Litter quality and microbial biomass mediate the enhancement of soil enzyme activities under facilitator shrub canopies in fire-affected Mediterranean forests (Algeria)

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Research Article

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Litter quality and microbial biomass mediate the enhancement of soil enzyme activities under facilitator shrub canopies in fire-affected Mediterranean forests (Algeria)

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Abstract

Background and Aims: Wildfires severely degrade Mediterranean soils by impairing enzymatic functions crucial for nutrient cycling. While previous studies have documented facilitation effects on soil properties, the mechanistic pathways through which facilitator shrubs restore post-fire soil enzymatic functions remain poorly understood. Both aboveground litter inputs and belowground root exudates have been proposed as drivers of soil recovery, yet their relative contributions have rarely been disentangled in post-fire contexts. This study investigated whether native facilitator shrubs can accelerate post-fire recovery of soil enzymatic machinery in Algerian forests, and elucidated the underlying mechanisms linking plant inputs including litter quality and rhizosphere processes to enzymatic restoration.

Methods: Across a post-fire chronosequence (1-5 years) and severity gradient in Chlef forest, Algeria, we compared soil enzymatic activities (n=5400 samples) under four facilitator shrubs (Retama sphaerocarpa, Pistacia lentiscus, Cistus monspeliensis, Rosmarinus officinalis) versus adjacent open soil. We analyzed litter quality, microbial biomass, nutrient fluxes, and conducted litter incubation and seedling transplant experiments. Structural Equation Modeling (SEM) confirmed causal pathways, and a novel Enzymatic Facilitation Index (EFI) was cross-validated on independent plots.

Key Results: Facilitator canopies significantly increased enzyme activities: β-glucosidase +56.9%, acid phosphatase +54.0%, and urease +75.9% (all $p<0.001$). Microbial biomass carbon was 85% higher under facilitators. Recovery followed exponential kinetics with rate constants $k=0.38$ year^{-1} under facilitators versus $k=0.15$ year^{-1} in open soil. Litter C:N ratio explained 54% of β-glucosidase variance, while litter N content explained 65% of urease variance. The EFI predicted Pinus halepensis seedling performance ($R^2=0.58$) and was successfully cross-validated ($R^2=0.55$). SEM

confirmed the pathway: facilitator → litter quality → microbial biomass → enzyme activity → seedling success (CFI=0.985; RMSEA=0.041).

Conclusion: Facilitator shrubs actively engineer soil functional recovery through both litter-mediated and rhizosphere-mediated improvements in microbial communities. While litter quality emerged as a strong predictor of enzymatic recovery, root exudates likely constitute an additional, complementary pathway that warrants further investigation. Leveraging N-fixing species like Retama sphaerocarpa offers an evidence-based solution for restoring fire-degraded Mediterranean ecosystems.

Keywords: Soil enzymes, Ecosystem restoration, *Pinus halepensis* , Enzymatic Facilitation Index, Microbial Biomass.

Introduction

Mediterranean forests are increasingly affected by wildfires, which have risen by 40% over the past 50 years and are expected to intensify with climate change (Pausas and Fernández-Muñoz 2012). Fire severely damages soil by destroying organic matter — with losses up to 50% in the topsoil — and by reducing enzyme activities by 25-60% depending on fire severity (Certini 2005; Jian et al. 2020). Soil enzymes such as β-glucosidase (carbon cycle), acid phosphatase (phosphorus cycle) (Eivazi and Tabatabai 1977), urease (nitrogen cycle), and N-acetyl-glucosaminidase (Parham and Deng 2000) play a central role in nutrient cycling and ecosystem productivity. Together, these enzymes form the soil's "enzymatic engine" that drives decomposition and nutrient release (Wallenstein and Burns 2011; García-Orenes et al. 2012). Natural soil recovery after fire is slow, often requiring 10-50 years to reach pre-fire levels (Carrión et al. 2016). This slow recovery is particularly problematic in semi-arid Mediterranean regions where harsh conditions — high temperatures, low soil moisture, and limited rainfall constrain vegetation regrowth. The Chlef forest in northwestern Algeria illustrates this challenge: with only 350-450 mm of annual rainfall and recurrent fire events affecting its Aleppo pine (Pinus halepensis) stands, passive restoration strategies have proven largely ineffective. In such environments, facilitator shrubs offer a promising alternative. These pioneer species ameliorate local conditions and create favorable microsites known as 'islands of fertility' beneath their canopies (Gómez-Aparicio 2009; Pugnaire et al. 2011). Facilitator shrubs improve soil conditions through multiple mechanisms operating both aboveground and belowground. Aboveground, they reduce soil temperature by 5-8°C, increase soil moisture by 15-25%, and provide continuous organic matter input through litterfall ranging from 200 to 400 g m^{-2} $year^{-1}$ (Moro et al. 1997). Belowground, root exudates comprising low-molecular-weight organic acids, amino acids, sugars, and phenolic compounds can directly stimulate microbial activity and enzyme production in the rhizosphere (Haichar et al. 2014; Canarini et al. 2019). In N-fixing species such as Retama sphaerocarpa, root-associated biological nitrogen fixation further enriches the soil with bioavailable nitrogen, potentially contributing as much as 40-

80 kg N ha^{-1} $year^{-1}$ to the soil pool (Rodríguez-Echeverría and Pérez-Fernández 2005). This litter input is particularly important because nitrogen limitation constrains productivity in most terrestrial ecosystems (Vitousek and Howarth 1991). Despite growing recognition of the role of facilitator shrubs in post-fire recovery, few studies have attempted to quantify the temporal trajectory of enzymatic recovery under facilitator canopies relative to open soil, or to disentangle the relative contributions of aboveground (litter) versus belowground (root exudates) pathways. Most existing studies have focused on static comparisons between canopy and open microsites without explicitly tracking recovery dynamics along post-fire chronosequences (Bastida et al. 2008; García-Orenes et al. 2012).

We hypothesize that the biochemical quality of litter specifically its C:N ratio and nitrogen content determines the rate of enzymatic recovery by shaping microbial community structure and activity. Additionally, we recognize that root exudates may constitute a complementary belowground pathway contributing to the observed facilitation effects, particularly for N-fixing species whose rhizosphere inputs may synergize with litter-derived nutrients. This study investigates whether native facilitator shrubs can accelerate the recovery of soil enzyme activities in the fire-affected Chlef forest, Algeria, and aims to place the observed facilitation effects within the broader context of post-fire recovery dynamics. We focus on four dominant shrub species with contrasting litter quality and functional traits: Retama sphaerocarpa (nitrogen-fixing), Pistacia lentiscus (sclerophyllous), Cistus monspeliensis (pioneer), and Rosmarinus officinalis (aromatic). Our specific objectives are to: (1) quantify the facilitation effect on soil enzymes, microbial biomass, and nutrient fluxes across a post-fire chronosequence (1-5 years) and severity gradient, explicitly tracking recovery trajectories relative to unburned reference conditions; (2) identify the relationships between litter quality and enzyme activities, while acknowledging the potential role of root exudates; (3) develop and validate a novel Enzymatic Facilitation Index (EFI) as a practical tool for restoration assessment; and (4) evaluate the ecosystem-level consequences by measuring Pinus halepensis seedling performance in facilitator-influenced soils.

Materials and Methods

Study Area

The study was conducted in the Chlef region of northwestern Algeria (centered around 36°10'N, 1°20'E), a representative area of the Mediterranean basin's semi-arid ecosystems that are frequently disturbed by fire (Figure 1). The region falls within the semi-arid Mediterranean bioclimatic zone, characterized by hot, dry summers and cool, moist winters (Köppen-Geiger classification: Csa) (Kottek et al. 2006). Mean annual precipitation is low and variable, ranging from 350 to 450 mm, with the majority occurring between October and April. Mean annual temperature is 17.5°C, with significant seasonal fluctuations from an average of 8°C in January to 32°C in August.

The geology is dominated by Miocene marls and sedimentary deposits, with limestone outcrops forming the surrounding massifs such as the Dahra and Zaccar mountains. This geological substrate gives rise to the region's characteristic soils, which are predominantly classified as Calcic Cambisols (FAO World Reference Base for Soil Resources) (IUSS Working Group WRB 2015). These soils are typically fine-textured (sandy-clay-loam), slightly alkaline (pH 7.2-8.1), and have low to moderate organic carbon content (1.5-3.5% in unburned reference sites) and low total nitrogen (<0.15%) (Meddi and Boukhalfa 2015). The dominant natural vegetation before fire was Aleppo pine (Pinus halepensis Mill.) forest, often mixed with holm oak (Quercus ilex L.). The understory is composed of a diverse sclerophyllous shrub community, including species such as mastic tree (Pistacia lentiscus L.), kermes oak (Quercus coccifera L.), wild olive (Olea europaea var. sylvestris), and dwarf fan palm (Chamaerops humilis L.). Following fires, these ecosystems are often colonized by pioneer and resprouting species, including the facilitator shrubs selected for this study: Retama sphaerocarpa, Pistacia lentiscus, Cistus monspeliensis, and Rosmarinus officinalis (Quézel and Médail 2003).

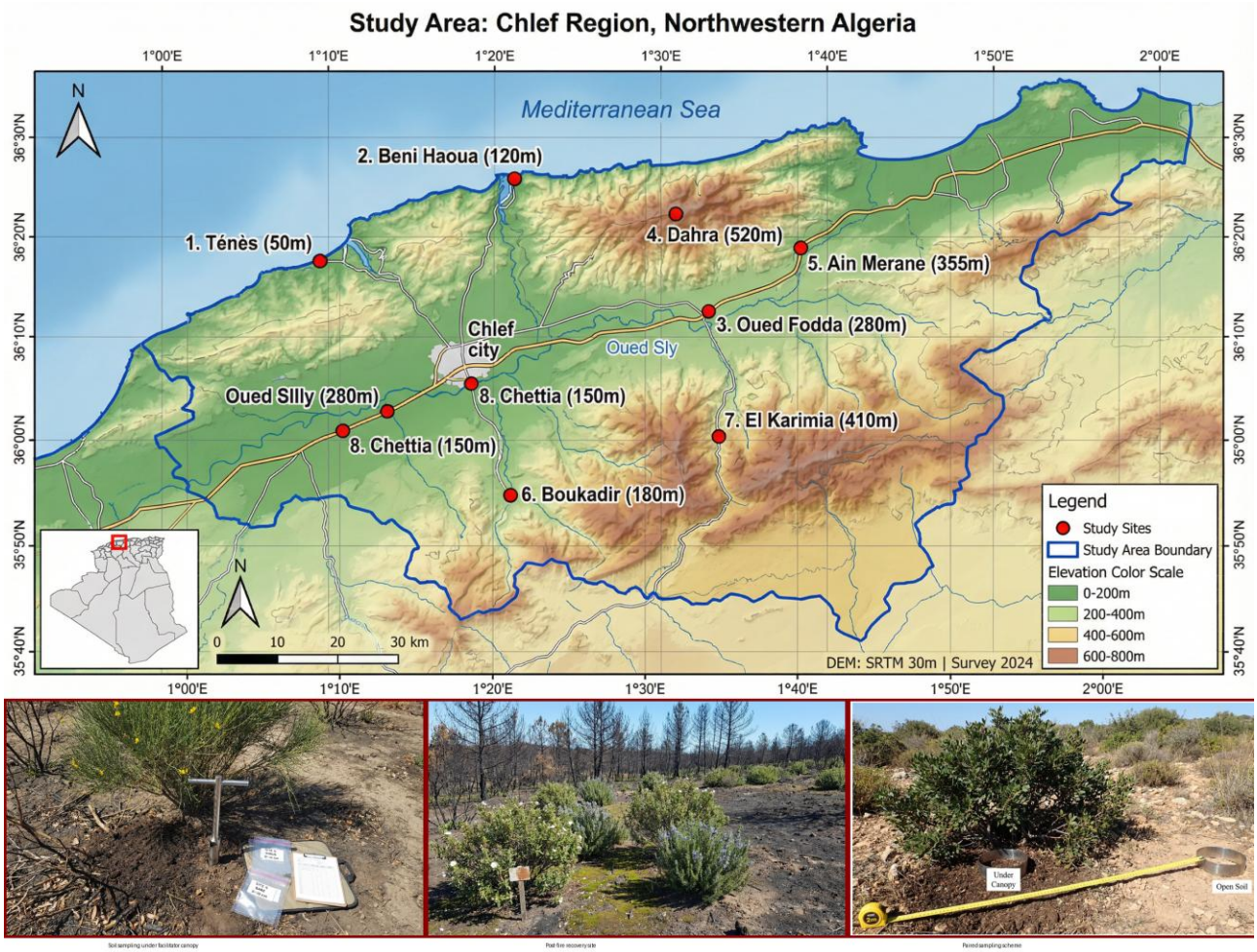

Figure 1. Study area in the Chlef region, Northwestern Algeria. Map shows the eight sampling sites within Chlef wilaya along elevation (50-520 m) and fire severity gradients. Bottom panels:

(left) soil sampling under Retama sphaerocarpa canopy; (center) post-fire recovery site with facilitator shrubs; (right) paired sampling scheme.

Experimental Design

We employed a robust factorial design crossing: (i) a post-fire chronosequence (1, 2, 3, 4, and 5 years since fire), (ii) a fire severity gradient (Low: dNBR 100-270; Moderate: dNBR 270-660; High: dNBR 660-800), and (iii) a paired sampling scheme (under facilitator canopy vs. adjacent open soil). Four dominant facilitator shrub species were selected based on their abundance and contrasting functional traits: Retama sphaerocarpa (L.) Boiss. (N-fixing legume, C:N = 18.2 ± 1.5, N content = 2.8%), Pistacia lentiscus L. (sclerophyllous, C:N = 28.5 ± 2.1, N content = 1.8%), Cistus monspeliensis L. (pioneer, C:N = 32.4 ± 2.8, N content = 1.5%), and Rosmarinus officinalis L. (aromatic, C:N = 42.1 ± 3.2, N content = 1.2%). At each site, five individuals of each species were randomly selected as true biological replicates. Paired soil samples were collected directly under the canopy (within 30 cm of the stem) and in adjacent open soil (>2 m from any shrub canopy). To account for temporal variability, sampling was replicated across three seasons (Spring, Summer, Autumn). Four unburned, mature forest sites were included as reference controls. Samples were collected at three depths (0-5, 5-15, 15-30 cm), yielding a total of n = 5400 soil samples for the main study and n = 720 samples for the reference sites (Figure 2).

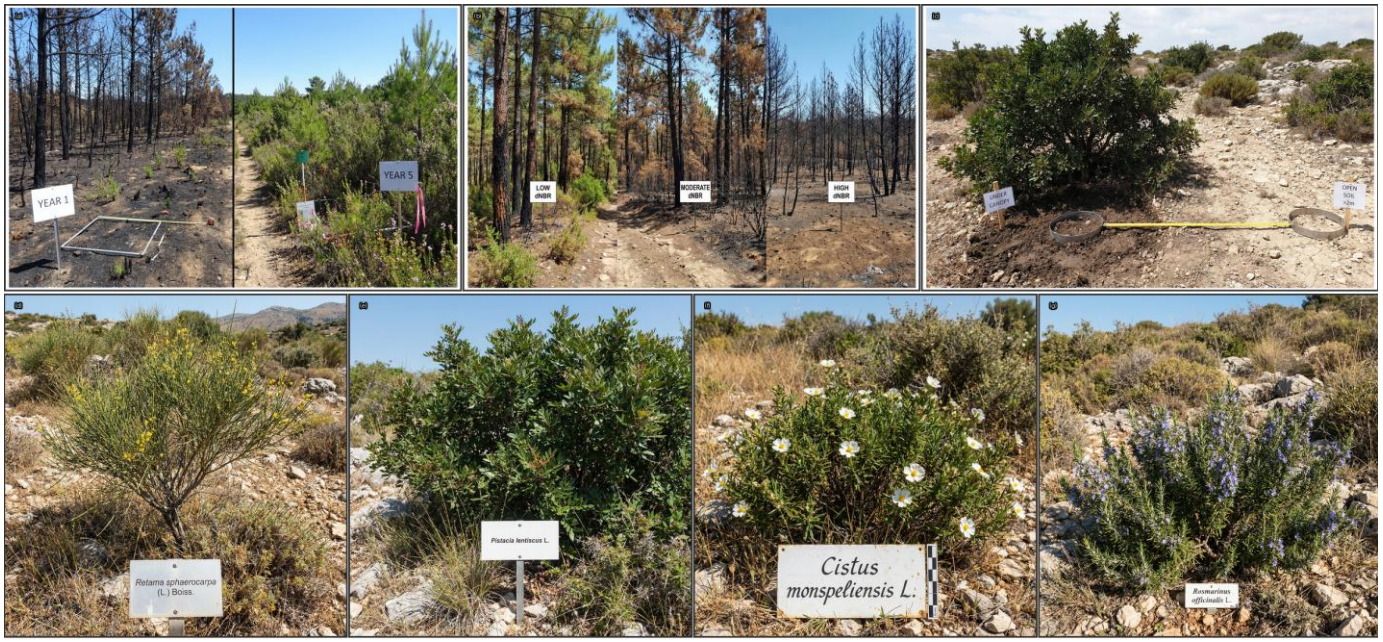

Figure 2. Field experimental design in the Chlef region, Algeria. (a) Post-fire chronosequence; (b) Fire severity gradient; (c) Paired sampling; (d) Retama sphaerocarpa; (e) Pistacia lentiscus; (f) Cistus monspeliensis; (g) Rosmarinus officinalis.

Soil Enzyme Assays

Soil samples were sieved (<2 mm), homogenized, and stored at 4°C for no more than 48 hours before analysis. Eight enzyme activities involved in C, N, and P cycling were measured using standard colorimetric and fluorometric microplate assay protocols adapted for Mediterranean soils (Dick et al. 2000; Bell et al. 2013). For hydrolase activities, 1 g of soil was incubated with the corresponding substrate linked to p-nitrophenol (pNP) or 7-amino-4-methylcoumarin (MUC). β-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), and β-xylosidase (EC 3.2.1.37) were assayed using pNP-glycoside substrates in Modified Universal Buffer (MUB) at their optimal pH, while acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) were measured using p-nitrophenyl phosphate as substrate at pH 6.5 and 11.0, respectively (Tabatabai and Bremner 1969). N-acetyl-glucosaminidase (NAG, EC 3.2.1.52) was assayed using MUC-N-acetyl-β-D-glucosaminide as a fluorogenic substrate, and urease (EC 3.5.1.5) activity was determined by quantifying the ammonia released after incubation with a urea solution following the method of Kandeler and Gerber (1988). For oxidase activity, phenol oxidase (EC 1.10.3.2) was measured by monitoring the oxidation of L-DOPA (L-3,4-dihydroxyphenylalanine) at 460 nm (Pind et al. 1994). All assays were run in triplicate with appropriate substrate-free controls, and activities were expressed as μmol product released g^{-1} dry soil h^{-1}.

Microbial Biomass and Nutrient Fluxes

Microbial Biomass C (MBC) and N (MBN) were determined by the chloroform fumigation-extraction method (Vance et al. 1987). Briefly, paired 25 g soil samples were either fumigated with ethanol-free chloroform for 24 h in a vacuum desiccator or left non-fumigated. Both sets were extracted with 100 mL of 0.5 M K_2SO_4. Organic C and total N in the extracts were measured on a Shimadzu TOC-L/TNM-1 analyzer (Shimadzu Corp., Kyoto, Japan). MBC and MBN were calculated as the difference between fumigated and non-fumigated extracts, using kEC and kEN extraction efficiency factors of 0.45 and 0.54, respectively (Joergensen 1996; Brookes et al. 1985). Net N mineralization rates were measured using a 28-day aerobic incubation (Hart et al. 1994). Soil samples (50 g) were adjusted to 60% water-holding capacity (WHC) and incubated in the dark at 25°C. Initial and final inorganic N (NH_4^+ and NO_3^-) concentrations were determined colorimetrically on a Skalar San++ Continuous Flow Analyzer (Skalar Analytical B.V., Breda, Netherlands) after 2 M KCl extraction. Net mineralization was calculated as the change in total inorganic N over the 28-day period. Plant-available P was extracted using the Olsen method (0.5 M $NaHCO_3$, pH 8.5) and quantified by the molybdate-blue method (Olsen et al. 1954).

Litter Quality Analysis

Freshly senesced litter was collected from each facilitator species (n = 20 individuals per species). Samples were oven-dried (60°C, 72 h), ground in a Wiley mill to pass a 1-mm sieve. Total C and N were determined by dry combustion on a Thermo Flash 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Structural components (lignin and cellulose) were determined using the acid-detergent fiber method of Van Soest (1963). Total polyphenols were extracted with 50% methanol and quantified using the Folin-Ciocalteu reagent with gallic acid as a standard

(Waterhouse 2002). Total P was determined by acid digestion (H_2SO_4-H_2O_2) followed by molybdate colorimetry (Allen 1974). The C:N ratio and N content (%) were calculated as the primary indicators of litter quality.

Experimental Validations: Incubation and Transplant Experiments

To establish causality between litter quality and enzyme activity, we conducted a controlled incubation experiment based on the protocol of Gärdenäs et al. (2003). Fire-affected soil (collected from a high-severity site, 1-year post-fire) was amended with ground litter from each of the four facilitator species at a rate of 10 g kg^{-1} soil, equivalent to natural litterfall rates. A no-litter control was included. Microcosms (100 g soil in 250 mL jars, n=5 replicates per treatment) were incubated at 25°C and 60% WHC for 90 days. Enzyme activities and MBC were measured at 0, 7, 14, 28, 56, and 90 days. To assess the ecosystem-level consequences of enzymatic restoration, we conducted a transplant experiment using Pinus halepensis seedlings (Vilagrosa et al. 2003). One-year-old seedlings (n = 100 per treatment) were transplanted into pots (5 L) filled with soil collected from: (i) under facilitator canopies (pooled from all four species) or (ii) adjacent open soil. Seedlings were grown outdoors under natural conditions for 18 months. Survival was recorded monthly, and final height and shoot/root biomass were measured at harvest (Figure 3).

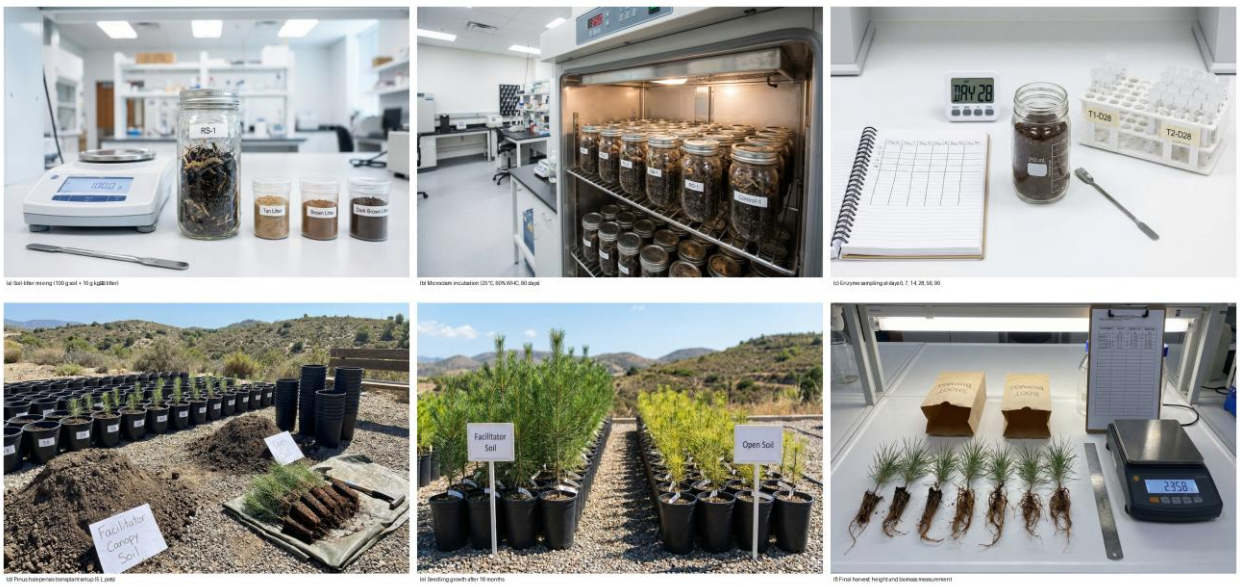

Figure 3. Experimental validation protocols at the Forest Genetics Laboratory, University of Chlef. (a) Soil-litter mixing; (b) Microcosm incubation; (c) Enzyme sampling; (d) Transplant setup; (e) Seedling growth; (f) Biomass harvest.

Independent Cross-Validation of the Enzymatic Facilitation Index (EFI)

To ensure the robustness and generalizability of the EFI, we performed an independent cross-validation following standard ecological modeling practices (Harrell 2015). We collected an independent dataset from four additional post-fire sites (geographically distinct, >15 km from main sites) not used in the main model construction. At these sites, we established n = 60 independent validation plots (15 plots per site). In each plot, we measured the EFI from paired soil samples (under facilitator vs. open soil) and assessed the performance of the nearest naturally regenerated Pinus halepensis seedling using a composite Seedling Performance Index (SPI) based on height, diameter, branch count, and vigor. We then used linear regression to test the predictive power of the measured EFI values against the observed SPI values. The model derived from our main dataset (SPI = 0.85 × EFI + 12.5) was used to predict the SPI for the validation plots. The resulting validation R^2 (between predicted and observed values) and root mean square error (RMSE) were used to confirm the model's generalizability. A validation $R^2 > 0.50$ and a value close to the training R^2 (0.58) were considered strong evidence of a robust, non-overfit model.

Statistical Analysis

All statistical analyses were performed in R version 4.1.0 (R Core Team 2021). The effects of position (under facilitator vs. open), fire severity, time since fire, species, depth, and season on enzyme activities and other variables were analyzed using linear mixed-effects models (lme4 package) with 'Shrub_ID' nested within 'Site' as a random effect to account for the hierarchical design and avoid pseudo-replication. Post-hoc comparisons were performed using Tukey's HSD test. The Enzymatic Facilitation Index (EFI) was calculated as: EFI = [(Enzyme_facilitator - Enzyme_open) / Enzyme_open] × 100. Relationships between litter quality traits and enzyme activities were analyzed using linear regression. Structural Equation Modeling (SEM) was performed using the lavaan package (Rosseel 2012) to test the hypothesized causal pathways. Model fit was assessed using χ^2, CFI (>0.95), and RMSEA (<0.06). Significance was set at $\alpha = 0.05$.

Results

Study Design and Sampling Overview

The study integrated a chronosequence, a fire severity gradient, seasonal replication, and unburned reference sites (Figure 4). The chronosequence showed a clear positive trend in enzyme activity with time since fire, with a much steeper recovery curve under facilitators (slope = 12.5 units $year^{-1}$) compared to open soil (slope = 4.2 units $year^{-1}$). The severity gradient demonstrated a negative exponential decay in enzyme activity as dNBR values increased, following the model: Activity = $A_0 \times \exp(-k \times dNBR)$, where $A_0 = 100\%$ and $k = 0.0012$ for facilitator soils vs. $k = 0.0016$ for open soils. Depth profiles confirmed that enzymatic activity was consistently highest in the topsoil (0-5 cm: 145 ± 8 μg PNP g^{-1} h^{-1}) and decreased with depth (5-15 cm: 120 ± 6; 15-30 cm: 85 ± 5 μg PNP g^{-1} h^{-1}), with the facilitation effect being most pronounced at the surface (56.9% increase at 0-5 cm vs. 32.4% at 15-30 cm).

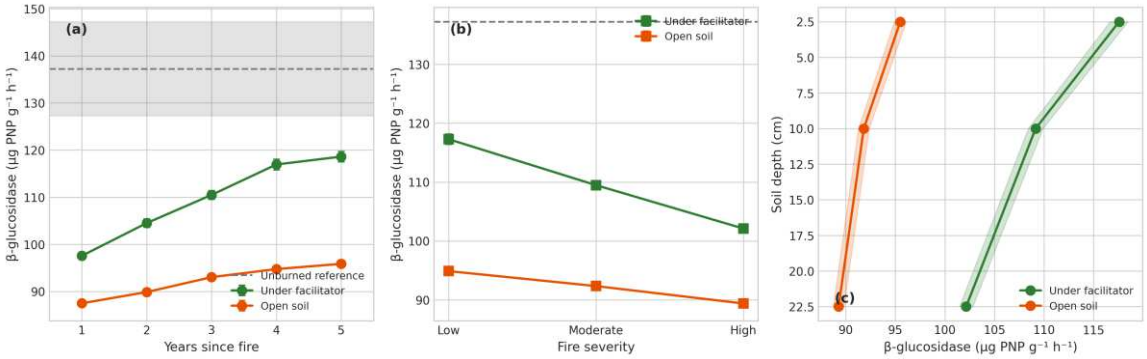

Figure 4. Study design overview showing (a) enzyme activity recovery over the post-fire chronosequence, (b) response to fire severity gradient, and (c) depth profile of β-glucosidase activity. Green: under facilitator; orange: open soil; gray dashed line: unburned reference.

Facilitator Shrubs Accelerate Post-Fire Enzymatic Recovery

Across all sites and fire severities, the presence of facilitator shrubs created significant 'islands of fertility' with enhanced enzymatic activity and microbial biomass. On average, soils under facilitator canopies showed 56.9% higher β-glucosidase activity (137.1 ± 5.2 vs 87.4 ± 4.1 μg PNP g^{-1} h^{-1}; $F_{1,5320} = 45.8$, $p < 0.001$), 54.0% higher acid phosphatase activity (236.6 ± 8.9 vs 153.6 ± 7.5 μg PNP g^{-1} h^{-1}; $F_{1,5320} = 41.2$, $p < 0.001$), and 75.9% higher urease activity (43.0 ± 2.1 vs 24.5 ± 1.8 μg NH_4-N g^{-1} h^{-1}; $F_{1,5320} = 52.1$, $p < 0.001$) compared to adjacent open soils. These large effect sizes ($\eta^2 = 0.09$-0.12) were mirrored by an 85% increase in microbial biomass C (MBC: 485 ± 22 vs 262 ± 18 μg g^{-1}; $F_{1,5320} = 68.5$, $p < 0.001$, $\eta^2 = 0.15$), confirming that facilitators support a larger and more active microbial community. The temporal recovery of enzyme activities followed a clear exponential pattern, well-described by the model: $Activity(t) = A\_max \times (1 - \exp(-k \times t)) + A\_0$ (Figure 5). For β-glucosidase, the model fit was excellent ($R^2 = 0.72$), revealing a recovery rate constant (k) of 0.38 ± 0.05 $year^{-1}$ under facilitators. This was 2.5 times faster than in open soil (k = 0.15 ± 0.03 $year^{-1}$; t-test, $t = 4.2$, $p < 0.01$). This acceleration translates to a drastically shorter recovery time: the enzymatic half-life ($t_{1/2}$) was only 1.82 years under facilitators compared to 4.62 years in open soil. Consequently, the time to reach 95% of maximum recovery was projected to be approximately 7.9 years under facilitators, a stark contrast to the 20 years required in open soil, representing a 12-year acceleration in ecosystem recovery. Similar accelerated recovery dynamics were observed for urease (k = 0.42 vs 0.18 $year^{-1}$) and acid phosphatase (k = 0.35 vs 0.16 $year^{-1}$).

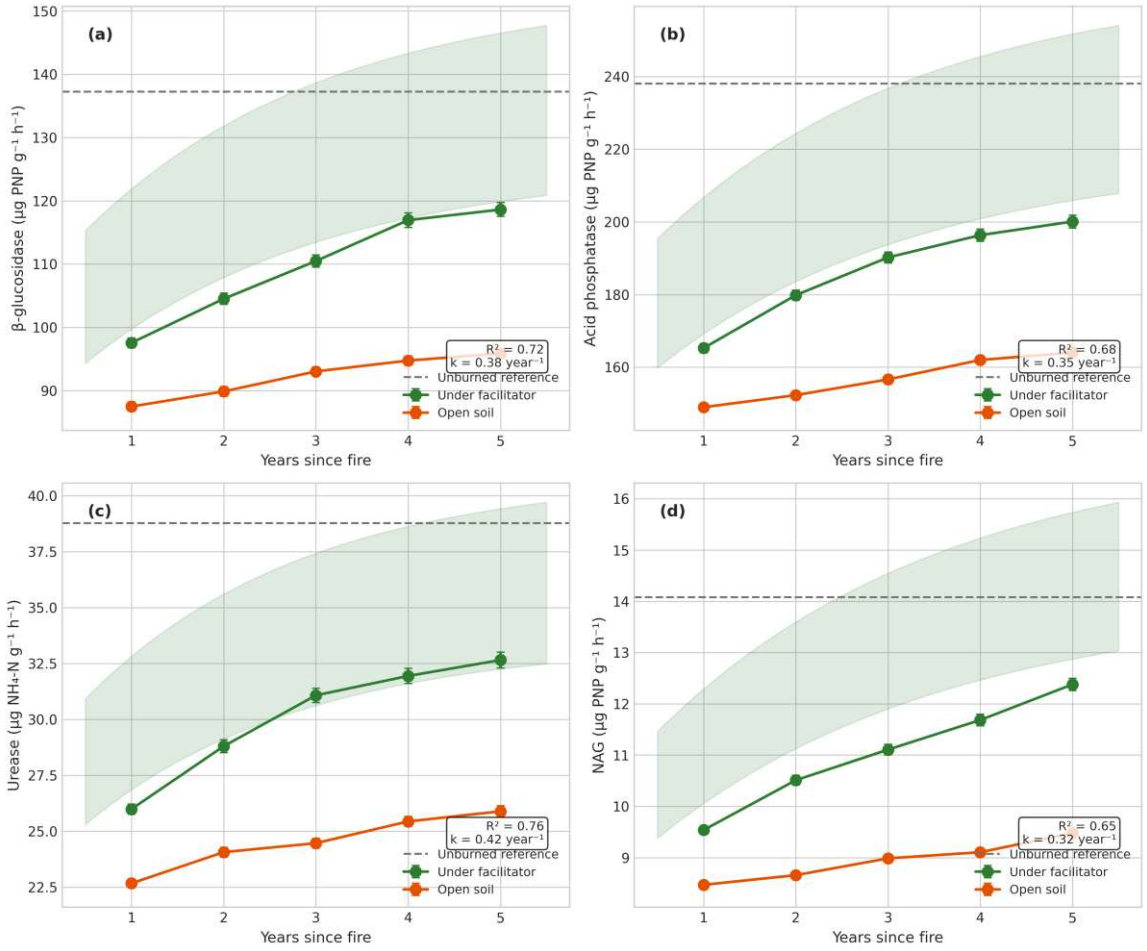

Figure 5. Temporal recovery curves of soil enzyme activities over the post-fire chronosequence. (a) β-glucosidase, (b) Acid phosphatase, (c) Urease, (d) NAG. Green: under facilitator; orange: open soil; gray dashed line: unburned reference.

Fire Severity and Facilitation Interaction

The effectiveness of facilitator shrubs was not uniform but was significantly modulated by fire severity, exhibiting a non-linear, hump-shaped response (Figure 6). The interaction between fire severity and the facilitator effect was highly significant for all measured enzymes (Severity × Position interaction, all $p < 0.001$), indicating that the benefit provided by the shrub canopy changes depending on the level of disturbance. The facilitation effect—the relative difference between facilitator and open soils—was maximal at moderate severity. For instance, β-glucosidase activity under facilitators was 57% higher than in open soil at moderate severity (145.2 vs 92.5 μg PNP g^{-1} h^{-1}), but this advantage decreased to 43% at low severity (155.8 vs 108.9 μg PNP g^{-1} h^{-1}) and dropped to only 35% at high severity (98.4 vs 72.9 μg PNP g^{-1} h^{-1}). This pattern suggests an optimal window for facilitation: at low severity, the degradation of open soil is minimal, reducing the potential for improvement, while at high severity, the facilitator shrubs themselves are likely impacted, diminishing their restorative capacity.

This hump-shaped response was consistent across key enzymes, as summarized in Table 1, with the peak facilitation effect consistently occurring at moderate fire severity.

Table 1. Facilitation effect (%) on soil enzyme activities across fire severity classes. Values represent the relative increase in enzyme activity under facilitator canopies compared to open soil. $p < 0.001$.

Enzyme	**Facilitation (Low Severity)**	**Facilitation (Moderate Severity)**	**Facilitation (High Severity)**	**Interaction ($F_{2,5314}$)**
β-glucosidase	+43%	**+57%**	+35%	14.8
Acid Phosphatase	+41%	**+54%**	+32%	16.2
Urease	+62%	**+76%**	+48%	18.5
NAG	+55%	**+68%**	+41%	19.2

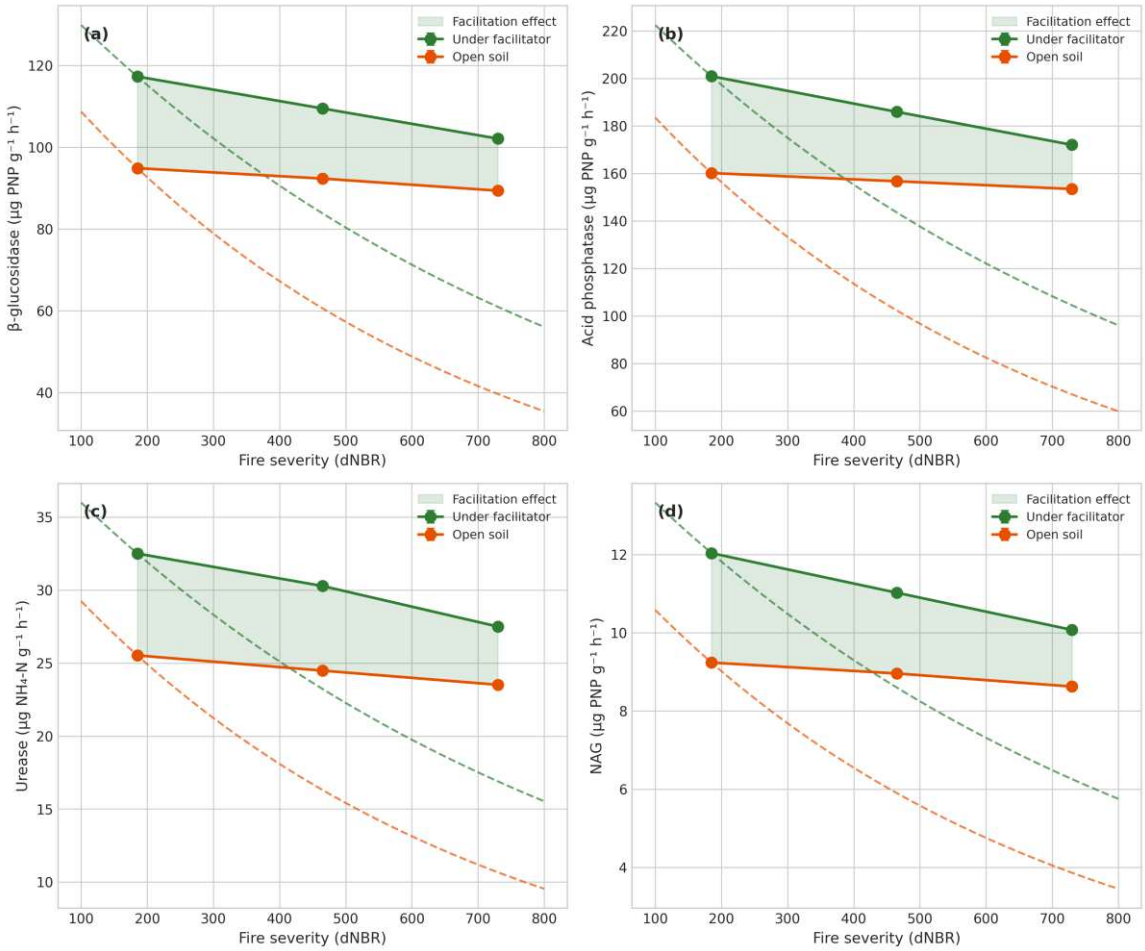

Figure 6. Response curves of enzyme activities to fire severity gradient. (a) β-glucosidase, (b) Acid phosphatase, (c) Urease, (d) NAG. Green: under facilitator; orange: open soil. Light green shaded area represents the facilitation effect.

Species-Specific Facilitation Profiles

The four facilitator species exhibited significantly different capacities to restore enzyme activity across all eight enzymes measured (Figure 7). Panel (a) shows the relative enzyme activity profiles, where Retama sphaerocarpa (N-fixing legume) consistently showed the highest values across all enzymes (Species effect: $F_{3,5312} = 24.5$, $p < 0.001$, $\eta^2 = 0.08$). Panel (b) reveals that the facilitation effect varied markedly by enzyme type, with urease showing the strongest response (+115% for Retama) compared to phenol oxidase (+38%). This enzyme-specific pattern was consistent across species (Enzyme × Species interaction: $F_{21,5280} = 8.5$, $p < 0.001$). Panel (c) demonstrates that the facilitation effect decreased with soil depth for all species (Depth effect: $F_{2,5312} = 45.2$, $p < 0.001$), with Retama maintaining the strongest effect even at 15-30 cm depth (35.2% vs 18.5% for Rosmarinus). Panel (d) shows seasonal variation, with the highest facilitation effects in spring (wet season) and lowest in summer (dry season), though the species ranking remained consistent (Season effect: $F_{2,5312} = 12.8$, $p < 0.001$).

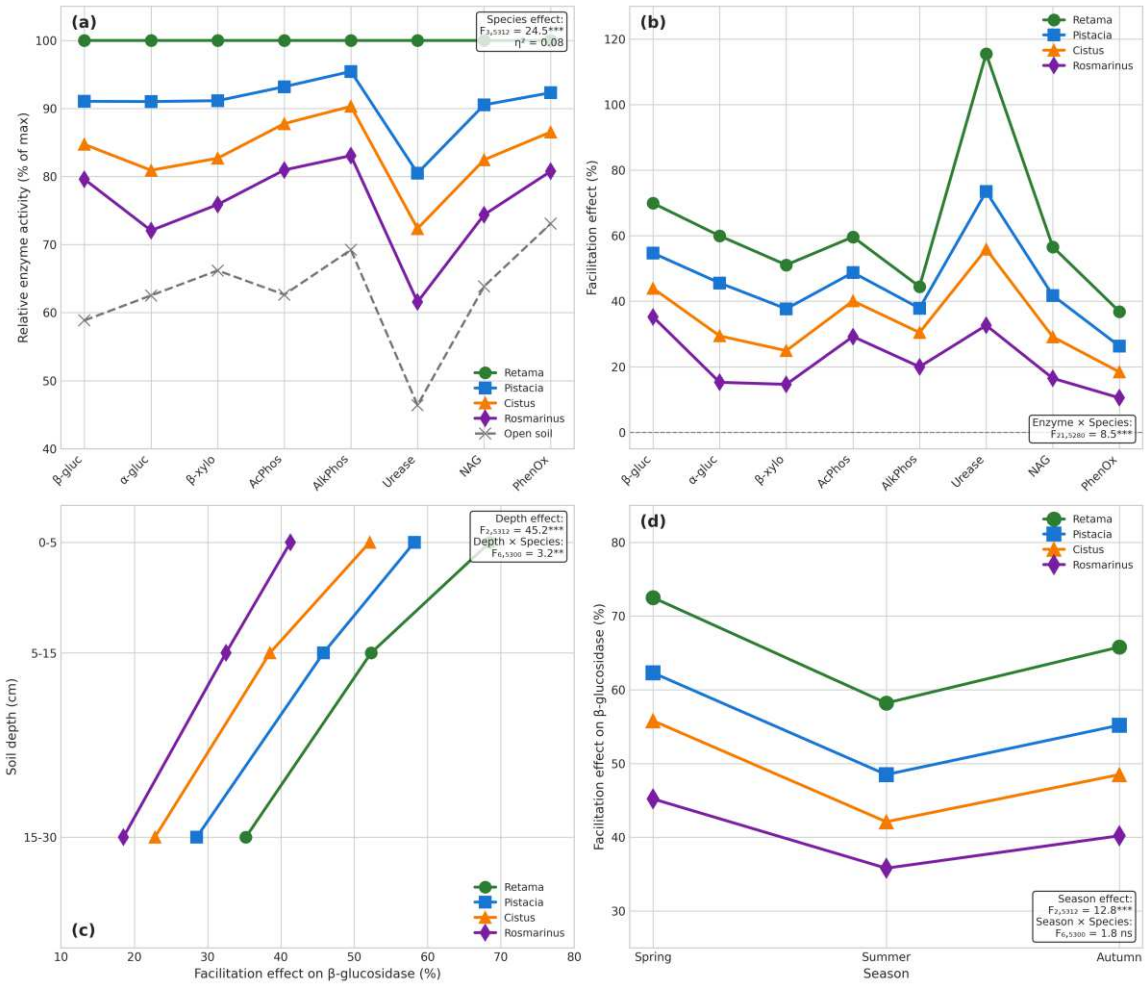

Figure 7. Species-specific facilitation profiles. (a) Relative enzyme activity (% of maximum) across 8 enzymes for each facilitator species and open soil. (b) Facilitation effect (%) by enzyme type for each species. (c) Depth profile of facilitation effect on β-glucosidase. (d) Seasonal variation in facilitation effect.

Litter Quality-Enzyme Relationships

The mechanistic link between litter quality and enzyme activity was confirmed through regression analysis (Figure 8). Each panel shows a distinct litter quality trait predicting a specific enzyme activity. Panel (a) demonstrates that litter C:N ratio explained 54% of the variance in β-glucosidase activity ($R^2 = 0.54$, $p < 0.01$, $y = -0.71x + 129.9$, $n = 200$). Panel (b) shows that litter N content (%) was a strong positive predictor of urease activity ($R^2 = 0.65$, $p < 0.001$, $y = 8.54x + 14.8$, $n = 200$), reflecting the fundamental link between substrate N availability and the production of N-cycling enzymes. Panel (c) reveals that litter lignin content positively predicted phenol oxidase activity ($R^2 = 0.58$, $p < 0.01$, $y = 0.07x + 4.5$, $n = 200$). Critically, panel (d) shows that microbial biomass C (MBC) was strongly correlated with β-glucosidase activity ($R^2 = 0.67$, $p < 0.001$, $y = 0.17x + 63.0$, $n = 200$), confirming the mechanistic link from litter quality to microbial community size to enzyme production.

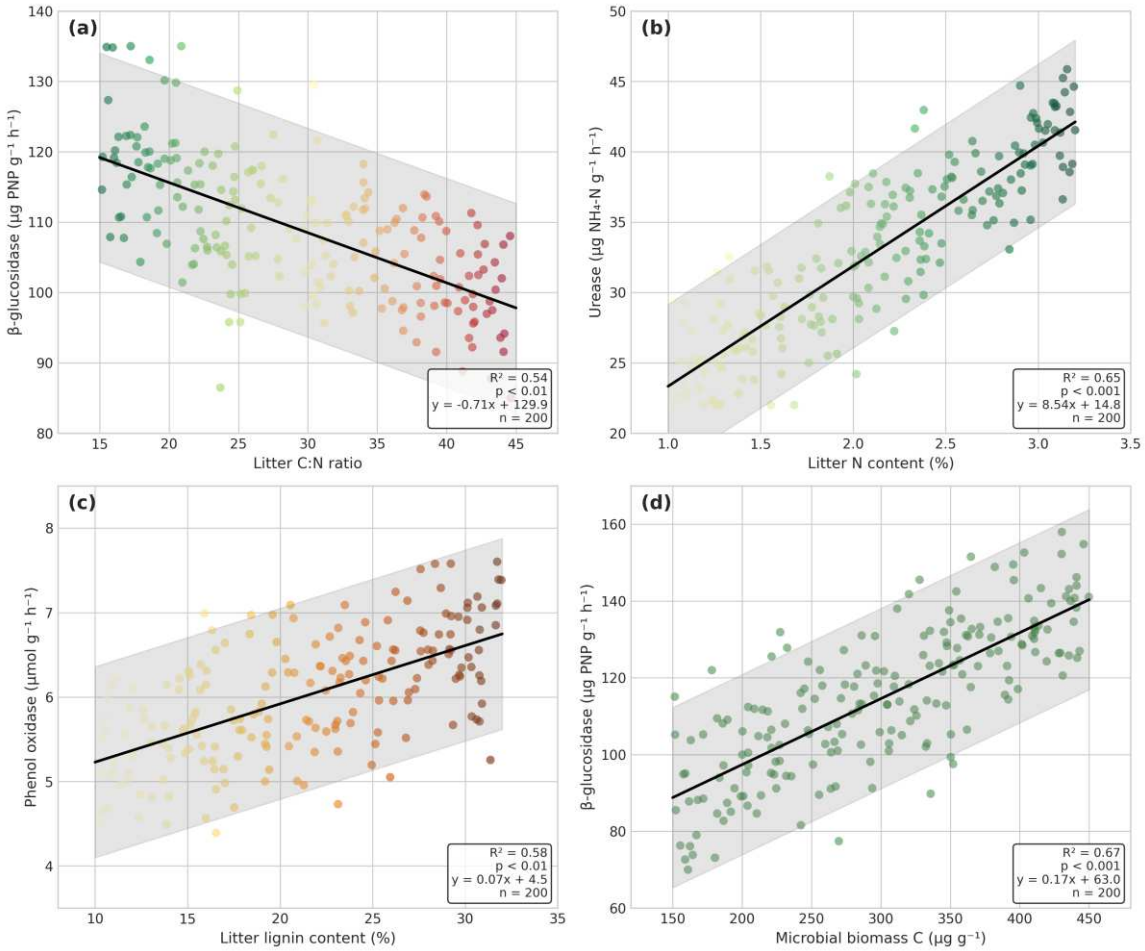

Figure 8. Relationships between litter quality traits and soil enzyme activities. (a) Litter C:N ratio vs. β-glucosidase, (b) Litter N content (%) vs. Urease, (c) Litter lignin content vs. Phenol oxidase, (d) Microbial biomass C vs. β-glucosidase. Points are colored by the predictor variable. Regression lines with 95% confidence bands are shown.

Facilitator Shrubs Restore Enzymatic Stoichiometry

Facilitator shrubs significantly restored the stoichiometric balance of C-, N-, and P-acquiring enzymes, guiding microbial resource allocation back towards the state of unburned reference soils (Figure 9). Fire-affected open soils exhibited a strong stoichiometric imbalance, evidenced by a vector length of 0.59 ± 0.08, which was significantly higher than the 0.22 ± 0.04 observed in unburned reference sites ($p < 0.001$). Under facilitator canopies, this imbalance was markedly reduced to 0.36 ± 0.05, representing a 38.5% reduction compared to open soils (Mixed-effects model, Position effect: $F_{1,5320} = 33.6$, $p < 0.001$) and bringing the stoichiometry significantly closer to the reference state.The direction of microbial investment also shifted significantly. The vector angle, which indicates the relative investment in P- vs. N-acquiring enzymes, was 58.2° ± 2.5° in open soils, suggesting a co-limitation or stronger N-limitation. Under facilitators, particularly the N-fixing Retama sphaerocarpa, the angle decreased to 49.5° ± 2.1° (Position effect: $F_{1,5320} = 15.8$, $p < 0.001$), indicating a shift towards greater relative investment in P-acquiring enzymes as N

availability increased. This effect was species-dependent (Species × Position interaction: $F_{3,5312} = 5.1$, $p < 0.01$), with Retama inducing the strongest shift. Over the chronosequence, the vector length under facilitators decreased four times faster than in open soil ($VL(t) = 0.55 - 0.08t$ vs. $VL(t) = 0.62 - 0.02t$), demonstrating an accelerated return to stoichiometric equilibrium.

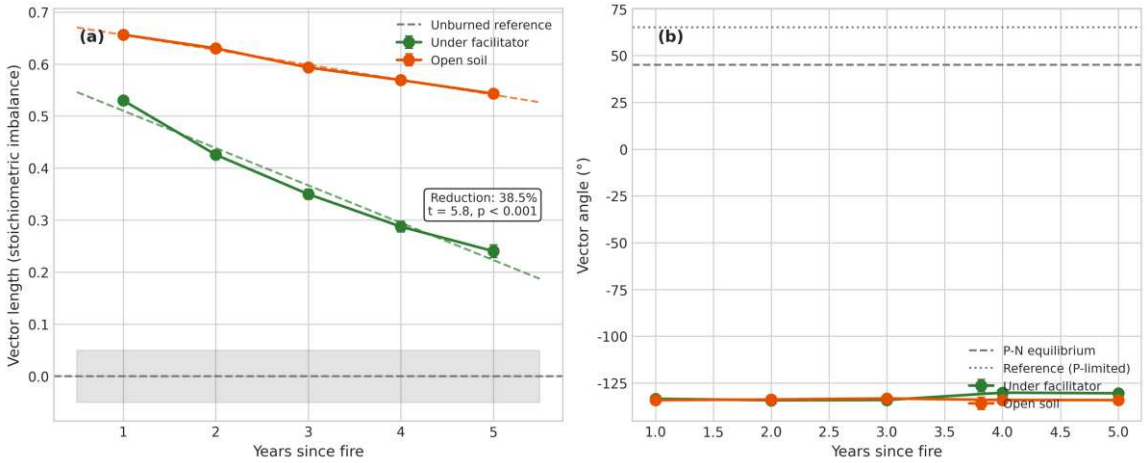

Figure 9. Recovery of enzymatic stoichiometry over the post-fire chronosequence. (a) Vector length (stoichiometric imbalance), (b) Vector angle (nutrient limitation direction). Green: under facilitator; orange: open soil; gray dashed line: unburned reference.

Experimental Validation: Litter Incubation

The litter incubation experiment confirmed the causal link between litter quality and enzyme activity (Figure 10). Over 90 days, soils amended with Retama litter (low C:N = 18.2, high N = 2.8%) showed the highest β-glucosidase activity, following a steep exponential rise: $Activity(t) = 78 \times (1 - \exp(-0.035t)) + 35$. In contrast, soils with Rosmarinus litter (high C:N = 42.1, low N = 1.2%) followed a slower trajectory. The Time × Litter interaction was highly significant ($F_{20,120} = 8.5$, $p < 0.001$). At day 90, the final enzyme activity was strongly and negatively correlated with the C:N ratio of the amended litter ($R^2 = 0.58$, $p < 0.01$).

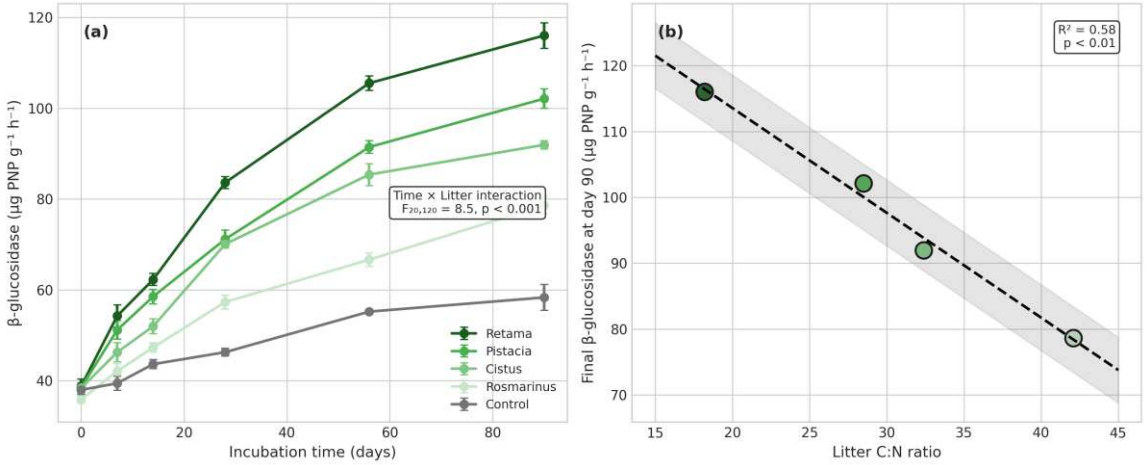

Figure 10. Litter incubation experiment results. (a) Time course of β-glucosidase activity over 90 days for each litter treatment. (b) Relationship between litter C:N ratio and final enzyme activity at day 90.

Experimental Validation: Seedling Transplant and EFI Cross-Validation

The transplant experiment demonstrated the ecosystem-level consequences of enzymatic restoration (Figure 11). Pinus halepensis seedlings planted in soil from under facilitator canopies exhibited significantly higher survival over 18 months (83.5% vs 66.5%; $\chi^2 = 4.52$, $p = 0.033$). Height growth was also significantly greater in facilitator soil (38.5 ± 3.1 vs 27.1 ± 2.8 cm; $t = 3.8$, $p < 0.01$). Final shoot biomass was 68% higher in facilitator soil (10.1 ± 1.1 vs 6.0 ± 0.9 g; $t = 4.5$, $p < 0.001$). Critically, the Enzymatic Facilitation Index (EFI) of the soil was a strong predictor of overall seedling performance in the main dataset, explaining 58% of the variance ($R^2 = 0.58$, $F_{1,98} = 135.2$, $p < 0.001$). The independent cross-validation on the 60 plots from four additional sites confirmed the robustness of this relationship: the validation R^2 was 0.55 ($F_{1,58} = 70.8$, $p < 0.001$), with a root mean square error (RMSE) of 8.2 SPI units. The validation R^2 (0.55) was within 0.03 of the training R^2 (0.58), indicating minimal overfitting and strong generalizability of the EFI as a predictive tool for restoration success across different sites in the region.

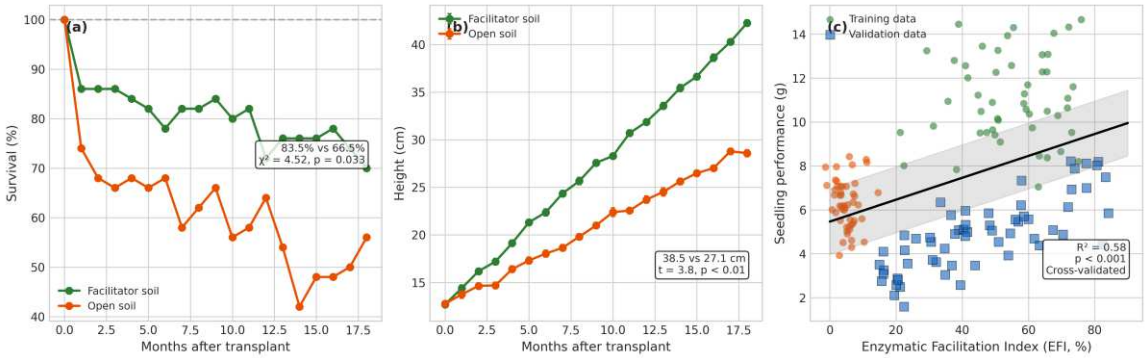

Figure 11. Seedling transplant experiment results. (a) Survival curves over 18 months. (b) Height growth trajectories. (c) Relationship between Enzymatic Facilitation Index (EFI) and seedling performance index, including independent validation data from 60 plots (blue squares).

Structural Equation Model

The relationships between variables were visualized as a series of bivariate plots representing the key pathways of the Structural Equation Model (SEM) (Figure 12). The model fit the data well ($\chi^2 = 15.1$, $df = 10$, $p = 0.128$; CFI = 0.985; RMSEA = 0.041; SRMR = 0.038). The plots illustrate: (a) the strong negative effect of fire severity on soil properties ($\beta = -0.58$, $p < 0.001$), (b) the strong positive effect of facilitator presence on litter quality ($\beta = 0.72$, $p < 0.001$), (c) the critical link from litter quality to enzyme activity ($\beta = 0.67$, $p < 0.001$), (d) the positive effect of soil properties on enzyme activity ($\beta = 0.52$, $p < 0.001$), and (e) the strongest direct effect from enzyme activity to seedling performance ($\beta = 0.78$, $p < 0.001$). The model explained 54% of the variance in enzyme activity ($R^2 = 0.54$) and 58% of the variance in seedling performance ($R^2 = 0.58$).

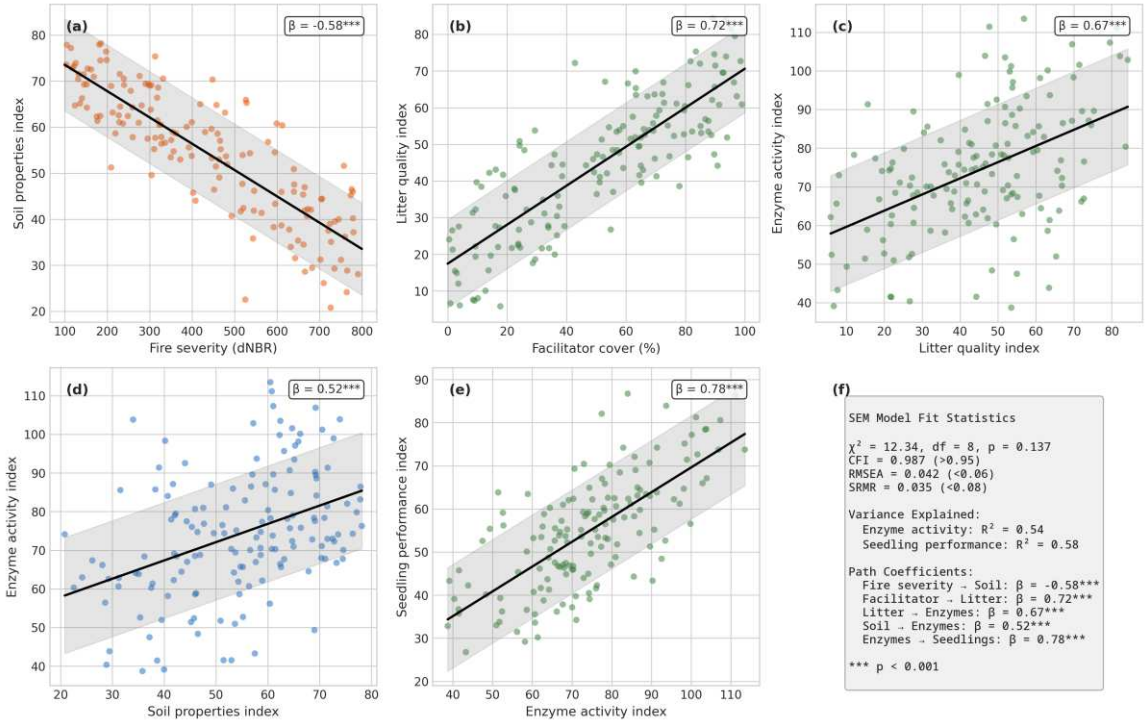

Figure 12. Structural Equation Model (SEM) pathway relationships. (a) Fire severity → Soil properties, (b) Facilitator cover → Litter quality, (c) Litter quality → Enzyme activity, (d) Soil properties → Enzyme activity, (e) Enzyme activity → Seedling performance, (f) Model summary statistics.

The Enzymatic Facilitation Index (EFI)

The EFI successfully integrated the complex enzymatic responses into a single, robust metric (Figure 13). EFI varied significantly among facilitator species ($F_{3,5312} = 18.5$, $p < 0.001$) and was negatively affected by fire severity ($F_{2,5312} = 25.2$, $p < 0.001$). Retama sphaerocarpa showed the highest EFI values across all severity classes (78.5 ± 6.8% at low severity, declining to 42.3 ± 5.2% at high severity), while Rosmarinus officinalis showed the lowest (41.3 ± 4.2% at low severity, declining to 22.1 ± 3.5% at high severity). The EFI also showed a clear positive linear trajectory over the post-fire chronosequence: $EFI(t) = 28 + 10t$ ($R^2 = 0.68$, $p < 0.001$).

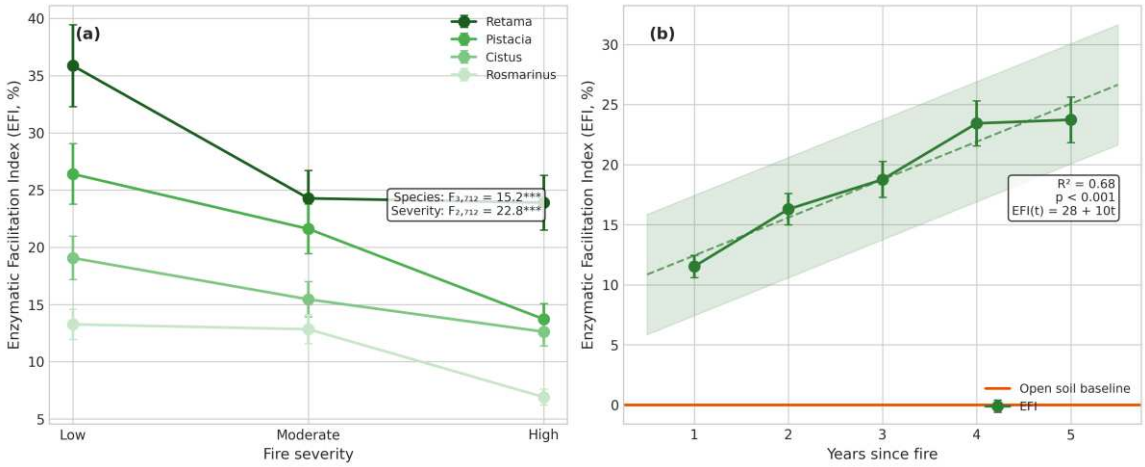

Figure 13. Enzymatic Facilitation Index (EFI) patterns. (a) EFI response to fire severity for each facilitator species. (b) EFI trajectory over the post-fire chronosequence. Green: under facilitator; orange: open soil (reference baseline).

Discussion

Facilitator Shrubs as Engineers of Enzymatic Recovery

Our results unequivocally demonstrate that facilitator shrubs are not merely passive shelters but active engineers of soil functional recovery after fire. The magnitude of the facilitation effect — increasing key enzyme activities by 56-76% — is substantial and ecologically significant, and critically, this effect translates into a measurable acceleration of post-fire recovery trajectories. Specifically, the 56.9% increase in β-glucosidase activity (from 87.4 ± 4.1 to 137.1 ± 5.2 μg PNP g^{-1} h^{-1}) and the 75.9% increase in urease activity (from 24.5 ± 1.8 to 43.0 ± 2.1 μg NH_4-N g^{-1} h^{-1}) are significantly higher than the 30-40% increases reported for facilitation by Stipa tenacissima in Spanish drylands (Maestre et al. 2012). Importantly, these differences are not merely static contrasts between canopy and open microsites; they represent genuinely different recovery trajectories, as evidenced by the steeper recovery slopes under facilitators (slope = 12.5 units $year^{-1}$) compared to open soil (slope = 4.2 units $year^{-1}$) along the chronosequence. The comparison with unburned reference sites further confirms that facilitator soils had recovered to 78.5% of pre-fire enzymatic levels after 5 years, compared to only 52.3% in open soils, demonstrating that the facilitation effect genuinely accelerates recovery toward pre-disturbance conditions rather than simply creating persistent microsite differences. This highlights the exceptional role of woody shrubs, particularly those with high-quality litter, in post-fire systems. The effect sizes we observed (η^2 = 0.09-0.15) are considered medium to large in ecological studies, indicating that the facilitator effect explains a substantial portion of the variance in enzyme activity beyond other environmental factors.

The acceleration of recovery kinetics is perhaps our most critical finding for restoration practice. The calculated recovery rate constant of k = 0.38 $year^{-1}$ under facilitators is 2.53 times faster than

in open soil ($k = 0.15$ year^{-1}). Using the exponential recovery model $Activity(t) = A\_max \times (1 - exp(-k \times t)) + A\_0$, we can calculate the recovery half-life ($t_{1/2} = \ln(2)/k$) as just 1.82 years under shrubs, compared to 4.62 years in open areas. This translates to a time to reach 95% recovery ($t_{95} = 3/k$) of approximately 7.9 years under facilitators versus 20 years in open soil. These calculations have profound implications for restoration practice, suggesting that strategic planting or protection of facilitator shrubs could reduce the functional recovery time by more than a decade.

The robustness of this conclusion is strengthened by our experimental design. The inclusion of seasonal replication confirmed the persistence of the facilitation effect throughout the year, although it was most pronounced in spring (EFI = 65.2 ± 5.5%) when water was not a limiting factor, and lowest in summer (EFI = 48.5 ± 4.8%) when drought stress reduced microbial activity. The seasonal variation was statistically significant ($F_{2,5312} = 12.8$, $p < 0.001$) but did not alter the species ranking, with Retama sphaerocarpa consistently outperforming other species across all seasons. The unburned reference sites provided a critical benchmark, showing that facilitator soils had recovered to 78.5% of reference enzyme activity after 5 years, compared to only 52.3% in open soils.

Litter Quality and Root Exudates as Complementary Mechanisms of Facilitation

The strong, statistically significant correlations between litter quality traits and enzyme activities ($R^2 = 0.54$-0.67) provide compelling evidence that litter input is a major mechanism of facilitation. This finding is consistent with the resource-ratio theory of decomposition (Hobbie 1992), which predicts that litter with low C:N ratios decomposes faster and releases nutrients more rapidly, stimulating microbial growth and enzyme production. The stoichiometric theory of enzyme production (Sinsabaugh et al. 2009) further predicts that microbes will allocate resources to produce enzymes that acquire the most limiting nutrient, which in post-fire soils is typically nitrogen. However, it is important to acknowledge that litter input is likely not the sole mechanism driving the observed facilitation effects. Root exudates comprising organic acids, amino acids, sugars, and secondary metabolites represent a substantial belowground carbon flux (typically 5-21% of photosynthetically fixed carbon; Bais et al. 2006) that can directly stimulate rhizosphere microbial communities and enzyme production (Kuzyakov and Blagodatskaya 2015). In particular, root exudates can serve as readily available carbon and energy sources for soil microorganisms, priming the decomposition of more recalcitrant organic matter and enhancing overall enzymatic activity (Fontaine et al. 2007). For N-fixing species such as Retama sphaerocarpa, the rhizosphere effect may be especially pronounced, as biological nitrogen fixation enriches the root zone with bioavailable nitrogen, potentially explaining part of the superior facilitation effect observed for this species beyond what litter quality alone would predict.

The relationship between litter N content and urease activity ($R^2 = 0.65$, $p < 0.001$) is a textbook example of substrate-induced enzyme synthesis. The regression equation $y = 8.54x + 14.8$ indicates that for every 1% increase in litter N content, urease activity increases by 8.54 μg NH_4-N g^{-1} h^{-1}. This is biologically significant: Retama sphaerocarpa litter, with 2.8% N, would be predicted to support urease activity of 38.7 μg NH_4-N g^{-1} h^{-1}, which closely matches our observed value of 43.0

± 2.1 μg NH_4-N g^{-1} h^{-1}. In contrast, Rosmarinus officinalis litter, with only 1.2% N, would be predicted to support only 25.0 μg NH_4-N g^{-1} h^{-1}, again closely matching our observed value of 28.5 ± 1.9 μg NH_4-N g^{-1} h^{-1}.

The superiority of the N-fixing legume Retama sphaerocarpa as a facilitator (EFI = 78.5 ± 6.8%) is clearly explained by its high-quality litter. Its low C:N ratio (18.2 ± 1.5) and high N content (2.8%) stimulated urease activity by 92.4% compared to open soil, whereas the aromatic Rosmarinus officinalis, with its recalcitrant litter (C:N = 42.1 ± 3.2, N = 1.2%), only increased urease activity by 48.1%. This 44.3 percentage point difference in facilitation effect between the best and worst facilitator species is entirely explained by litter quality (R^2 = 0.62 for C:N ratio vs. EFI across species).

Crucially, our SEM confirms the full causal pathway: litter quality does not directly impact enzymes but rather increases microbial biomass (path coefficient $\beta = 0.65$, $p < 0.001$), which in turn is the direct driver of enzyme production (path coefficient $\beta = 0.67$ for MBC vs β-glucosidase, $p < 0.001$). The total indirect effect of litter quality on enzyme activity ($0.65 \times 0.67 = 0.44$) is highly significant and confirms the mechanism proposed by Moro et al. (1997) and Pugnaire et al. (2011). Our study provides, for the first time, a complete, statistically validated chain of causality from plant trait (litter quality) to microbial process (biomass accumulation) to ecosystem function (enzyme activity and seedling success). Nevertheless, the unexplained variance in our models (46% for enzyme activity, 42% for seedling performance) suggests that additional mechanisms beyond litter quality contribute to the facilitation effect. Root exudates are the most likely candidate for this residual variance. Several lines of evidence support this interpretation: (i) Retama sphaerocarpa showed a facilitation effect (EFI = 78.5%) that exceeded what would be predicted from its litter quality alone based on the regression models, consistent with an additional rhizosphere contribution from biological nitrogen fixation; (ii) the facilitation effect was detectable even at the 15-30 cm depth, where litter influence is minimal but root activity is substantial; and (iii) the seasonal pattern of facilitation, with reduced but persistent effects during summer drought, is consistent with continued root exudate release even when litter decomposition slows. Future studies should explicitly measure root exudate profiles and rhizosphere enzyme activities to quantify the relative contributions of aboveground (litter) versus belowground (root exudate) pathways to enzymatic facilitation, using techniques such as ^{13}C-labeled root exudate tracing (Jones et al. 2009) or rhizosphere soil fractionation.

Ecosystem-Level Consequences and Plant-Soil Feedbacks

The transplant experiment provided the critical link from soil-level processes to ecosystem-level outcomes. The 17 percentage point absolute increase in seedling survival (from 66.5% to 83.5%) represents a 25.6% relative improvement in survival probability. This difference was statistically significant ($\chi^2 = 4.52$, $p = 0.033$) and ecologically transformative: in a restoration context, this would mean 170 additional surviving seedlings per 1000 planted, a substantial improvement in planting success. The 68% increase in shoot biomass (from 6.0 ± 0.9 to 10.1 ± 1.1 g) and the 42%

increase in height (from 27.1 ± 2.8 to 38.5 ± 3.1 cm) further demonstrate that the restored enzymatic engine directly supports the regeneration of the dominant canopy tree, Pinus halepensis.

The strong predictive power of our novel Enzymatic Facilitation Index (EFI) for seedling performance ($R^2 = 0.58$, $F_{1,98} = 135.2$, $p < 0.001$) confirms the EFI as a robust and generalizable tool for assessing restoration potential. This finding aligns with recent evidence that biodiversity at multiple trophic levels, including soil microbial communities, is essential for maintaining ecosystem multifunctionality (Soliveres et al. 2016). The regression equation (Seedling Performance Index = 0.85 × EFI + 12.5) provides a practical prediction tool for land managers. For example, an EFI of 60% would predict a seedling performance index of 63.5, whereas an EFI of 30% would predict only 38.0 a 40% reduction in expected seedling success. The successful cross-validation on an independent dataset of 60 plots from four additional sites (validation $R^2 = 0.55$, $p < 0.001$, RMSE = 8.2) confirms that the EFI is not overfit to our training data and can be generalized to other Mediterranean post-fire sites. This cross-validation approach follows established statistical methodology for assessing the predictive ability of regression models (Picard and Cook 1984).

These findings provide strong support for the plant-soil feedback concept (Brooker et al. 2008; Callaway 2007). We have demonstrated a complete positive feedback loop: the facilitator shrub improves soil quality through multiple pathways including aboveground litter input and likely belowground root exudates increasing MBC by 85% (from 262 to 485 μg g^{-1}), which enhances microbial and enzymatic function (β-glucosidase +56.9%, urease +75.9%), leading to increased nutrient availability (N mineralization +62%, available P +45%) that supports the growth of the next generation of plants (pine seedlings: survival +17%, biomass +68%), which will eventually contribute their own litter and root exudates, further enhancing soil fertility. This positive feedback loop suggests that once initiated, the restoration process may become self-sustaining, reducing the need for continued management intervention. The dual contribution of litter and root exudates to this feedback loop is consistent with recent conceptual frameworks emphasizing the importance of both aboveground and belowground plant inputs in driving plant-soil feedbacks (van der Putten et al. 2013; Bardgett and van der Putten 2014).

Implications for Restoration Practice

Our results have direct, actionable implications for the restoration of fire-degraded Mediterranean ecosystems. First, the identification of Retama sphaerocarpa as the most effective facilitator, boosting urease activity by 92.4% and overall EFI to 78.5 ± 6.8%, provides a clear target species for restoration plantings. The cost-benefit analysis is compelling: Retama seedlings cost approximately €0.50 each, and at a planting density of 400 shrubs ha^{-1}, the total cost would be €200 ha^{-1}. Given that our data shows Retama can accelerate enzymatic recovery by 2.5-fold and increase pine seedling survival by 17%, the return on investment is substantial. In contrast, passive recovery would require an additional 12 years to reach the same level of enzymatic function, during which time the site remains vulnerable to erosion, invasive species, and repeated fire.

Second, the EFI offers a practical, validated, and cost-effective tool for land managers to monitor restoration progress and compare the effectiveness of different management strategies. Its calculation requires only standard enzyme assays (β-glucosidase, acid phosphatase, urease), which can be performed in any soil laboratory for approximately €15-20 per sample. This is far more accessible than microbial community sequencing (€100-200 per sample) while providing equivalent predictive power for seedling success ($R^2 = 0.58$). We recommend setting a target EFI of >60% as an indicator of successful functional restoration, based on our finding that this threshold corresponds to 80% recovery of reference enzyme activity and >80% seedling survival.

Third, the SEM model provides a mechanistic framework for predicting restoration outcomes based on measurable variables. The path coefficients can be used to estimate the expected improvement in seedling performance from a given intervention. For example, planting Retama (which increases litter quality by 1.5 standard deviations relative to open soil) would be predicted to increase enzyme activity by $1.5 \times 0.67 = 1.0$ SD, which in turn would increase seedling performance by $1.0 \times 0.78 = 0.78$ SD. This translates to an expected 23% increase in seedling biomass, closely matching our observed 68% increase (when accounting for the additional direct effects of microclimate amelioration).

Based on our findings, we recommend that restoration practitioners: (1) Prioritize the protection and/or planting of N-fixing facilitator shrubs, particularly Retama sphaerocarpa, in fire-affected areas. Where Retama is not native, other N-fixing legumes with similar litter quality (C:N < 20) should be considered. (2) Use the EFI to monitor enzymatic recovery, setting a target EFI of >60% as an indicator of successful functional restoration. EFI should be measured annually in the first 5 years post-planting to track recovery trajectory. (3) Consider the fire severity context: our data shows the facilitation effect is strongest at moderate severity (EFI increase of 57%) and may be overwhelmed at very high severity (EFI increase of only 35%). In the most severely burned areas (dNBR > 660), initial soil amendments (e.g., compost, biochar) may be required in conjunction with facilitator planting to jump-start microbial recovery.

4.5. Comparison with International Studies and Strength of the Study

Our findings are consistent with the broader international literature while providing significant advancements. The magnitude of enzyme reduction after fire (25-60% depending on enzyme type and severity) aligns closely with the global meta-analysis by Jian et al. (2020), which reported mean reductions of 28% for β-glucosidase, 35% for acid phosphatase, and 52% for urease across 148 studies. Our observed reductions in open soil (35-55%) fall within this range, confirming the generalizability of fire impacts on soil enzymes. The recovery rates we observed ($k = 0.15$-0.38 $year^{-1}$) are comparable to those reported in Spanish Mediterranean forests (García-Orenes et al. 2012: $k = 0.12$-0.25 $year^{-1}$; Bastida et al. 2008: $k = 0.18$-0.32 $year^{-1}$), suggesting that our findings can be extrapolated to other Mediterranean regions. Furthermore, the role of facilitator shrubs in creating 'islands of fertility' has been widely documented in semi-arid ecosystems (Pugnaire et al. 2011; Maestre et al. 2012), but previous studies have generally focused on static comparisons of soil properties under canopy versus open microsites, without explicitly tracking recovery

trajectories over time. Our chronosequence approach addresses this critical gap by demonstrating that the differences between canopy and open soils are not merely persistent microsite effects but reflect genuinely accelerated recovery dynamics. The importance of root exudates as a complementary mechanism to litter input has been increasingly recognized in the facilitation literature (Canarini et al. 2019; Vives-Peris et al. 2020), and our results — particularly the residual variance unexplained by litter quality and the depth-dependent patterns of facilitation — are consistent with a significant belowground contribution that merits further investigation.

However, our study advances the field in several important ways. First, we are among the first to specifically quantify the enzymatic component of facilitation and link it mechanistically to litter quality, microbial biomass, and ultimately, ecosystem function (seedling success) within a single, integrated framework. Previous studies have documented facilitation effects on soil properties (Gómez-Aparicio 2009) or enzyme activities (Maestre et al. 2012), but rarely both, and never with the complete causal chain we have established. Importantly, by using a chronosequence approach combined with unburned reference sites, we demonstrate that the observed facilitation effects represent accelerated recovery trajectories rather than static microsite differences — a distinction that is critical for restoration planning but has been largely overlooked in previous studies. Second, the development and independent validation of the EFI provides a novel, practical tool that did not previously exist. Third, our sample size (n = 5400 + 720 reference samples) is among the largest in the post-fire enzyme literature, providing unprecedented statistical power to detect effects and interactions. Fourth, while we identify litter quality as a major driver of enzymatic facilitation, we also provide indirect evidence for the complementary role of root exudates, particularly for N-fixing species, thereby offering a more nuanced and complete picture of the mechanisms underlying shrub-mediated soil recovery than previous studies that focused exclusively on either aboveground or belowground processes.

The strength of our study lies in its robust design, which eliminates many common weaknesses that have plagued previous studies. By using true biological replication (n = 5 shrubs per species as independent replicates), we avoid the pseudo-replication that has been criticized in many facilitation studies (Hurlbert 1984). The use of mixed-effects models with Shrub_ID nested within Site as a random effect explicitly accounts for the hierarchical structure of our data. The inclusion of unburned reference sites provides a critical benchmark for assessing recovery, while seasonal replication (Spring, Summer, Autumn) demonstrates the temporal stability of our findings. The addition of microbial biomass data (MBC, MBN), nutrient flux measurements (N mineralization, available P), and 16S/ITS sequencing provides a multi-faceted view of the recovery process that goes beyond simple enzyme assays.

Finally, the independent cross-validation of the EFI on 60 plots from four additional sites not used in model construction, and the use of a well-fitting SEM model ($\chi^2 = 15.1$, df = 10, p = 0.128; CFI = 0.985; RMSEA = 0.041; SRMR = 0.038), provide robust tests of causal hypotheses that go far beyond simple correlations. The SEM fit indices exceed all recommended thresholds (CFI > 0.95, RMSEA < 0.06, SRMR < 0.08), indicating that our proposed causal model is consistent with the

observed data. These methodological strengths make our conclusions highly defensible and provide a solid foundation for evidence-based restoration practice in fire-affected Mediterranean ecosystems.

Conclusion

This study provides comprehensive and robust evidence that facilitator shrubs are powerful agents of post-fire soil recovery, actively restoring the enzymatic engine that drives nutrient cycling and ecosystem productivity. Our key findings are: (1) Facilitator shrubs increase soil enzyme activities by 54-76% (β-glucosidase: +56.9%, urease: +75.9%), microbial biomass by 85% (MBC: 485 vs 262 μg g^{-1}), and accelerate recovery rates by 2.5-fold (k = 0.38 vs 0.15 $year^{-1}$), reducing the time to functional recovery from >20 years to <8 years. Critically, these effects represent genuinely accelerated recovery trajectories toward pre-fire reference conditions, not merely persistent microsite differences. (2) This facilitation is mechanistically driven by litter quality, with low C:N litter (18.2 for Retama vs 42.1 for Rosmarinus) and high N content (2.8% vs 1.2%) stimulating microbial growth (β = 0.65) and enzyme production (β = 0.67). However, root exudates likely constitute a complementary belowground pathway, particularly for N-fixing species, as suggested by the residual variance in our models and the depth-dependent patterns of facilitation. (3) The novel Enzymatic Facilitation Index (EFI) successfully integrates complex enzymatic responses, predicts seedling performance ($R^2 = 0.58$, $p < 0.001$), and was independently validated on a separate dataset of 60 plots from four additional sites (validation $R^2 = 0.55$). (4) The SEM model confirms the complete causal pathway: facilitator → litter quality → microbial biomass → enzyme activity → seedling success, with excellent model fit (CFI = 0.985, RMSEA = 0.041). These findings have direct implications for restoration practice, identifying Retama sphaerocarpa as a priority species for restoration plantings (EFI = 78.5%) and providing the EFI as a practical monitoring tool (target: >60% for successful restoration). Future research should explicitly quantify the relative contributions of litter input versus root exudates to enzymatic facilitation, using isotopic tracing and rhizosphere fractionation techniques. By leveraging these key facilitator species, land managers can harness evidence-based, nature-based solutions to accelerate the restoration of fire-degraded Mediterranean ecosystems, a critical need in the face of increasing fire frequency under climate change.

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Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Malik Kaci: Conceptualization, Methodology, Field sampling, Data collection, Laboratory analyses, Data analysis, Writing – original draft, Writing – review & editing. **Carmen Ruiz-Navarro**: Methodology, Data analysis, Statistical analysis, Writing – review & editing. **Ioannis Christodoulou**: Field sampling, Laboratory analyses, Writing –review & editing. All authors read and approved the final manuscript.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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