



Effect of Different Tree Plantations on the Chemical Properties and Microbial Activity in Galician Forests Soils

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OPEN ACCESS

Edited by:

Isabel Miralles Mellado,
University of Almería, Spain

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Received: 29 May 2025

Accepted: 14 October 2025

Published: 24 October 2025

Citation:

Barreiro A, Míguez-González A, Cela-Dablanca R, Díaz-Raviña M, Núñez-Delgado A, Fernández-Sanjurjo MJ and Álvarez-Rodríguez E (2025) Effect of Different Tree Plantations on the Chemical Properties and Microbial Activity in Galician Forests Soils. *Span. J. Soil Sci.* 15:14988. doi: 10.3389/sjss.2025.14988

Forest soils are crucial carbon sinks, with the soil microbial community playing a key role in the stabilization of organic matter and harboring numerous ecosystems services. These ecosystems can be affected, among other factors, by the different tree species present in the forest canopy. This study is focused on forests soils located in Galicia (on the north-west of Spain). Different soil properties and microbial activity were analyzed in 54 forest plantations, with different plant covers: birch, chestnut, eucalyptus, walnut, pines, oak and shrublands. These forest soils have in general an acid pH and a high organic matter content, but a small amount of phosphorus. These properties are mainly related to the parent material and the overall climatic conditions of this region, namely the high rainfall scores. The soil under eucalyptus and birch plantations were the driest (13% and 14% on average respectively) vs. the moistest with 27% on average under shrublands. The results regarding microbial activity showed that soils under walnuts have the biggest respiration rates whereas the smallest were under eucalyptus but there were no differences regarding the β -glucosidase enzyme activity. These results show that the forest management, specifically, which tree species are cultivated, has an impact on the soil microbial respiration and should be considered when elaborating forestry exploitation plans, especially in the current scenario of climate change where the C amount that healthy forest soils will be able to fix become crucial.

Keywords: forestry management, forest soils, soil respiration, β -glucosidase, soil C

INTRODUCTION

Forests all over the world are endangered by different factors such as fragmentation, landscape change, deforestation, pollution or inadequate management (Birdsey and Pan, 2015; Tin et al., 2018; Ruiz-Chután et al., 2025), having detrimental effects on biodiversity (Díaz-Martin and Karubian, 2021) or even on climate change (Delabre et al., 2020). These ecosystems are crucial carbon sinks (Pandey, 2002), both above and below-ground, even though the soil section is still sometimes excluded from the C pools estimations, despite the enormous amount of C stored in it (Robinson, 2007). The soil microbial community plays a key role in the stabilization of organic matter in the forest soil systems, even acting as a C sink in the case of fungi (Cairney, 2012), and harbors numerous ecosystems services (Delgado-Baquerizo et al., 2016).

The soils of Galicia are generally acidic as a result of a humid climate and a strongly subtractive system, which lead to intense leaching and, frequently, an acidic parent material (Macías et al., 1982; Macías, 1986). Furthermore, vegetation plays an important role in determining the physicochemical and biological soil properties (Chandra et al., 2016; Waymouth et al., 2020; Sui et al., 2022). Therefore, it would be crucial to increase the understanding of the effect due to different tree species on soil properties in order to select the most appropriate plants for a given area.

Globally, forest structure has been subjected to enormous changes in the last decades, with a marked decrease in extension since 1990, whereas the area occupied by forest plantations has increased (Birdsey and Pan, 2015). The species used for the plantations differed depending on the specific pedoclimatic conditions, but in temperate regions natural broadleaved forests have been replaced by trees mainly belonging to the family Pinaceae (Sawada et al., 2021) or Eucalyptus (Tomé et al., 2021). Some authors have noted that natural forest stands have better control of the nutrient cycle and superior soil quality than forest plantations (Chandra et al., 2016; Sui et al., 2022). Soil degradation processes can be related to the conversion from natural to plantation forest (Widyati et al., 2022), involving C and N concentrations decrease (Liao et al., 2012) or modifications in both bacterial and fungal soil communities (Sawada et al., 2021; Wang Q. et al., 2021; Sui et al., 2022).

Several studies have been conducted to understand the interrelationship between tree species, soil microbiota, and the physicochemical properties of forest soils (Mahía et al., 2006; Álvarez et al., 2009; Chandra et al., 2016). The forest soil microbiome can be affected by the different forestry activities such as logging and clear-cutting (Hartmann et al., 2014; Chen et al., 2021), irrigation (Hartmann et al., 2017), fertilization (Addison et al., 2021) or canopy disturbance (van Nuland et al., 2020). Contrasting results have been published for other practices like thinning, with some authors describing a negative impact of this practice on microbial carbon use efficiency (Xue et al., 2023), meanwhile others did not detect and impact on the soil fungal community (Castaño et al., 2018).

The natural primary drivers of the soil microbiome in forest include soil composition and nutrient availability, plant community structure, microbial interactions within the soil, disturbances, succession, and temporal dynamics (Onet et al., 2025). One of the key factors that determines the soil microbial community composition and function of forest soil is the different tree species present in the forest canopy, since a high microhabitat specificity of bacterial communities interacting with forest type has been described (Rodríguez-Rodríguez et al., 2023).

The current study is focused on the analysis of the effect of different forest species on the soil microbial activity and soil properties of forests located in Galicia (on the north-west of Spain) with a temperate-oceanic climate, which favors that the forests in this region are very productive, and this has a clear impact in the tree species that grow on then naturally and the species planted with commercial purposes. We hypothesized that the variability on the different physicochemical and biological soil

parameters will be determined by the different tree species, with the bigger differences between the native broadleaf species and the intensive plantations (pines or eucalyptus), in soils developed over the same parent material and under the same climatic conditions.

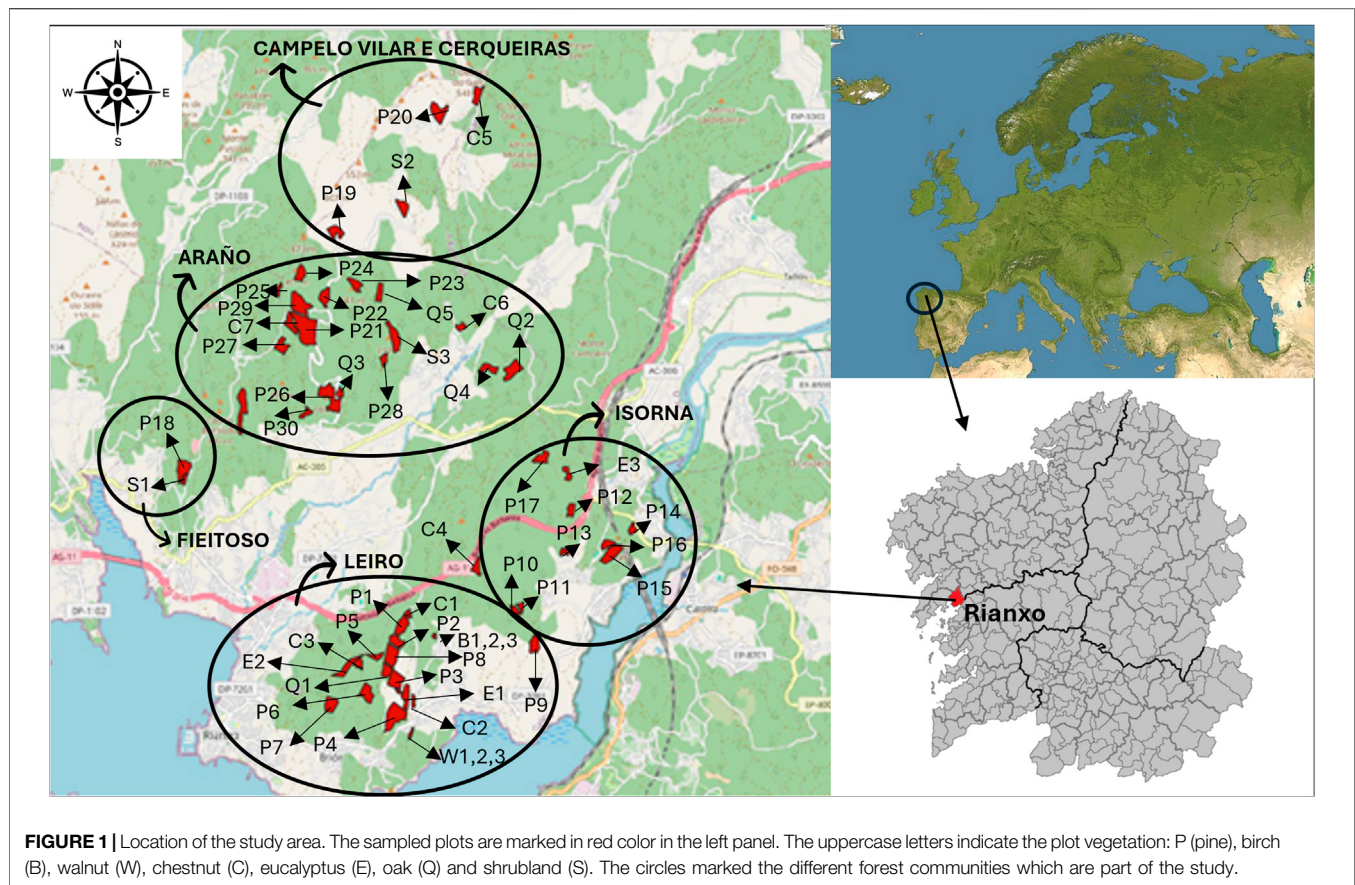
MATERIALS AND METHODS

Study Area and Soil Sampling

The sampling area is located in Rianxo municipality (Galicia, north-west of Spain, **Figure 1**). The climatic classification applied to this region defines the bioclimate region as typical temperate, oceanic and superior thermo-temperate (Rodríguez-Gutián and Ramil-Rego, 2007). The average temperature in the study area in 2024 was 15.7 °C with an accumulated precipitation of 1774 L m², according to a weather station located in the area (42.580074 | -8.804707 WGS84) belonging to Meteogalicia (Xunta de Galicia meteorological network). The parent material in this area is mainly granitic, and the soils are mostly classified as Leptosols and Umbrisols (WRB IUSS Working Group, 2022).

The type of property of these forests is communal, meaning that villagers are the group owners of specific areas of the forest usually near their village, which entails different challenges such as obtaining authorizations to access the property. The sampling was performed in collaboration with the forest owner associations in five different forest communities (Campelo Vilar e Cerqueiras, Araño, Fieitoso, Isorna and Leiro) within Rianxo municipality. Totally, 54 different forest plots were selected with different plantations established, 4 in Campelo Vilar e Cerqueira, 17 in Araño, 2 in Fieitoso, 9 in Isorna and 22 in Leiro (**Figure 1**). The number of replicates sampled differed between the different tree species, depending on what was available in the studied area and the agreement with the forest owners' associations to sample, but the minimum number was three.

Pinus was one of the most representative forest plantations in this area and 30 soil samples under this tree type were collected along the five forest communities (**Figure 1**). The pine stands had different ages: 4 *Pinus pinaster* stands located in Isorna were 40–45 years old; 10 *P. pinaster* and 2 *Pinus radiata* stands, dispersed through the sampling area, were 14–17 years old; and 14 samples, also dispersed, were recent *P. pinaster* plantations (2–4 years old) (**Figure 1**; **Table 1**). The non coniferous species were less represented in this area and the following forest plantations were sampled: 3 soil samples under 4-year old birch plantations (*Betula spp*) and 3 under 4-years old walnut plantations (*Juglans regia*), both located in Leiro; 7 soil samples under 1–17 years old chestnut plantations (*Castanea x hybrida*) located in Leiro, Campelo and Araño; 3 soil samples under 6–14 years old eucalyptus plantations (*Eucalyptus globulus*) located in Leiro and Isorna; 5 soil samples under 2–17 years old oak plantations (mixed of *Quercus rubra* and *Quercus robur*) located in Leiro and Araño; and 3 soil samples under shrublands (*Ulex europaeus*, *Pteridium aquilinum*, *Erica cinerea*, *Pterospartum tridentatum*, *Callunavulgaris*, *Davoecia cantabrica* and *Cytisus scoparium*) located in Fieitoso, Campelo and Araño (**Figure 1**; **Table 2**). Totally, 30 samples



were collected under coniferous forest, 18 under deciduous forest, 3 under eucalyptus and 3 under shrubland at different altitudes (50–485 m above the sea level) (Tables 1, 2).

The soil depth, estimated during the soil sampling, was 50–150 cm, being the deeper soils under the more mature forest stands (Tables 1, 2). This area has a history of wildfires, specifically the communities of Leiro and Isorna which had an important fire event in 2019. The management of the plots in the last 5 years is also shown in Tables 1, 2. In each of the 54 forest stands, 10 subsamples were collected in a zig-zag transect making a composite sample per plot, sampling the 0–20 cm depth soil layer with an auger in the summer of 2024. The samples were immediately transported to the laboratory, sieved (<2 mm) and hand homogenized (quartering method, 5 times). A sub-sample was stored at 4 °C for the microbial analysis, and the rest was dry at 40 °C for the analysis of the soil physicochemical properties.

Soil Physicochemical and Microbial Analyses

Soil physicochemical properties were analyzed using standard procedures (Gutián Ojea and Carballas, 1976; Tan, 1986). Soil texture was determined using the international method of Robison Pipette. Soil water content was determined by the gravimetric method, where soils were oven-dried at 105 °C until constant weight. The pH values were obtained in water (1:2.5), using a pH-

meter CRISON, model 2001. Total C and N were determined by elemental analysis using LECO equipment, model TRUSPEC CHNS. Extractable P was measured in soil samples using the Olsen method (Olsen and Sommers, 1982). Soil exchange cations (Ca^{2+} , Mg^{2+} , Na^{+} , K^{+} , Al^{3+}) were extracted using NH_4Cl 1 mol L^{-1} (Peech et al., 1947) in 1:10 solution. Ca^{2+} , Mg^{2+} and Al were measured by atomic absorption spectrophotometry; Na^{+} and K^{+} were quantified using emission spectrophotometry. The sum of all these exchange cations was considered the effective cation exchange capacity (eCEC) (Kamprath, 1970).

Regarding microbial analysis, soil microbial activity was estimated by the respiration rate (CO_2 production) by transferring 1 g of soil to a 20 mL glass vial, which was then sealed and incubated for 24 h at room temperature and without light. The CO_2 concentration was determined using a gas chromatograph equipped with FID and using a standard of CO_2 . Additionally, the β -glucosidase soil enzyme activity, one enzyme from the C-cycle, was analyzed according to the method of Eivazi and Tabatabai (1988). Briefly, the released p-nitrophenol was determined colorimetrically after incubation of the soil with p-nitrophenyl β -D-glucopyranoside for 2 h at 37 °C.

Data Analysis

The effect of the different forest cover was analyzed by means of standard one-way ANOVA. In cases of significant F statistics, Tukey's significant difference test was used to separate the means.

TABLE 1 | Environmental properties of the pine (P) plots.

Forest community	Trees	Age (years)	Altitude (masl)	Parental rock	Soil depth (cm)	Wildfires (Years)	Management (last 5 years)
Leiro	P1	3	160	Gneis	50	2019	Plantation
	P2	4	180	Gneis	50	2019	-
	P3	3	170	Schists/Gneis	50	2019	Plantation
	P4	14	125	Schists/Gneis	100	-	-
	P5	15	145	Schists/Gneis	50	-	-
	P6	5	110	Schists/Gneis	20	2019	-
	P7	4	90	Granite	50	2019	-
	P8	3	180	Granite	50	2019	Plantation
	P9	14	115	Granite	100	-	-
Isorna	P10	3	175	Granite	50	2019	Plantation/Clearing
	P11	3	140	Granite	50	2019	Plantation/Clearing
	P12	3	105	Granite	100	2019	Plantation/Clearing
	P13	40	50	Granite	150	-	Clearing
	P14	45	65	Granite	150	-	-
	P15	45	70	Granite	150	-	-
	P16	45	80	Granite	150	-	-
	P17	14	165	Granite	100	-	-
Feitaso	P18	17	155	Schists/Gneis	100	-	Clearing, acacia removing, extensive grazing
Campelo	P19	14	450	Granite	50	-	Clearing, acacia removing
	P20	2	485	Granite	50	-	Plantation
Araño	P21	17	290	Granite	100	-	Clearing, trituration
	P22	2	330	Granite	50	-	Plantation
	P23	3	325	Orthogneis	50	-	Plantation
	P24	3	480	Granite	50	-	Plantation
	P25	3	445	Granite	50	-	Clearing, trituration
	P26	17	160	Granite	150	-	Plantation
	P27	17	315	Granite	100	-	Clearing, trituration
	P28	17	150	Granite	150	-	Clearing, trituration
	P29	17	360	Granite	100	-	Clearing, trituration
	P30	17	145	Granite	150	-	Clearing, trituration

TABLE 2 | Environmental properties of the birch (B), walnut (W), chestnut (C), eucalyptus (E), oak (Q) and shrubland (S) plots.

Forest community	Trees	Age (years)	Altitude (masl)	Parental rock	Soil depth (cm)	Wildfires (Years)	Management (last 5 years)
Leiro	B1	4	85	Granite	50	2019	Plantation
	B2	4	85	Granite	50	2019	Plantation
	B3	4	85	Granite	50	2019	Plantation
Leiro	W1	4	75	Schists/Gneis	50	-	Clearing/Plantation
	W2	4	75	Schists/Gneis	50	-	Clearing/Plantation
	W3	4	75	Schists/Gneis	50	-	Clearing/Plantation
Leiro	C1	4	150	Gneis	50	2019	Plantation
	C2	5	85	Gneis	100	2018	Plantation
	C3	3	110	Schists/Gneis	50	2019	Plantation
	C4	4	125	Granite	50	2019	Plantation
Campelo	C5	14	455	Granite	100	-	-
Araño	C6	1	125	Granite	100	-	Clearing/plantation
	C7	17	295	Granite	100	-	Clearing, trituration
Leiro	E1	6	130	Granite	50	2019	-
	E2	15	120	Schists/Gneis	50	2019	-
Isorna	E3	14	90	Granite	50	2019	-
Leiro	Q1	17	160	Schists/Gneis	50	2007/2010/2016	Plantation
Araño	Q2	2	190	Granite	50	-	Eucalyptus removal
	Q3	17	135	Granite	150	-	Clearing, trituration
	Q4	2	140	Granite	100	-	Eucalyptus removal
	Q5	17	195	Orthogneis	100	-	Clearing
Feitaso	S1	-	150	Schists/Gneis	50	-	Intensive grazing
Campelo	S2	-	375	Granite	50	-	Clearing
Araño	S3	-	200	Granite	100	-	Eucalyptus removal (2023)

TABLE 3 | Soil physicochemical properties under different types of forest cover. Average $\pm$ SE.

Forest cover	pH	SOM (%)	N (%)	C/N	P (mg kg ⁻¹)	Na ⁺ (cmol kg ⁻¹)	K ⁺ (cmol kg ⁻¹)	Al ³⁺ (cmol kg ⁻¹)	Sat Al ³⁺ (%)
Birch	4.92 $\pm$	12.9 $\pm$	0.45 $\pm$	16.5 $\pm$	0.2 $\pm$	0.15 $\pm$	0.03 $\pm$	0.07 $\pm$	63 $\pm$
Chesnut	4.93 $\pm$	15.5 $\pm$	0.54 $\pm$	17.0 $\pm$	1.1 $\pm$	0.19 $\pm$	0.05 $\pm$	0.02 $\pm$	83 $\pm$
Eucalyptus	4.98 $\pm$	12.1 $\pm$	0.41 $\pm$	17.4 $\pm$	1.8 $\pm$	0.14 $\pm$	0.03 $\pm$	0.13 $\pm$	72 $\pm$
Walnut	5.06 $\pm$	20.9 $\pm$	0.4 $\pm$	17.6 $\pm$	0.1 $\pm$	0.14 $\pm$	0.02 $\pm$	0.05 $\pm$	75 $\pm$
Oak	4.72 $\pm$	15.4 $\pm$	0.61 $\pm$	14.8 $\pm$	0.8 $\pm$	0.15 $\pm$	0.04 $\pm$	0.05 $\pm$	79 $\pm$
Shrubland	4.63 $\pm$	14.2 $\pm$	0.54 $\pm$	15.4 $\pm$	1.6 $\pm$	0.09 $\pm$	0.01 $\pm$	0.02 $\pm$	90 $\pm$
Pine	4.75 $\pm$	15.1 $\pm$	0.54 $\pm$	16.3 $\pm$	0.4 $\pm$	0.14 $\pm$	0.02 $\pm$	0.01 $\pm$	86 $\pm$

There are no significant differences between groups ($p > 0.05$). Kruskal-Wallis test was used for pH and K and ANOVA test for the rest ($n = 54$).

For the properties with non-normal distribution Kruskal-Wallis test was used. The correlation between the different soil physicochemical properties and the microbial activity parameters was analyzed using Pearson correlation coefficients at the $p < 0.05$ level. The values corresponding to all the physicochemical and biological soil properties were subjected to a redundancy analysis (RDA) to elucidate the main differences between the different forest covers and explore the relationship with the environmental variables. The statistical analyses were performed using the R software package (R studio, version 4.1.0).

RESULTS AND DISCUSSION

Soil Physicochemical Properties

The dominant soil texture in this area was sandy, with 76% of soils having a sandy loam texture, 22% had a loamy sand texture and 2% were sandy soils, in accordance with the granite material from which they originate (Macías et al., 1982). These forest soils have, on average, an acid pH (between 4.63 and 5.6), a high content of soil organic matter (SOM) (between 12.1% and 20.9%), with N concentrations ranging 0.41%–0.69% and C/N ratio between 14.8 and 17.6, but containing small phosphorus concentrations (between 4.0 and 8.4 mg P kg⁻¹) (Table 3). The statistical analysis showed no significant differences for the physiochemical properties of these soils between the different forest species, but it was observed that the soils under oak, pine and shrublands had the most acidic pH, meanwhile soils under birch and eucalyptus showed the lower amounts of C, SOM and P (Table 3). The low available P content in all these forest soils can be related to the acid pH, since at these values P is retained by the variable charge soil components which are positively charged, as happens for most of the forest soils in Galicia (García-Rodeja and Gil-Sotres, 1997; Eimil-Fraga et al., 2014).

These properties are mainly related to the climatic conditions of this region, namely the elevated precipitation since the humid climate favors the process of leaching and acidification, and are also due to the acidic nature of the parent material (Macías et al., 1982; Macías, 1986; García-Rodeja et al., 2023). There are numerous studies on the influence of different tree species on soil properties, but the results are inconsistent. Some authors point out that conifers acidify the soil and are therefore associated with degradation and podzolization processes, especially in cold regions (Matzner and Ulrich, 1983; Augusto et al., 1998; van Breemen et al., 2000, among others). More recent articles also describe a better quality of deciduous forest soils compared to coniferous forests and relate this to the quality of the leaf litter (Sui et al., 2022).

Conversely, in other areas, some authors indicate that vegetation may be a secondary factor influencing soil properties, subordinate to climate and parent material (Macías et al., 1982). Priha and Smolander (1999) state that it takes a long time for trees to cause changes in the soil, which could explain the lack of differences in the previously mentioned soil properties, since more than half of the forest stands analyzed in the present study were recent plantations (<6 years) (Tables 1, 2). To note

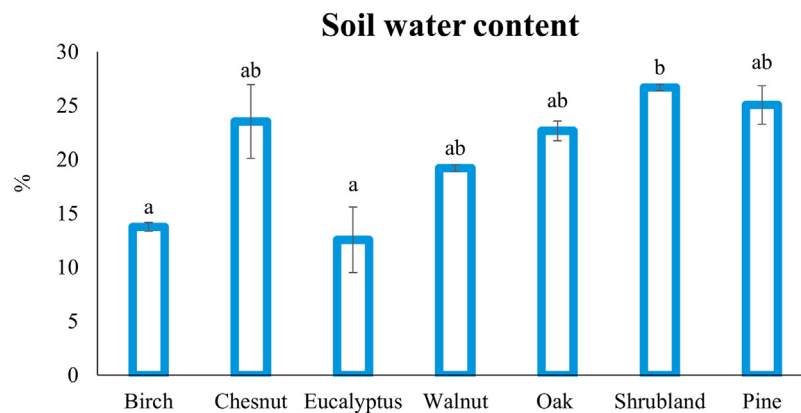


FIGURE 2 | Soil water content (%) under the different type of forest cover. Different letters indicate significant differences, Kruskal-Wallis test ($p < 0.05$) ($n = 54$).

that the soil solution is more sensitive than the solid phase for the detection of possible changes caused by anthropogenic activities (Álvarez et al., 2002; Álvarez et al., 2005). In this sense, these authors observed a significant effect of tree species on the pH of the liquid phase of Galician soils and on the most labile forms of aluminum (labile monomeric aluminum, and Al-F and Al-OH complexes), which were higher in soils under pine.

Soil moisture showed significant differences between some of the different forest plantations (Figure 2). Low soil water content was detected under eucalyptus and birch (13% and 14% on average, respectively) in contrast with higher values detected in the soils under shrubland (27%) ($p = 0.042$ and $p = 0.035$, respectively). The rest of the samples presented intermediate values, namely 19% for walnuts, 23% for oaks, 24% for chestnuts and 25% for pine (Figure 2). The ability of eucalyptus trees to extract a high amount of soil water has been described in the literature (Robinson et al., 2006), but other authors stated that eucalyptus water depletion is a myth and is more related to an inappropriate management of the plantation than to the trees themselves (Medeiros et al., 2025). The capacity of birch to decrease the water content of the topsoil has been well described, compared to coniferous trees (spruce) (Špulák et al., 2021), but in the mentioned study the stands were older than the ones in the current research.

Both birch and eucalyptus stands were established over shallow soils (~50 cm) that suffered the impact of a wildfire in 2019, whereas for the other species there were wildfire events in just some of the field replicates (pines, oaks and chestnuts) or a complete lack of these events in the case of walnut and shrubs (Table 2). If the disturbance of vegetation by fire is substantial enough, the resulting perturbations to soils can persist for years, resulting in lower water contents (Cooperdock et al., 2020). The soils under these trees showed the lower SOM content, 12.1% under eucalyptus and 12.9% under birch (Table 3) compared with the other species. The low water content of these samples might be related to the fire event, the soil management for the tree's establishment or the low SOM content which would increase water retention under the other species (Rawls et al.,

2003). Indeed, we found a significant positive correlation between soil water content and soil organic matter ($r = 0.29$, $p < 0.05$) (Figure 3).

The bigger soil moisture in the soils under shrubland might be related to the afforestation process in terms of tree water consumption (Herron et al., 2022; Farley et al., 2004), being the differences in tree physiology, plantation design and management and the forestry operations the factors that affect the most to forest hydrology (van Dijk and Keenan, 2007). The permanent soil cover in the shrubland and the shrub roots (Gao et al., 2021) favor the high-water content in those soils. The lack of previous fire events in these plots (Table 2) might be another positive factor for the biggest soil water content.

On the other hand, the soils under shrubs showed the lowest effective cation exchange capacity, meanwhile soils under birch, oak and walnut had the highest values (Figure 4A). Conversely, other authors detected bigger eCEC scores under pine compared with oak trees in acid forest soils (Gruba and Mulder, 2015). The highest eCEC values coincide with the higher level of organic matter and/or higher pH (Table 3), which favors the increase of the negative charges in variable charge components (Gruba and Mulder, 2015). There were no differences regarding the concentration of Mg^{2+} , Ca^{2+} , Na^{+} , K^{+} and Al^{3+} (Table 3; Figures 4B,C) among the different plantations.

The amount of Al^{3+} (on average between 4.7 and 7.43 cmol kg^{-1}) was quite high, which is common in these forest granitic soils (Macías et al., 1982). These Al^{3+} concentrations represented on average between 63% and 90% saturation of the exchange complex (Table 3), with practically all samples having values > 60% Al^{3+} saturation, indicative of allic soils, that can limit plant growth (Kochian et al., 2004; Gupta et al., 2013). The percentage of Al^{3+} saturation was higher under shrublands (90%), pines (86%) and chestnut (83%), even though this difference was not significant. In addition, an elevated Al^{3+} saturation under pine has been previously described (Álvarez et al., 2002).

However, despite the high Al^{3+} saturation of these forest soils, timber production in Galicia is high in the context of the temperate-humid region (Corbelle-Rico and Tubío-Sánchez,

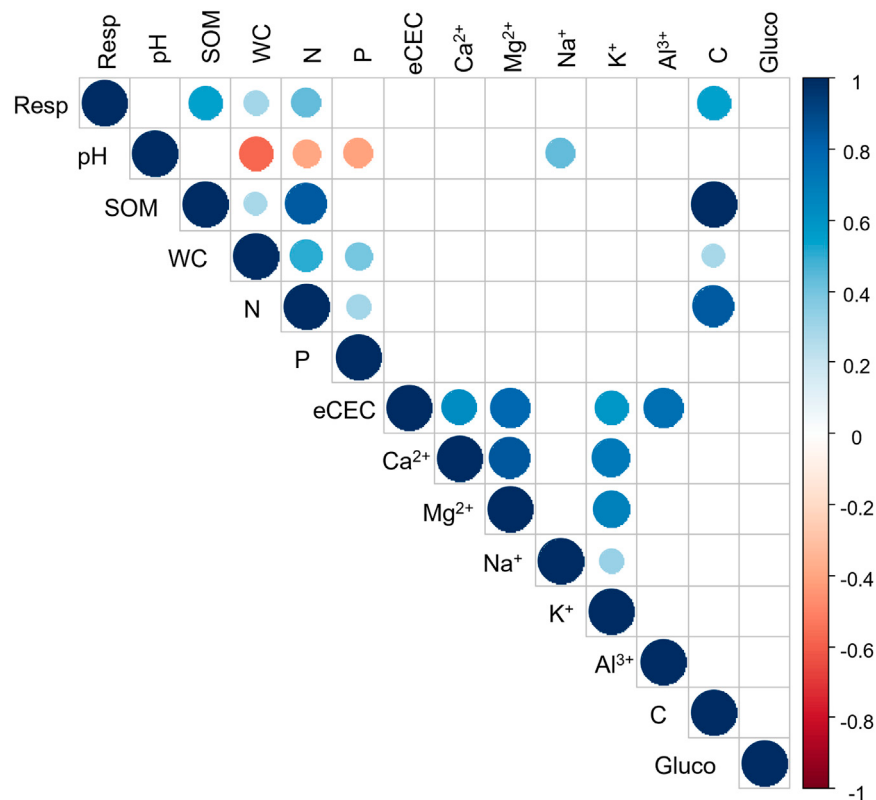


FIGURE 3 | Pearson correlation of the different soil physicochemical properties. Blue dots mean significant positive correlations and red dots mean significant negative correlations, the size of the dot is proportional of the correlation coefficient ($p < 0.05$) ($n = 54$). Resp: respiration; SOM: soil organic matter; WC: water content; eCEC: effective cation exchange capacity; Gluco: β -glucosidase activity.

2018). This may be related to different causes (species adaptation to these soils, mycorrhization, etc.), but with respect to Al^{3+} , it has been shown that organic matter plays an important role in reducing the toxicity of this element, complexing Al^{3+} in the form of polymers or monomers (Álvarez et al., 2002; Álvarez et al., 2005; Eimil-Fraga et al., 2015; Eimil-Fraga et al., 2016). In addition, anions such as sulfate or fluoride can also bind to Al (Al-SO_4 , Al-F), decreasing its toxicity (Álvarez et al., 2005; Eimil-Fraga et al., 2016).

It was also observed that soils under chestnut, shrubland and pines had the lowest concentration of Mg^{2+} and Ca^{2+} (Figures 4B,C), even though these differences were not significant. To cope with the Al toxicity some species demand large quantities of Ca^{2+} and Mg^{2+} to reach high levels of productivity (Rocha et al., 2019). In fact, the Ca/Al ratio in soils, leaves and roots has been used as an index to evaluate the toxicity of this element (Cronan and Grigal, 1995; Álvarez et al., 2005; Eimil-Fraga et al., 2016). Kinraide (2003) and Kinraide et al. (2004) referred that the addition of Mg^{2+} to the external medium relieved Al toxicity in many plants. We detected a correlation between the exchangeable Ca^{2+} and Mg^{2+} in our samples with $r = 0.85$, $p < 0.05$ (Figure 3). The same trend was described in base-poor soils by other authors (Rocha et al., 2019). The lower concentrations of Ca^{2+} and Mg^{2+} in the soil under chestnuts,

shrubland and pines could be a result of the major requirements of these plants to tolerate these high Al concentrations (Table 3).

Soil Microbial Activity

The results regarding microbial activity showed that soil under broadleaf species (birch, chestnut, walnut and oaks) have bigger respiration rates than soil under pines, shrublands and especially under eucalyptus (Figure 5B). The only significant differences, when divided at the species level, were between the soil respiration under eucalyptus and walnuts, with average values of $537 \pm 211 \text{ ppm CO}_2 \text{ g}^{-1}$ and $1440 \pm 97 \text{ ppm CO}_2 \text{ g}^{-1}$, respectively, with the soil respiration under the rest of the tree species being between those values (Figure 5A).

The soil under walnuts showed the biggest amount of SOM (20.9%) meanwhile the soils under eucalyptus had the lowest values (12.1%) (Table 3). Even though these average values were not significantly different, soil respiration was positively correlated with soil organic matter ($r = 0.54$), total nitrogen ($r = 0.43$) and total C ($r = 0.54$) (Figure 3). On the other hand, walnut leaf litter has a big amount of phenolic compounds (Mungai and Motavalli, 2006) which can inhibit Gram-positive bacteria but do not affect fungi (Pereira et al., 2007), which are the main responsible of respiration on forest soils (Fransson, 2012).

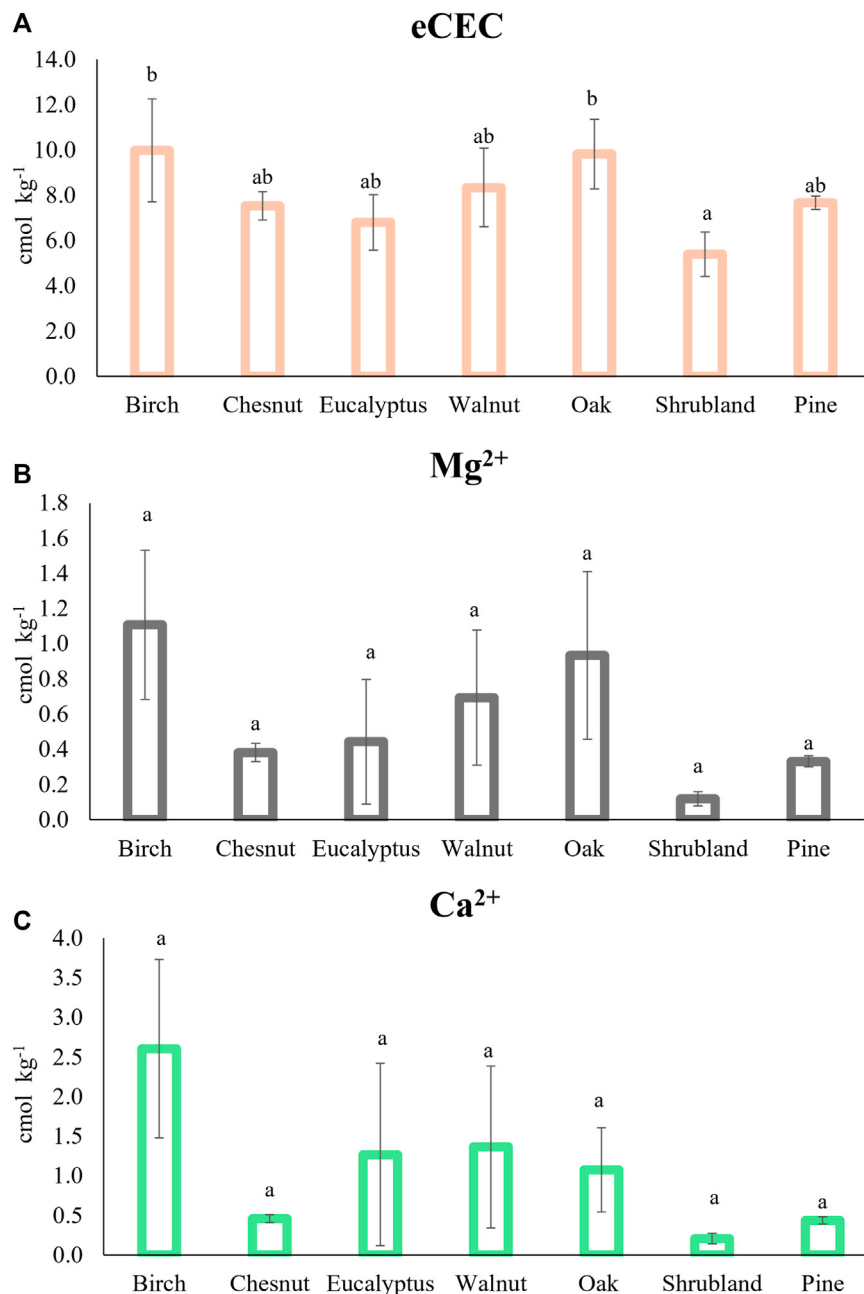


FIGURE 4 | Effective cation exchange capacity (eCEC) **(A)**, exchangeable Mg²⁺ **(B)** and Ca²⁺ **(C)** under the different the different type of forest cover. Different letters indicate significant differences ($p < 0.05$) Krustal-Wallis test was used for Mg²⁺ and Ca²⁺ and ANOVA test for eCEC ($n = 54$).

The walnut plots did not suffer wildfires recently, whereas all the eucalyptus plots were burnt in 2019, therefore the smaller values in respiration for the latter could be a consequence of the wildfire event 5 years ago. However, recent metanalysis studies reveal that generally after 5 years total soil respiration recovers to pre-fire values (Zhou et al., 2023) and specifically for temperate forests the recovery time will be around 3 years (Gui et al., 2023). In general, microbial activity changes can be transitory, and their values can reach pre-fire ones, but

diversity changes seem to be maintained at a longer time (Barreiro and Raviña, 2021).

When the values of soil respiration under de deciduous trees were pooled together (**Figure 5B**), it was observed that the respiration under eucalyptus was significantly lower than under the deciduous forests, but the coniferous values were between them. These results are in agreement with other authors (Raich and Potter, 1995; Hibbard et al., 2005) that did not find statistical differences in soil respiration between

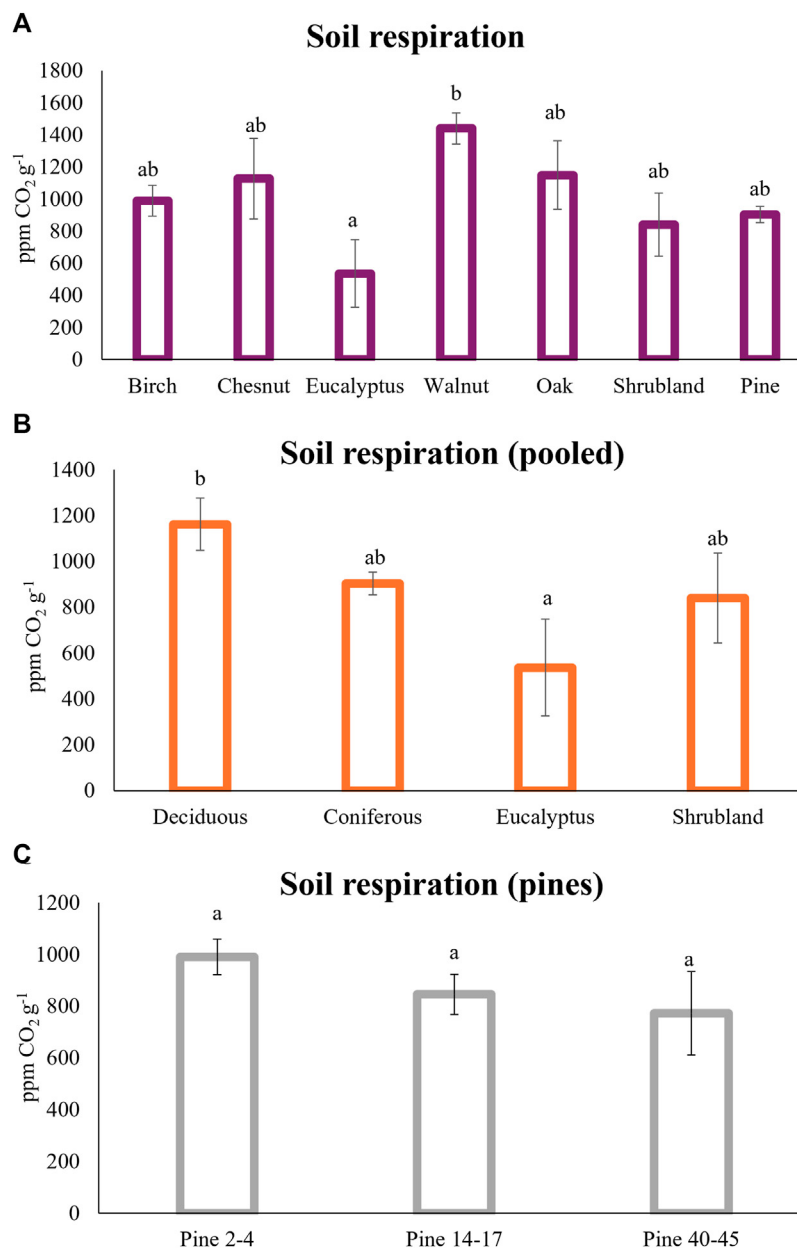


FIGURE 5 | Soil respiration (ppm CO₂ g⁻¹) under the different tree plantations **(A)**; with the deciduous species pool together (oak, birch, walnut and chestnut) **(B)**; and under pine plantations with different ages **(C)**. Different letters indicate significant differences, ANOVA test ($p < 0.05$).

coniferous and deciduous forests. The litter input provided by the deciduous trees had a strong positive impact on soil respiration (Zhang et al., 2020). Similarly, soil respiration could increase under deciduous tree species, compared with evergreen tree species, due the bigger root exudation rates and annual root exudate carbon fluxes of the deciduous trees (Wang Y. et al., 2021).

The forest plantations with lower soil water content were birch and eucalyptus, and correlations between soil respiration and soil water content were detected ($r = 0.29$, $p < 0.05$)

(Figure 3). However the soil respiration was lower under the eucalyptus plantation, meanwhile the values under birch were in the same order as the rest of the broadleaf species (Figure 5A). The dependency on the soil water content of soil respiration under eucalyptus has been described (Epron et al., 2004), and these authors also found a correlation of the soil respiration with both leaf and total aboveground litter in eucalyptus plantations but this was not analyzed in the current study. Other authors have described a modification in the microbial community structure within eucalyptus plantations, specifically a decrease

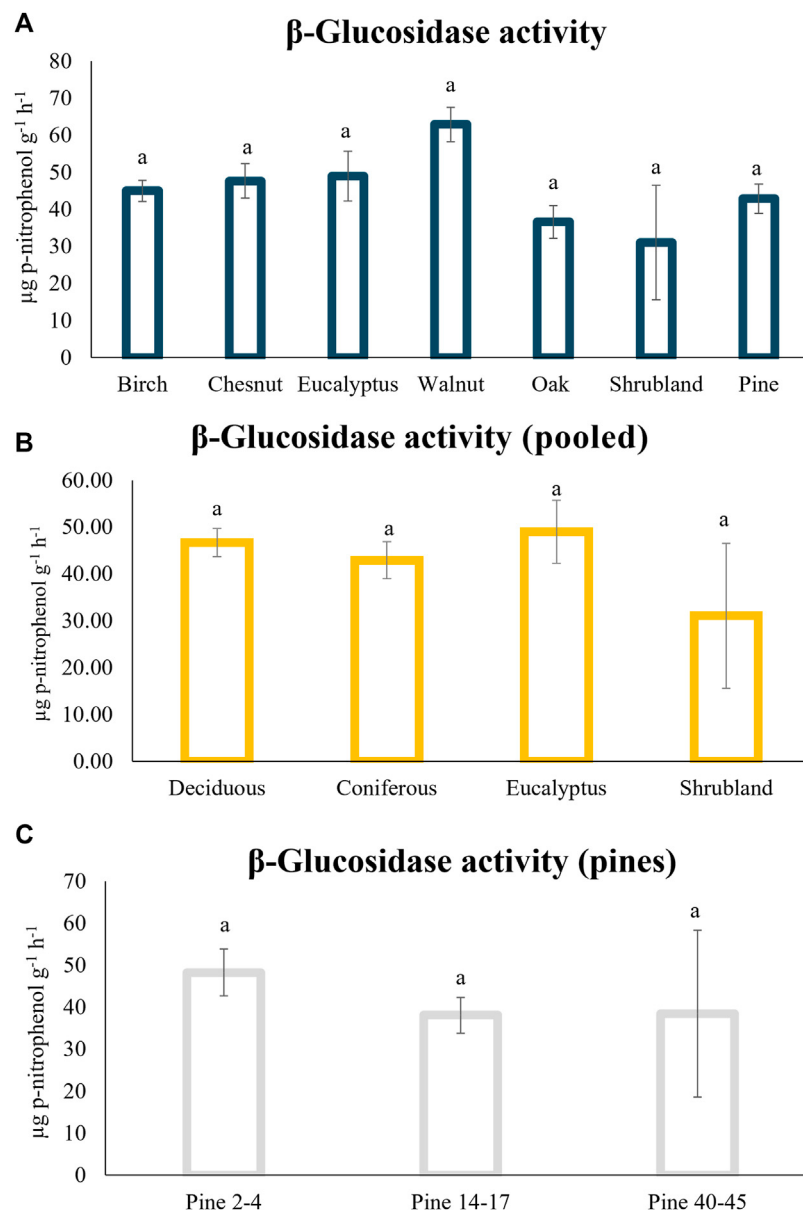


FIGURE 6 | β -Glucosidase soil enzyme activity under the different tree plantations **(A)** with the deciduous species pool together (oak, birch, walnut and chestnut) **(B)**; and under pine plantations with different ages **(C)**. Different letters indicate significant differences, ANOVA test ($p < 0.05$).

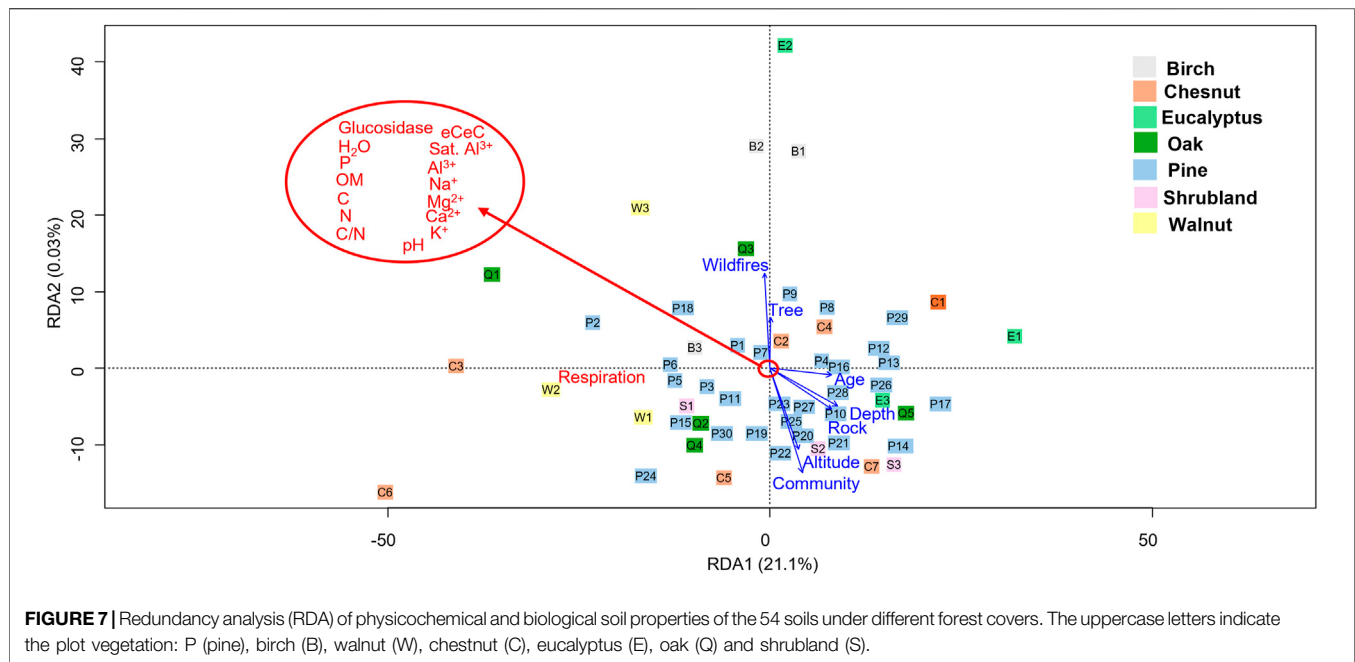
in the fungal dominance (Behera and Sahani, 2003), which could explain the lower soil respiration rates under this forest plantation (Figure 5A).

For the pine plantations, soil respiration was also compared between stands of different ages, but no differences were observed (Figure 5C), even though the average values tended to decrease with the tree longevity, from $991 \pm 69 \text{ ppm CO}_2 \text{ g}^{-1}$ for the 2–4 years pine stand to $774 \pm 162 \text{ ppm CO}_2 \text{ g}^{-1}$ for the older pine stands (40–45 years). A similar decrease with age stands under pine was detected by Zhao et al. (2016).

In the analysis of the β -glucosidase enzyme activity no differences were detected for the soils under the different forest plantations, with average values between $60 \pm 5 \mu\text{g}$

p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$ for soils under walnuts and $31 \pm 15 \mu\text{g}$ p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$ for soils under shrublands. No differences were found neither when the values for the deciduous trees were pooled together for the distinct age stands in the case on the pine plantations (Figures 6A–C). Other studies described bigger β -glucosidase values under pines compared with oak (Błońska and Lasota, 2017).

Within our pine plantations, the young stands (2–4 years old) showed slightly higher average activity ($48 \pm 6 \mu\text{g p-nitrophenol g}^{-1} \text{h}^{-1}$) than the older stands (38 ± 4 and $38 \pm 20 \mu\text{g p-nitrophenol g}^{-1} \text{h}^{-1}$ for 14–17 and 40–45 years old stands respectively), even though this difference was not significant (Figure 6C). Wang et al. (2019), using plantations within the



same age range also found bigger β -glucosidase activity in the younger stands (3–6 years old) compared with stands that were 12–18 years old. However, these authors detected an increase in this enzyme activity in the soils under the oldest tree stands that was not found in our samples.

The role of this enzyme is key in the cellulose degradation (Zang et al., 2018), since β -glucosidases complete the final step of cellulose hydrolysis by converting cellobiose to glucose. This enzymatic activity is key in the C-cycling and can be used as a soil quality indicator (Stott et al., 2010). This decomposition process is stand-specific with a particularly negative effect in the case of evergreen tree litter (Joly et al., 2017) that was not detected in our study.

Soil enzyme activity in general, including β -glucosidase activity, usually correlated with soil pH, soil organic carbon and total nitrogen and edaphic properties, had higher importance in explaining enzyme variability than climate or stand properties (Oliveira et al., 2025). The limited variability observed in most of the edaphic properties within our samples (Table 3) might explain the lack of differences between the various forest stands (Figure 6A) or the non-significant correlation with such properties (Figure 3).

The values of this activity were lower than others described under oak and eucalyptus in the same region and with similar physicochemical properties (Lombao et al., 2015), but the referred soil had double the amount of moisture compared to the soil of the present study. In this case, the sampling was performed in summer, where a marked decrease in precipitation occurs every year, leading to a decrease in the water content, which might be the responsible for the lower enzyme activity detected in our samples. This suggest that β -glucosidase was sensitive to changing soil moisture regimes, as reported by other authors (Zhang et al., 2011). However, other studies in coniferous forest soils detected an impact of seasonality in the β -glucosidase gene pool, but not in

the activity itself (Pathan et al., 2017). Different studies that identify differences between the soil β -glucosidase activity under different forest stands (Salazar et al., 2011) usually refers to adult trees, meanwhile in our study most of the trees are <17 years old.

Figure 7 represents the redundancy analysis (RDA) of the whole data set. Despite of the differences described for water content, eCEC and soil respiration between different forest plantations, when all the physicochemical and biological properties were analyzed together in the RDA analysis no structural differences were detected. The permutation test for the RDA reduced model showed that the age of the stand was the only significant factor ($p < 0.05$) affecting the soil properties, meanwhile wildfire events were marginally significant ($p = 0.056$). For the other environmental factors considered, community where the forest belongs, altitude, tree species, parental rock, and soil depth had no significant effect on the analyzed soil properties ($p > 0.05$). The soil depth and parental rock were correlated as the deeper soils developed generally over granitic rocks; as well as the altitude and the community, since some communities were located at higher altitudes than others (Figure 7; Tables 1, 2).

The first component, which explained 21.1% of the variability, was related to the soil respiration, bigger in the soil samples with the high amount of SOM (Figure 7), in agreement with what has been previously published about the relation of CO_2 efflux and carbon pools in roots and soils (Zhou et al., 2013). The rest of the soil properties did not have a clear impact on the distribution of the different samples in the RDA analysis. The influence of the stand age might be hindering the effect of the forest type, which has legacy effects in defining soil community composition (Rodríguez-Rodríguez et al., 2023). Forest age and structure have a noticeable effect on the soil nutrients and metals that tend to accumulate in soil rather than the litter (Lucas-Borja et al., 2019).

Regarding the soil microbial activity, younger stands have lower soil enzymatic activities and respiration, related with soil moisture, litterfall, soil organic matter and water holding capacity, compared with older stands (Lucas-Borja et al., 2016). However clear correlations between the age of the stand and the soil respiration and β -glucosidase activity were not detected in our study, probably due to the young age of most of the stands analyzed. Our hypothesis regarding the influence of the tree species in the soil properties was not fulfilled, most likely due to the different age of the stands which acted as a confounding factor.

CONCLUSION

The results of the current research indicate that tree species do not significantly affect soil pH, SOM, C, N, P or exchangeable cations, likely due to the analyses being restricted to the solid soil phase and the different ages of the plantations.

In contrast, three species influenced soil moisture and microbial respiration, with soils under eucalyptus exhibiting the lowest values for both parameters. These findings are critical for informing forestry management plans, particularly under current climate change scenarios, where productive forests serve as significant C sinks, yet management practices can markedly alter soil C pools.

Considering the entire spectrum of soil properties, stand age and historical wildfire events emerged as primary drivers of the observed soil variability. Further research is needed to elucidate species impact on soil properties and to guide forest management under specific edapho-climatic conditions. Integrating microbial community analysis with functional assessments, alongside evaluating long-term wildfire and management impacts is essential in the context of climate change.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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AUTHOR CONTRIBUTIONS

AB and AM-G participated on software, data curation, writing-Original draft preparation, visualization, and investigation. RC-D participated in software, data curation, visualization and investigation. MD-R, AN-D, and MF-S participated in conceptualization, methodology, writing-Original draft preparation, visualization, supervision, and validation. EÁ-R participated in visualization, supervision, validation, writing-reviewing and editing. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declare that financial support was received for the research and/or publication of this article. Laboratorio Ecosocial do Barbanza has the support of Fundación Biodiversidad from Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO) within the Plan de Recuperación, Transformación y Resiliencia (PRTR), funded by European Union – NextGenerationEU.

CONFLICT OF INTEREST

The authors declare that the research 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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