, at the sampling point itself (Fig. 81).

Figure 81. Filtering of samples and details of the material.

eDNA sampling was carried out considering the following specifics for each aquatic medium:

- <u>Water Samples from Rivers</u>: Five liters of water are collected from the river in five different collections. Every 12 minutes, a 1-liter sample of water is taken using wide-mouth plastic bottles (HDPE, QUIAGEN, 1000 ml). Once the 5 liters are collected in 5 containers, 300 ml from each of them is filtered, resulting in a final sample present in the filter obtained from filtering a composite sample of 1.5 liters of water.

- Biofilm Samples from Rivers: The biofilm sampling for eDNA analysis is done in conjunction with the sampling of diatoms, as previously described, and chlorophyll a (see below). For eDNA analysis, a total volume of 10 ml is filtered.

- Water Samples from Lakes and Ponds: In each lake/pond, 200 ml of water is taken at 5 different points within the same body of water, creating a composite sample in a 1-liter bottle. From this sample, a volume of 450 ml is filtered to obtain the sample (filter) for eDNA analysis.
- Sediment Samples from Lakes and Ponds: Similar to the water samples, an integrated sample is collected from 5 points on the lakebed. This sample is diluted in 50 ml of distilled water in a Falcon tube and then filtered.
- Water Sample from Watering Places: A sample of 450 ml of water is taken in a previously sterilized container, being careful not to disturb or cloud the water. Finally, the total volume of the sample is filtered.
- Biofilm/Sediment Sample from Watering Places: The water from the watering place is removed, and 450 ml of water is collected in a sterilized container. Between 50-100 ml of this sample is filtered depending on the turbidity and saturation of the filter, noting the final filtered volume for each sample.

To prevent degradation and ensure the proper preservation of the filtered genetic material, all filters (i.e., samples) are stored in vials with absolute ethanol and kept cold until they are placed in the freezer at -32ºC upon arrival at the laboratory.

11.2.2. Sample processing in the laboratory

Once in the laboratory, a series of sequential steps must be followed, which are described below.

1. <u>DNA Extraction.</u> The extraction of DNA is based on the release of DNA molecules through a process of both physical and chemical/enzymatic lysis of the membranes and cell walls of the cells contained in the sample to be analyzed. Subsequently, isolation and purification of the obtained DNA fragments is performed. To achieve this, each filter is cut in half with a sterilized blade, placing each half on sterilized aluminum foil, and then dividing it into smaller fragments to facilitate more effective extraction. The phenol-chloroform technique is used for DNA extraction, which allows for the acquisition of high-quality DNA and extraction using a commercial kit called DNeasy PowerSoil Pro Kit-QIAGEN. Additionally, in cases where necessary, inhibitors were removed using a commercial kit.
2. <u>Primer Design and PCR Amplification.</u> The correct identification of species or groups of organisms present in an eDNA sample depends on the quality and specificity of the primers used. Primers are very short fragments of sequences used to amplify a specific region of DNA through the PCR technique. Depending on the study's objective, different types of primers are used, both specific and universal. In this case, both types were used, following a dual strategy: 1) the use of universal primers to generally characterize the biodiversity present in different aquatic environments and 2) the use of specific primers to discriminate DNA

sequences from target species present in the sample, referred to as "key elements." These include:

- ✓ Conservation-Interest Species: Primers have been included for fish species *S. trutta*, *S. salar*, *Petromyzon marinus*, *Alosa alosa*, and *Rutilus arcasii*; mammals *Lutra lutra*, *Mustela lutreola*, and *Galemys pyrenaicus*; and the fern *Woodwardia radicans*.
- ✓ Pathogenic Bacteria: Pathogenic bacteria for fish (*Aeromonas salmonicida*, *Flavobacterium psychrophilum*, and *Yersinia ruckeri*), crustaceans (*Aphanomyces astaci*), and amphibians (*Batrachochytrium salamandrivorans*, *B. dendrobatidis*, and *Ranavirus* spp.). Pathogens of livestock, such as tuberculosis (*Mycobacterium tuberculosis*, *M. bovis*) and brucellosis (*Brucella* sp.) were also included.
- ✓ Invasive Exotic Species: Invasive exotic species such as the diatom *D. germinata*, the mammal *Neovison vison*, the snail *Potamopyrgus antipodarum*, and the crayfish *Pacifastacus leniusculus* and *Procambarus clarkii*.

For these species, both published primers and new primers specifically designed using mitochondrial genomes (mtDNA) available in the public repository GenBank were used. Mitochondrial genes have more copies per cell and are more likely to be detected in environmental DNA samples. The goal is to use conserved regions of the target genome that are variable in their close taxa, so that the new primers are specific to each species in those specific regions. Additionally, the target amplified region comprises a size of amplification between 200 and 400 base pairs. Initially, individual amplifications were carried out for each specific primer with a total of 7980 individual PCRs from the 380 samples of water, sediment, and biofilm obtained. The PCR products were visualized using electrophoresis on agarose gels (Sybrgreen staining). The presence of PCR-amplified DNA is considered a positive presence, while the absence of a band indicates absence. Next, multiplex PCRs were performed using specific primers along with universal primers that amplify the entire community. Presence or absence is determined by sequencing the PCR products to confirm the presence of target species and to characterize the overall biodiversity.

3. <u>Amplicon Sequencing and Taxonomic Identification.</u> The samples taken from the 13 monitoring sites (see Fig. 64) in 2018 and 2019 were analysed using Illumina sequencing technology (Miseq600), while the 127 sampled locations in 2020 and 2021 (Fig. 67) were analysed using Oxford Nanopore Technologies (ONT).Third generation sequencing with ONT provides several advantages over short fragment sequencing methods like Illumina, as it: 1) determines the sequence in real time, allowing for specific decisions to be made during sequencing, 2) has no size limit on the sequenced molecule, allowing for increased taxonomic

resolution, and 3) offers very advantageous characteristics in terms of portability, handling, and market price of the sequencer. In recent years, the quality of the obtained sequences (new Q20 chemicals >99%) has improved considerably, and specific bioinformatics workflows have been developed for data analysis, increasing their use and applicability. To carry out the sequencing, libraries were prepared from the PCR products (SQK-LSK110 ligation sequencing kit). The samples were sequenced in batches of 20 to 24 samples per flow cell using an individual identifier (barcode) for each sample (PCR kit 1-96 EXP-PBC096). In both cases, the steps specified in the manufacturer's protocols were followed. Sequencing was carried out until 2 million sequences per run were obtained. Subsequently, high-accuracy base calling was performed to determine the specific sequences of each amplification by sample. The sequences were aligned to recover coverage and fully resolved consensus sequences using specific algorithms and functions. For the first round of taxonomic identification, the latest version of the SILVA database was used, which contains taxonomic information for the domains of Bacteria, Archaea, and Eukarya.

11.2.3. Universal primers for the characterization of indicator communities

The universal COI marker demonstrates a high taxonomic precision, capable of identifying aquatic invertebrates until the species taxonomic level. Moreover, this marker has more comprehensive and curated molecular databases compared to other markers (Devloo-Delva et al., 2018). To evaluate the effectiveness of COI against other approaches, an analysis of the invertebrate communities sampled in 2019 at 13 points in the monitoring network of the Picos de Europa NP was conducted, following three different approaches:

1. Traditional taxonomy.
2. Through the analysis of eDNA from water samples and biofilm (COI eDNA).
3. Through the analysis of eDNA from the preservative alcohol of invertebrate samples, using the mitochondrial marker COI (BF2/BR2) and Illumina (Miseq600). The taxonomic assignment of the extracted and amplified sequences was performed using the GenBank database (Benson et al. 2013).

After conducting a normalized analysis of variance (ANOVA), significant differences were found in the number of taxa identified in each of these 3 approaches ($F_{2.36} = 10.06$, $p < 0.001$). The method that identified the greatest number of taxa was the COI primer from the invertebrate sample's alcohol (62%, 8 of 13 points), followed by the traditional method (39%, 5 of 13 points). The highest number of species was identified at the Cares site for both, molecular techniques (67 species COI_alcohol, 45 COI eDNA), and traditional taxonomy (42; Fig. 82). These data indicate that the power of molecular techniques focused on identifying aquatic invertebrates reaches its optimum when sampling methods are directed towards these communities. Due to the diversity of behaviours and habitats of invertebrate communities, the sampling method carried out with environmental eDNA samples (water and biofilm) does not capture all the taxa identified by traditional methods. On the other hand, these data indicate that when sequences are present in the sample, the taxonomic resolution of molecular techniques is greater than that obtained from applying traditional identification methods.

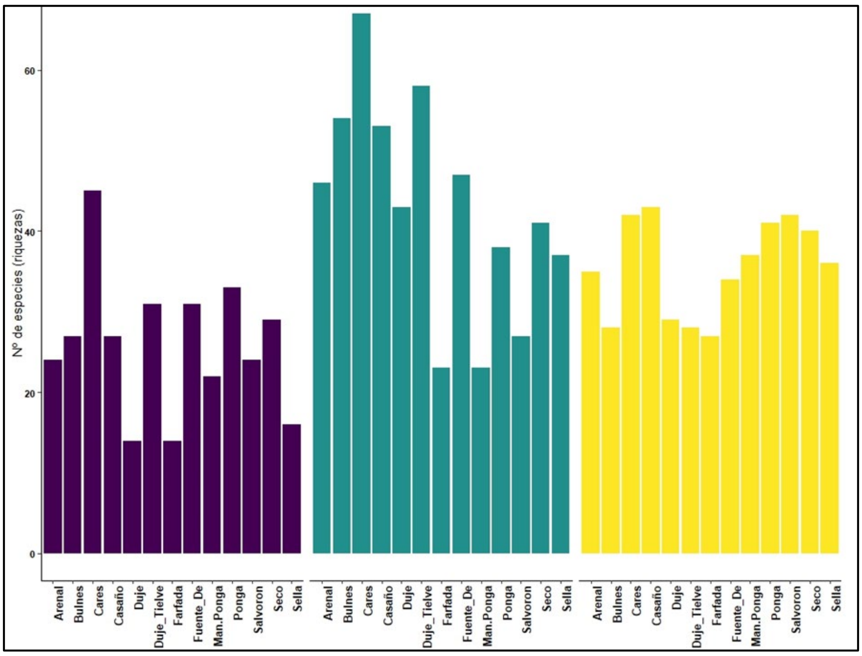


Figure 82. Number of taxa identified in the 13 sites according to technique used: purple - COI_eDNA / green - COI_alcohol / yellow - traditional sampling.

11.2.4. Universal primers for the characterization of aquatic diversity

Primers that amplify regions of ribosomal genes (e.g., 18S for eukaryotes) have lower taxonomic resolution, typically reaching the order and family level for most eukaryotic taxa, and even to the genus level in some cases. However, these primers allow for the detection of a broader taxonomic range, enabling the study of the configuration of biological diversity at different levels and taxonomic groups.

During the sampling conducted in 2018, 13 samples from the monitoring network were analysed using the universal primer Euka02 sequenced with Illumina technology (Miseq600). The taxonomic assignment of the extracted and amplified sequences using the SILVA database revealed the wide diversity of taxonomic groups recorded with this primer. In general, the results showed 21 phyla belonging to different kingdoms: plants, macroalgae groups from the phyla Ochrophyta and Phragmoplastophyta; animals, including both vertebrates (Vertebrata) and invertebrates (e.g., Arthropoda, Cnidaria, Tardigrada, Mollusca, Brachiopoda, Porifera); fungi (e.g., Ascomycota, Basidiomycota); and Protista (e.g., Cryptomonadales, Dinoflagellata, Cercozoa). The largest number of sequences belonged to the phylum Ochrophyta, followed by the phylum Phragmoplastophyta (Fig. 83).

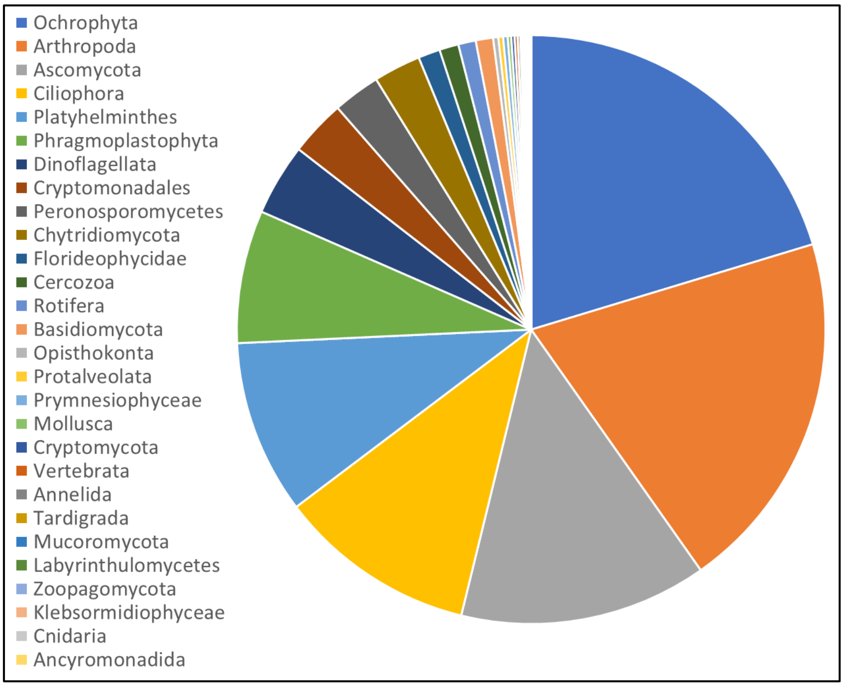


Figure 83. Relative abundance of sequence reads matching the major taxa assigned with the SILVA database.

From these data it is possible to determine global patterns of biodiversity and the spatial and temporal trend of taxonomic richness, α, β or γ biological diversity, as a whole or by taxonomic group. The detection of this large number of taxa using universal primers also makes it possible to identify the presence of taxa hitherto not identified in a certain area or of groups.

11.2.5. Identification of key elements

The following are examples of the application of the eDNA technique to identify the presence and distribution of certain target species, also referred to as key elements, in the Sella and Deva-Cares watersheds.

Iberian desman (*G. pyrenaicus*)

The Iberian desman, a species listed in the Spanish List of Threatened Species (category "Vulnerable") and in the Annex of species of community interest under the Habitats Directive, was detected at 50 of the 127 sites where analyses were conducted in 2020 and 2021. This includes 41 rivers/streams from the sub-basins of the Sella, Cares, Duje, Urdón, and Deva, as well as in the Liordes lagoon and 8 lakes located within the boundaries of the Picos de Europa NP. Notably, the detection of this species occurred in two consecutive sampling campaigns (summer and spring 2020), as well as in both sampled media (water and sediment) in the Liordes Lagoon and Bajero Lake, as well as in the Besnes River, a tributary of the Cares River (Fig. 84).

In general, it is important to highlight the detection of this species in different types of rivers, ranging from small streams with very low flow to the main axes of the described basins, as well as in various lakes and lagoons, covering a wide altitudinal range and, therefore, different niches. Our results indicate that the distribution of this species in this area may be greater than previously described in studies where its presence was identified through genetic analysis of feces using mitochondrial markers (Igea et al. 2013). The higher number of positives using eDNA samples may suggest that this technique is less influenced by sampling effort and the identification of feces (success rates between 30-50%, depending on the abundance of the species in the surveyed stretch; Igea et al. 2013).

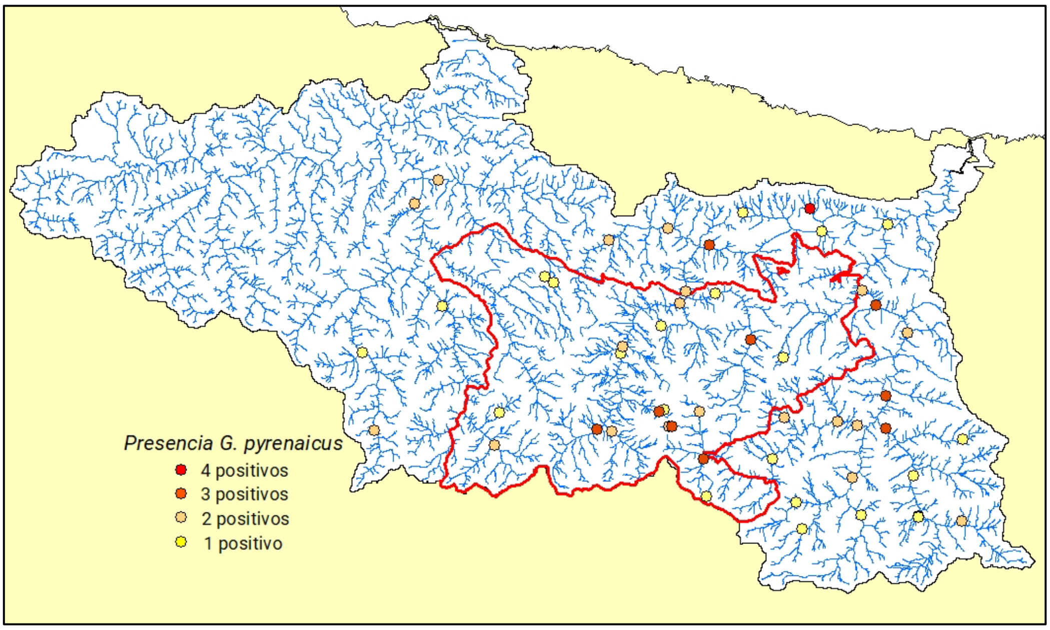


Figure 84. Distribution of *G. pyrenaicus* in Sella and Deva-Cares basins using eDNA. Occurrence is shown by colour gradation in number of positives by sample type (water, biofilm/sediment) and campaign (summer and spring 2020 and spring 2021).

<u>Pathogenic species of the fish community.</u>

This section presents the results of bacteria most commonly implicated in infections affecting salmonids in the described area. These are the gram-negative bacteria *Flavobacterium psychrophilum*, *Yersinia ruckeri*, and *Aeromonas salmonicida*, known to be the etiological agents of cold-water disease, enteric redmouth disease, and furunculosis, respectively. The transmission of these diseases occurs through the high number of bacteria released into the water by an infected or carrier fish. These bacteria can survive for long periods in the water or as hosts in carrier specimens, remaining undetectable until the disease manifests, for instance, in a stressful situation caused by poor water quality or changes in water temperature. eDNA can be used as an early warning tool to detect such diseases before a pandemic episode affects a large number of individuals in a specific fish population.

Following the described protocols, the presence of *F. psychrophilum* has been detected at 35 of the 127 sampled points, while *A. salmonicida* tested positive at 28 of these points and *Y. ruckeri* at 21 (Fig. 85). The highest occurrence recorded is for *F. psychrophilum* at the lower points in the Sella basin, where it appears in samples of both water and biofilm in all three campaigns conducted (spring and summer 2020 and summer 2021). In this area, the presence of *A. salmonicida* has also been recorded (spring 2020 and 2021 in water and biofilm) and *Y. ruckeri* (spring 2020 and 2021 in water)

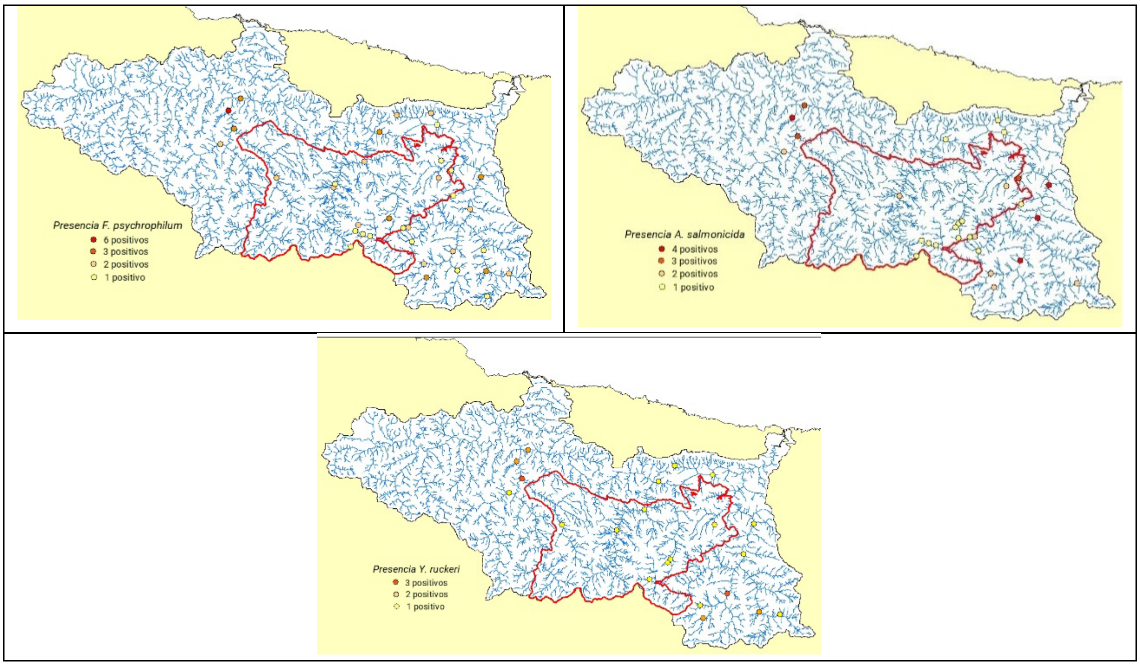


Figure 85. Distribution of *F. psychrophilum*, *A. salmonicida* and *Y. ruckeri* in Sella and Deva-Cares basins using eDNA. The occurrence is shown by colour gradation in number of positives by sample type (water, biofilm/sediment) and campaign (summer and spring 2020 and spring 2021).

<u>Alien species</u>

One of the main challenges regarding invasive alien species (IAS) is determining their distribution and developing early warning tools to identify their presence before their spread and population size can have negative effects on native populations. eDNA is a very promising technique to tackle these challenges (Jerde et al. 2011).

In the Sella and Deva-Cares watersheds, this technique was applied to determine the presence of several IAS, including the american mink (*N. vison*), the New Zealand snail (*P. antipodarum*), and the signal (*P. leniusculus*) and red crayfish (*P. clarkii*) (Figure 83). The results place these species outside the boundaries of the Park (Fig. 86). In the case of the american mink, its presence has been recorded at 3 points in the Deva basin. On the other hand, the presence of *P. antipodarum* has been recorded at 3 points in the upper Deva area and in the Güeña River, a tributary of the Sella. Finally, the two invasive crayfish species have only been located at this same point in the Güeña River.

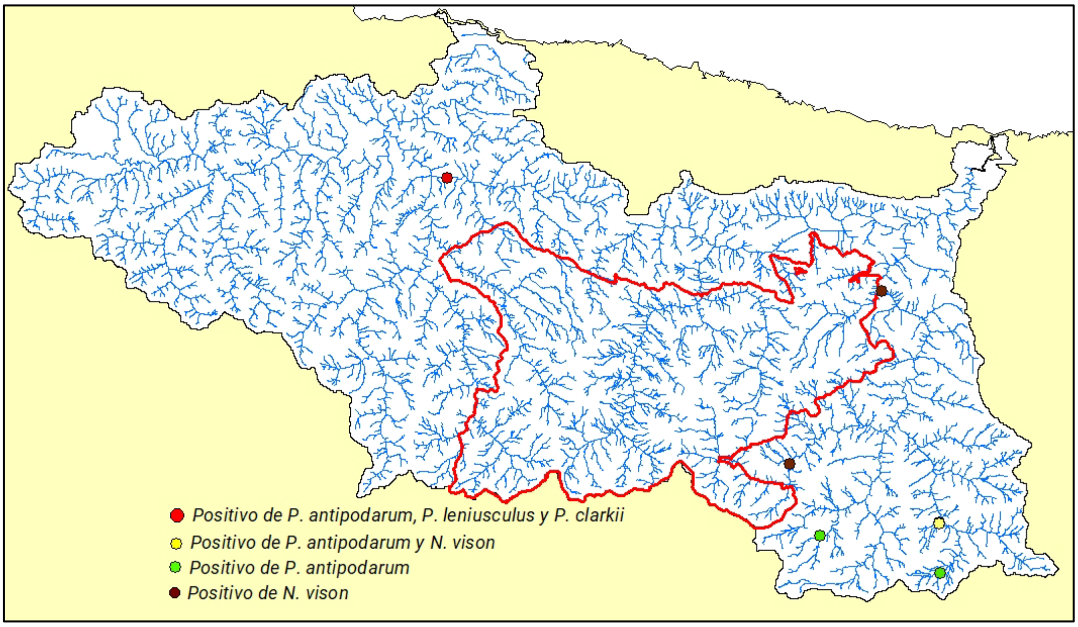


Figure 86. Distribution of *N. vison*, *P. antipodarum*, *P. leniusculus* and *P. clarkii* in the Sella and Deva-Cares basins using eDNA

12. Trophic resources

In the summer campaigns for characterizing the river environment, conducted annually at the aforementioned network of 13 sampling sites (see Fig. 64), different variables indicative, among other things, of the trophic resources available for the primary and secondary consumer communities in the rivers, were analysed. These variables are:

- **Chlorophyll *a***: To determine the concentration of chlorophyll *a* in the benthos, 3 stones from the riverbed in the fast-flowing areas and another 3 stones in pool areas were collected at each study site, aiming for stones approximately 10 cm x 10 cm in size. Stones must be completely submerged and preferably located in the middle of the channel, in areas with current flow and avoiding isolated arms or areas located beneath infrastructures (e.g., bridges). Once these 6 stones were selected, they were brushed separately onto a tray, using about 100 ml of water from the same river to wash off the biofilm scraped onto a plastic tray. From the volume deposited on each tray, both from pools and fast-flowing areas (run), 10 ml was extracted in each case. A sample is taken from this volume for benthic eDNA and to determine the structure and composition of the diatom community (see above). The remaining volume on each tray was measured with a plastic graduated cylinder and diluted with 500-1000 ml of water, noting the final added volume. Finally, a sample for chlorophyll *a* analysis was taken from a volume of 50 ml that is suctioned with a syringe. Once the sample is in the syringe, a GF/F glass fibre filter (0.7 μm), 25mm 100/PK, was placed on the filter holder. The syringe plunger was then pressed over the filter holder until the filter becomes clogged, noting the filtered volume. Each filter was stored wrapped in aluminium foil, previously sterilized in a muffle furnace, and appropriately labelled (Pool/Fast - Point Name - Date - Claw).

Once in the laboratory, the pigments were extracted by placing the filter inside a small glass tube with 5 ml of 90% acetone. Subsequently, another 5 ml was added, and the samples were kept at 4 ºC in the dark. Next, the tubes were centrifuged for 30 minutes at 1000 rpm. To measure in the spectrophotometer, 3 ml was transferred to a glass cuvette, and the optical density was measured at 750, 665, and 664 nm. Finally, 3 drops of hydrochloric acid are added, and after waiting 90 seconds, the optical density was measured again (750 nm, 665 nm, and 664 nm).

- **Epilithic Carbon**: The field protocol is the same as that previously described for benthic chlorophyll sampling; however, in this case, the final filter, wrapped in aluminium foil, was labelled as follows: Pool/Fast - Point Name - Brush. Once in the laboratory, all the Cepil filters to be analysed were placed on an aluminium tray. They were dried in an oven (70 ºC - 72 hours). They were then cooled in a desiccator for 30 minutes and weighed on a precision balance. After this, they were placed back on the aluminium tray and burned in a muffle furnace at 500 ºC for 2 hours. Once burned, the filters were removed, rehydrated with 2 or 3 drops of distilled water, and dried again at 70 ºC for a minimum of 72 hours until a constant weight is obtained. Finally, the filters were cooled in a desiccator for 30 minutes and weighed again, thus obtaining the ash-free dry weight.
- **Organic Matter**: To estimate the organic matter distributed in the riverbed at these 13 sites, organic remnants included in the invertebrate samples collected with a Surber net from pools and fast-flowing areas, as described above, were analysed. When extracting invertebrates from these samples in the laboratory, the organic remnants appearing in the sample itself were also separated and stored in independent vials with 70% alcohol, distinguishing 10 categories: 1) leaves, 2) wood and branches, 3) fruits and flowers, 4) moss, 5) algae, 6) fungi, 7) roots, 8) macrophytes, 9) particulate material (those >1 mm in size but cannot be classified in the previous categories due to their small size; APOM), and 10) fine organic particulate material (FPOM), which has a size between 0.12 and 1 mm. Subsequently, the classified organic matter in each of these categories was placed in a separate ceramic crucible and dried in an oven at 70 ºC for 72 hours. After this time, they were cooled in a desiccator and weighed, thus obtaining the dry weight (DW) of each one of these classes of organic matter. After weighing, they are burned in a muffle furnace at 500 ºC for 4 hours (Fig. 87), cooled in a desiccator, and weighed again, thus obtaining the weight of the ashes (AW). Finally, the ash-free dry weight (AFDW) of each of these classes is obtained by subtracting the AW from the DW.

Figure 87. Different stages to obtain the ash-free dry weight of the benthic organic matter.

These data allow us to determine, for example, whether the disturbances observed in the fluvial environment of the Picos de Europa NP influence the amount of available resources in the river, or to understand how consumers, such as invertebrates, are structured in relation to the available trophic resources. For this, the invertebrate community is reclassified, not by taxonomy, but grouped according to their biological traits into Functional Feeding Groups (FFG). The groups considered in this classification are: predators, gatherers, scrapers, filterers, shredders, and gatherer-scrapers.

As an example, the results up to 2016, presented at the XVIII Congress of the Iberian Limnology Association (AIL) held in Tortosa, showed, through a Friedman test (a non-parametric analysis for dependent or paired data), how the abundance of certain resources varied significantly between control and impact points. Chlorophyll a and epilithic carbon reached significantly higher concentrations at impact points compared to control points. On the other hand, these same results did not show significant differences for Total Benthic Organic Matter (BOM), Fine Particulate Organic Matter (FPOM), and Coarse Particulate Organic Matter (CPOM) between treatments (see Table 9).

	MOB g/m^2	*X^2; df=1*	MOPF g/m^2	*X^2; df=1*	MOPG g/m^2	*X^2; df=1*	CLO *a* mg/m^2	*X^2; df=1*	CEPIL g/m^2	*X^2; df=1*
Control	52,5 ±13,2	1,6	3,2 ±1,5	2,4	**47,9** ±13,1	2,4	26,3 ±2,0	*8,0***	6,2 ±0,4	*4,5**
Impacto	**22,8** ±7,6		**8,9** ±0,7		19,7 ±7,1		**44,4** ±5,0		**8,0** ±1,9	

Table 9. Results for Friedman test with blocking. Year was the repeated measure effect, while treatment (Control/Impact) was the blocking factor. The sites were considered as replicates within each block (*p<0.05 and **p<0.01). Total Benthic Organic Matter (MOB), Fine Particulate Organic Matter (MOPF) and Coarse Particulate Organic Matter (MOPG).

If we examine the relationship between trophic resources and the structure of the invertebrate communities based on their functional feeding groups (FFG), we can see that communities developing in impacted sites showed significant correlations in most cases, whereas no significant correlation was observed at control sites (Table 10). The same pattern is seen when correlating the taxonomic richness, density and Shannon diversity index of the invertebrate community. In control communities, only Shannon diversity was significantly correlated with Fine Particulate Organic Matter (FPOM; Table 10).

		CEPIL	Clo *a*	BOM	FPOM	CPOM
CONTROL	Predators	-0.09	-0.34	-0.03	0.02	-0.03
	Collectors	-0.31	-0.19	0.41	0.38	0.39
	Scrapers	0.15	0.27	-0.13	-0.06	-0.13
	Filters	-0.30	-0.16	-0.19	-0.30	-0.18
	Shredders	-0.01	0.26	0.28	0.01	0.28
	Gatherers	0.21	0.35	-0.19	-0.21	-0.19
	Richness inv.	0.10	0.33	0.10	-0.11	0.11
	Density inv.	-0.04	0.16	0.11	0.10	0.10
	Shannon diversity inv.	0.13	0.13	0.24	-0.51*	0.28
IMPACT	Predators	-0.16	0.74**	0.83**	0.95**	0.60*
	Collectors	-0.24	0.63**	0.72**	0.81**	0.52*
	Scrapers	0.43*	0.80**	0.54*	0.73**	0.29
	Filters	-0.13	0.59**	0.56*	0.77**	0.28
	Shredders	0.11	0.91**	0.86**	0.94**	0.65*
	Gatherers	-0.08	0.68**	0.57*	0.80**	0.29
	Richness inv.	0.03	0.56*	0.50*	0.44	0.47
	Density inv.	-0.16	0.70**	0.72**	0.85**	0.48
	Shannon diversity inv.	-0.03	-0.20	-0.16	-0.27	-0.04

Tabla 10. Coeficiente de correlación de Pearson (r) entre los recursos disponibles y los grupos funcionales de alimentación de la comunidad de invertebrados. También se incluyen otros descriptores de esta comunidad como la riqueza taxonómica, la densidad y la diversidad de Shannon (*<0,05; **<0,01).

13. Functioning

River metabolism is a key element to evaluate river ecosystem functioning. Understanding river metabolism helps us comprehend the biological processes of carbon fixation and mineralization in these ecosystems, revealing how the flows of matter and energy behave. This analysis has an integrative character, encompassing numerous processes, and its use is becoming increasingly common to assess the functioning and conservation status of these ecosystems (Battin et al., 2023).

River metabolism depends on:

1. **Gross Primary Production** (GPP), which represents the total fixation of inorganic carbon into organic carbon by the ecosystem's autotrophic organisms through photosynthesis.
2. **Ecosystem Respiration** (ER), which indicates the mineralization of organic carbon back into inorganic carbon by all organisms in the ecosystem, both autotrophic and heterotrophic.

The balance between these two processes (GPP and ER) is known as Net Ecosystem Production (NEP). In river ecosystems, ER is tied to the consumption of dissolved oxygen in the water, and this measure reflects the aerobic respiration of the ecosystem's biological communities. Thus, NEP is the sum of a positive GPP value and a negative ER value. Positive NEP values indicate that the ecosystem, such as a specific river segment, is fixing more carbon (C) than it is respiring. As a result, the river segment stores or exports some amount of carbon downstream as organic matter, meaning it would be considered autotrophic (Bernhardt et al., 2018). On the other hand, negative NEP values indicate that the segment is mineralizing more carbon than it is fixing, meaning it "breathes" (i.e., consumes) more carbon than it produces. In this case, the river segment would be classified as heterotrophic, relying on external sources of organic matter (e.g., leaf litter from riparian forests or organic matter from upstream). A ratio of GPP/ER >1 characterizes autotrophic ecosystems, whereas GPP/ER <1 indicates heterotrophic ecosystems.

In freshwater ecosystems, changes in GPP and ER, as well as their balance, are determined by different processes at the basin or segment scale. Changes in light incidence, water flow, temperature, nutrient concentration, and organic matter significantly influence metabolism (Roberts et al., 2007). In turn, the balance between GPP and ER strongly affects dissolved oxygen levels, organic matter availability for primary consumers, and other factors related to water quality. Because of the relationships between metabolism, water quality, and human-induced alterations to aquatic ecosystems (e.g., degradation of riparian forests) and adjacent terrestrial ecosystems (e.g., changes in land use), metabolism estimates are increasingly used as an indicator of ecosystem integrity. Furthermore, GPP and ER estimates enhance our understanding of matter and energy flows in food webs and the capacity of aquatic ecosystems to process and eliminate excess nutrients, thus preventing potential impacts like eutrophication.

There are several approaches (methods, instruments, etc.) to determining metabolism in aquatic environments, though all of them are based on measuring the day-night variation in dissolved oxygen concentration in the water over a sufficiently representative period (Fig. 88). Typically, the following variables need to be characterized: 1) dissolved oxygen, 2) solar time, 3) water temperature, 4) flow rate, 5) depth, 6) dissolved oxygen saturation, and 7) Photosynthetically Active Radiation (PAR). These variables should be measured in situ, although some, such as PAR or atmospheric pressure (used to determine dissolved oxygen saturation), can be derived from remote sensing databases.

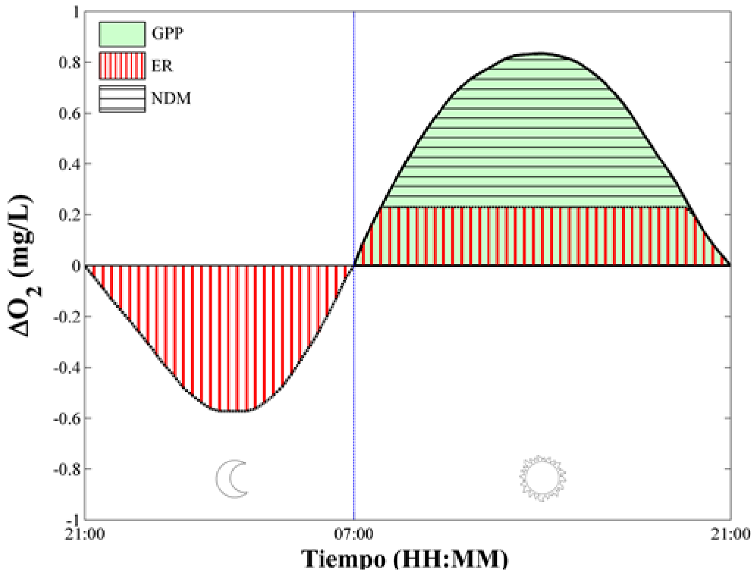


Figure 88. Scheme of the rate of change curve derived from changes in the concentration of dissolved oxygen during a day. GPP = Gross Primary Production, ER = Ecosystem Respiration and NDM = Net Daily Metabolism.

To characterize river metabolism in Picos de Europa NP, the single-station method has been employed. This method is applied to homogeneous river sections to ensure the collection of reliable data that corresponds to the mass balance approach. To do this, it's crucial to avoid river sections with excessive physical reaeration (e.g., due to the presence of waterfalls and bubbling) and ensure that no significant changes in water flow occur upstream of the characterized section, such as from water extractions or contributions from tributaries or springs. When applying this method, in each characterized river section (13 sections/study sites; see Figure 64), the following equipment must be installed:

- A dissolved oxygen and water temperature logger (e.g., MiniDOT–PME, HOBO–OnSet, etc.).
- A light sensor (e.g., HOBO Pendant Temperature/Light Data Loggers–UA-002-64).
- A barometric pressure sensor (e.g., HOBO–OnSet).

Additionally, it is recommended to use a PAR (Photosynthetically Active Radiation) sensor to verify the light data recording.

In applying the single-station method, it's essential to estimate the integrated upstream distance from which the oxygen logger will capture the signal. According to Grace & Imberger (2006), this distance can vary from 1 to 20 km, depending on water velocity and the reaeration coefficient, following this relationship. This ensures that the metabolism measurements accurately reflect the section of the river being studied.:

$$Distance = \frac{3\nu}{k_{O2}}$$

Therefore, using different combinations of aeration coefficients and velocities, the integrated distance can be estimated for different types of currents (Table 11), which must be applied for an adequate selection of the river sections to be characterized.

	K_{O2} (d^{-1})	velocity (m s^{-1})	velocity (km d^{-1})	DI (km)
Fast flow	80	0.8	69.1	2.6
Intermediate flow	20	0.3	25.9	3.9
Slow flow	5	0.1	8.6	5.2

Table 11. Estimation of Integrated Distances (ID) for different currents using average aeration coefficients (KO2) and the average water velocity in the reach.

Once the study section has been selected, the oxygen logger must be placed at a representative point within the section, following these conditions:

1. The oxygen logger must remain fully submerged throughout the entire recording period.
2. It should be located where the water flow is laminar or similar, meaning there should be no turbulence (Fig. 89).
3. The logger should be placed preferably in the middle of the section, avoiding spots near the riverbanks.
4. Stagnant water or deep pools should be avoided, and the logger should never be located downstream from rapids, waterfalls, or bubbling flows, as these areas promote physical reaeration of the water, leading to oxygen oversaturation (above 100%). This oversaturation would later prevent accurate metabolism calculations.
5. Ensure the logger's sensor is positioned facing the direction of the water flow, to avoid false readings caused by air bubbles and to minimize the likelihood of algae growing on the sensor.

Figure 89. Location of an oxygen probe/logger in the center of the channel in a river habitat with laminar flow and without bubbling and detail of the fixation used to secure the probe with steel cable.

To prevent vandalism or damage from flooding, the oxygen logger should be protected and securely attached to a stable surface, such as a tree root along the riverbank or a rock in the riverbed. Similarly, the light sensor should be installed and secured horizontally in a location outside the watercourse but with similar lighting conditions to the river section being studied. This ensures that the light readings are representative. The barometric pressure logger should be placed in a safe, less visible location to avoid potential theft or vandalism. The light sensor is typically placed on a branch of a riverside tree. In addition to these setups, to calculate the river's metabolism, it's also necessary to estimate the water depth and flow rate of the section. This can be done by taking measurements at various transects perpendicular to the river's flow. At each transect, periodic measurements of flow velocity and depth should be taken, for example, every 50-100 cm, depending on the width of the section and the bed's topography. Another useful variable to improve and adjust light data recorded by the sensor is the shade coverage from the canopy and valley walls over the river, which is crucial for estimating gross primary production (GPP). To measure this shade, a digital camera with a wide-angle "fish-eye" lens can be mounted on a tripod in the river's center, ensuring it is completely level and pointing towards the sky. The top of the image must always be oriented to the north. Once the images are captured (Fig. 90), they can be processed using software like GAP Analyzer to calculate the percentage of the river section that is in sunlight or shade.

Figure 90. Images taken with a wide-angle "fisheye" lens show a canopy closed by the abundant riparian vegetation and a more open canopy, mainly limited by the valley walls. Photos taken in the Seco River (left) and at the head of the Duje River (right).

Once all the field information was generated, the estimation of the GPP and ER can be carried out using different approaches, by using different analytical and statistical techniques to solve the fundamental equation developed by Odum (1956; or one of its variations) that relates dissolved oxygen to GPP and ER. One of the most used equations is:

$$\frac{dO_{i,d}}{dt} = \left(\frac{GPP_d}{\bar{z}_{i,d}} \; x \; \frac{PPFD_{i,d}}{\overline{PPFD}_d}\right) + \left(\frac{ER_d}{\bar{z}_{i,d}}\right) + f_{i,d}(K600_d)\left(Osat_{i,d} - O_{i,d}\right)$$

Where oxygen concentration measured in a given moment (mg l^{-1}) depends on diary GPP (g O_2 m^{-2} d^{-1}), wáter Depth in that momento (m), density of the active photosintethyc radiation (PPFD, µmol m^{-2} s^{-1}), the ER and the rate gas exchange between water and atmosphere (K600, d^{-1}), which is the physical function depending on flow (m^3 s^{-1}) and the difference among the measured oxygen and oxygen in the saturation point.

There are two main types of analytical methods to solve this equation:

1. Direct methods: require empirical data for all the necessary parameters to estimate gross primary production (GPP) and ecosystem respiration (ER). Their main disadvantage is the complexity involved in empirically estimating the gas exchange rate (K600), which requires adding an inert gas to the water and measuring its disappearance rate.
2. Indirect methods, based on inverse modelling techniques developed and improved in recent years, do not require prior measurement of the K600 rate. The functionality of inverse models is based on iteratively estimating unknown parameters until finding the best fit for the observed oxygen data. Although these models demand significant computational power, various studies have demonstrated that they are more accurate and flexible than direct estimation methods, leading to their widespread use in recent years. One of the recommended indirect methods, implemented in the R package *streamMetabolizer* (Appling et al., 2018), uses a Bayesian inverse model with Markov Chain Monte Carlo (MCMC) to simultaneously estimate the parameters of GPP, ER, and K600 and solve the equation mentioned earlier.

14. Ecosystem services

According to the Millennium Ecosystem Assessment, Europe is one of the most fragmented and altered territories in the world. As mentioned earlier, the history of urbanization, agricultural exploitation, river alterations and infrastructure development has led to significant changes in biodiversity patterns, as well as the structure and functioning of natural ecosystems. Maintaining the integrity of these ecosystems is crucial for the development of human societies, as they provide valuable benefits, known as ecosystem services (ES). These services can be classified as follows:

- **Regulation services:** These arise from key ecosystem functions that help reduce several environmental risks, such as climate regulation, water cycle management, soil erosion control, and pollination.
- **Provisioning services:** These refer to the goods or raw materials provided by ecosystems for human use, such as wood, water, or food.
- **Cultural services:** These are related to leisure, recreation, and broader cultural or intangible values associated with ecosystems and their components.

In this regard, mountain regions are particularly important, as they are source of different physical and biological processes that impact downstream areas in the river basin

(Ferrier & Jenkins, 2021). These regions provide vital ES to the lowlands by storing water, regulating the climate, the hydrological cycle, and mass flows (Löffler et al., 2011). However, global change affects precipitation patterns (both rain and snow) and the resulting infiltration, runoff, and pollutant dissolution patterns, thereby altering the stability of slopes and natural processes controlled by hydrometeorological factors, such as erosion, floods, and droughts (Beniston, 2006). In this regard, the abandonment of traditional agricultural practices in many mountain areas might reduce vulnerability to these growing risks. The increased ecosystem maturity associated with land abandonment dynamics, as habitats succeed to more climactic stages (e.g., from grasslands to forests), typically leads to a greater capacity to provide regulation services (Odum, 1969). However, it could also jeopardize other provisioning and cultural services tied to the traditional management of these areas, such as grazing, aesthetic value, or cultural identity (Körner et al., 2005). In this context, an analysis was conducted to assess how land-use changes and environmental conditions impact the provision of ES in the Sella and Deva-Cares river basins, with the aim of guiding the management and conservation of their terrestrial and aquatic ecosystems. This ES analysis is presented in two parts: 1) the definition and conceptualization of the general framework, and 2) the specific development of mapping based on the structural landscape patterns and connectivity of aquatic ecosystems.

14.1. General framework for ecosystem services characterization

Currently, the framework described is used by a wide range of stakeholders, including scientists, economists, policymakers and land managers. However, this broad use often leads to ambiguities in the use of certain terms and concepts, making it difficult to compare different projects or temporal and spatial contexts, while also reducing its practical application potential (Fisher et al., 2009). Therefore, to ensure proper management of the resources in the described area, it is essential to develop a clear conceptualization of the framework used for modelling. Answering key questions such as what ES are, where and how they are provided, who uses them, and how they can be enhanced is necessary for creating effective mapping in the territory. This requires a clear and precise understanding of the fundamental principles that support the ES framework. The first step is defining the classification system used to name, define, and group the various considered ES. Next, it is important to address the spatial and temporal dynamics needed to understand how these services are provided in the landscape and how they relate to one another (Fisher et al., 2009).

14.1.1. Cascade model of ecosystem services

The idea of "*cascade of services*" can be used to summarize much of the logic underlying the contemporary paradigm of ES and the key elements of its conceptual foundation (Fig. 91; Potschin & Haines-Young, 2011). This model illustrates how biophysical structures and ecological processes support the functions of ecosystems, whose outcomes are transferred as socioeconomically valued services.

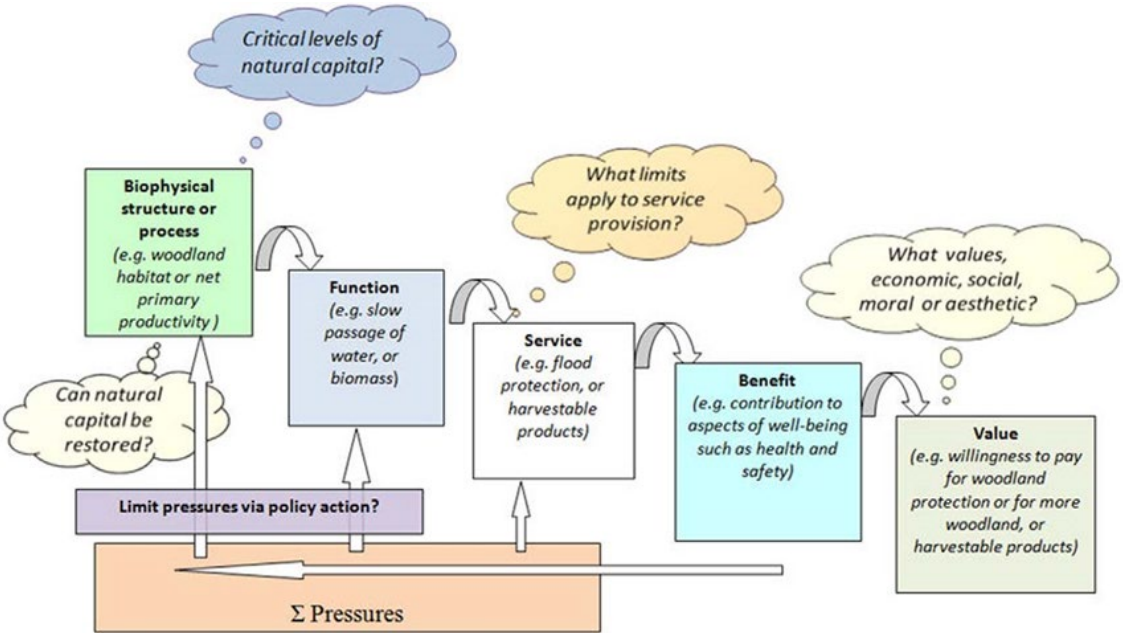


Figure 91. Conceptual graph of Potschin & Haines-Young's (2011) SE waterfall model.

Biological components are involved in a large number of physicochemical cycles and interactions that occur within and across the boundaries of the ecosystem, providing various ecological processes and functions, such as productivity and nutrient recycling. More specifically, each ecological function originates from the biophysical interaction between the biological component and the physical processes that control abiotic flows (e.g., flows of water, energy, or matter; Kremen & Ostfeld, 2005). At the landscape level, humans benefit from ecosystem functions in the form of a wide range of ES when the biophysical interaction occurs at the spatial scale required by the specific process. For example, vegetation provides regulatory ES, such as flood mitigation or erosion protection, by retaining some of the water and sediment flows in the areas draining the watershed into the river network. Thus, the cascade model of ES reflects a "production chain" logic that links bioecological structures and processes on one hand, and human well-being on the other. This framework should also help frame a series of questions regarding the relationships between human societies and nature, including: 1) whether there are critical levels or reserves of natural capital necessary to maintain the flow of ES, 2) whether that capital can be restored once it is damaged, 3) what the limits of ES supply are in different situations, and 4) how we value the contributions that ES make to human well-being (Potschin & Haines-Young, 2011).

14.1.2. Spatial and temporal dynamics of ecosystem services

ES are typically delivered within process-related landscape units such as watersheds, specific habitats or natural/functional units. A notable advantage of the ES paradigm is that it shows the conditions under which nature generates benefits. However, the areas that provide ES may differ from those in which society benefits from such services, and three types of areas in the landscape can be differentiated in relation to these ES flows (Fig. 92; Syrbe & Walz, 2012):

- **Service Providing Areas (SPA):** Spatial units that are sources of ecosystem services (ES) in a given landscape. These are the areas where the biophysical interaction led by ecosystems occurs to generate ES.
- **Service Connector areas (SCA).** Spatial units that connect the service providing areas with the beneficiary areas in a specific landscape.
- **Areas Benefiting from services (SBA).** Spatial units where the benefits of ES are required/consumed in a given landscape.

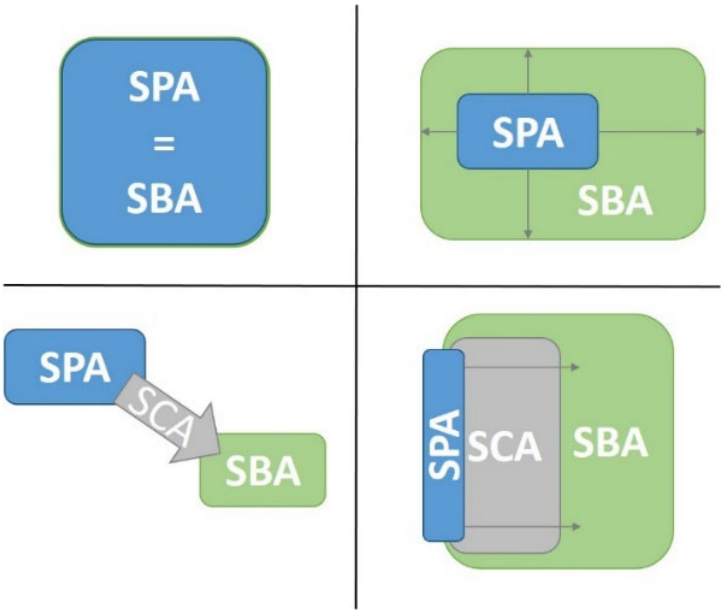


Figure 92. Spatial Relationships between Service Providing Areas (SPA) and Service Beneficiary Areas (SBA). Upper Left: In situ situation, where SPA and SBA are identical; the service and benefits occur in the same area. Upper Right: "Omnidirectional" situation, where SBA expands SPA without directional bias. Lower Left: "Directional" situation dependent on slope, where SBA is located downstream from SPA, with service provided by gravity. Lower Right: "Directional" situation without slope dependency; SBA is located "behind" SPA concerning the effects of greater directional range.). Figure from Syrbe & Walz (2012).

On the other hand, the ES provided by a given biological component can also fluctuate over time. Sometimes this variation is due to changes in the abiotic and biotic flows within the functional unit, affecting the interaction that generates the ES (e.g., reduction in grass production in winter due to lower solar radiation). In other instances, the change in ES provision is driven by changes in demand, and there may also be a temporal lag between the generation of the ES and its final delivery.

To work on the valuation of ecosystem services in the Sella and Deva-Cares basins, within the framework of the LIFE DIVAQUA project, the Common International Classification of Ecosystem Services (CICES; Haines-Young and Potschin, 2018) was used. This classification incorporates a nested hierarchical structure (i.e., hierarchy: Section > Division > Group > Class) and distinguishes between provisioning, regulating, and cultural ecosystem services. For the development shown below, the analyses were primarily based on the "Class" level. However, some tasks required a more specific framework, so a higher level of detail was used in those cases.

14.2. Methodological framework for ecosystem services modelling

The modelling of the potential provision of ES requires a geospatial framework able of simultaneously considering the interactions between different ecosystems and the abiotic flows through the watershed, which ultimately flow into the river network. In this regard, the framework developed by Pérez-Silos (2021) has proven capable of accounting for these functional connections (i.e., abiotic flows) between terrestrial and riverine ecosystems. Likewise, different previous experiences position it as a more effective approach than others based solely on the use of predefined models, especially when higher levels of precision in the analysis are required. This approach (Pérez-Silos, 2021) considers the spatial interaction between abiotic flows and the ecosystems of a watershed, as well as how this interaction produces ES in areas where they are in demand, i.e., in SBA (Socio-Beneficiary Areas). Specifically, the theory of meta-ecosystems allows for conceptualizing how flows of energy, matter, water, and/or temperature are driven by physical forces that link the different patches of ecosystems within the watershed, from the upper slopes to the lower areas of the river network. These abiotic flows are modified by biological communities as they traverse different patches of the landscape matrix through biophysical interactions, changing the input/output balance of resources and energy depending on the type of biological community and its location within the watershed and river network. In this sense, every biological function is produced by the biophysical interaction between the physical process driving the abiotic flow and the habitats present within a functional unit (Křováková et al., 2015).

Functional units account for the spatial scale required for each biophysical interaction to potentially generate an ES. The regulation of these flows and the provision of derived resources produced in SPA (Service Production Areas) could potentially generate societal benefits, provided they are properly connected along the river network to the SBA. According to Petersen (1999), four types of functional units are essential for determining the abiotic flows produced in a watershed, with specific hydrological functions and ecological potential within the basin (Fig. 93). These are: drainage wings, riparian zones, floodplains, and the river reaches themselves. All of these are landforms capable of incorporating biotic-abiotic interactions at the required functional scale, as well as ensuring the necessary connectivity between the different ecosystems. From a conceptual perspective, this spatial discretization allows for tracing the potential flow of ES between the biological function generated in the terrestrial or riverine environment (SPA) and the final ES delivered in the river network (SBA).

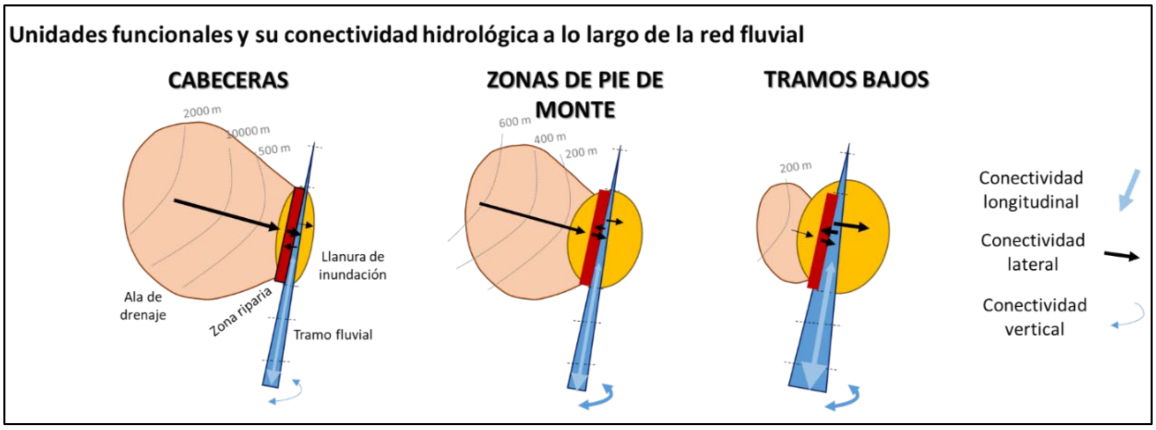


Figure 93. Plan view of the functional units and their relative longitudinal, lateral and vertical hydrological connectivity in an idealized river network (headwaters, foothills and lowland reaches).

From a modeling perspective, functional units also allow to simulate the existing connections between the different biological and physical components of the landscape, and to aggregate the modelled processes at the pixel level into a spatial unit that has full functional significance within the landscape (Fig. 94). Consequently, within this digital framework, each river segment is gravitationally connected to the terrestrial environment through these functional units, as well as to the rest of the river network. Thus, while drainage wings unidirectionally connect the slopes with riparian zones and floodplains, riparian zones and floodplains serve as the ecotone between terrestrial and aquatic domains. Lastly, the river segments are also bidirectionally connected to one another. Although most of the sediments, water, and materials flow downstream, it is also important to account for the reverse movement carried out by different organisms, such as fish, which can shift the predominant flow of matter and energy downstream (Tonkin et al., 2018).

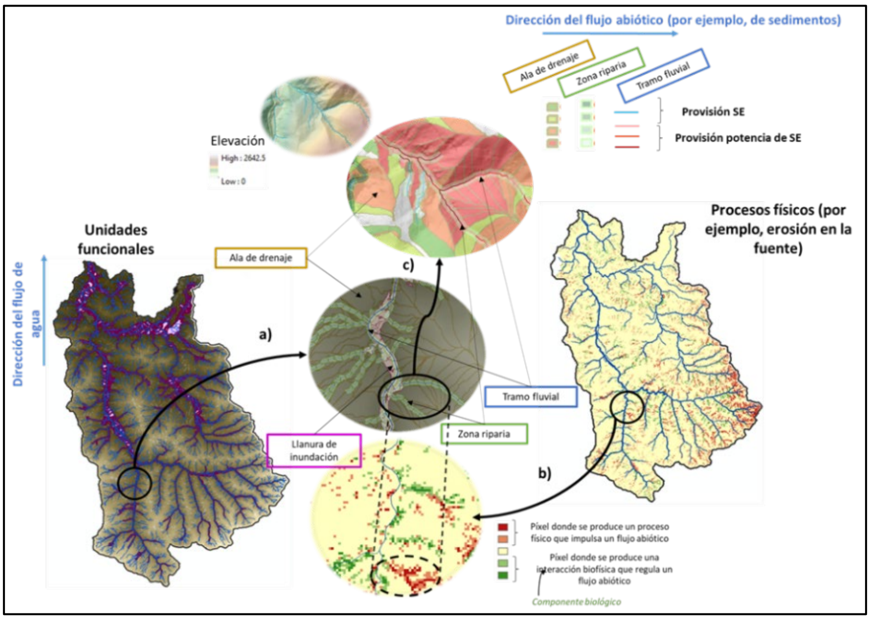


Figure 94. Digital framework to model and conceptualize ES provision in basin systems, applied to the Pas basin (Cantabria, northern Spain).

According to this approach, the modelling of ES is based on identifying priority areas where both, the provision and potential demand for these services, occur. ES are determined by the interaction of terrestrial vegetation with topographic, geomorphological and climatic factors. Therefore, the generation of ES within the landscape matrix is based on the effect that the presence of ecosystems has on ES generation, compared to the abiotic potential that would determine its intensity in the absence of the ecosystem. In this way, for the entire DIVAQUA area, zones within the Sella and Deva-Cares watersheds were identified where the provision of a specific ES is deficient due to the degraded state of the ecosystem (potential ES provision areas). Additionally, areas where the presence of the ecosystem in a healthy state ensures the correct provision of ES were also identified (current ES provision areas; Fig. 94). By aggregating the modelled information into these functionally homogeneous areas, it is possible to establish complex relationships to simulate the flows of matter, energy, and organisms within the watershed. This approach allows for correlating the interactions that occur in areas of the watershed that generate an ES (i.e., the Service Production Area, SPA) with the functional units that benefit from these services (i.e., the Socio-Beneficiary Area, SBA). This process maintains the original information (pixels) which spatially and explicitly identifies, with greater resolution, the areas where the interactions providing the ES occur.

14.3. ES Modelling and mapping in Sella and Deva-Cares basinss

In DIVAQUA (Sella and Deva-Cares basins) models related to the following ES have been developed following a process of initial theoretical conceptualization and subsequent methodological development, generating spatially explicit outputs for each case.

14.3.1. Hydrologic services

The hydrological response of the watershed is closely related to two key ES: flood risk mitigation (through hydrological response regulation via hillside runoff and floodplain water storage) and freshwater provision. The hydrological response refers to the movement of water within the watershed, migrating from areas with higher hydraulic potential to lower ones after a precipitation event and/or snowmelt. The water's pathways through the different biophysical components of the watershed determine the spatial and temporal patterns of flood and drought events within the river network. Watershed and river network drying limit the amount of water available for human consumption, directly linking this process to the quantitative aspect of the freshwater supply ES.

The precipitation and temperature regime is the primary factor controlling water inputs into the watershed. However, the hydrological response of the watershed also depends on river connectivity, which is intrinsically controlled by topo-geological, edaphic (soil-related), and biological factors (Graf & Lecce, 1988; Tooth & Nanson, 2011). These factors determine the pathways and proportions in which water entering the watershed is transferred to the river network, influencing the likelihood of flooding during intense or sustained precipitation, or the resilience to drought periods in response to scarce or absent rainfall. In this context, flood generation is linked to runoff mechanisms, while drought prevention is related to the proportion of precipitation that can be stored in a watershed and subsequently contribute to water flow over time (Haines-Young & Potschin, 2012). In watersheds with low regulation capacity, rainfall water quickly drains from the drainage wings to water bodies via surface runoff, increasing short-term water flow in the river segments and, in turn, the risk of flooding during intense precipitation, while also reducing water supply during dry seasons (Martínez-Retureta et al., 2020). On the other hand, storage mechanisms in a watershed, such as aquifers, wetlands, snowpacks, or mature forests, generate water flow during periods of reduced precipitation (Mcamara et al., 2011). Therefore, the ES of hydrological response regulation and water provision depend on storage mechanisms, which are also linked to key hydrological factors such as soil characteristics, topographic relief, and land cover (Bracken, 2013).

The vegetation component of ecosystems largely controls the availability of water resources and their spatial-temporal distribution. Vegetation influences water flow through processes such as interception, evapotranspiration, surface runoff, and subsurface flows. Empirical evidence shows that forests improve the infiltration capacity of surface soils and water retention (Ilstedt et al., 2007), reducing runoff and slowing the watershed's hydrological response, suggesting that mature forests could be the main provider of ES related to hydrology. Another highly relevant component involved in regulating hydrological flows is floodplains. The lateral connection between the river and its floodplain naturally reduces flooding, moderating peak flows by allowing overflow and smoothing the flood wave, thereby mitigating downstream flood risk (Jacobson et al., 2015). Vegetation, especially riparian forests, increases the roughness of the floodplain, enhancing water storage during floods by increasing water residence time in the floodplain. Well-functioning floodplains also support alluvial recharge during post-flood events, delaying the drying phase of the river network during droughts. By reducing flow velocity in the flooded area, water residence time in the floodplain increases, promoting infiltration rates and aquifer recharge (Opperman et al., 2010).

Runoff regulation service

Following this conceptual framework, the potential of a hillside to generate runoff has been considered based on slope and soil permeability. Additionally, according to Maetens et al. (2012), areas with high annual precipitation tend to have a more uniform distribution throughout the year, leading to seasonal soil saturation and a greater likelihood of producing runoff. An index has been constructed with these components, considering each cell's contribution to the frequency and intensity of peak flows, as well as its potential mitigation through vegetation.

On one hand, susceptibility to runoff generation was quantified by considering the process of soil saturation excess, as Hewlettian runoff is expected to predominate due to the humid climatic characteristics of both study watersheds (Hewlett & Hibbert, 1967). Three factors are considered:

- Slope and Soil Saturation: The slope is multiplied by the inverse of the saturated water content of the soil's top layer, normalized by the formula ([valor-min]/[max-min]; factor 1).
- Lateral Flow Travel Time: As the second factor, the SPHY model formulation for lateral flow travel time is incorporated (also normalized from 0 to 1). SPHY assumes that the travel time of lateral flow depends on field capacity, saturated water content, and saturated conductivity (see SPHY in section 8 of this document). Since a longer travel time for lateral flow results in a smoother streamflow hydrograph, its inverse value is included in the index..
- Precipitation Distribution: The spatial distribution of precipitation is also considered, using a normalized grid of average annual precipitation for both current and future simulation scenarios. This grid was obtained from a climate regionalization conducted for the study area (Predictia SL, unpublished data obtained from the following climate grid: AEMET Climate Repository). These climate data have a spatial resolution of 1 km and a daily temporal resolution.

These three factors are combined with equal weight to generate an index of the watershed's potential hydrological response. Finally, the ability of native forests to regulate water flows (i.e., the "sponge effect"; Belmar et al., 2018) is incorporated. For each simulation scenario, natural forest coverage pixels were extracted from vegetation maps and reclassified based on the potential hydrological response using the following rule: if a pixel is covered by natural forest, the value of the index is fixed as positive; otherwise, the value is multiplied by -1. This creates a continuous hydrological response index for each 50-meter pixel ranging from -1 to 1, where negative values indicate a contribution to runoff and an increase in peak flows and flood risk, while positive values indicate the regulation of peak flows due to the presence of forest. The closer the index is to zero, the lower the pixel's contribution to peak flows and the less significant the forest's role in flow regulation. Finally, the results were then aggregated to the functional unit scale, specifically at the level of drainage wings.

The analysis revealed that the mountainous areas of the Sella and Deva-Cares basins are concentrated in regions with the steepest slopes and the greatest flood risk, meaning that these areas generate most of the watershed’s runoff (Fig. 79). However, the high permeability of the karstic massif of Picos de Europa results in low runoff generation in the highest areas. In this regard, the headwaters of the Cares and Sella watersheds, along with some mid-valleys and lower-altitude mountain ranges with high precipitation, stand out as areas with the highest potential for providing this ES. In these regions, mature deciduous forests that cover the slopes are able to regulate runoff over much of the territory (Fig. 79), although about 30% of the area is still susceptible to generating high levels of surface runoff. In future scenarios, these values are projected to decrease by at least 10%, primarily due to significant forest expansion expected in the study area (Fig. 95).

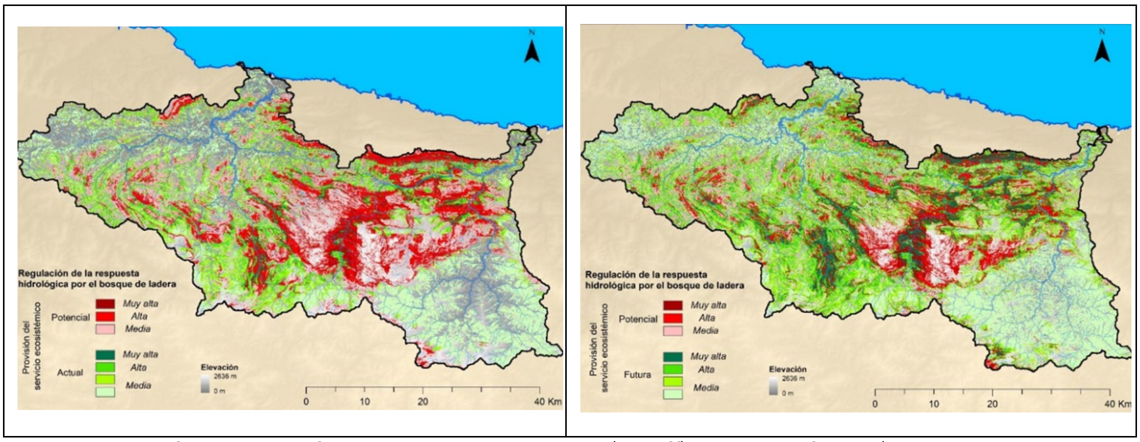


Figure 95. SE of regulation of the hydrological response (runoff) by hillside forest (hydrological regulation index) in the current (left) and future (right) scenario.

Floodplains water storage service

The regulation of hydrological response by floodplains was quantified based on their water storage capacity, that is, the volume of water they can potentially hold temporarily if in floods. Both, the delimitation of the floodplain and the potential water storage volume during a flood event, were estimated using the "Valley Floor" and "Flood Storage" tools in the NetMap software (Benda et al., 2007). Floodplains were delineated following a geomorphological criterion based on the valley surface that lies at a height of "n" times the total depth of the river channel (Fernández et al., 2012). In this case, a multiplier of 3 was used, as it is associated with a flood with an approximately 100-year return period (Ilhardt et al., 2000). River channel depth was estimated for each segment of the river network through a regional regression based on drainage area, mean annual precipitation, and field measurements of channel depth (for more detailed information, see Benda et al., 2011).

Stored water volume is defined from a reference plane below which the water is temporarily stored. The reference plane was considered the average height the water would reach when the entire floodplain polygon is inundated. In this case, water level is defined as the mean elevation above the channel of all the pixels contained within the floodplain polygon, corresponding to three times the channel depth. Thus, for each functional floodplain unit, Flood Storage tool calculates the volume stored between the reference plane's height plus its average elevation over the channel segment and the mean altitude of the channel segment. After calculating the volume for each floodplain polygon, they were grouped into larger, hydrologically independent units following the criteria described in Benda et al. (2011).

Using this methodology, it was estimated that the floodplains in the Sella and Deva-Cares watersheds have a total current water storage capacity of 121.26 hm^3, with an average capacity of 0.152 hm^3. The floodplains with the highest storage capacity are located in the lower areas of the watershed (Fig. 80), while those in the higher areas have lower capacity, mainly due to the orography of the watersheds themselves.

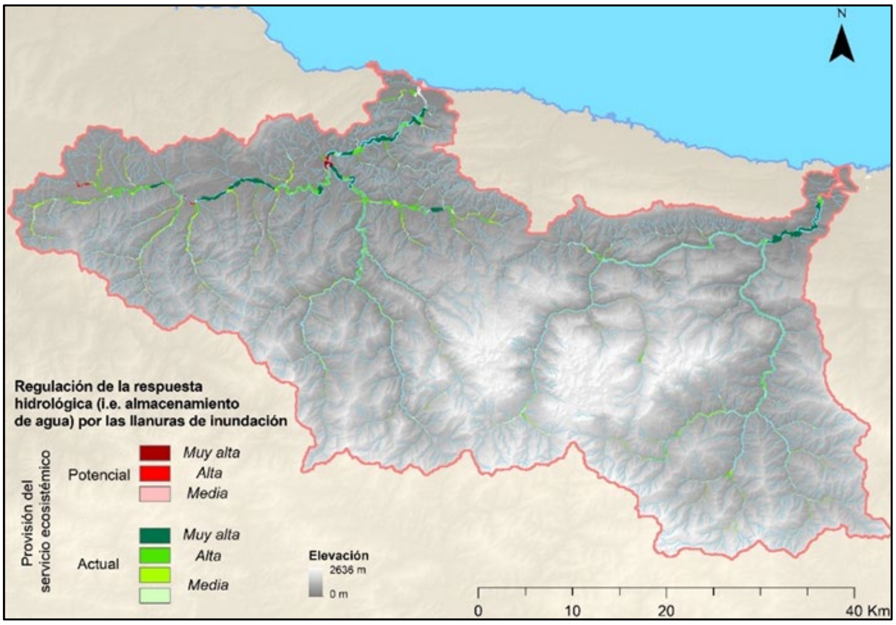


Figure 96. Map of the Sella and Deva-Cares basins for the SE regulation of the hydrological response volume of water storage in the floodplains.

Most of the river channels in both watersheds do not exhibit significant hydromorphological alterations. However, there are five river sections where channelization or bank stabilization operations have been carried out, reducing their capacity to store water and, consequently, limiting the provision of this ecosystem service. In these cases, the water storage potential of the floodplains ranges between 0.21 hm^3 and almost 1.9 hm^3. This means that if the original river channel were restored in these sections, their regulation capacity would be significantly increased during flood events.

Freshwater provision sevice

To calculate freshwater provision, the SPHY hydrological model (Terink et al., 2015; see model description in section 8 of this document) was applied. Of all the results provided by this model, surface flow was used to quantify freshwater provision. Freshwater provision service was evaluated in two spatial situations: 1) in river channels and 2) in spring captures or small streams (watering holes). Models for both scenarios were conducted at a spatial resolution of 100 meters and a daily time step. Model inputs included a Digital Elevation Model (DEM), a map of land uses and land cover, and soil characteristic maps. The main dynamic inputs consisted of spatially explicit precipitation and temperature data. In this regard, the available water in surface flow was quantified for each pixel as the sum of surface runoff, lateral flow, and base flow (from superficial, subsuperficial, and groundwater deposits, respectively). Finally, the average value for the summer months (July, August, and September) of this daily available flow result was obtained independently for both current and future scenarios.

The results indicate a significant decrease in the ecosystem service of freshwater provision in the future scenario (Fig. 97), which becomes more pronounced as one descends to lower basin areas due to the cumulative effect of the flow. Headwater streams and watering holes would experience an even more marked reduction, thus increasing their intermittency (i.e., the number of days without flow, meaning dry periods).

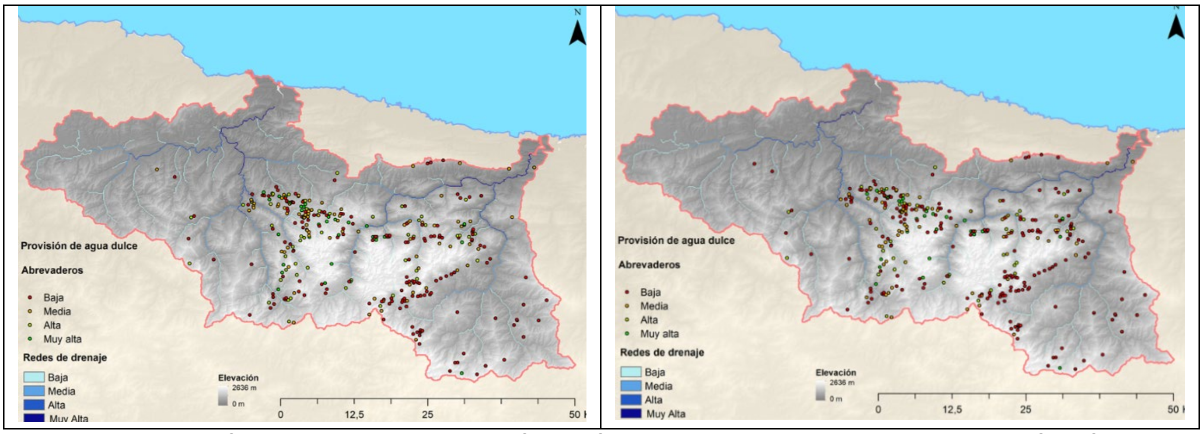


Figure 97. Map of the Deva and Sella basins for the freshwater provision SE, measured as surface flow in drainage networks and troughs, in the current (left) and future (right) scenario.

14.3.2. Erosion regulation, transport and delivery of sediments to aquatic systems service

Soil erosion consists of three main phases: 1) the initial detachment of particles, 2) their subsequent transport by agents such as wind, water, or gravity, and 3) the final deposition as sediment in terrain depressions or water bodies. The ES of erosion regulation encompasses the mitigation of these 3 phases through two interrelated processes:

1. The regulation of erosion at the source, which prevents soil loss and avoids the generation of sediments that could be transported.
2. The filtering of erosion, which retains some particles once the transport flow has begun, thereby decreasing the amount of sediment that ultimately reaches the river network during the transport process or at final deposition.

In this context, peaks in sediment concentration in river networks significantly affect the functioning of these systems. At base flow, suspended solids make water bodies murkier and hinder light penetration in the water column, which can also affect various aquatic biological communities and the functioning of the biofilm (Wood & Armitage, 1997). Pesticides, some metals, and other pollutants and nutrients can adhere to the suspended sediments in the water, thus increasing the concentration of contaminants and toxins. Lentic systems are particularly sensitive to these processes of organic enrichment, which can lead to eutrophication of the system. On the other hand, high sediment concentrations combined with high flow velocities during flood periods promote erosion and hinder the development of benthic communities (Ryan, 1991).

The detachment of sediments is not solely the result of the impact of raindrops, but also originates from the flow of water as an erosive agent (Römkens et al., 2002). Both factors generate shear stress on the soil surface, and if this exceeds the cohesive resistance of the soil, sediment detachment occurs, which can take different forms such as surface or deep landslides, gullies, surface erosion and erosion on the slopes and channel in the case of the river's own erosive action (Merritt et al., 2003). In humid environments, such as the Sella and Deva-Cares basins, sediment detachment is typically driven by slope and convergence of the hillsides (Dietrich & Dunne, 1978). The sediment flow in the drainage areas is highly correlated with runoff routes and is primarily directed by the slope. If not previously retained by any biophysical structure, such as depressions or wetlands, the sediment flow ultimately connects to the river network through the riparian zone.

Over short time scales (100 years), vegetation is an important agent for stabilizing sloped areas covered by erodible material, due to the support provided by its root system and, to a lesser extent, its action through transpiration, canopy interception and the redistribution of rainwater that allows the development of diffuse flow pathways through living and dead root systems (Gonzalez-Ollauri & Mickovski, 2017). Consequently, vegetation acts as a provider of this ES by preventing soil erosion in drainage areas, mitigating the impact resulting from the combination of the erosive power of precipitation and the biophysical conditions of a given area. Thus, from an ecological perspective, a more complex and mature vegetation cover contributes to greater soil protection. In fact, woody cover stabilizes the soil surface and prevents its movement, resulting in lower soil loss compared to other types of vegetation cover (Marden, 2012). Furthermore, vegetation also plays a crucial role in promoting sedimentation and, therefore, filtering sediment flows from upland areas. In a watershed context, this sediment filtering ecosystem service becomes even more important in riparian zones where sediments are transferred from drainage areas to the river network. The riparian area typically presents a break in slope, being a less steep area that facilitates sedimentation. Thus, riparian vegetation cover further increases the sediment retention rate before reaching the river network, potentially being 75% more effective in retaining sediments in agricultural areas (Lind et al., 2019) and between 50% and 95% in foothill areas (White et al., 2007) than in its absence.

To develop the corresponding models for this ES, the erosive potential in the basin and the probability that generated sediments will reach the river network or other water bodies was estimated using a dimensionless topographic index on a DTM with a resolution of 5 m. This index employs slope and topographic convergence based on the relationship taken from Miller & Burnett (2007), where higher values indicate greater erosive potential. This index is:

$$GEP = (AI * S) / b,$$

- *GEP* = Generic Erosion Potential (0 to 1.

- *b* is a measure of local topographic convergence that defines the length of an elevation contour traversed by the flow of a pixel, where lower values imply a convergent slope.
- *Al* is a measure of the local contributing area, within a pixel length.
- *S* = slope.

In humid environments, the highest density of landslides and surface erosion caused by precipitation events occurs on slopes ≥35º. Therefore, this slope was related to the GEP value to determine the threshold for high probability of erosion in the study basins (around a GEP value of 0.5).

To calculate the potential input of sediments into river channels and wetlands, the flow path was traced from each pixel to the first channel or water body using the Delivery tool from the NetMap software. The calculated probability that sediment transfers to each downstream cell decreases monotonically based on the gradient and topographic convergence. In this sense, the probability that sediment reaches its respective functional unit of the riparian zone was assigned to the source cell as a multiplicative factor of the GEP of the pixel, estimating not only the erosive potential of the drainage area but also the likelihood that this sediment will be potentially transferred to a channel.

The effects of the forest as a provider of the ecosystem service of erosion regulation were considered in two ways

1. Regulation of erosion at source potentially transported to lentic and lotic systems (reduction in the amount of sediment generated).
2. Sediment filtration (reducing the amount of sediment delivered to a body of water).

Firstly, when a pixel is covered by forest class, it is considered that erosion is much lower than in pixels without this type of vegetation. In this case, the probability of erosion (i.e., GEP) that is prevented at the source by the presence of tree cover in the pixel was quantified. Additionally, these pixels do not accumulate towards the channel. Secondly, those river stretches where the amount of sediment arriving from the drainage areas is predominantly filtered by the riparian forest were identified. For this, two buffer widths were considered based on the average slope of the adjacent hillsides: 15 m for slopes of 0-20% and 30 m for slopes greater than 20%. If the riparian forest covers more than 70% of the buffer, it would be filtering most of the sediment that would reach the river stretch or lentic water body.

After applying the criteria outlined, the results obtained for the current moment show significant erosion in the upper areas of the central massif and in the western and southern areas with steeper slopes (Fig. 98). The hillside forest that counteracts this effect is widely distributed in the southern area and appears to a lesser extent in the west, being scarcely represented in the high mountain zone. The model developed for the future scenario indicates that the pattern of forest expansion follows the aforementioned, so in this scenario, the main erosive problems are only observed in the central massif area, as new forest masses are expected to evolve in the western and southern slopes of the area (Fig. 98).

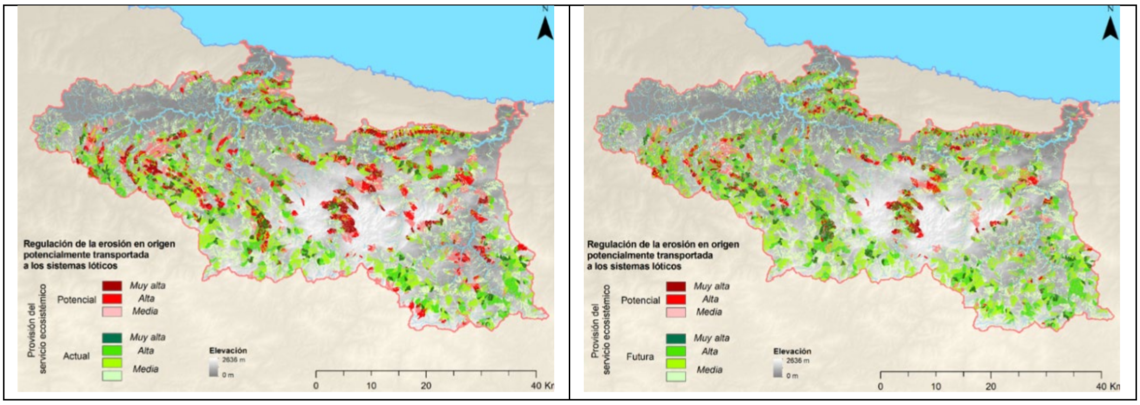


Figure 98. Result of the erosion regulation SE at source potentially transported to lotic systems carried out by the hillside forest in the current (left) and future (right) scenario. “Very high” and “high” categories = 10th and 25th percentiles of potential provision (the ES could be provided, but the associated ecosystem is not present) and current (the ES is being provided when the associated ecosystem is found). Middle category = 50th percentile for potential SE provision and 50th and 75th percentiles for current provision.

Regarding lentic systems, in the current moment assessment, it is again the high mountain area that presents the highest potential for erosion generation, with few forest masses capable of counteracting this effect (Fig. 99). If we observe the modelled future situation, most of the erosive production decreases significantly due to the expansion of forest masses, and only in the higher altitude areas of the Picos de Europa massif does it remain high (Fig. 99).

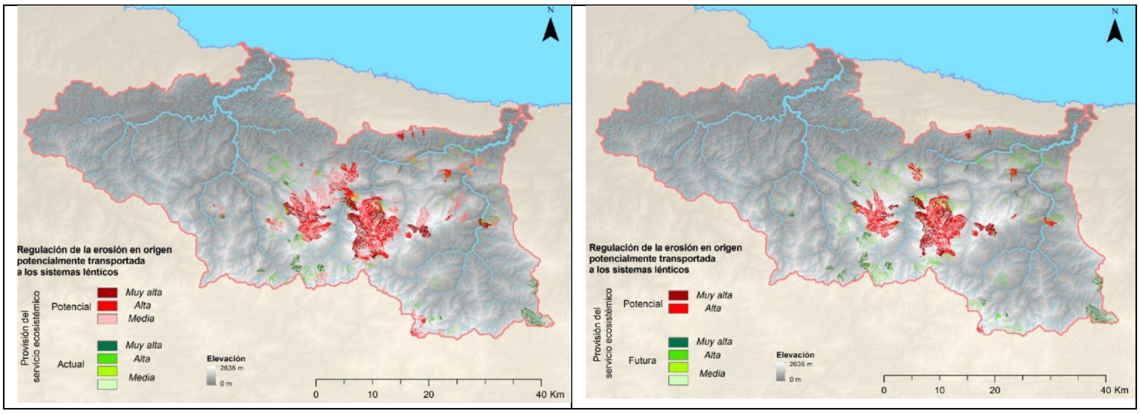


Figure 99. Result of the regulation of erosion SE of at source potentially transported to lentic systems carried out by the hillside forest in the current (left) and future (right) scenario. The categories represent the same values as in Fig. 98.

Finally, in the study of riparian vegetation as a sediment filtering element produced on slopes to prevent their entry into the rivers and streams of these basins, it is observed that, at present, there are few riparian areas that can perform this function optimally (Fig. 100). These are mainly associated with the western lowland areas, with some elements in both the central zone and near the mouth. However, many surfaces are candidates for improvement to potentially provide this ecosystem service, mainly those located in the central and eastern areas of the region. In the future assessment, the pattern is repeated, with fewer candidate areas and a larger riparian surface capable of developing the sediment filtering function before they reach the lotic water bodies (Fig. 100).

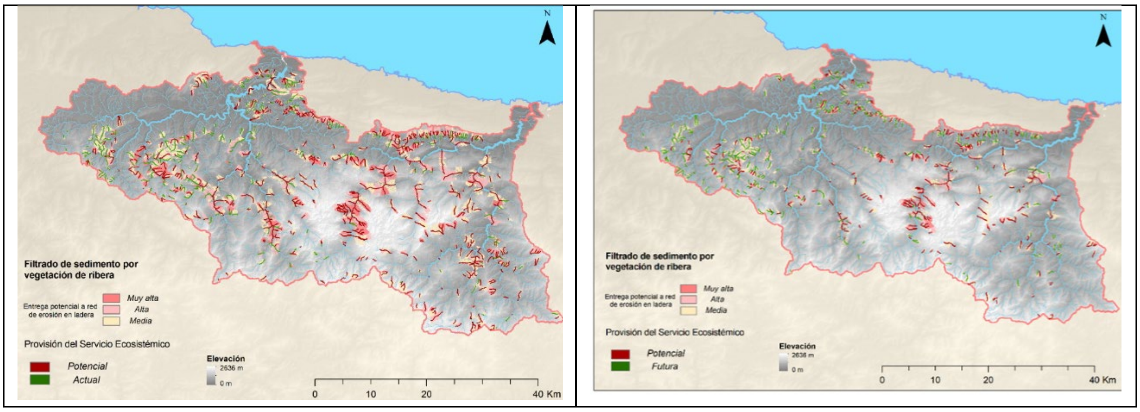


Figure 100. SE result of sediment filtration by riparian vegetation in lotic water bodies in the current (left) and future (right) scenarios. The categories represent the same values as in Fig. 98.

14.3.3. River water temperature regulation service

River water temperature is regulated by dynamic flows of energy (heat) and hydrological processes at the air-water and water-riverbed interfaces (Hannah et al., 2008). Rivers are hierarchical systems, meaning that, at a specific point, the water temperature is initially determined by the mixing of contributions from various inputs (snowmelt, runoff, subsurface flows, etc.) and, subsequently, by the energy gained or lost through the interfaces of the water surface and the riverbed as it flows downstream (Hannah & Garner, 2015). In this sense, the ecosystem service of thermal regulation in rivers involves the buffering of both energy transfer processes within the river network: 1) direct exchange with incident solar radiation, and 2) indirect transfer from water flows draining into the river network.

Water temperature regulates almost all biochemical processes occurring in these ecosystems, affecting several ecological and physiological patterns, as well as the structure and composition of biological communities. Therefore, the influence of water temperature is evident at all levels of biological organization, from individuals to ecosystems, and across trophic levels, from primary producers (Gudmundsdottir et al., 2011) to secondary consumers (Hawkins et al., 1997).

According to the conceptual model proposed by Hannah & Garner (2015), the factors controlling temperature dynamics in rivers are organized as follows:

1. Climate drives the thermal regime of rivers and is therefore the first-order control over regional patterns in the magnitude and timing of seasonal dynamics.
2. Catchment characteristics are second-order controls; water sources, topography, geography, and land use can moderate the influence of climate and thus modify the timing and magnitude of sub-seasonal temperature dynamics.

3. Specific reach conditions interact with the water column as it moves through the catchment, including notable factors such as topographic and riparian shading, hyporheic exchanges, and localized contributions from groundwater. At this scale, the flow of the current also influences the thermal regime of the water through the mixing of water from different sources, including heat exchanges from the streambed.

In relation to vegetation, plant cover is located at the atmosphere-soil-water interface and therefore plays a fundamental role in energy flow exchanges between these systems at meso and microscale. On one hand, the removal of vegetation can warm shallow aquifers in the catchment by increasing the downward heat flow from the heated land surface (Kurylyk et al., 2015). The decrease in surface shading and albedo increases net radiation, leading to direct heating of the surface and subsurface of the drainage areas. Furthermore, the removal of tree canopies can decrease transpiration, thereby increasing the energy available to heat the soil surface. Consequently, the temperature regulation ES provided by vegetation in the drainage areas to shallow groundwater and subsurface flow is particularly important for the thermal regimes of streams and rivers dominated by groundwater. In fact, some studies have observed an increase of about 2ºC in the long-term average annual temperature of groundwater in response to deforestation (Kurylyk et al., 2015). Although these processes are not directly considered in the ES modeling analyses conducted, they have been taken into account for the Sella and Deva-Cares catchments through the model developed to simulate water temperature in the river network. On the other hand, the direct shade provided by riparian vegetation to the river channel has a positive effect on regulating stream temperatures. The removal of vegetation decreases shading, which increases solar radiation levels and consequently the water temperature in the channel (Trimmel et al., 2018). Moreover, the removal of riparian vegetation can lead to the erosion of banks, generating changes in channel geometry towards a wider and shallower form, which would also contribute to an increase in water temperature.

Starting from this conceptual description, the water temperature regulation ES aims to assess, first, the thermal potential in the channels through topographic criteria, and then to estimate the role played by forest cover as a buffer for incident thermal radiation. Unlike modelling water temperature in the river course, modelling incident thermal radiation does not require local calibration or consideration of other factors, such as groundwater flow, substrate conditions, or hyporheic flow. Therefore, the thermal potential, measured in watt-hours/m², was directly estimated in each reach as the average hourly radiation, which is the sum of direct and diffuse radiation during the daytime on June 20, the day of the summer solstice in the Northern Hemisphere. A 5 m pixel DEM and the Solar Radiation Area tool from ArcMap software were used for this purpose.

To simulate the effect of the riparian forest, the attenuation of incident radiation caused by the presence of tree and shrub vegetation in the riparian zone was modeled (Beer's law; Monteith & Unsworth, 2013). For this, incident radiation was recalculated using a digital surface model (DSM), which considers not only the topography of the terrain but also the roughness generated by vegetation concerning its height and density. The DSM was obtained at 5 m resolution from remote sensing data (LiDAR PNOA). The reduction in radiation due to the presence of forest was obtained by subtracting the incident radiation on the DEM obtained for the pixels of the channel from the incident radiation on the DSM for those same pixels. Finally, the result was aggregated at the river reach level.

In DIVAQUA study area riparian forest mainly correspond to: 1) forests dominated by poplar (*Populus* spp.), willow (*Salix* spp.) and elm (*Ulmus* spp.) and 2) forests dominated by alder *(A. glutinosa*), ash (*F. excelsior*) and tree-like willows, mainly white willow (*S. alba*). These forests reduce, on average, about 782 W·h/m^2 of the radiation incident on the river bed, eliminating a total of 4062·106 W·h/m^2 in the entire river network (Fig. 102).

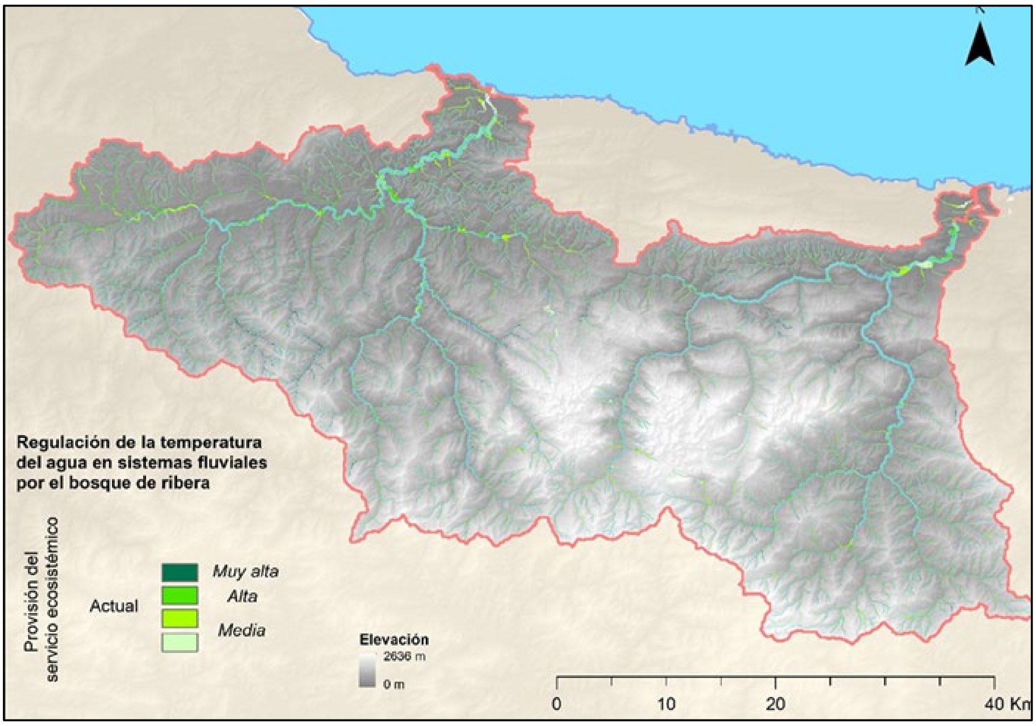


Figure 102. Model for river water temperature regulation by riparian forest in the current scenario (buffering of incident solar radiation). The "very high", "high" and "medium" categories correspond respectively to the 25th, 50th, 75th and 100th percentiles of SE provision.

14.3.4. Pasture production service

Grass is defined as the plant resource that serves as food for livestock, whether through grazing or forage, originating from anthropogenic sources, either through seeding or naturally, consisting of spontaneous flora. Its presence is indispensable with a certain level of management by humans and their livestock. In general, natural pastures have the following common characteristics:

- ✓ They are complex systems that must be managed as a multi-specific community, rather than as grouped individuals.
- ✓ They are communities that stabilize thanks to use, primarily through livestock grazing.
- ✓ They exhibit a high degree of temporal variability, both intra-annual and inter-annual.
- ✓ Their production and diversity are maximized when the grass is managed.

Extensive mountain livestock farming practiced in the Picos de Europa and its surroundings is characterized by the seasonal occupation of grasslands following a terminal altitudinal gradient. This activity is highly relevant, making pastures a significant socioeconomic resource, while also playing an essential role in the conservation of biodiversity in this space. They constitute a semi-natural ecosystem where numerous cataloged species, both animal and plant, thrive (Busqué, 2015). Rural depopulation and the loss of traditional trades have led to changes in pastoral systems that can trigger, in turn, degradation problems and a decline in grass production, both due to undergrazing in less accessible areas and overgrazing in more accessible ones. In the first case, a process of shrub encroachment often occurs, while overgrazing typically leads to reduced plant productivity and, consequently, a decrease in overall production.

The potential grass production in a territory primarily depends on the factors that determine the photosynthetic yield of herbaceous and shrub systems. These factors are mainly climatic (precipitation, temperature, and humidity), lithological and edaphological (composition, soil structure, nutrient limitations), and topographical (Houérou & Hoste, 1977). Specifically, altitude, geomorphological relief, and exposure to solar radiation are the three most important factors, as they imply a primary scale of diversity that manifests in the presence of various biogeographical, morphoclimatic, and land-use levels (Kathleen et al., 1996). These levels offer a wide variety of resources and pastoral potential. Moreover, within each level, slope and exposure create very distinct microenvironments, contributing to a highly complex mosaic of land uses and cover types, where each patch constitutes a landscape unit with different utilization possibilities.

The extensive livestock farming system practiced in the studied watersheds, along with its altitudinal gradient and the diversity of geological substrates present, has resulted in a notable diversity of grass types existing in these watersheds. Near urban areas, we find historically more productive pastures, maintained with organic matter input in the form of fertilizer and through grazing in spring and autumn, and they are also mowed at least once before summer. The plant diversity of this type of pasture is usually low, and its productivity tends to be threatened by the trend of overgrazing in recent decades. At the highest altitudinal extremes, we find alpine pastures, with plant communities of great ecological value and summer utilization by livestock in a semi-free range system. In the intermediate altitudinal situations, there is a large area of communal plots where shrubby grasslands with low forage value usually develop, along with scattered meadows with huts in mid-mountain situations, providing a seasonal complement to the forage from low meadows and alpine pastures. Here, we can observe a remarkable floristic diversity.

To assess the grass provision ES, the total above-ground biomass of shrubs and grasslands was mapped using the "Pastures and Livestock" modeling library (PaL; Torres et al., 2021). PaL is the integrated adaptation of a mechanistic model that simulates grass dynamics (Puerto model; Busqué, 2014) within the ARIES platform (Villa et al., 2014). The Puerto model establishes relationships and biophysical constants between the life cycle of vegetation, livestock grazing processes, and the nitrogen cycle, proving to be a robust tool for modeling grasslands in Cantabria. Within the framework of the DIVAQUA project, a standardized measure of grass production was obtained across the described territory, avoiding the introduction of variability due to context-dependent management practices at the local level (livestock, fertilization, etc.).

To get the results shown below (Fig. 103), only the modules of moisture, temperature, radiation and nitrogen were considered to quantify the climatic and edaphic control over the potential growth of vegetation. Additionally, the vegetation growth, senescence, and leaf litter fall modules were used to simulate the interaction of abiotic parameters with the entire life cycle of vegetation over time. These modules require spatially explicit data on land cover and habitat distribution for simulation scenarios (current and future), soil properties, topography, and climate (precipitation and temperature, for both simulation scenarios), as well as parameters that capture the main physiological characteristics of the vegetation. A spatial resolution of 20 m and a daily time step were established for both simulation periods, which have been averaged into an estimation of the grass provision service at the present time and in a future climate scenario RCP8.5, along with a persistence scenario or BAU (Business As Usual) for land use in the year 2050. The results obtained with the described model for the current time (Fig. 86) show a distribution that primarily responds to the distribution of different physiognomic units of land use or dominant types in the landscape. In this regard, areas dominated by grassland communities exhibit the highest values of grass productivity and biomass throughout the study area. Moreover, several abiotic gradients related to altitude, slope, exposure, and lithological dominance are clear determining factors in the spatial variability of this service. Greater accumulations of biomass and plant productivity are observed in valleys of flat areas and regions of lower altitude.

Regarding the future scenario, the dominant processes of abandonment of traditional agricultural and livestock activities, the consequent forest expansion (shrub and forest) over abandoned grasslands, and the more severe climatic scenarios in the year 2050 for an RCP8.5 have minimized the potential capacity of the territory for the production of quality pastures (Fig. 103). High values are only observed in areas of higher altitude that meet the topographical conditions required for the generation of productive and palatable pastures for livestock, meaning they display flat topography that allows for soil accumulation and relatively low altitudes that avoid the more rigorous climate and lower temperatures during the vegetative growth period.

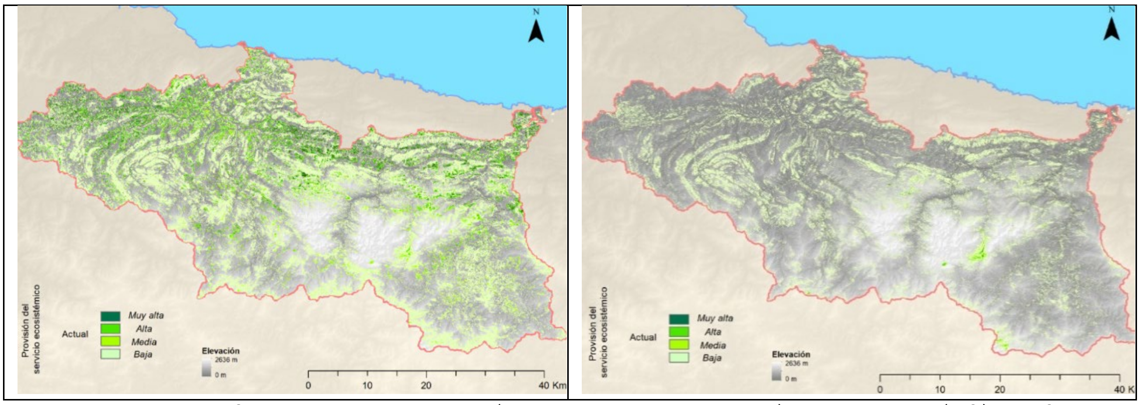


Figure 103. Model for pasture provision ES (aerial biomass production) in the current (left) and future (right) scenario. The categories “very high”, “high”, “medium” and “low” correspond respectively to the 10th, 25th, 50th and 75th percentiles.

15. Future recomendations

Once the monitoring program conducted in the Picos de Europa NP to assess the conservation status of its aquatic ecosystems and preserve its biodiversity against different long-term environmental changes has been described, a series of recommendations for its future maintenance and improvement are proposed.

Recently, the **Picos de Europa NP has been established as the geographical base of a new node that is part of the LTER Spain network, and therefore of LTER Europe and LTER International**. The LTER network (Long Term Ecological Research) was launched in 1993 with the goal of identifying and characterizing ecological phenomena within the context of long-term global change. Thus, the LTER network is based on four pillars:

1. Long-term monitoring.
2. Generation of in situ data.
3. Identification of environmental processes and dynamics.
4. Focus on systems with different environmental and socio-economic components.

The development of this network in Europe was boosted by the ALTER-Net project (2004-2009; Framework VI project of the EU), which promoted the development of a single network at the European level. This allowed for the establishment of the governance structure of the LTER Europe network, the definition of criteria for the inclusion of new nodes, the concept of the LTSER platform (Long Term Socio-Ecological Research), as well as the definition of variables for harmonized monitoring and integrated data management.

Within LTER Europe, the LTER Spain network currently (as of 2023) has 13 nodes, with the Picos de Europa National Park being the only node located on the northern Atlantic façade of Spain, except for the node corresponding to the Atlantic Islands of Galicia National Park (see: https://lter-spain.csic.es/).

The European Strategy Forum on Research Infrastructures (ESFRI) presented its roadmap for large-scale scientific infrastructures in 2018, which identifies new pan-European research infrastructures of priority, among which the LTER-Europe network appears. In turn, the Horizon 2020 Advance Europe LTER project facilitated the development of a strategic report on the future LTER-Europe infrastructure, which had the direct support of 17 countries (including Spain) and 161 research institutions that signed the scientific memorandum of the Europe LTER-Research Infrastructure (E-LTER_RI). The inclusion of E-LTER in the ESFRI roadmap has allowed it to become an infrastructure eligible for funding and management derived from public programs, marking a significant achievement for the maintenance of European long-term ecosystem research infrastructures and socio-ecological research platforms (E-LTER-RI). This offers a range of services, results, and information on European environmental trends to end users (governments, managers, and researchers). Additionally, E-LTER-RI provides access to highly instrumented spaces with expert personnel, long-term environmental observation data, and additional services such as data synthesis and modeling, support for research and technological development, and various training programs through its thematic centers.

All these funding and development opportunities mean that the inclusion of the Picos de Europa NP as a new node of the LTER Spain network represents an unparalleled opportunity to maintain and qualitatively advance the long-term monitoring of the area included in the Socio-Ecological Research Platform Picos de Europa (LTSER-Picos de Europa) node. It is worth noting that this LTSER node not only includes the area delineated within the Picos de Europa NP but also encompasses the entire surface area drained by the Sella and Deva-Cares river basins, from their headwaters to their mouths.

During the process of developing and defending the candidacy of LTSER Picos de Europa, before to its incorporation as a new node within the LTER Spain network, a consortium of institutions was established that, in one way or another, has worked and continues to work in the sociological and environmental monitoring of this area. This has allowed for the consolidation of all monitoring programs established in this National Park, beyond those related to aquatic ecosystems, including those described in this document. The institutions involved are:

- Consorcio Interautonómico del Parque Nacional de los Picos de Europa, organismo gestor de este PN con representación de las 3 administraciones autonómicas donde se asienta el Parque: Asturias, Cantabria y Castilla y León (CIPNPE).
- Fundación Instituto de Hidráulica Ambiental de Cantabria (FIHAC; IHCantabria).
- Universidad de Cantabria (UC; IHCantabria).

- Universidad de Valladolid. Grupo de Investigación Reconocido Patrimonio Natural y Geografía Aplicada (GIR-Pangea).
- Universidad de Oviedo (UniOvi-IMIB)
- Centro Superior de Investigaciones Científicas (CSIC-IMIB)

The collaborative effort made by all these institutions to prepare the candidacy of the LTSER Picos de Europa node has served to highlight all the work developed so far in this area concerning its environmental and socio-ecological monitoring. In summary, the various monitoring programs carried out to date by all these institutions can be synthesized as follows:

1. Monitoring of the Cryosphere: glaciers, ice caves, geomorphological processes associated with ice, snow quantity and
2. Monitoring land use and cover.
3. Monitoring of Aquatic Ecosystems: water quantity, the integrity of riverine and lacustrine ecosystems (described in detail in this document).
4. Monitoring of Unique Biological Populations and Communities: chamois (*Rupicapra pyrenaica parva*), wolf (*Canis lupus signatus*), brown trout (*Salmo trutta*), mountain birds, lepidopterans, bumblebees, threatened flora, amphibians and their emerging diseases and toxicity and epizootics.
5. Socio-Economic Monitoring: livestock, visitors to the Picos de Europa NP and activities and social perceptions.

Thus, a large amount of information is currently available from different institutions involved in the management and research activities of this area, much of which derives from monitoring systems that have been operational for several decades, continuously generating new data. The collective effort made by all these institutions has also helped to identify some weaknesses that need to be addressed in the future to ensure the continuity of the monitoring system for the node itself and to improve the results obtained from it, with the aim of facilitating an integrative management of the conservation of this area that, in turn, allows for the compatibility of conservation with other socio-economic uses. The most relevant weaknesses that are considered to require future attention are as follows:

1. Lack of Funding: There is a need for funding to guarantee the long-term continuity of the described monitoring systems.
2. Absence of a Common Plan: There is a lack of a common plan to improve the management, digitalization, and utilization of generated data.
3. Independence in Design and Execution: The design and execution of the various monitoring plans described are independent, lacking a common plan among different institutions for integrative monitoring of the NP and its surroundings.

4. Low Representativity: There is low representativity of various environmental variables, habitats, and ecosystems relevant to this area in the currently established monitoring systems.

5. Lack of Standardization: There is a lack of standardization of environmental monitoring conducted in different National Parks in Spain and Europe to achieve analogous objectives and identify common environmental patterns.

Next, a series of recommendations are proposed to advance the improvement of the described weaknesses.

15.1. Incorporate the LTSER Picos de Europa node into ESFRI

Currently, the different nodes included within LTER-Europe are divided into three categories based on the quality of the monitoring systems they implement in their areas. Among these, Category 3 includes nodes with less comprehensive monitoring systems, while Category 1 groups nodes with higher quality monitoring systems, meaning they monitor more variables, habitats, and ecosystems with more frequent data collection intervals. The category in which a particular node is grouped is essential for its inclusion in ESFRI, as it is currently necessary to receive a rating of Category 1 or 2 to be considered by ESFRI. Being recognized within ESFRI is crucial for a node to qualify for various European programs and possibly for future lines of funding promoted at the national level by the relevant ministries of the Spanish Government.

Following the defense of the candidacy of the LTSER Picos de Europa node and its inclusion within the LTER-Spain network, efforts are being made to complete the information required by LTER-Europe to establish the classification of the node within these three categories. Thanks to the extensive work carried out in the Picos de Europa National Park over the past decades, LTER-Europe has preliminarily included the LTSER Picos de Europa node in the aforementioned Category 2, which will facilitate its future inclusion in ESFRI. However, some deficits have been detected that could hinder maintaining this category, so it would be advisable to implement the necessary efforts to remain within the criteria established by LTER-Europe. Currently, LTER-Europe is undergoing significant dynamism, resulting in changes in the classification criteria for the mentioned categories, as well as in the management of the network itself, both in Spain and in Europe, etc. Since the acceptance of the LTSER Picos de Europa node into the LTER-Spain network, active collaboration has been established with the management of this network to ensure, among other things, access to and maintenance of Category 2 status for the LTSER Picos de Europa node. Additionally, attendance at the upcoming SPF05 meeting to be held in Lunz, Austria, in October 2023, is planned, where the criteria and decisions regarding this matter will be defined.

Therefore, it is very important to maintain presence and participation in these management bodies of LTER-Spain and LTER-Europe, as well as to adopt the recommendations that arise from them to guarantee the participation of the LTSER

Picos de Europa node in ESFRI, which, in turn, will enhance the prospects for the continuity and improvement of the monitoring established in the node by increasing its potential for funding through external resources that may be obtained from future lines of funding from European and national programs.

15.2. Digitize the Picos de Europa NP monitoring systems

The sharing of all the information generated by the institutions conducting environmental monitoring programs in the Picos de Europa area has highlighted the absence of a common database that would allow for the adequate management of all the data obtained from such programs. This limitation restricts the ability of the results to be applied to improve the management and conservation of this space and its biodiversity, as well as the research activities conducted on it. To advance in the digitalization and unification of the environmental information obtained over the years in different environments of this space (aquatic and terrestrial ecosystems, social dynamics, wild populations and communities, etc.), the development of a digital database is proposed, following the approach known as "digital mirror." Digital mirrors, also known as data lakes, are repositories of data that store large amounts of structured and unstructured information. These digital mirrors are particularly useful for data management in big data environments, with the capacity to collect and store data from diverse sources. In the context of monitoring and conservation of protected natural areas, digital mirrors can play a very relevant role in collecting, organizing, and analysing databases derived from both biotic variables (e.g., populations and communities of organisms) and abiotic variables (e.g., physical environmental variables such as the quantity and quality of water, ice, etc.). Among other benefits, the main advantages provided by digital mirrors can be summarized as follows:

- **Data Collection**. Digital mirrors can integrate data from multiple sources and types, such as those derived from remote sensors, surveillance cameras, or weather stations that continuously collect in situ data, along with other data obtained from field sampling and subsequent analysis, for example, in a laboratory. This allows for the effective grouping and management of a wide variety of data regarding biodiversity, environmental conditions, and other relevant aspects for the management and research of the natural environment in relation to long-term processes and dynamics, such as those associated with global change.
- **Enhanced Data Analysis** Capabilities. Digital mirrors facilitate the analysis of large databases, allowing for the identification of the most relevant information based on the analysis that is to be performed. The use of different data processing techniques, such as data mining, machine learning, or the application of various statistical algorithms, enables the identification and definition of distinct environmental patterns and trends, as well as their correlation with the most relevant determining factors. This can help natural space managers

improve their understanding of ecosystems and make informed decisions that favor the conservation of various elements.

- **Real-Time Monitoring and Data Querying**. Digital mirrors allow for the continuous real-time monitoring of natural spaces. Data collected by measurement instruments (e.g., probes to measure water quality) can be analysed in real time to detect environmental changes, provided that these instruments have the capacity to transmit data. This serves as an exceptional early warning tool, allowing for the prediction of events such as wildfires, floods, variations in water and air quality, the emergence of invasive species, or the migratory movements of different wildlife species. This capability allows for a rapid and efficient response to emergencies or environmental problems, improving the effective management of the monitored space and organisms.
- **Collaboration and Data Management**. The implementation of a digital mirror in the described space would allow for real and coordinated collaboration among different institutions interested in monitoring the area (e.g., institutions that are part of the LTSER Picos de Europa node). The information stored in the database managed by the digital mirror can be shared securely, fostering collaboration, knowledge exchange, and evidence-based decision-making. This is also very relevant for establishing collaborations among different institutions working in various natural spaces with similar characteristics and pursuing common objectives. For example, among different mountain protected natural areas (PNN) in Spain or other regions of the world, to confirm the existence of a specific identified temporal environmental pattern in any of these areas.

All these capabilities mean that the implementation of a digital mirror in the LTSER Picos de Europa node, or in the area exclusively limited within this National Park, improved the potential of the environmental monitoring carried out until now in it, strengthening some of the previously mentioned weaknesses. Described

15.3. Standardization of NNPP monitoring programs

In this case, it is not a recommendation to be applied in a specific area, such as the Picos de Europa NP, but rather a recommendation directed at the national body responsible for these areas, such as the Autonomous Organization of National Parks (OAPN) under the Ministry for Ecological Transition and the Demographic Challenge of the Spanish Government. It has recently been noted that one of the main problems in conducting long-term environmental monitoring of aquatic ecosystems in the mountain protected natural areas (PNN) of Spain is the lack of harmonization among the different systems implemented in each of the five analysed peninsular parks (Peñas et al., 2023). This study highlights the absence of a common national strategy to monitor the potential environmental impacts that global change may be generating over the long term in the rivers, lakes, and wetlands of these mountain spaces.

The lack of a coordinated national strategy means that the conclusions drawn from pooling all the information generated by each monitoring system are less effective and weaker compared to the possibility of obtaining conclusions from a multiple and simultaneous program that generates a common database obtained through methods and approaches agreed upon by all monitored areas (Tydecks et al. 2019).

The analysis conducted on the monitoring systems of aquatic ecosystems in Spain's mountain PNN has also identified a general weakness related to the lack of space-time designs (Peñas et al., 2023), which would be necessary to statistically discern the changes attributable to global change from those arising from the natural variability inherent to the aquatic environment itself (e.g., dry years vs. wet years; Downes 2002). Thus, in most cases, the best design does not go beyond the reference condition approach, while approaches based on Control-Impact (CI) designs or so-called Before-After/Control-Impact (BACI) designs would help to more effectively discriminate between dynamics determined by anthropogenic causes and those generated by the natural variability typical of the mountain environment. The ability to have Control data makes the comparability conditions between controls and impacts dynamic, as opposed to the static value employed by the reference condition approach. This possibility is currently a reality in the monitoring system of river ecosystems implemented in the Picos de Europa NP, as a Control-Impact approach has been adopted in designing the network of study points used in this monitoring system (see description in section 9.1 of this document).

15.4. Improve monitoring of scarcerly characterized variables, environments and communities

The sharing of the different monitoring systems carried out to date in the Picos de Europa National Park, as well as the review work conducted to characterize the monitoring systems of global change on the aquatic ecosystems of the mountain protected natural areas (PNN) in Spain (Peñas et al., 2023), has allowed for the identification of elements that need to be strengthened to adequately monitor these ecosystems in the Picos de Europa NP and determine the long-term impacts of global change. Below is a synthetic proposal of the elements on which to increase efforts to improve the monitoring and conservation of the aquatic ecosystems of the Picos de Europa NP and its biodiversity.

15.4.1. Climatic and meteorological variables.

Although the Picos de Europa NP has several meteorological stations that characterize different basic climatic variables such as temperature, precipitation, atmospheric pressure and air humidity, this area lacks instruments capable of measuring variables related to snow. This absence currently prevents the characterization of the snow accumulation regime and the melting processes of snow and their relationship with the hydrological regime of the aquatic ecosystems in this area (e.g., flow rates in rivers and streams or water volume in lakes, lagoons, and wetlands). As described in this document, changes in the timing of the transition from snow and ice to flowing water directly influence runoff processes in the watershed and the hydrological regime of mountain aquatic ecosystems. For this reason, additional efforts are recommended to improve the current understanding of these processes. As a reference, attention can be drawn to the Global Snow Monitoring System implemented in Sierra Nevada National Park (www.uco.es/dfh/snowmed; Polo et al. 2019), which has additional instrumentation that allows for the characterization of snowfall, snow coverage in the characterized area, as well as various specific components of the energy balance.

15.4.2. Lakes and lagoons biological communities

As recently described by Peñas et al. (2023), several monitoring systems established in mountain lakes in Spain and European NNPP conduct more or less exhaustive tracking of the planktonic communities that develop in these lentic ecosystems (i.e., phytoplankton and zooplankton). Both communities are considered good biological indicators for characterizing and assessing the conservation status of lakes and lagoons, with the benthic phytoplankton community being incorporated—through the calculation of its biovolume—as a quality element for monitoring the ecological status of lakes classified as water bodies in Spain, in accordance with the Water Framework Directive (R.D. 817/2015). Therefore, the continuous analysis of the structure and composition of phytoplankton and zooplankton communities provides valuable information for determining changes in their integrity concerning various long-term dynamics generated by global change (e.g., water quality, changes in temperature regimes, etc.). Although several studies have characterized both communities in the Picos de Europa National Park, it is recommended that this analysis be maintained over time to determine how the integrity of these lentic ecosystems varies in the long term and the possible responses of both planktonic communities to different environmental dynamics.

15.4.3. Improving weatlandas and peatlands monitoring

Another aspect that should be improved in the monitoring of the aquatic ecosystems of the Picos de Europa NP is the characterization of the dynamics of wetlands and peatlands. Mountain wetlands and peatlands are considered particularly fragile habitats, as they are highly dependent on the quantity and quality of water and, therefore, on various meteorological processes (e.g., precipitation regime, temperature, snowmelt phenology, etc.). Furthermore, these ecosystems are especially affected by certain traditional uses that occur in this area, primarily extensive livestock grazing, which uses these spaces as water sources, degrading them through trampling and manure deposition. The importance of conserving these types of ecosystems lies in their very nature, as many of them are considered habitats of community interest, which must be maintained in a favourable conservation status, as indicated by the Habitats Directive (Directive 92/43/EEC). They are also very important aquatic habitats in mountain environments due to their ability to host organisms that find their ideal habitat here, with many of these taxa also considered of community interest under the Habitats Directive.

To implement a monitoring system for the wetlands of the Picos de Europa NP, it is recommended to follow the guidelines established by the Ministry for the Ecological Transition (Silva-Sánchez et al., 2019).

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