frontiers in Forests and Global Change

TYPE Original Research
PUBLISHED 25 February 2026
DOI 10.3389/ffgc.2026.1715128

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OPEN ACCESS

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RECEIVED 28 September 2025
REVISED 09 January 2026
ACCEPTED 26 January 2026
PUBLISHED 25 February 2026

CITATION
Alfaro-Saiz E, Cruz Ramón P, Cortón-Gracia B, Rodríguez-Fernández S, Encina A, Lobato Pérez S, Paniagua-García AI, Díez-Antolínez R, Acedo C and Fernández-Salegui AB (2026) Birch sap extraction and short-term physiological responses in *Betula celtiberica* forests of north-western Spain. *Front. For. Glob. Change* 9:1715128. doi: 10.3389/ffgc.2026.1715128

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Birch sap extraction and short-term physiological responses in *Betula celtiberica* forests of north-western Spain

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Introduction: In recent years, birch sap has gained attention due to its applications in different sectors. However, the ecological impact of sap extraction and its implications for ecosystem sustainability remain insufficiently explored. This study focuses on the sustainable management of *Betula celtiberica* sap extraction in the Reserva de la Biosfera de los Valles de Omaña y Luna (RBVOyL).
Methods: The effects of sap extraction on tree physiology were assessed by measuring water potential and chlorophyll fluorescence. In parallel, the biochemical composition of the sap was analyzed to characterize its biocompounds and evaluate their potential commercial uses. Practices for sustainable and responsible sap collection were also considered.
Results: Sap extraction did not produce negative effects on tree physiological status, as neither water potential nor chlorophyll fluorescence showed adverse responses 1 month after extraction. In addition, the volume of sap obtained and the concentrations of biocompounds detected support its potential use as a food and cosmetic resource.
Discussion: Birch sap could represent a viable, sustainable and natural product for small-scale production in the study region, contributing to the diversification of local economic activities. Nevertheless, further research is required to assess the long-term effects of sap extraction and its broader potential as a forest resource.

KEYWORDS
biocompounds, birch groves, birch sap, chlorophyll fluorescence, endogenous resources, ethnobotany

1 Introduction

The sustainable use of wild plants has long been recognized and promoted by international organizations as a key strategy for biodiversity conservation. Within the Kunming–Montreal Global Biodiversity Framework [Convention of Biological Diversity (CBD), 2022], the sustainable use of wild species (Target 9) and the implementation of biodiversity-friendly management practices in agriculture and forestry (Target 10) are considered fundamental pathways for achieving equitable benefit-sharing, enhancing ecosystem resilience, and securing the long-term socio-economic and ecological functions of landscapes. Non-timber forest products represent a crucial component of this vision, as their responsible extraction can simultaneously support rural livelihoods, foster bioeconomic diversification, and help maintain biodiversity, directly contributing to several United Nations Sustainable Development Goals (SDGs), including SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), and SDG 15 (Life on Land) (United Nations, 2015).

The Somos Agua II project (Fundación Biodiversidad, 2022) aligns with these global objectives by fostering the development of a micro-entrepreneurial network that promotes a transformative circular bioeconomy in a depopulated mid-mountain area within the Reserva de la Biosfera de los Valles de Omaña y Luna (RBVOyL), Castilla y León, Spain. Building upon traditional ecological knowledge and other endogenous resources, the initiative integrates scientific and technical analyses of natural resources' bioactive potential with biocultural processes supported by innovative tools for social participation.

The birch forests of the Cantabrian Mountains are boreal remnants largely dominated by *Betula celtiberica*, serving as living testimonies of past climatic changes and biodiversity reservoirs (Vicente Orellana et al., 2008). Biologically adapted to expand successfully when conditions are favorable, birch established itself on the siliceous soils of the Cantabrian Mountains and the Omaña region during the glacial and interglacial cycles of the Quaternary period (Blanco Castro et al., 1997). Changes in traditional land uses, such as the decline of livestock farming and timber extraction, have facilitated the natural recovery of these native forests (García de Celis et al., 2004). However, this regeneration is not always perceived positively by local communities, as it is often associated with the loss of extractive activities, including the reduction of grazing areas for livestock. Developing alternative and sustainable livelihoods may help reshape local perceptions and relationships with forest ecosystems. Studies have shown that the traditional use of wild plants plays a significant role in preserving local culture and identity (de Pardo Santayana and Gómez Pellón, 2002; Pilgrim et al., 2008), which can foster a more favorable attitude toward nature. Forest conservation and wild plant use extend beyond provisioning and regulating ecosystem services; they also encompass cultural services that are interconnected and generate a broad range of socio-ecological benefits (Reyes-García et al., 2015). In certain urban contexts, wild plant gatherers have been identified as effective environmental stewards, contributing to biodiversity conservation and informing policy development, thereby linking resource sustainability with conservation biology (Teixidor-Toneu et al., 2022).

In this context, birch sap harvesting serves as an illustrative example of how non-timber forest resource use can be aligned with global conservation targets, delivering social and economic benefits while safeguarding forest ecosystem services.

The genus *Betula* L. includes tree and shrub species widely distributed in temperate and boreal regions of Europe, Asia and America (Vladimirov et al., 2019). Many of these species play a crucial role in forests, as pioneer species (Hynynen et al., 2010). In the Iberian Peninsula three species of birch can be found: *Betula celtiberica*, *Betula pubescens* Ehrh. and *Betula pendula* Roth subsp. *pendula* (Ashburner and McAllister, 2016). This article focuses on the first of these species, as it is the most common in the Iberian Peninsula and the one present in the study area. *B. celtiberica* was recognised as a distinct species in a paper on the systematis of European birches. It was described from many of the mountain ranges of northwest Spain and eastern Portugal. It is probably a stabilised population derived from *B. pubescens* heavily introgressed with *B. pendula* (Ashburner and McAllister, 2016). *B. celtiberica* is a tree up to 20 m tall with leaves ranging from rhomboidal to ovate-rounded with an acute apex and irregularly toothed margin. It grows in moist, preferably siliceous, loose and fresh soils. It forms forests in areas near freshwater streams or in waterlogged places up to 1800 m above sea level (m asl) (Menéndez Valderrey, 2008). Birches (*Betula* spp.) stand out for their multiple applications in the forestry industry, reforestation of degraded lands or their use as ornamental trees (Rastogi et al., 2015), to mention a few examples. There is evidence of the use of almost all parts of the plant: leaves, bark, wood, sap and buds, for various purposes. In this article, we will focus on birch sap extraction, a traditional practice that has recently gained interest due to its potential applications in food, cosmetic and pharmaceutical industries (Keita et al., 2021; Svanberg et al., 2012). Birch sap extraction and collection has traditionally been performed in many northern hemisphere countries (Svanberg et al., 2012), with *B. pendula* and *B. pubescens* being the most commonly used species (Grabek-Lejko et al., 2017). The sap extracted from birch is a solution composed of approximately 97% water (Bilek et al., 2015), in addition to other bioactive substances such as inorganic ions and vitamins that confer significant nutritional value (Sancho et al., 2022). In terms of organic compounds, sugars and organic acids stand out for their quantity (Kallio and Ahtonen, 1987). Regarding sugars, the sap is mainly composed of glucose, fructose, sucrose and galactose in that order of abundance (Łuczaj et al., 2014). Among the organic acids, malic acid stands out, giving the sap a moderately low pH, between 5.5 and 6.0. It also contains traces of free amino acids (mainly glutamate and aspartate) and high molecular weight proteins. The presence of these nitrogenous components, although in low concentrations, can cause Maillard browning during heating or concentration of the sap, contributing to the sensory characteristics of birch syrup (Kallio et al., 1987). As for inorganic ions, those found in the highest concentrations are calcium (Ca^{+2}) and potassium (K^{+}) although others such as zinc (Zn^{+2}), magnesium (Mg^{+2}), manganese (Mn^{+2}), copper (Cu^{+1}/Cu^{+2}), cadmium (Cd^{+2}), iron (Fe^{+2}/Fe^{+3}) or sodium (Na^{+2}) can also be found (Grabek-Lejko et al., 2017). In Grabek-Lejko et al. (2017), they concluded that the concentration of heavy metals in the soil does not impact the concentration of heavy metals in the sap. However, pollution as well as other environmental stresses, such as traffic pollutants, could increase the antioxidant activity of birch sap.

The optimal time for sap extraction is considered to be the beginning of spring, when the sap starts to rise through the xylem, before leaf development (Mingaila et al., 2024). This occurs because birch roots absorb water from the soil through osmosis and pump it upwards, without the need of transpiration even before the leaves develop. This flow generates a positive pressure in the xylem (root

pressure) that causes birch sap to sprout if the trunk is cut or pierced during this period (Zajączkowska et al., 2019). However, the seasonality depends on various factors such as geographical location and climatic conditions (Mingaila et al., 2024; Westhoff et al., 2008). In central Europe, extraction is usually carried out between late March and early April, but may be delayed or advanced depending on latitude (Kūka et al., 2013). Extraction can take two to four weeks, and the total yield varies depending on the species (Grabek-Lejko et al., 2017). In addition, other factors such as tree location in the forest, orientation, age, size, edaphic conditions or habitat type influence the quantity and quality of extracted sap (Grabek-Lejko et al., 2017; Hölttä et al., 2018; Łuczaj et al., 2014; Mingaila et al., 2020; Mingaila et al., 2024; Sancho et al., 2022; Staniszewski et al., 2020; Westhoff et al., 2008; Zajączkowska et al., 2019). The most common method used to extract sap is to drill a hole of small diameter in the birch trunk, where a tap, tube or spigot is inserted (Mingaila et al., 2024). In some Nordic countries and in the United States, specially in the maple region, vacuum pumps are used to extract the sap without contaminating it (Wilmot et al., 2007).

Numerous articles are investigating the most efficient way to extract birch sap, such as Mingaila et al. (2020), Westhoff et al. (2008) or Zajączkowska et al. (2019); or evaluating compounds present in it, such as Kallio et al. (1987); Sancho et al. (2022) or Vladimirov et al. (2019). However, there is hardly any information on the damage or stress caused to these trees during sap extraction. To analyze the possible stress caused to birch, various physiological parameters such as xylem water potential and chlorophyll a fluorescence of photosystem II can be used (Juenger and Verslues, 2023; Maxwell and Johnson, 2000). Water potential is defined as the variation of the chemical potential of water in a given system with respect to the value of the chemical potential of pure water (Kramer and Boyer, 1995). It is a thermodynamic parameter that describes the potential energy of water in any system. It is used to determine the direction and intensity of water movement in soil–plant-atmosphere systems, being key in processes such as water absorption by roots, its transport by xylem and transpiration in leaves (Kramer and Boyer, 1995). Therefore, it can be used as a tool to analyze the physiological response of plants against stress factors such as water deficit or mechanical damage, as it allows a direct measure of plant water status (Juenger and Verslues, 2023). On the other hand, the measurement of chlorophyll a fluorescence from photosystem II, it is an effective and non-destructive method to quantify the efficiency of the photosynthetic process in terms of the conversion of light energy into chemical energy (Maxwell and Johnson, 2000; Roháček, 2002). This method generally consists of taking two measurements with a fluorimeter to obtain the maximum quantum yield, which corresponds to the photochemical conversion at the photosystem II level; and the effective quantum yield that takes into account all photochemical and non-photochemical processes, such as electron transport, ATP synthesis and Calvin cycle, that occur after light absorption in photosystem II (Roháček, 2002).

The present work has the following objectives:

- To quantify sap yield from *Betula celtiberica* and evaluate the effect of perforation number on extraction volume.
- To analyze the sugar and organic compounds composition of birch sap.
- To evaluate the impact of sap extraction on the physiological status of birch trees, monitoring water potential and chlorophyll fluorescence as stress markers.

2 Materials and methods

The experimental design explained in this section is summarised schematically in the Methodological Abstract (Figure 1).

2.1 Study area

The study area is located in the Reserva de la Biosfera de los Valles de Omaña y Luna (RBVOyL) in León, Castilla y Leon, Spain. Three forest stands classified as Public Utility Forests (MUP) were selected based on their accessibility and the existence of homogeneous characteristics. The three birch groves were located in the municipality of Murias de Paredes (León). The birch grove considered as 'A' is located in the town of Vivero de Omaña (42.870252, −6.216087), birch grove 'B' in Fasgar (42.806911, −6.233011) and birch grove 'C' in Murias de Paredes (42.845795, −6.200203) (Figure 2). The three birch groves have a predominant north-west orientation and mean recorded altitudes of 1,478 m asl, 1,425 m asl and 1,331 m asl, respectively.

It is important to note that obtaining the appropriate authorization is mandatory in each case for birch sap extraction. In this case, as the groves were MUP of León, a permit was requested from the Consejería de Medio Ambiente, Vivienda y Ordenación del Territorio de la Junta de Castilla y León.

2.2 Selection of individuals and sap extraction

Within each birch grove, 18 birches were selected based on criteria of similarity in diameter at breast height (DBH 14–20 cm), trunk straightness and low branching. The trunks of the trees were marked at a height of approximately 130 cm, and a cross was made on the north face to indicate the area where the drilling was to be carried out. The diameter and exact location of each tree were recorded, and a color code was used to differentiate each sap exudation treatment applied to each tree (Treatments I, II and III below). Sap extraction took place between April 8th and 16th, 2024. In each tree, boreholes were drilled at the previously marked height on the trunk, using a drill and a 10 mm diameter drill bit. A depth of 2–3 cm was calculated with an upward inclination. The number of boreholes varied according to the treatment assigned to each birch: treatment I was the control treatment, with no boreholes drilled; treatment II had two boreholes drilled, one on the north side and one on the south side; and treatment III had four boreholes drilled, one in each cardinal point of the trunk. Therefore, in each birch tree grove, six control trees (treatment I) were selected, and 12 trees were drilled, six with two holes (treatment II), and six with four holes (treatment III). Exceptionally, the birch grove 'C' only had five trees for treatments II and III, because there were no suitable specimens for the established diameter range without being too far away from the sampling area.

Once the trunk was drilled, a 10 mm diameter food grade PVC hose (Arin Cristal, Todogoma S. A.) connected to a 5 or 8 L reservoir previously labelled and secured to the tree with rope was inserted into each hole. The lid of the reservoir was provided with a hole that facilitated air entry to allow continuous sap flow. In treatment II trees, both hoses went to the same reservoir, while in treatment III trees, each pair of hoses went to a different reservoir in the expectation of greater sap extraction. The total sap extraction time was 10 days. After this, the collected sap was removed, and the trunk punctures were sealed with a branch carved from the same birch tree and potassium soap.

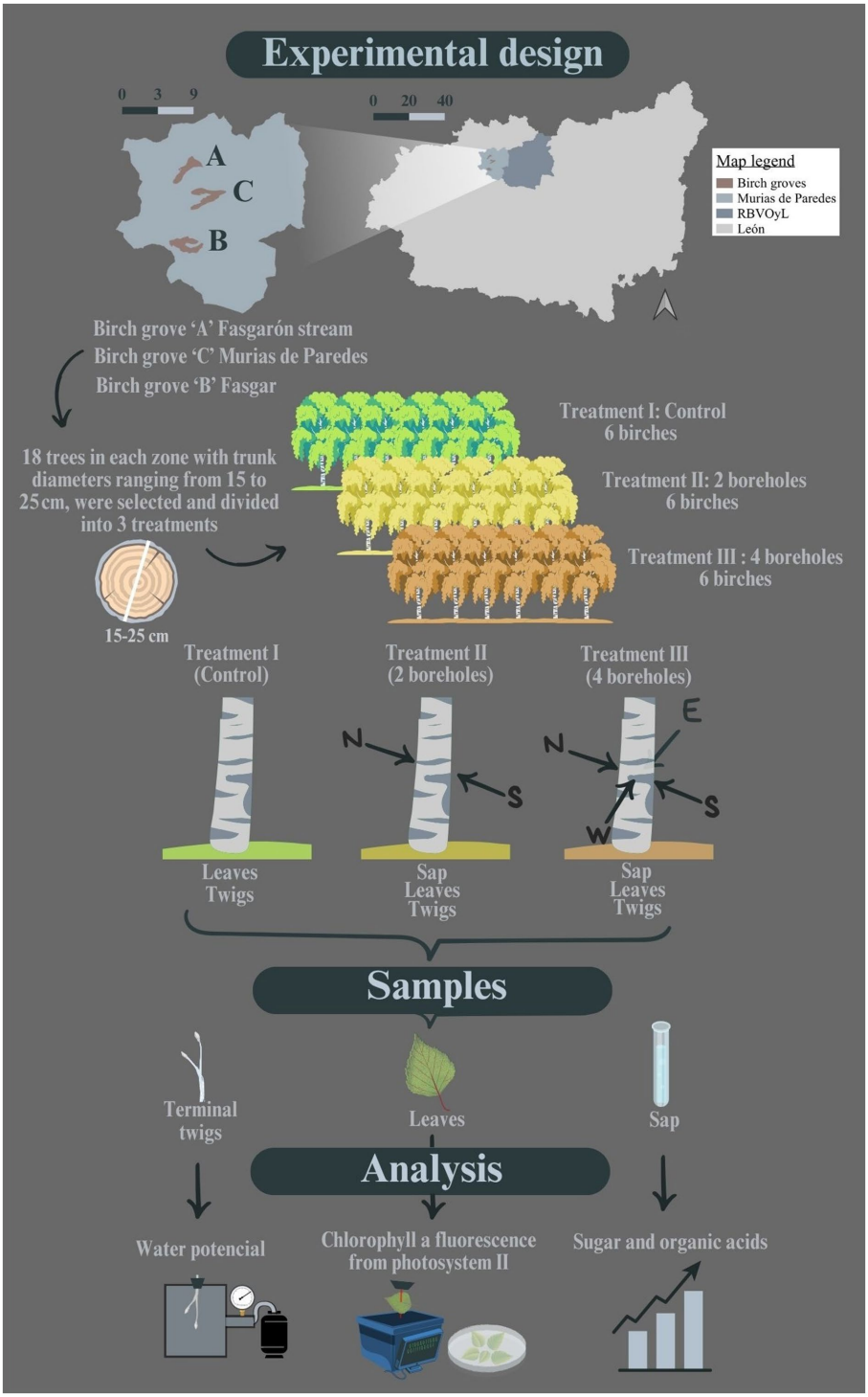


FIGURE1
Methodological abstract of the experimental design explained in "section 2. Materials and methods".

For more information on how to extract birch sap in the most sustainable and environmentally friendly way possible, see Appendix 1 in Supplementary material.

2.3 Sap analysis

Once the sap was extracted, the sap samples of each tree (six trees per treatments II and III) were analyzed at the Instituto Tecnológico Agrario de Castilla y León (ITACyL) to determine the content of its main organic compounds: sugars and organic acids. The analysis was performed using an Agilent 1,200 HPLC equipment (Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector (RID, G1362A, Agilent Technologies). Separation was performed with an Aminex HPX-87H cation exchange column (300 mm × 7.8 mm) (Biorad, Hercules, CA, USA) operated at 60 °C. The mobile phase consisted of 5 mM H_2SO_4, with a flow rate of 0.6 mL/min. The injection volume was set at 20 µL. All samples were filtered through 0.22 µm nylon syringe filters (Agilent Technologies) before analysis.

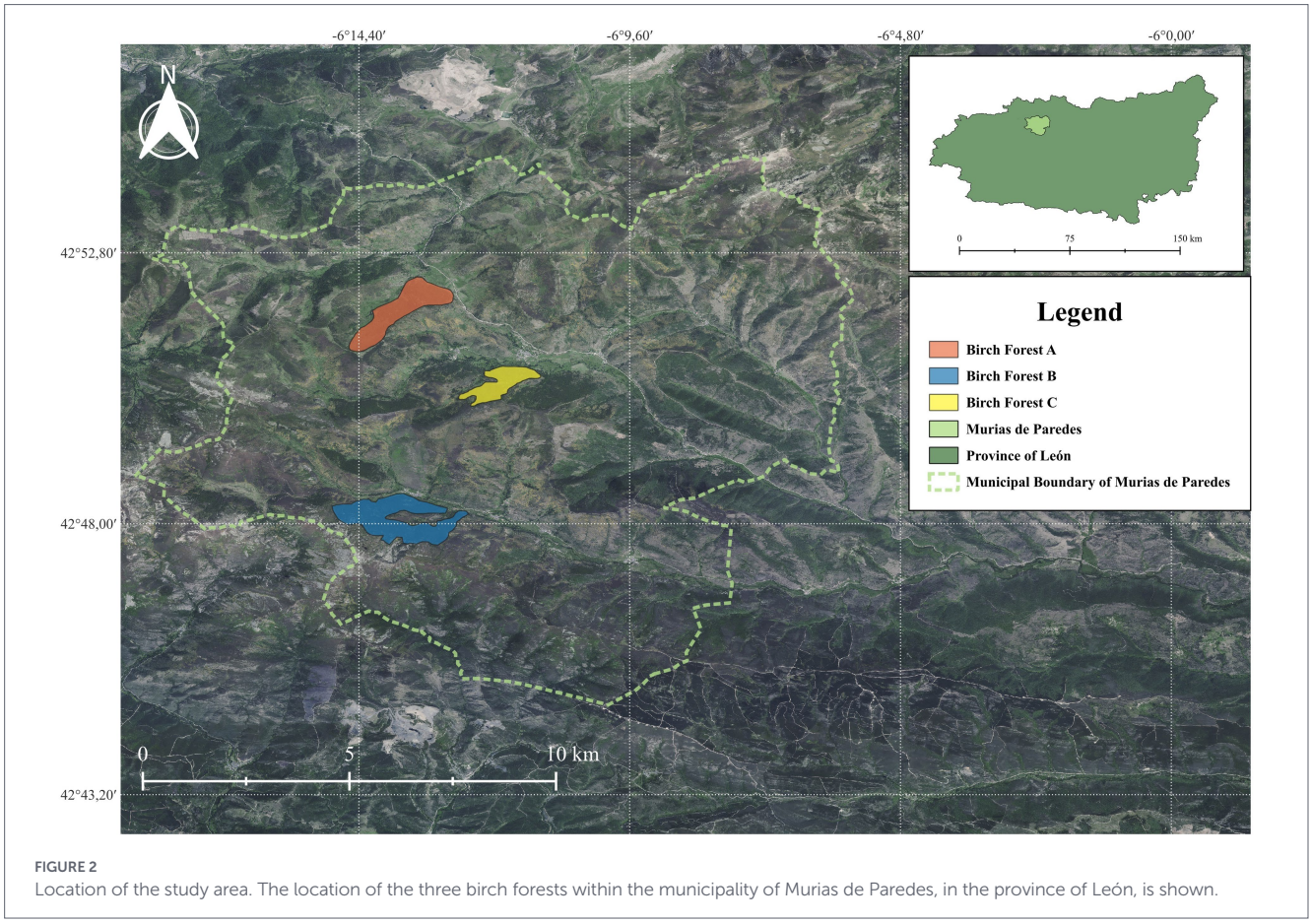


FIGURE 2
Location of the study area. The location of the three birch forests within the municipality of Murias de Paredes, in the province of León, is shown.

2.4 Water potential measurement

To measure the water potential of birch xylem, the method described by Scholander et al. (1965) was followed using a pressure chamber or Scholander chamber (model 600, PMS Instrument Co.). The methodology consisted of collecting 3 to 5 terminal twigs of the year with 3–5 mm diameter evenly distributed throughout the tree canopy. Sampling was always done at night to avoid fluctuations in water potential during daylight hours. Tree twigs were stored in hermetically sealed bags, kept cold to conserve moisture, and analyzed immediately afterwards. Twig water potential measurements were taken at three different times about the start of sap extraction: at 2 days, 7 days, and 30 days.

2.5 Measurement of chlorophyll a fluorescence from photosystem II

A fluorometer (Teaching PAM 210, Walz) was used to measure chlorophyll of a photosystem II. The methodology consisted of collecting 5 leaves per birch tree, 1 month after sap extraction, coinciding with the onset of spring leaf development. The leaves were stored in hermetically sealed bags and kept cold to conserve humidity until they reached the cabinet, where they were placed in petri dishes so that measurements could be taken immediately afterwards. Two measurements were made. The first measurement was performed under dark conditions to determine the maximum quantum yield. The second measurement was performed the following day, after a minimum period of 2 h of exposure of the leaves to sunlight, to determine the effective quantum yield.

2.6 Statistical analysis

Statistical analyses were performed using the R programming language (R Core Team, 2024) in the RStudio development environment (Posit, 2024). Prior to analysis, the assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk test and Levene's and Bartlett's tests, respectively. As these assumptions were met, no data transformations were required, and all analyses were conducted on the original data scale.

Student's t-test was used to assess the effect of the number of trunk perforations (two vs. four) on the total volume of sap extracted per tree. A one-way analysis of variance (ANOVA) was applied to evaluate differences in sap chemical composition among locations. The relationship between glucose and fructose concentrations was examined using Pearson's correlation coefficient.

To evaluate the impact of sap extraction on tree physiological status, generalized linear models (GLM) with Gaussian distribution were fitted. For water potential, models included treatment (extraction vs. control), locality (A, B, and C), and their interaction, and were fitted separately for each measurement time (48 h, 1 week, and 1 month). For chlorophyll fluorescence (maximum and effective quantum yield), GLMs included the amount of sap extracted (L) and locality as additive effects, assuming no interaction between these variables.

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Model selection was based on comparison of the Akaike Information Criterion (AIC), and the models with the lowest AIC values were selected for inference (Appendix 2 in Supplementary material). For all GLMs, estimated coefficients, standard errors, t-values, and p-values are reported, allowing evaluation of both the magnitude and direction of effects. Detailed model outputs are provided in Appendix 3 in Supplementary material. A significance level of $\alpha = 0.05$ was used throughout. Graphical representations were generated using the ggplot2 package (Wickham et al., 2016).

3 Results

3.1 Quantities of extracted birch sap

In total, 323.4 L of sap were collected from the 34 birch trees: 162.25 L in birch grove "A", being the largest amount; 85.65 L in grove C and 75.5 L in grove B. Each birch tree produced 9.5 L on average over 7 days, equivalent to 1.3 L per tree per day. Individually, the birch from which the most liters of sap were collected was located in grove "A", was assigned treatment III and produced 28.5 L of sap in total, equivalent to 4 L per day. On the other hand, the birch from which the least sap was collected in grove "B" was also assigned to treatment III and produced a total of 1.6 L of sap, 0.2 L per day. No significant differences were observed between treatments II and III, so our results indicate that the difference between drilling the tree trunk at two or four points does not affect sap extraction yield.

3.2 Compositional analysis of birch sap: sugar and organic acids

Compounds such as sugars (glucose and fructose) and organic acids (specifically succinic, acetic, malic and citric acids) present in sap samples were analyzed at ITACyL. No significant differences were found in the amount of glucose and fructose in the sap samples from birch trees collected at the different locations (glucose: $M = 1.826 \pm 0.855$ g/L; fructose: $M = 3.457 \pm 1.095$ g/L). A significant moderate positive Pearson correlation ($r = 0.4015$, $p < 0.05$) was found between the two, so that higher levels of glucose tend to be associated with higher levels of fructose (Figure 3). From highest to lowest concentration, citric acid ($M = 0.052 \pm 0.045$ g/L) showed significant evidence among the three locations ($p = 0.028$). Birch C ($M_C = 0.081 \pm 0.068$ g/L) showed more variable and higher values, ranging from 0.02 to 0.24 g/L. Birch A ($M_A = 0.031 \pm 0.018$ g/L) showed the lowest values, ranging from 0 to 0.06 g/L, and birch B ($M_B = 0.048 \pm 0.029$ g/L) showed intermediate values, ranging from 0.02 to 0.11 g/L (Figure 4). Malic acid ($M = 0.101 \pm 0.134$ g/L) was found in birch trees A and B at concentrations below 0.39 g/L and in concentrations equal to zero in birch trees C (Figure 5). Succinic and acetic acids ($M = 0.009 \pm 0.032$ g/L and $M = 0.001 \pm 0.005$ g/L, respectively) were not major components of birch sap; they were detected only in grove "C", in one tree (succinic acid) and three trees (acetic acid), with concentrations consistently below 0.14 g/L.

3.3 Water potential

For all locations and times of measurement of water potential, the mean values ranged from −0.23 MPa to −0.77 MPa (Figure 6). It could be observed that, regardless of the sap extraction treatment

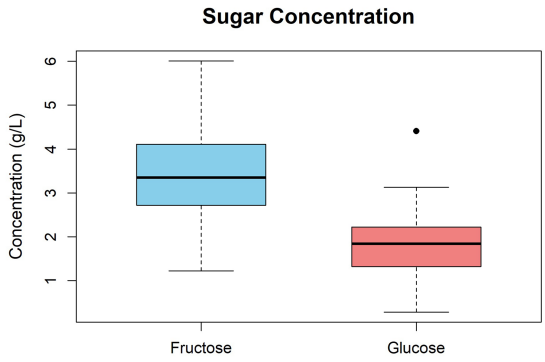


FIGURE 3
Box-and-whisker plots showing the distribution of fructose and glucose concentrations (g/L) in birch trees (n = 34). Boxes represent the interquartile range (IQR), the horizontal line indicates the median, and whiskers indicates the standard deviation. Individual points denote outliers.

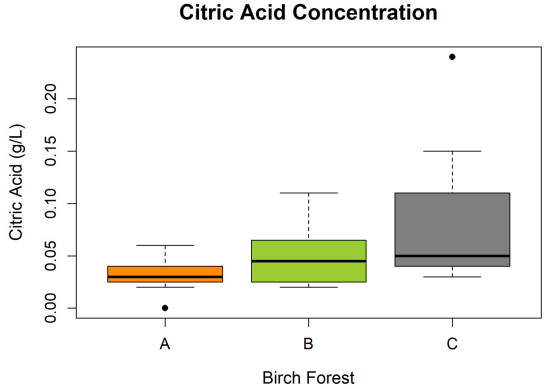


FIGURE 4
Box-and-whisker plot of citric acid concentration (g/L) in the three birch groves: **(A)** Vivero de Omaña (n = 12), **(B)** Fasgar (n = 12), and **(C)** Murias de Paredes (n = 10). Boxes represent the interquartile range (IQR), the horizontal line indicates the median, and whiskers indicates the standard deviation. Individual points denote outliers.

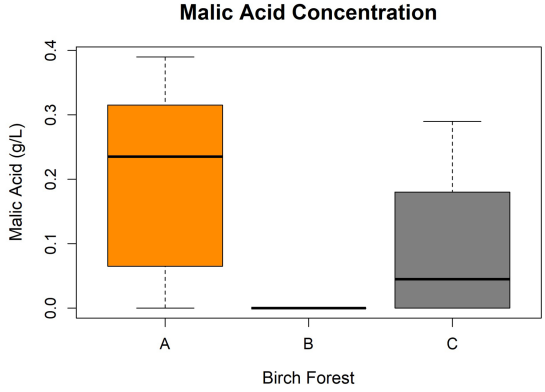


FIGURE 5
Box-and-whisker plot of malic acid concentration (g/L) in the three birch groves: **(A)** Vivero de Omaña (n = 12), **(B)** Fasgar (n = 12), and **(C)** Murias de Paredes (n = 10). Boxes represent the interquartile range (IQR), the horizontal line indicates the median, and whiskers indicates the standard deviation.

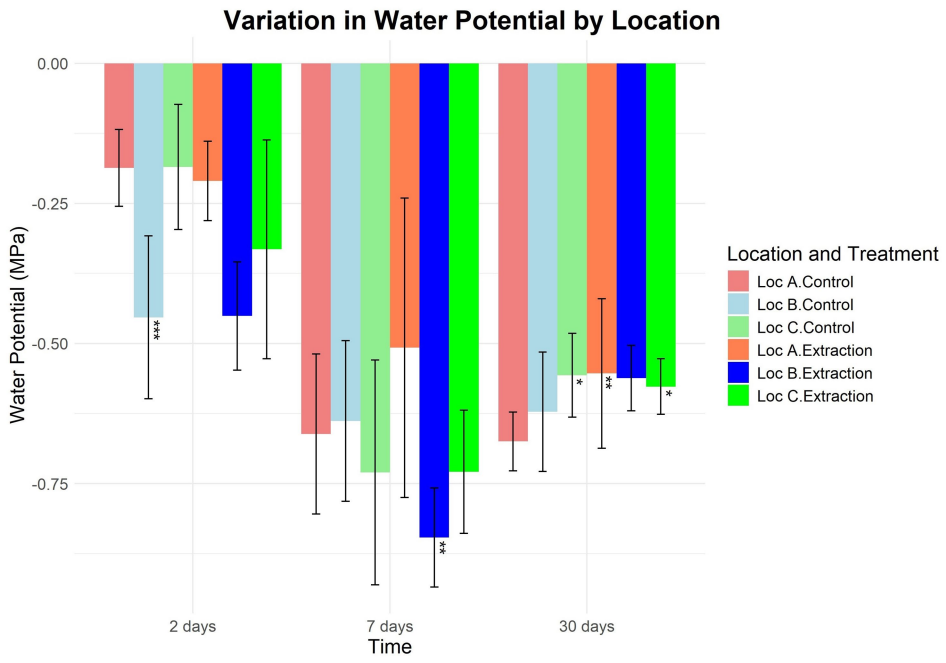


FIGURE 6
Variation of water potential (MPa) in different locations and treatments over time. The average values of water potential are presented with their respective error bars. The treatments correspond to "Blank" (no extraction) and "Extraction," and were applied in three different locations: A, B, and C. Asterisks (*) show results with significant differences $p < 0.05$, (**) highly significant $p < 0.01$, and (***) very highly significant $p < 0.001$.

used, the water potential decreases after 1 week and 1 month, compared to the value at the beginning of the study.

Two days after sap extraction, no significant differences were found between the extracted birches and the control birches. However, it was observed that birch B had significantly lower water potential levels compared to birch A ($p = 0.0005$). At 1 week from the start of tapping, a non-significant increase in the water potential of trees with sap tapping was observed in birch grove A ($p = 0.0849$). One week after the star of tapping, the water potential of the extracted trees in Grove B was significantly lower ($p = 0.0057$) than that of the control trees. However, no significant differences in water potential were found when the control and extracted trees from A and C groves were compared (see Figure 6).

At 1 month, a significant increase in the mean water potential was observed in trees subjected to sap extraction in grove A ($p = 0.0085$). Additionally, control trees at grove C exhibited significantly higher water potential than those at grove A ($p = 0.0249$). The interaction term for extraction and grove C was also significant ($p = 0.0304$), indicating that the effect of sap extraction on water potential differed between localities A and C.

Detailed results of the generalized linear model (GLM) for water potential at 48 h, 1 week and 1 month as a function of treatment and location are in Table 1 through Appendix 3 in Supplementary material.

3.4 Chlorophyll fluorescence

Extracted sap showed a marginally positive relationship for both maximum quantum yield ($p = 0.0669$) and effective quantum yield ($p = 0.0590$) (Figure 7). For both parameters, there are significant differences between locations, with higher values observed in birch grove "B"' and lower values in birch grove "A", in general.

Detailed results of generalized linear models (GLM) for maximum quantum yield and effective quantum yield as a function of sap amount and locality are in Table 2 through Appendix 3 in Supplementary material.

TABLE 1 Comparative table of the Akaike Information Criterion (AIC) with the different GLM models for each physiological parameter studied.

Group 1 models	AIC
GLM Treatment*Locality~Wat. Pot. 48 h	−61.27*
GLM Extracted sap*Locality~Wat. Pot. 48 h	−56.26
GLM Treatment*Locality~Wat. Pot. 1 week	−25.41*
GLM Extracted sap*Locality~Wat. Pot. 1 week	−19.61
GLM Treatment*Locality~Wat. Pot. 1 month	−95.26*
GLM Extracted sap*Locality~Wat. Pot. 1 month	−89.25

Group 2 models	AIC
GLM Treatment + Locality~Wat. Pot. 48 h	−61.77
GLM Extracted sap + Locality~Wat. Pot. 48 h	−59.72
GLM Treatment + Locality~Wat. Pot. 1 week	−20.60
GLM Extracted sap + Locality~Wat. Pot. 1 week	−20.51
GLM Treatment + Locality~Wat. Pot. 1 month	−93.88
GLM Extracted sap + Locality~Wat. Pot. 1 month	−92.15

The GLMs chosen for use in the present work are marked with an asterisk (*).

4 Discussion

The results obtained in this study of sap quantity per tree per day (1.3 L/day) differs from other studies, for example, in Germany a sap quantity of 8.5 L/day was obtained (Westhoff et al., 2008), in Lithuania 6.3 L/day (Mingaila et al., 2020), in Romania with *B. pendula* it is 0.9 L/day (Enescu, 2017) and 3.5 L/day (Peev et al., 2010) and in Russia, with *B. pendula* and *B. pubescens* it was 4.5 L/day (Zyryanova et al., 2010). It is important to emphasize that the diameter of the birch trees used in this

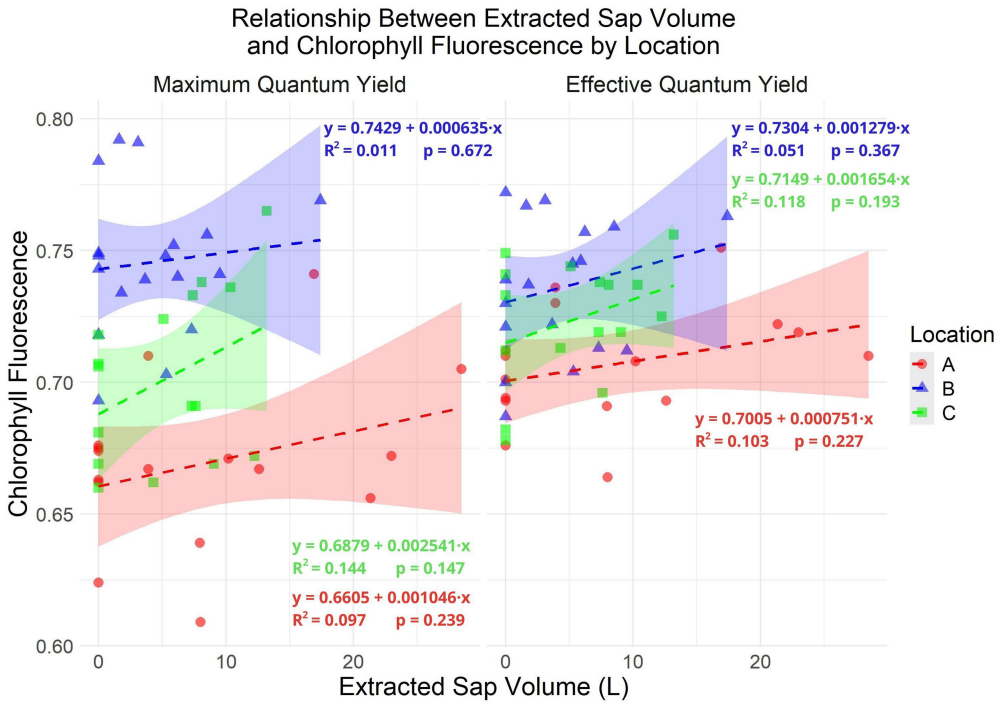


FIGURE 7
Relationship between extracted sap volume (L) and chlorophyll fluorescence parameters in *Betula celtiberica* at three study locations (A, B, and C). Maximum quantum yield is shown in the left panel and effective quantum yield in the right panel. Points represent individual trees, colored by location. Dashed lines indicate linear regression fits for each location, with shaded areas representing 95% confidence intervals. Regression equations, coefficients of determination (R^2) and *p*-values are shown for each location.

study was smaller (DBH: 14–20 cm) than in the other works. Considering that diameter is directly proportional to the amount of sap extracted (Enescu, 2017), larger amounts of sap can likely be extracted by choosing birch trees with larger diameters. Moreover, a large variability in the amounts of sap extracted has been observed among the different birches studied, ranging from 0.2 L to 4 L per day. This suggests that, apart from tree diameter, other factors may be limiting in sap extraction, such as height, number of trunk branches or distance between individuals (Staniszewski et al., 2020; Westhoff et al., 2008). On the other hand, there were no significant differences in the number of punctures made for sap extraction, suggesting that a single puncture per tree is sufficient (Bilek et al., 2015) and thus also reduces the likelihood of bacterial and/or fungal infections of the tree (Trummer and Malone, 2008).

Regarding sap components, previous studies state that the concentration of sugars and organic acids varies during the extraction season (Kallio and Ahtonen, 1987; Semjonovs et al., 2014), which could explain the differences observed between this study and the others. On the other hand, the concentration of citric acid (0.052 g/L), does fall within the parameters established by other studies, such as the one carried out in Finland with <0.1 g/L in *B. pubescens* (Kallio and Ahtonen, 1987) and Korea with 0.04 ± 0.001 g/L in *B. platyphylla* var. *japonica* (Miq.) H. Hara (currently *B. pendula* subsp. *mandshurica* (Regel) Ashburner and McAll.) (Jeong et al., 2013). Other organic acids, such as malic, succinic and acetic, presented low mean concentrations (0.101, 0.009 and 0.001 g/L, respectively) and were not found in all the studied individuals nor the three studied birch trees, as in other previous studies (Carpintero et al., 2023; Jeong et al., 2013; Kallio et al., 1987). The glucose concentration showed mean values of 1.83 ± 0.86 g/L. A similar concentration has been reported in Korea for *B. platyphylla* var. *japonica* sap (2.5 g/L, Jeong et al., 2013), while another study in Finland with *B. pubescens* found 2.5–4.3 g/L (Kallio et al., 1987), partially overlapping with the present results. Lower glucose levels (0.54 g/L) were documented by Carpintero et al. in *B. pendula* sap collected only 33 km north-east of our study area (Asturias, Spain). In contrast, markedly higher concentrations have been reported in other *B. pendula* studies, including 4.46 g/L in Latvia (Kūka et al., 2013), 3.32 g/L (Semjonovs et al., 2014), 3.8–4.7 g/L in Finland (Kallio et al., 1985), and 4.45 g/L in Poland (Bilek et al., 2016). Such variability

TABLE 2 Comparative table of the Akaike Information Criterion (AIC) with the different GLM models for each physiological parameter studied.

Maximum quantum yield				
	Coefficient	Standard error	*t*-value	*p*-value
Intercept	0.6590	0.0092	71.4374	<0.001***
Quantity of sap (L)	0.0012	0.0007	1.8766	0.0690
Locality B	0.0814	0.0105	7.7464	<0.001***
Locality C	0.0359	0.0106	3.3766	0.0015**
Effective quantum yield				
Intercept	0.6984	0.0073	96.0540	<0.001***
Quantity of sap (L)	0.0010	0.0005	1.9362	0.0590
Locality B	0.0331	0.0083	4.0021	0.0002***
Locality C	0.0200	0.0084	2.3905	0.0210*

The GLMs chosen for use in the present work are marked with an asterisk (*).

among studies may be related to species-specific traits as well as environmental and edaphoclimatic differences across regions. Fructose presented mean concentrations (3.46 ± 1.1 g/L) similar to those reported by Carpintero et al. (2023) in Asturias (3.5 g/L) which are also comparable with studies with *B. pendula* in Latvia (5.39 g/L, Kūka et al., 2013, 4.67 g/L, Semjonovs et al., 2014), Finland (3.5–4.5 g/L, Kallio et al., 1987) and Poland (4.7 g/L, Bilek et al., 2016); with *B. platyphylla var. japonica* in Korea (3.3 g/L, Jeong et al., 2013); and with *B. pubescens* in Finland (2.3–4.3 g/L, Kallio et al., 1985). In general, it is observed that the studied concentrations of organic acids and sugars in the sap of *B. celtiberica* do not differ from those obtained in other studies with other species of the genus *Betula*. Therefore, no clear differences between species and/or varieties of the genus can be established (Grabek-Lejko et al., 2017). Variations in sap composition could be due to environmental factors, genetics, different extraction techniques or even different laboratory analysis techniques. In addition, further analysis of the sap composition of the studied territory would be necessary to include more components, such as amino acids, proteins and inorganic ions.

As for water potential, values closer to 0 indicate a better water status; therefore, well hydrated trees, but more negative values reflect a higher degree of dehydration. In this study, values were obtained in a range between −0.08 and −1.05 MPa. According to Gu et al. (2007), these values would represent well-hydrated specimens since the hydric deficit would occur with values below −1.5 MPa. This would indicate that, during the time period used for water potential measurement, no birch reached the critical water stress level. It is worth mentioning that temporal variability in water potential was observed, possibly related to tree phenology. At 2 days, the values recorded were close to 0 MPa, coinciding with the state of vegetative rest. At 1 week, a slight decrease in water potential was observed, coinciding with the moment when most of the trees were already showing buds close to bud break, which could be attributed to an increase in water demand. Finally, after 1 month, the water potential stabilized while the trees were fully leafed and likely prepared for optimal transpiration and photosynthesis. According to this physiological parameter, no significant effects of the impact of sap extraction on birch trees were observed during the first 48 h, although trees at location B showed more negative water potential values, suggesting that they suffered greater water stress than those at locations A and C. At 1 week, water potential values continued to decline, especially at location B, possibly due to less favorable starting conditions. One month after sap extraction, a significant recovery of water potential was observed, suggesting that the birches studied were able to adjust their water balance during this period.

Concerning chlorophyll fluorescence, the ranges of values obtained for maximum (0.61–0.79) and effective (0.66–0.77) quantum yields are within the thresholds established for plants without any stress by other authors, such as in *B. pendula* (Akinyemi et al., 2024) and *Corylus avellana* L., a species belonging to the Betulaceae Gray family (Catoni et al., 2019). No significant effect of extracted sap volume was observed on either maximum quantum yield ($p = 0.067$) or effective quantum yield ($p = 0.059$) but the effect of locality was observed, suggesting that other environmental or edaphic factors could be modulating this parameter in birch trees, for example, it has been observed that trees subjected to irradiance stress, present lower quantum yields (Sellin et al., 2008). It is noteworthy that trees from which a greater volume of sap was extracted showed a proportionally higher quantum yield and although the cause of this relationship is not clear, this suggests that those trees with a better general physiological state have higher aquatic yields and greater sap availability.

5 Conclusion

Based on the physiological parameters considered in this study, no indications of stress were detected in birch trees 1 month after sap extraction. In addition, the composition of the extracted sap was comparable to that reported in other regions where birch sap is traditionally exploited, suggesting similar potential uses. These results should be interpreted in the context of the seasonal nature of birch sap flow, which is restricted to a short phenological period prior to budburst. While no short-term physiological effects were observed within this timeframe, the limited duration of sap flow and the one-year scope of the study constrain the evaluation of possible cumulative impacts. Further multi-year studies will therefore be required to assess the long-term sustainability of repeated sap extraction in *Betula celtiberica*.

This work contributes to the definition of a list of good practices for sustainable birch sap extraction.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://github.com/EstrellaAlfaroSaiz/Betula-data/blob/74506ad063e678540edea13d5d0b7e66292601d6/Dataset.xlsx.

Author contributions

EA-S: Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. PC: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. BC-G: Investigation, Methodology, Supervision, Visualization, Writing – review & editing. SR-F: Investigation, Methodology, Supervision, Visualization, Writing – review & editing. AE: Investigation, Supervision, Validation, Writing – review & editing. SL: Investigation, Methodology, Supervision, Writing – review & editing. AP-G: Investigation, Methodology, Supervision, Writing – review & editing. RD-A: Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. CA: Methodology, Supervision, Writing – review & editing. AF-S: Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The work was funded by the project Somos Agua II: Research applied to the development and

diversification of the bioeconomy associated with traditional knowledge and other endogenous resources of the Reserva de la Biosfera de los Valles de Omaña y Luna (RBVOyL) which is supported by the Fundación Biodiversidad of the Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO) in the framework of the Plan of Recovery, Transformation and Resilience (PRTR) funded by the European Union - NextGenerationEU.

Acknowledgments

Thanks to the students, Ana Paula Melero Castellot and Clara Rubio Reinares, for their assistance in the fieldwork. To Javi, from "Esencias de Luna", for teaching us everything he knew about sap extraction and accompanying us in our first steps. To the staff of the Murias de Paredes Town Hall, for welcoming us into their home and making our stay more comfortable, especially Mª del Carmen Mallo Álvarez., Pilar Álvarez, Antonio Sabugo Valcarce, as well as the staff of the Fasgar Neighborhood Council, Félix García Rubio and Rosa María Fernández. Last but not least, thanks to the staff at RBVOyL, our partners in Somos Agua II project, especially Natalia Castro Nicolás, Estela González Román y Guillermo Castiñeira Lera.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2026.1715128/full#supplementary-material

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