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# Soil amelioration induced by nurse shrubs in coal mines reclaimed to pastures and their synergistic effects with grazing

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## ABSTRACT

Native shrubs facilitate the establishment of oak seedlings in the opencast coal mines rehabilitated to pastures in Northern Spain, under a Mediterranean sub-humid climate. We evaluate soil changes as one of the possible facilitative effects of nurse shrubs. We hypothesize that nurse shrubs on mining soils can improve edaphic properties directly and indirectly by reducing the negative effects of trampling and grazing by ungulates. Thus, we assessed the combined effects of nurse shrubs (plots with vs without shrubs) and grazing (plots with vs without a fence for preventing browsing and trampling) upon soil properties in a reclaimed coal mine. Chemical properties such as electrical conductivity, $K^+$, cation exchange capacity, and C/N ratio reached higher values beneath shrubs' canopies, so did total organic matter, total N, total organic C, total P, available P, and $Mg^{2+}$ under shrubs but only with grazing. In contrast, pH was higher outside the shrubs; also $Ca^{2+}$, though only in the absence of grazing. With grazing, $Na^+$ decreased under shrubs. Among physical properties, bulk density increased and porosity decreased only in grazed plots, whereas sand content increased under shrubs in the grazed plots, and clay decreased in such locations. Water holding capacity and available water were the highest in ungrazed areas. Overall, we found that shrubs have a positive direct effect on soil fertility, especially relevant in grazed areas because nurse shrubs and grazing have synergistic effects, and a positive indirect effect on physical properties because they attenuate negative effects of grazing, particularly soil compaction, by reducing livestock and wild ungulates trampling. Therefore, these results demonstrate how nurse shrubs contribute to soil amelioration, helping to facilitate plant establishment in reclaimed mines, which has relevant restoration implications for pasture and forest recovery.

## 1. Introduction

Mine reclamation seeks the restoration to an acceptable state of the physical, chemical, and biological quality or potential of air, land, and water regimes disturbed by mining (Cooke and Jonson, 2002). However, restored mine soils are often shallow, unstructured, and highly drained, which are limiting conditions for plant establishment (Bradshaw, 1997; Vickers et al., 2012; Alday et al., 2014) and the recovery of ecosystem services (Laurence, 2001; Doley et al., 2012; Tibbett et al., 2012). Therefore, reclaimed mines tend to be firstly occupied by ruderal and stress-tolerant species (Grime et al., 1988; Sigcha et al., 2018; López-Marcos et al., 2020). All these species gradually modify the environment and generate ecological niches, helping others to establish; indeed, some of these species may nurse others (Callaway and Walker, 1997; Padilla and Pugnaire, 2006; Brooker et al., 2008). These nurse plants colonize barren and disturbed soils, facilitating the establishment of other plant species in mining areas (Anthelme and Dangles, 2012; Navarro-Cano et al., 2018). Some studies have suggested that the main facilitation mechanism of nurse plants is the improvement of abiotic conditions under them (Pugnaire et al., 1996a,1996b; Tewksbury and Lloyd, 2001; Domínguez et al., 2015). Some shrub species are known to act as nurse species by improving microclimatic conditions (Pugnaire et al., 1996a; Moro et al., 1997a, 1997b; Prieto et al., 2011), and/or soil properties (Pugnaire et al., 1996a, 1996b, 2004; Prieto et al., 2011). Nurse plants develop microclimatic islands under their canopies where conditions are milder (e.g., less insolation and lower desiccation risk)

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and facilitate seedling survival and growth (Navarro-Cano et al., 2018). Nurse plants also constitute fertility islands that facilitate the establishment of other plant species (Aguiar and Sala, 1999; Mihoč et al., 2016; Navarro-Cano et al., 2018) and contribute to the plant community structure (Badano and Cavieres, 2006; Michalet et al., 2011; Cavieres et al., 2014). Nurse plants can also have many indirect effects such as protection from herbivores, attracting pollinators, providing shelter for animals, and altering the edaphic and mycorrhizal fauna (Callaway and Walker, 1997).

Herbivory has many positive effects on soils such as enhancing nutrient availability (Abdalla et al., 2018; Castillo-García et al., 2022). However, herbivores have the potential to affect negatively soil physicochemical properties (Snyman and du Preez, 2005; Castellano and Valone, 2007; Allington and Valone, 2010; Abdalla et al., 2018; Castillo-García et al., 2022). For instance, biomass removal by browsing (Gizicki et al., 2018; Sigcha et al., 2018) and soil compaction by trampling (Gizicki et al., 2018) can reduce water infiltration (Castellano and Valone, 2007; Pulido et al., 2018) and increase runoff and erosion (Gizicki et al., 2018). This change in the physical properties also has the potential to drastically modify the accumulation and spatial distribution of nutrients in the soil (Allington and Valone, 2014) affecting, among others, pasture production (Pulido et al., 2018) and forest reclamation (Löf et al., 2019). However, herbivory consequences depend largely on the site conditions and grazing management practices (Reynolds et al., 2007; Pulido et al., 2018).

Therefore, soil improvement under the nurse shrubs' canopy in pastures can be mediated not only by direct effects of shrubs on the soil properties (Navarro-Cano et al., 2018) but also by indirect effects, particularly because shrubs protect the space they cover by preventing trampling and browsing (see Torroba et al., 2015; Alday et al., 2016).

Nurse species effects commonly exert a more positive influence in harsh environments (Anthelme and Dangles, 2012; Mihoč et al., 2016). According to the stress gradient hypothesis (SGH), positive interactions (e.g., facilitation) tend to be more important than negative ones (e.g., competition) in plant communities under high abiotic stress or high consumer pressure (Bertness and Callaway, 1994). This is the reason why mine reclamation has been using the engineering capacity of the nurse plants (Brooker et al., 2008). In mining lands, fertility islands associated with nurse shrubs have been demonstrated to promote soil microbial activity (Parraga-Aguado et al., 2013; Navarro-Cano et al., 2018; Ezeokoli et al., 2020), suggesting that the use of nurse plants is a suitable option for the management of these degraded environments.

It is important to study how nurse shrubs and grazing exclusion affect soil properties, especially in mining degraded ecosystems. This can help us understand when and where herbivores have a negative impact on the soil (Sigcha et al., 2018), and when nurse shrubs have a positive effect. However, few studies have analyzed the combined effect of both factors. For instance, Allington and Valone (2010) found a strong "fertile islands" pattern for nitrogen and organic carbon under shrub canopies in arid grazed areas, but not in ungrazed areas, suggesting that livestock grazing itself may play a role in the creation or perpetuation of this pattern (Allington and Valone, 2014).

Specific to coal mines of northern Spain reclaimed for pasture use, the nursing effect of leguminous shrubs has been demonstrated to promote oak establishment (Torroba-Balmori et al., 2015; Alday et al., 2016; Martínez-Ruiz et al., 2021). These previous studies also support the practice of using nurse shrubs as ecosystem engineering species to create a rapid and heterogeneous vegetation cover that provides favorable microsites for the establishment of late-successional species, such as *Quercus petraea* (Matt.) Lieb. and *Quercus pyrenaica* Willd. (Torroba-Balmori et al., 2015; Alday et al., 2016). Mine soil constraints (López-Marcos et al., 2020) and grazing (Sigcha et al., 2018) have been identified as some of the main factors determining the successional dynamics of vegetation in such "mine sites", but the underlying causes have not been disclosed yet. Soil improvement promoted by shrubs can have a positive effect on the establishment of trees, late-successional species, in these highly degraded environments, opening up great prospects for forest regeneration in areas with similar limitations. On the other hand, it's necessary to assess the role of shrubs that can play as a physical barrier to livestock trampling and browsing and how it modifies soil properties.

Therefore, it is necessary to identify and assess the underlying facilitation mechanisms involved. There is a need to explore the causes of such facilitation by assessing the combined effects of shrubs and grazing upon soil properties in reclaimed mines. There is a need to deepen into the differentiation of the main plant-to-plant facilitation via, such as the direct soil improvement by nurse shrubs (e.g., by litterfall production, N fixation, nutrients uptake), and the indirect soil amelioration by shrubs through the avoidance of the negative effects of ungulates' trampling.

In this context, this study aimed to find out whether nurse shrubs and grazing by livestock and wild ungulates improve soil properties in reclaimed coal mines of northern Spain. We herein hypothesized that (1) native shrubs, colonizers of lands mined for coal and reclaimed for livestock use in northern Spain, have a positive effect on mine soil properties; (2) shrubs' positive effect is stronger in grazed areas because they attenuate the livestock negative impact on soil; and (3) a greater effect of grazing on the soil physical properties is expected rather than on the chemical properties.

## 2. Materials and methods

### *2.1. Site description*

The mining area where the experiment was deployed is a 17 ha reclaimed opencast coal mine located in the 'Montaña Palentina' Guardo-Cervera de Pisuerga coal basin, central Cantabrian range (Northern Spain; latitude 42° 48' N, longitude 4° 52' W, ca. 1200 m a.s.l.; Fig. 1A). The dominant geologic material is Paleozoic limestone alternated with carboniferous coal layers. The coal deposits are arranged in layers in an unfavorable mountain relief and the open-pit exploitation is carried out with contour mining, by excavating the mountains along the contour lines, until the economic limit of the mineral deposit is reached.

The climate is sub-humid Mediterranean, with an annual mean temperature of 9.3 °C and means annual precipitation of 977 mm (Torroba-Balmori et al., 2015). The rainfall is irregularly distributed all over the year, concentrated in autumn (October and November) and spring (April and May), and a dry period from July to August (with only 8% of the annual rainfall; Milder et al., 2013).

The dominant soil class surrounding this mine is a Typic Dystroudept (Soil Survey Staff, 2014), with a sandy clay loam texture, acid pH (4.3–4.8), electrical conductivity of 0.0082 S $m^{-1}$, without evidence of carbonates, high soil organic matter content, and low available phosphorous content (see López-Marcos et al., 2020).

The mine is surrounded by deciduous broadleaved forests dominated by the sub-sclerophyll, sub-Mediterranean *Q. pyrenaica* oak, with an understory composed of diverse shrubs, e.g. *Crataegus monogyna* Jacq., *Sorbus* spp. (Milder et al., 2013). Eurosiberian *Q. petraea* oak forests are also common, though they show a more fragmented distribution. The degradation of such forests in the study area generates shrublands, namely broomlands, dominated by leguminous species such as *Cytisus scoparius* (L.) Link and *Genista florida* L. (Milder et al., 2013; Martínez-Ruiz et al., 2021).

The opencast mine was reclaimed in October 2000. The open pit was filled up with coal mining wastes from nearby mines to regrade the mine gap to the original contour (Martínez-Ruiz et al., 2021). Then, wastes were covered with a mixture of topsoil and fine-textured materials from deeper parts of the neighboring opencast pits (Martínez-Ruiz et al., 2021), also amended with cattle manure that contained a very poor seed bank (González-Alday et al., 2009) and finally, hydroseeded (Torroba-Balmori et al., 2015) with a mostly perennial grassland species mixture: *Festuca rubra* L. (20%), *Phleum pratense* L. (20%), *Lotus*

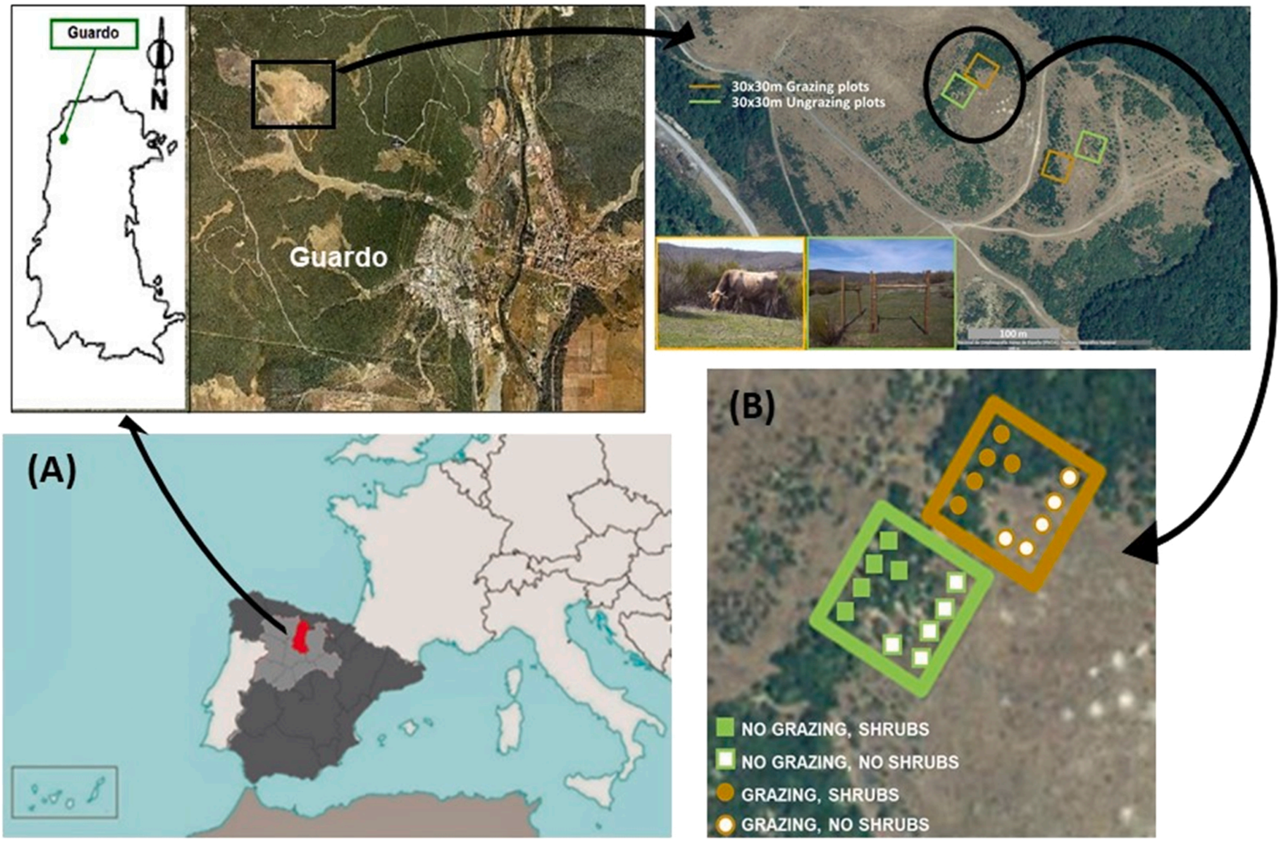

**Fig. 1. (A)** Location of the studied coal mine near Guardo in the 'Montaña Palentina' (Northern Spain). **(B)** Experimental design and plots and subplots location in the field.

*corniculatus* L. (18%), *Bromus sterilis* L. (10%), *Poa trivialis* L. (10%), *Trifolium repens* L. (8%), *Avena sativa* L. (7%) and *Secale cereale* L. (7%) to contribute to the establishment of a permanent plant cover (Alday et al., 2011).

The topsoiling mixture had a clay loam texture, with a pH of 6.5, electrical conductivity of 0.0114 S $m^{-1}$, easily oxidizable carbon of 1.98%, available phosphorous of 9.7 mg $kg^{-1}$, and an effective depth of 10–15 cm (López-Marcos et al., 2020). The mine soils after the reclamation process are Lithic Udorthents (Soil Survey Staff, 2014) and they have a very low water holding capacity compared to the natural soil in the forest ($2.27 \pm 0.36$ vs. $19.87 \pm 1.52$ g $cm^{-2}$; López-Marcos et al., 2020).

After the pasture reclamation process, wild ungulates such as deer (*Cervus elaphus* L.), roe deer (*Capreolus capreolus* L.) and wild boar (*Sus scrofa* L.), and cattle livestock (light stocking rate $<$ 10 AU 100 $ha^{-1}$, AU= animal unit; Teague et al., 2011) freely feed on the vegetation established in the study area (Milder et al., 2013). Native herb species from neighbouring areas (e.g., *Crepis vesicaria* L., *Plantago lanceolata* L., *Poa bulbosa* L., *Trifolium* spp., *Vulpia* spp.) gradually replaced hydroseeded ones (López-Marcos et al., 2020). Also, during the 19 years after the reclamation works, native shrub species coming from the surrounding areas, mainly *C. scoparius* and *G. florida*, naturally colonized the mine reclaimed to pasture (López-Marcos et al., 2020).

### 2.2. *Experimental design*

Our experimental set-up, previously used to demonstrate the positive short-term effects of nurse shrubs and herbivory exclusion on seedling establishment of the two *Quercus* species (Torroba-Balmori et al., 2015), was revisited to ascertain the causes of such facilitation by evaluating the combined influence of nurse shrubs and grazing on soil properties. Therefore, the combinations to be tested were: (a) grazing without shrubs (d) grazing with shrubs (c) no-grazing without shrubs, (d) no-grazing with shrubs. Through this experimental set-up, we also explored the shrub-induced soil differentiation, as potentially being one of the main plant-to-plant facilitation mechanisms, such as the direct soil improvement by nurse shrubs (e.g., by litterfall production, nutrients uptake) and the indirect soil amelioration from shrubs through the avoidance of the negative effects of ungulates' trampling.

This experimental set-up, established in a flat area of the reclaimed mine in February 2011, consisted of four 30 m x 30 m permanent plots (Fig. 1B): two of them surrounded by 2-m-height fences (5 cm × 15 cm mesh hole) for the exclusion of wild ungulates and livestock, and another two non-fenced plots in their proximity (Torroba-Balmori et al., 2015). Within each plot, 10 permanent sub-plots (5 with shrubs and 5 without shrubs) were allocated randomly ca. 4 m apart from each other (with each sub-plot measuring ca. 2 ×2 m, the sub-plots with shrubs included 2–3 mature, naturally recruited shrub plants each) to assess the combined influence of nurse shrubs and grazing upon soil properties. The nurse shrubs, *C. scoparius* and *G. florida*, are two common native species that often colonize degraded sites (Milder et al., 2013), forming mixed stands. Both species are non-thorny leguminous shrubs that share most of their structural and functional characteristics (e.g., structure, leaf-related traits, phenology, and atmospheric N-fixation; Talavera and Castroviejo, 1999). In addition, both species' populations within the reclaimed mine also had very similar ages and sizes (mean ± se height: $2.22 \pm 0.066$ m), and the number of individuals per species was balanced so they were not differentiated in the experiment.

### 2.3. *Soil sampling and laboratory analysis*

The soil survey was done in the spring of 2019. During this survey, one disturbed soil sample and one undisturbed soil sample were collected per subplot. The 40 disturbed soil samples were taken by collecting 25 cm × 25 cm topsoil samples with variable depth depending on the A horizon thickness, with a 15 cm maximum depth. The 40 undisturbed soil samples were collected using steel cylinders (diameter: 8 cm, depth: 5 cm; volume = 251.33 $cm^3$).

The disturbed soil samples were air-dried and sieved (2 mm mesh) before physical and chemical analyses. Physical analyses included weight percentage of coarse fraction (> 2 mm; %CF) and fine-earth fraction (< 2 mm; %FF), and particle size distribution of the fine fraction (sand, clay and silt percentage) determined by the Bouyoucos-method (Day, 1965). Soil chemical properties were measured as follows: soil pH and electrical conductivity (EC) using a conductivity meter in a 1:2.5 soil:deionized-water-slurry (Allen, 1989); total organic matter (TOM) using K-dichromate oxidation method (Walkley, 1947) and oxidizable organic matter (oxOM) using the redox volumetric method (Walkley and Black, 1934); total organic carbon (TOC) and oxidizable organic carbon (oxOC) were obtained dividing TOM and oxOM respectively by Van Bemmelen factor (1.724; Van Bemmelen, 1890); total nitrogen (TN) concentration using the Kjeldahl-method (Bremner and Mulvaney, 1982); available phosphorus (avP) concentration using the Olsen method (Olsen and Sommers, 1982); total phosphorus (TP) was extracted by microwave digestion in a closed high-pressure vessel (ETHOS EASY Milestone Microwave) using a mixture (9:1) of concentrated Nitric and Hydrogen Peroxide, and measured on an optical ICP (Spectro Genesis); cation exchange capacity (CEC) using barium chloride and triethanolamine at pH= 8.1 (Rhoades, 1982) and finally exchangeable cations as calcium ($Ca^{2+}$), sodium ($Na^+$), potassium ($K^+$) and magnesium ($Mg^{2+}$) were determined using an atomic absorption/emission spectrometer after extraction with 1 N ammonium acetate at pH= 7 (Schollengberger and Simon, 1945). From the sum of exchangeable cations concentrations, we obtained the sum of bases (SB, López-Marcos et al., 2018), and by dividing SB by CEC we obtained the base saturation (V; Garrido Valero, 1993).

The undisturbed soil samples were oven-dried at 105 °C for 24 h before being weighed ( ± 0.001 g) to calculate the soil bulk density (BD), the real density (RD) estimated by the pycnometer method to calculate soil porosity (Porosity=[1-(BD/DR)]x100; MAPA, 1994), and the available water (avW) determined by the MAPA (1994) methods. The avW was determined as the difference between water content at field capacity (water remaining in a soil after it has been thoroughly saturated and allowed to drain freely for 2 days) and the permanent wilting point (soil water content retained at 1500 kPa using Eijkelkamp pF Equipment). The WHC was calculated as the product of avW, BD, % FF (fine-earth fraction), and soil thickness (see López-Marcos et al., 2019).

### 2.4. Data analyses

In order to assess the potential effects of nurse shrubs (and their synergistic effects with grazing) on the soil physicochemical properties we built linear-mixed models (LMM; Pinheiro and Bates, 2000) with the fixed effects of shrub cover and grazing regime (and their interaction) on each soil property. During the preliminary analysis, all different random effects, nesting structures, heteroskedasticity and autocorrelation variations were tested by maximum likelihood LMM (ML; Richards, 2005) in order to get the most accurate results possible. The Akaike information criterion (AIC) was used to verify whether the alternative model was more parsimonious, i.e. smaller values of AIC (Pinheiro and Bates, 2000), and the ANOVA was applied to test the significant differences between the null and the alternative models. Accounting for spatial dependence, the final models included as random effects the intercepts of the plot, and the sub-plot nested within it. No clear evidence of autocorrelation and/or heteroscedasticity was found for the different variables studied. We used LMM with the restricted maximum likelihood method (REML; Richards, 2005) for the final models. The method used to test the significance of fixed effects was the likelihood ratio, and the corresponding p-value significance (Pinheiro and Bates, 2000).

Finally, working over the model matrix, multiple pairwise comparisons (Pinheiro and Bates, 2000) were calculated to test differences between treatments when both fixed factor levels had significant effects. The Bonferroni correction was used to adjust the significance level for each t-test, thus preventing Type I error inflation (Sokal and Rohlf, 1995).

Following the LMM results, two principal component analyses (PCA) were applied to the data matrices of soil chemical or physical properties that were significant in the LMM. Then, we fitted the LMM fixed factors, nurse shrub and grazing, to the soil samples scores in the first two dimensions of each PCA using the vegan ‘envfit’ function (Oksanen et al., 2020) with 9999 permutations. The "ordiellipse" function (Oksanen et al., 2020) was used to draw the standard deviation ellipses (95% confidence limits) of the (weighted) centroids of the soil samples within the four groups resulting from the combination of factors shrub and grazing.

All these statistical analyses were implemented in the R software environment (4.1.2; R-Core Team, 2021), using the nlme package for linear mixed models (LMM; 3.1–152; Pinheiro et al., 2021), and the vegan package for multivariate analyses (2.5–7; Oksanen et al., 2020).

## 3. Results

### 3.1. Effect of shrub and grazing on soil chemical properties

Among all the soil chemical properties analyzed, EC, CEC, $K^+$, oxOC/TOC, and C/N were only affected by shrubs’ simple effect (Table 1A). The values of EC (0.012 ± 0.001 vs. 0.009 ± 0.001), CEC (22.86 ± 0.90 vs. 20.28 ± 0.68), $K^+$ (0.47 ± 0.03 vs. 0.38 ± 0.02), and C/N (10.87

**Table 1**
Linear Mixed Models (LMM): F-values and significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) of shrub and grazing simple and interaction fixed effects upon soil **(A)** chemical and **(B)** physical properties.

| | Grazing | Shrub | Grazing x Shrub |
|---|---|---|---|
| **(A) Chemical properties** | | | |
| **pH** | 19.005 * ** | 11.599 * * | 0.011 |
| **EC (S $m^{-1}$)** | 0.617 | 6.409 * | 4.031 |
| **CEC ($cmol_+$ $kg^{-1}$)** | 3.875 | 6.141 * | 1.202 |
| **SB ($cmol_+$ $kg^{-1}$)** | 5.958 * | 0.318 | 5.460 * |
| **V (%)** | 6.212 * | 2.902 | 5.384 * |
| **$Mg^{2+}$ ($cmol_+$ $kg^{-1}$)** | 0.0001 | 7.196 * | 6.253 * |
| **$Na^+$ ($cmol_+$ $kg^{-1}$)** | 0.302 | 4.829 * | 14.788 * ** |
| **$Ca^{2+}$ ($cmol_+$ $kg^{-1}$)** | 6.341 * | 0.754 | 5.056 * |
| **$K^+$ ($cmol_+$ $kg^{-1}$)** | 0.599 | 5.504 * | 0.278 |
| **TN (%)** | 0.059 | 9.469 * * | 14.391 * ** |
| **TP (%)** | 2.218 | 5.405 * | 6.192 * * |
| **avP (mg $kg^{-1}$)** | 1.476 | 3.007 | 6.101 * |
| **TOM (%)** | 0.039 | 18.039 * ** | 12.547 * * |
| **oxOM (%)** | 0.213 | 3.335 | 16.168 * ** |
| **TOC (%)** | 0.039 | 18.062 * ** | 12.567 * * |
| **oxOC (%)** | 0.213 | 3.333 | 16.164 * ** |
| **oxOC/TOC** | 0.567 | 9.456 * * | 0.968 |
| **C/N** | 2.165 | 9.507 * * | 1.714 |
| **(B) Physical properties** | | | |
| **Sand (%)** | 6.52 * | 4.38 * | 18.83 * ** |
| **Clay (%)** | 2.76 | 5.60 * | 19.16 * ** |
| **Silt (%)** | 0.49 | 0.37 | 0.41 |
| **CF (%)** | 0.09 | 0.10 | 2.60 |
| **FF (%)** | 0.09 | 0.10 | 2.60 |
| **BD (g $cm^{-3}$)** | 6.22 * | 1.52 | 2.02 |
| **Porosity (%)** | 3.79 * | 0.15 | 0.30 |
| **avW (%)** | 4.63 * | 3.36 | 4.51 * |
| **WHC (g $cm^{-2}$)** | 17.37 * ** | 7.43 * * | 8.25 * * |

EC= electrical conductivity; CEC=cation exchange capacity; SB=sum of bases; V= base saturation; TN=Total Nitrogen; TP=Total phosphorus; avP=available phosphorus; TOM= total organic matter; oxOM = easily oxidizable organic matter; TOC=total organic carbon; oxOC= easily oxidizable organic carbon; C/N = TOC/TN; CF=coarse fraction; FF=fine fraction; BD=bulk density; avW= available water; WHC=water holding capacity.

± 0.25 vs. 9.58 ± 0.40) were significantly higher under than outside shrubs, whereas the ratio oxOC/TOC was significantly higher outside than under shrubs (0.85 ± 0.04 vs. 0.70 ± 0.02). The pH was the only property significantly affected by both (shrub and grazing) simple effects, i.e. no interaction effect (Table 1A); pH was significantly higher outside than under shrubs (5.73 ± 0.13 vs. 5.33 ± 0.10), and without than with grazing (5.79 ± 0.13 vs. 5.27 ± 0.09).

The remaining soil chemical properties were significantly affected by the shrub x grazing interaction effect (Table 1A). The values of SB, V, and $Ca^{2+}$ were significantly higher outside than under shrubs in the no-grazing plots but similar in the grazing plots (Table 2A). $Na^{+}$, instead, was significantly higher outside than under shrubs in the grazing plots though similar in the no-grazing plots (Table 2A). $Mg^{2+}$, TN, TP, avP, TOM, and TOC values were significantly higher under than outside shrubs in the grazing plots but similar in the no-grazing plots (Table 2A). The oxOM and oxOC showed an opposite pattern with and without grazing (Table 2A), being higher under shrubs in the grazing plots but outside shrubs in the no-grazing plots.

The PCA applied to the matrix of the significant soil chemical properties (Fig. 2) showed the sub-plots clustered in four groups following the combined effect of shrub and grazing. The first dimension, PC1, was related to shrub canopy (sub-plots under shrubs on the right and outside shrubs on the left), and the second dimension, PC2, was related to grazing (sub-plots without grazing on the upper end, and with grazing on the bottom). Moreover, subplots with and without grazing (brown and green colors, respectively) were closer under shrub canopies (solid lines) than outside the shrubs (dashed lines), suggesting an important effect of shrubs on the soil chemical properties. Indeed, the 'envfit' analysis showed a significant effect of shrub and shrub x grazing interaction but not the simple effect of grazing in explaining differences in soil chemical properties (Table 3A).

**Table 2**

Mean value ± standard error of soil **(A)** chemical and **(B)** physical properties for which there is a significant shrub x grazing interaction (see Table 1). Different letters indicate significant differences between pair-wise comparisons with Bonferroni's test ($p < 0.05$). Abbreviations as in Table 1.

| | No grazing | | Grazing | |
|---|---|---|---|---|
| | Shrub | No shrub | Shrub | No shrub |
| **(A) Chemical properties** | | | | |
| **SB (cmol$_+$ kg$^{-1}$)** | 8.63 ± 1.91 **b** | 12.45 ± 0.39 **a** | 8.50 ± 0.85 **b** | 6.16 ± 1.56 **b** |
| **V (%)** | 37.17 ± 8.62 **b** | 57.33 ± 2.22 **a** | 36.31 ± 3.34 **b** | 33.22 ± 4.20 **b** |
| **$Mg^{2+}$ (cmol$_+$ kg$^{-1}$)** | 1.14 ± 0.09 **b** | 1.12 ± 0.08 **b** | 1.40 ± 0.16 **a** | 0.86 ± 0.07 **c** |
| **$Na^{+}$ (cmol$_+$ kg$^{-1}$)** | 0.08 ± 0.02 **ab** | 0.06 ± 0.009 **b** | 0.03 ± 0.006 **c** | 0.09 ± 0.02 **a** |
| **$Ca^{2+}$ (cmol$_+$ kg$^{-1}$)** | 6.94 ± 0.77 **b** | 10.87 ± 1.88 **a** | 6.60 ± 1.46 **bc** | 4.86 ± 0.31 **c** |
| **TN (%)** | 0.41 ± 0.02 **b** | 0.43 ± 0.03 **b** | 0.53 ± 0.06 **a** | 0.32 ± 0.03 **c** |
| **TP (%)** | 0.08 ± 0.002 **b** | 0.08 ± 0.004 **b** | 0.10 ± 0.01 **a** | 0.07 ± 0.003 **b** |
| **avP (mg kg$^{-1}$)** | 7.61 ± 0.92 **b** | 10.46 ± 2.54 **b** | 21.88 ± 7.67 **a** | 5.60 ± 0.90 **c** |
| **TOM (%)** | 7.64 ± 0.70 **b** | 7.19 ± 0.54 **b** | 10.03 ± 0.46 **a** | 5.05 ± 1.19 **c** |
| **oxOM (%)** | 5.23 ± 0.27 **b** | 6.33 ± 0.70 **a** | 7.02 ± 0.78 **a** | 4.07 ± 0.45 **c** |
| **TOC (%)** | 4.44 ± 0.41 **b** | 4.18 ± 0.31 **b** | 5.83 ± 0.27 **a** | 2.93 ± 0.69 **c** |
| **oxOC (%)** | 3.04 ± 0.16 **b** | 3.68 ± 0.41 **a** | 4.08 ± 0.46 **a** | 2.37 ± 0.26 **c** |
| **(B) Physical properties** | | | | |
| **Sand (%)** | 54.62 ± 0.75 **b** | 56.94 ± 1.77 **b** | 61.74 ± 1.74 **a** | 55.10 ± 1.42 **b** |
| **Clay (%)** | 26.94 ± 0.98 **a** | 24.59 ± 1.84 **a** | 19.88 ± 2.18 **b** | 27.76 ± 1.61 **a** |
| **avW (%)** | 11.08 ± 1.42 **b** | 20.35 ± 1.62 **a** | 11.01 ± 4.01 **b** | 10.33 ± 1.77 **b** |
| **WHC (g cm$^{-2}$)** | 0.22 ± 0.03 **b** | 0.45 ± 0.06 **a** | 0.17 ± 0.04 **c** | 0.16 ± 0.03 **bc** |

### 3.2. *Effect of shrub and grazing on soil physical properties*

Some soil physical properties such as silt, CF, and FF percentages were not affected by shrub or grazing (Silt: 18.11 ± 0.96; CF: 72.32 ± 2.67; FF: 27.68 ± 2.67). BD and porosity were only affected by grazing (Table 1B) and showed opposite patterns: BD was significantly higher with grazing (1.01 ± 0.06 vs. 0.81 ± 0.05) and porosity without grazing (60.93 ± 2.68 vs. 52.28 ± 3.55). The remaining properties responded significantly to the shrub x grazing interaction (Table 1B). The sand percentage was significantly higher under than outside shrubs in the grazing plots but similar in the no-grazing ones, whereas clay showed the opposite pattern: higher clay percentage was measured outside than under shrubs canopy in the grazing plots but similar in the no-grazing plots (Table 2B). WHC and avW showed the same behavior, being significantly lower with than without grazing, both under and outside shrubs for WHC and outside shrubs for avW (Table 2B).

The PCA applied to the matrix of the significant soil physical properties (Fig. 3) showed how sub-plots clustered in four groups because of the combined effect of shrub and grazing. Again, PCA1 and PCA2 were respectively related to shrub canopy (with shrubs with positive PC1 and without shrubs with negative PC1), and grazing (without grazing with positive PC2 with grazing with negative PC2). Moreover, subplots with and without shrubs (solid and dashed lines, respectively) were closer without grazing (green color) than with grazing (brown color), suggesting an important significant effect of the grazing on the soil physical properties. Indeed, the 'envfit' analysis showed a significant effect of grazing and shrub x grazing interaction but not a simple effect of the shrub to explain differences in soil physical properties (Table 3B).

## 4. Discussion

Our results demonstrated that the leguminous nurse shrubs, *G. florida* and *C. scoparius*, facilitators of the oak establishment (Torroba-Balmori et a, 2015; Alday et al., 2016), promote soil amelioration and have positive synergistic effects with grazing in reclaimed opencast coal mines. These native nurse shrubs had positive effects on the physical and chemical mine soil properties, that for the physical properties were more pronounced in grazed areas likely by attenuating the negative impact of livestock and wild ungulates trampling, as hypothesized. Therefore, not only the nurse shrub protection under their canopy from desiccation, browsing and trampling but the amelioration of soil properties is also a likely cause for better plant establishment.

### 4.1. *Influence of shrubs and grazing on soil chemical properties*

Shrubs litterfall (Alegre et al., 2004) and belowground roots turnover (Fu et al., 2016) contributed to soil organic matter; not necessarily to TOM or OxOM, but the ratio among them. Lower oxOC/TOC ratio in soils under shrubs indicates a lower proportion of labile forms of carbon (López-Marcos et al., 2020), which is related to the coarser biomass size fractioning of shrubs compared to grass, and their composition, e.g. higher lignin content in shrubs' woody tissues (Condron and Newman, 1998). Accordingly, we found higher C/N ratios in soils under shrubs at grazed areas that indicate a slower rate of TOM decomposition (Wang et al., 2016a, 2016b), compared to the non-shrub plots.

On the other hand, grazing, through trampling, browsing, and excreta, contributes to accelerate litter (Wang et al., 2016a, 2016b) and soil organic matter decomposition (Burke et al., 1989) and therefore high labile carbon, measured through oxOC and oxOC/TOM ratio. Thus, low grazing pressure in shrub-cover pastures can promote vegetation production through accelerated decomposition and nutrient cycling and, as our results suggest, to higher TOM. Meanwhile, despite the low

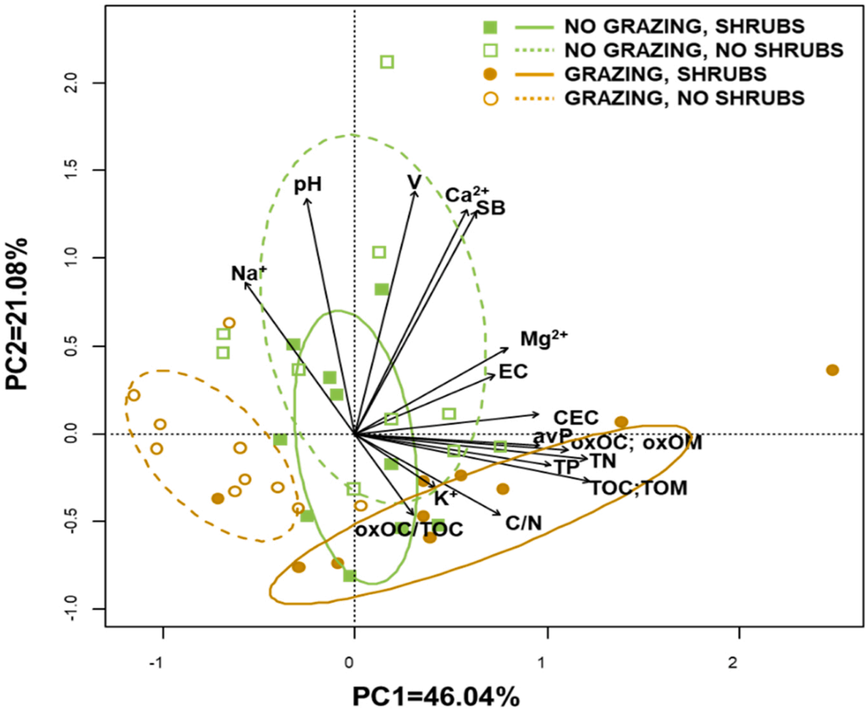

**Fig. 2.** PCA biplot of sub-plots (dots) and significant soil chemical properties (vectors), with the projection of the standard deviation ellipses (95% confidence limits) that group the subplots according to the combined effect of shrub and grazing on soil chemical properties (abbreviations as in Table 1).

**Table 3**
Goodness of fit (R$^2$) and significance (*p-value*) of the effect of shrub-cover, grazing-regime, and their interaction on the PCA ordinations for soil **(A)** chemical and **(B)** physical properties that were significant in the LMM (see Table 1).

| Effects | R$^2$ | *p* |
|---|---|---|
| **(A)** | | |
| Grazing | 0.0656 | 0.0713 |
| Shrub | 0.1344 | 0.0018 |
| Grazing x Shrub | 0.3121 | 0.0001 |
| **(B)** | | |
| Grazing | 0.1160 | 0.0085 |
| Shrub | 0.0057 | 0.8123 |
| Grazing x Shrub | 0.2092 | 0.0064 |

ungulate density, herbivores' continuous trampling and browsing during the vegetative growth period produced soil compaction and low aboveground biomass and TOM in the grazed area without shrubs, as suggested by Pinheiro Machado Filho et al. (2021) for pastures. As a result of the balance between both processes: biomass production and decomposition, increased differences in TOM content and fractioning in areas with vs without nurse shrubs, whereas grazing exclusion lead to intermediate values of TOC and in oxOC between sites with and without shrubs and small differences among them.

The high TN content in soils under shrubs in the presence of livestock is explained by the greater shrubs litterfall and its high nitrogen content, the higher grass production under shrubs, and dung nutrients (Tessema et al., 2011). On the other hand, without grazing, there were no differences in TN with and without shrubs. Other authors argue that higher TN content found under the stands of leguminous species (e.g., *Robinia pseudoacacia* L. forests) is a consequence of the N fixation capacity of many of the species of this group (Wang et al., 2016a, 2016b). TN plays an important role in vegetation growth and development (Wang et al., 2016a, 2016b), so its availability has been proven relevant in the productivity and functioning of ecosystems (LeBauer et al., 2008).

We observed TN and TOC were higher under shrub canopies in grazed areas, as reported in arid environments by Allington and Valone (2010). A similar trend was also found for total phosphorus (TP). Greater contents of soil TP are commonly associated with higher levels of soil carbon (He et al., 2021; Sato et al., 2019), because TP and TOC are stabilized and retained through similar processes in the soil and, therefore, they may have similar behavior (Doetterl et al., 2015). Available phosphorus is also higher in grazed soils under shrubs, which may be related to livestock excrements, litter accumulation, and higher surface phosphorus mobility due to livestock trampling (Zarekia et al., 2012).

The total cation exchange capacity (CEC) of our mine soils reached significantly higher values under shrubs because the TOM provides CEC, being responsible for between 30% and 65% of the total CEC in other studies (Arranz-González, 2011; Garrido Valero, 1993).

Also related to the CEC and nutrients mobility, pH values indicate that the soils of the mining area are acidic (pH = 5.53 ± 0.14; Garrido Valero, 1993), with similar values to the natural soils of the surrounding *Q. pyrenaica* forests (5.1 ± 0.18; López-Marcos et al., 2020), and much lower than the (6.5 ± 0.07) pH measurements done by López-Marcos et al. (2020) to the topsoiling material. This evidences that pH of the topsoil decreased strongly, with approximately 1 pH unit, in the first 19 years, getting closer to the pH of the natural soils, very likely as a result of the organic matter decomposition (Ritchie and Dolling, 1985) and of nutrients leakage (Crawford et al., 1995). However, the decrease in the pH of the mine soil was greater under shrubs (regardless of grazing), which contribute to more organic matter, and more recalcitrant. Some studies also detected lower pH values under the canopy of other leguminous shrub species: for instance, under *C. multiflorus Q. pyrenaica)* and *Quercus ilex* subsp. *ballota* Samp 'dehesas' of the Salamanca province (Costa et al., 2017), and under *Retama sphaerocarpa* L. in shrublands of Badajoz (Rodríguez-Echeverría and Pérez-Fernández, 2003). Furthermore, the pH variability was also lower under shrubs (lower standard error), suggesting that woody vegetation can restrain alterations of soil pH (Rodríguez-Echeverría and Pérez-Fernández, 2003). On the other hand, the decrease in the pH of the mine soil with grazing (regardless of shrub cover) could be stimulated by plant growth in general, root

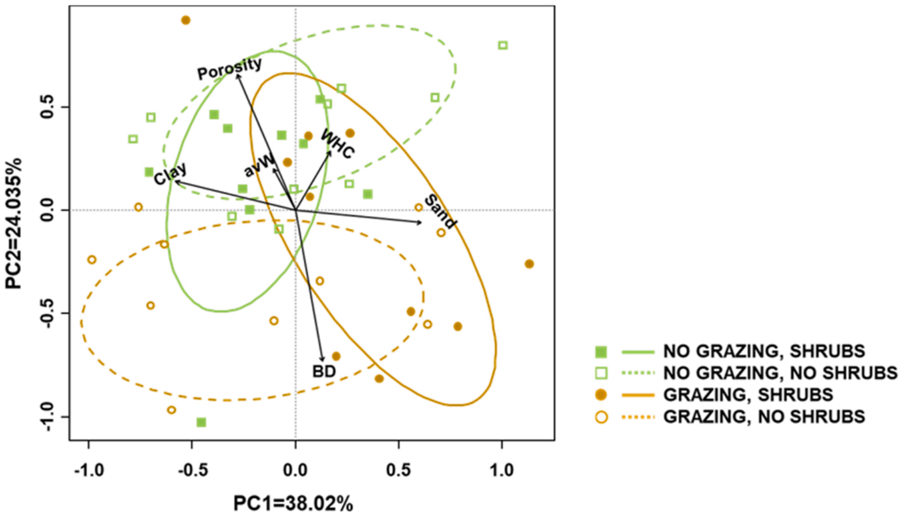

**Fig. 3.** PCA biplot of sub-plots (dots) and significant soil physical properties (vectors), and projection of the standard deviation ellipses (95% confidence limits) that grouped the subplots according to the combined effect of shrub and grazing on soil physical properties (abbreviations as in Table 1).

production, and organic matter decomposition. Manure addition and urine nitrification might also have contributed to the pH decrease (Posselt Martins et al., 2014). The relationship between the amount of organic matter and soil acidity as a result of its decomposition could explain the reduction in pH, both under shrubs and with grazing (Posselt Martins et al., 2014; Talore et al., 2016); in our study, the TOM was higher beneath shrub canopy in grazed areas where we found the lower pH values.

Unlike pH, electrical conductivity (EC) increased under shrubs as found by Moro et al. (1997a), (1997b) and Pugnaire et al. (1996a), (1996b) for *R. sphaerocarpa* in semi-arid environments of the Iberian Peninsula, and by Ghorbanian and Jafari (2007) for *Salsola rigida* Pall. in desert areas of Iran. EC increase is likely to be related to leguminous N fixation and root exudates (Bruning et al., 2015). Since EC is an indirect measure of the salinity of the soil, soils with higher EC have a higher salt content (Garrido Valero, 1993). The EC of our mining substrates is not high, contrary to what is frequently found in coal mine soils (Arranz-González, 2011), so it does not impede good plant development (Garrido Valero, 1993). Current EC in the mine is somewhere in the middle ($\text{EC}=0.0105\ \text{S m}^{-1}$) among original reclamation topsoiling ($\text{EC}=0.0114\ \text{S m}^{-1}$) materials and forest soil EC ($\text{EC}=0.0082\ \text{S m}^{-1}$), being shrub soils less evolved and with larger EC than soils without shrubs (0.012 ± 0.001 vs. 0.009 ± 0.001). Indeed, in grazed areas in our reclaimed mine, although there were no significant differences, the sum of bases (SB) and the percentage of base saturation (V) were higher under shrubs, in agreement with higher EC values (Garrido Valero, 1993). On the contrary, without grazing, the trend was the opposite one, suggesting a synergistic effect of shrubs and grazing on the soil salinity of reclaimed mines.

Among exchangeable cations, Wang et al. (2016a), (2016b) found higher available $K^+$ under leguminous, as we also found in our study. Other Mediterranean leguminous shrubs, such as *R. sphaerocarpa*, also have a positive effect on many nutrients, including potassium (Rodríguez-Echeverría and Pérez-Fernández, 2003). Potassium increases plant resistance to drought, mainly due to osmotic adjustments and a reduction in transpiration rates, which involves a higher water-use efficiency (Gómez-Aparicio et. al, 2005). Thus, the increase in available $K^+$ could be an important nurse shrub-soil effect in areas where drought is the main limiting factor for plant survival (Gómez-Aparicio et al., 2005). Therefore, the lower nutrient values (e.g., TN, $K^+$, TP) in open sites could be due to the CEC processes of belowground biomass (Osem et al., 2004).

The higher $Na^+$ content with grazing outside shrubs could be due to livestock excreta, which are more frequent in these sites (Zarekia et al., 2012). This cation can negatively affect soil structure by preventing the formation of aggregates, thus contributing to a decrease in soil porosity, making the soil more impermeable to water and air (Garrido Valero, 1993). However, the concentration of $Na^+$ in our soil samples might be too low to cause problems.

The content of exchangeable $Mg^{2+}$, another essential cation for plant growth and development (López-Marcos et al., 2019), was also higher in soils under shrubs in grazed areas. However, $Ca^{2+}$ was higher out of shrubs in ungrazed areas, suggesting higher uptake by shrubs as well as a positive effect of livestock exclusion. Teague et al. (2011) have demonstrated a negative effect of the increasing livestock stocking rate on calcium content in the soil (Teague et al., 2011).

In our study we demonstrated that *C. scoparius* and *G. florida* forming mixed stands contribute to TOM and CEC-related properties, thus potentially raising fertility, while livestock and wild ungulates contribute to TOM decomposition and nutrient cycling. Our results also showed a positive synergistic effect of leguminous shrubs, *C. scoparius* and *G. florida*, and grazing on increasing the content and availability of limiting nutrients for plant growth in mining substrates. The situation contributes to both soil properties improvement, and higher fertility should be the combination of both: nurse shrubs and grazing (Alday et al., 2014).

### *4.2. Influence of shrubs and grazing on soil physical properties*

Our results indicate that *C. scoparius* and *G. florida* shrubs also have a positive effect on soil physical properties, with a statistically significant improvement for some of them.

Texture regulates many important factors for plant growth: water and nutrient storage capacity, infiltration, ease of tillage, etc. (Arranz-González, 2011) by both organic matter and textural fractions involved.

Our mining soils under shrubs contain a significantly higher percentage of sand and a lower percentage of clay compared to the soil outside shrubs canopy, but only in the grazed areas, so there is a combined effect of shrubs and grazing on soil textural fractions. Livestock trampling is likely contributing to reducing fine particles (clay) movement, maintaining a low relative proportion of sand particles (Li et al., 2003; Wasson and Nanninga, 1986). On the other hand, woody vegetation may intercept sand particles and thereby decrease the relative proportion of clay beneath shrub cover. However, herbs also reach relatively high cover under shrubs, so we firmly believe that the main process is not related to intercepting particles, but the mobilisation from neighbouring corridors and gaps by trampling. Woody cover can also decrease the erosion of fine particles by wind and surface runoff during heavy rainfall events (Wasson and Nanninga, 1986). López-Marcos et al. (2020) obtained a similar percentage of sand and a lower percentage of clay in shrublands soils than in grasslands soils in the same coal mine ten years before excluding grazing. Therefore, the effect of shrubs on soil texture requires some time to become significant, even in grazed areas, and more time is likely needed for the textures of mining soils to approach those of the surrounding natural soils (Thurman and Sencindiver, 1986).

In the soil profile of the reclaimed study mine, there is a lack of soil structure except in the most superficial part where elements with granular structure have been linked to the root system. They are incipient soils of very low evolution, with A-C type profile (López-Marcos et al., 2020). Since an improvement in soil structure favors water retention (Arranz-González, 2011), higher values of available water and water holding capacity are expected to be found under shrubs.

Directly related to soil structure and porosity, bulk density is a widely used measure to estimate the degree of soil compaction (Arranz-González, 2011). In general, mining soils have a high degree of compaction (higher bulk density) due to the use of heavy machinery in mining and rehabilitation works (Martínez-Ruiz and Fernández Santos, 2001). Our results showed that grazing increased the bulk density of mine soils due to the trampling, which increases soil compaction (Chaichi et al., 2005). Although the effect of shrubs on bulk density was not significant, bulk density values were higher outside shrubs. According to Chaichi et al. (2005), this could be possible not only because of the higher trampling in areas without woody cover but also because of the lower organic matter content in the topsoil of grazed areas, whose structure is less stable (Mapfumo et al., 2000).

In general, soil compaction is a stress factor that negatively affects plant growth, in many cases reducing root development, which can be a disadvantage for plants under water stress conditions, but its effects vary between species and with the range of soil compaction. In the study area, despite the higher TOM content under nurse shrubs, available water, and water holding capacity were similar in soils under and outside shrubs. Nevertheless, grazing reduces water holding capacity (because of the vegetation cover reduction by livestock browsing), and soil porosity, because of the compaction increase by trampling (Lai and Kumar, 2020). These effects relate to the lower organic matter content (Arranz-González, 2011) and higher $Na^+$ content in grazed areas especially outside of shrubs (Garrido Valero, 1993). Also, Shrestha and Lal (2008) found lower water holding capacity in grazed areas that related to the high bulk density, low porosity, and low content in some chemical properties such as TOC.

## 5. Conclusions

There was a synergistic effect of native leguminous nurse shrubs and low-intensity grazing on the physical and chemical properties of coal-mine soils in northern Spain. Nurse shrubs had a positive effect on mine soil properties, which was more noticeable in grazed areas. Nurse shrubs directly contributed to increasing soil fertility by accumulating organic matter, and indirectly by avoiding trampling and browsing by livestock and wild ungulates in the spaces they covered. Livestock and wild ungulates have a negative effect on soil physical properties; they modified soil textural properties and increased compaction by reducing water holding capacity. These negative effects of livestock trampling and browsing on the soil were attenuated by the presence of shrubby vegetation. Therefore, the nurse shrub protection function affects not only other plant species but it is extended to soil properties.

The herein confirmed soil amelioration promoted by nurse shrubs can have important consequences for the nutrient dynamics, the whole ecosystem functioning, and the restoration of the ecosystem services of the reclaimed mine, particularly in combination with low grazing intensity, whose synergistic effects contribute to more intense soil amelioration. The fact that shrub-promoted soil improvement and their role as a physical barrier to livestock trampling and browsing could have a positive effect on the establishment of tree species in these highly degraded environments opens up great prospects for forest regeneration in areas with similar constraints. The colonization of herbaceous pastures by nurse shrubs should therefore be promoted to ensure the mining soil amelioration necessary for the establishment of other species in rehabilitated coal mines.

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## CRediT authorship contribution statement

**Elena Muñoz-Cerro:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Juan García-Duro:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – review & editing. **Carolina Martínez-Ruiz:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Daphne López-Marcos:** Investigation, Methodology, Resources, Supervision, Visualization, Writing – review & editing.

## Declaration of Competing Interest

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

## Data Availability

Data will be made available on request.

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