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Fire-Induced Alterations in Soil Function and Regeneration Dynamics of Subtropical Chir Pine (*Pinus roxburghii*) Forests

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SUMMARY

Sub-tropical chir pine (*Pinus roxburghii*) forests are highly susceptible to forest fires due to the resinous nature of the species and the accumulation of dry needle litter on the forest floor. Recurrent fires can significantly alter vegetation structure, soil properties, and natural regeneration processes, thereby affecting long-term ecosystem stability. This study aimed to evaluate the impacts of forest fire on soil physicochemical properties and natural regeneration in sub-tropical chir pine forests of District Buner, Khyber Pakhtunkhwa, Pakistan. The study was carried out in four naturally burnt forest patches and four adjacent unburnt patches. Soil samples were collected at a depth of 10–20 cm using an auger, with five representative samples obtained from each patch, resulting in a total of 40 samples. Laboratory analyses were conducted to assess soil potassium (K), phosphorus (P), pH, electrical conductivity (EC), water holding capacity (WHC), and organic nitrogen. To evaluate the effects of fire on regeneration, twenty circular plots (8.4 m radius) were established in both burnt and unburnt areas, yielding a total of 40 plots. Within each plot, all naturally regenerated chir pine seedlings with a height of 4 feet or less were counted. Soil physicochemical properties and regeneration dynamics were significantly changed by forest fire. Burnt soils exhibited significantly higher potassium concentrations (20.54 vs. 15.75 mg kg^{-1}), reduced water holding capacity (12.62% vs. 25.47%), lower electrical conductivity (0.06 vs. 0.46), higher pH (6.49 vs. 6.35), and lower organic carbon content (0.256% vs. 0.585%), while phosphorus showed no significant change ($p = 0.145$). Forest regeneration was also significantly reduced in burnt areas (34.6 vs. 44.0 seedlings). The findings highlight that forest fire has pronounced effects on soil health and regeneration dynamics in subtropical chir pine forests. Understanding these changes is essential for sustainable forest management and ecosystem restoration in fire-affected landscapes. The study recommends the implementation of firebreaks, fuel load reduction, erosion control measures, and community-based fire management strategies to mitigate fire impacts and promote long-term forest resilience.

Keywords: Forest fire; natural regeneration; *Pinus roxburghii*; soil physicochemical properties; sub-tropical pine forest; sustainable forest management

INTRODUCTION

Forest fires are recognized as a major global threat to biodiversity, ecosystems and human Livelihoods (Mehmood *et al.,* 2024). Fire is a natural process and is associated with vegetation, as green plants provide both the oxygen and fuel necessary for combustion, making fire an important component of terrestrial ecosystems (Pausas & Keeley, 2009). Fire has played a fundamental role in structuring the shape of vegetation, nutrient cycling in the Earth system, and has even been used by humans to modify landscapes (Bond & Keeley, 2005). However, in recent decades, forest fires have become more frequent and severe, largely due to human interventions. It is estimated that over 80% of fires are attributed to human activities (Phillips et al., 2022). The growing concerns of climate change at the global level, land-use alterations, and invasive species are further deteriorating the situation, creating conditions that exceed the adaptive capacity of many ecosystems (Kelly *et al*., 2020; Kelly *et al.,* 2023). Climate projection indicates that subtropical forests are likely to experience longer and more intense fire seasons, worsening ecological degradation subsequently leading to biodiversity conservation issues (Flannigan *et al.,* 2013). Forest fire not only affects vegetation, but it also significantly modifies soil properties, nutrient availability, thereby influencing ecosystem recovery processes. Forest fire results in nutrient losses through volatilization and soil erosion and redistributing nutrients through ash deposition in the soil (Kellman *et al.,* 1985; Boerner, 1982). High temperatures resulting from forests fire may further modify the chemical properties of the soil, affecting post-fire regeneration (Certini, 2005). However, this overall impact varies on the intensity of forest fire, vegetation composition, and fuel characteristics (Kutiel & Shaviv, 1989). The impact on regeneration is complex and dependent on fire frequency and severity. However, evidence suggests that while fire may initially accelerate seedling emergence, it may negatively affect sapling survival (Sharma *et al*., 2023). Likewise, species richness and density increase due to low fire frequency, whereas high fire frequency can suppress regeneration (Bargali *et al*., 2023). Forest fire with enhanced frequency in subtropical Chir pine forests, have been indicated to severely affect vegetation composition, diversity, and regeneration capacity (Hussain *et al.,* 2021). The subtropical Chir pine (*Pinus roxburghii*) forests, distributed across the Himalayan foothills from Pakistan to northeastern India, are ecologically and socio-economically important ecosystems, providing critical ecosystem services. The services include watershed protection, biodiversity conservation, and Livelihoods for local communities (Tewari, 1994). Unfortunately, these forest ecosystems are highly vulnerable to forest fires due to numerous reasons why the accumulation of resinous needles is particularly critical because it forms a highly flammable ground layer. In regions like Buner, fire occurrence is further intensified by anthropogenic activities (Kumar *et al.,* 2013; Muhammad *et al.,* 2024). Despite the fact that forest fire has been recognized as the main ecological driver, there is lack of empirical, site-specific studies assessing its impacts on vegetation and soil properties in Pakistan. The subtropical Chir pine forests of District Buner have received limited scientific attention, especially their impact on regeneration and soil properties in post fire scenario. Although remote sensing and GIS have been used widely for fire monitoring and management, their application in Pakistan remains limited (Attri *et al.,* 2020). Consequently, the lack of localized data restricts the development of management strategies in post fire scenarios (Nafees *et al*., 2009). The present study aims to evaluate the impacts of forest fire on vegetation structure and regeneration, as well as on selected

physiochemical soil properties, in subtropical Chir pine forests of District Buner. By comparing burnt and unburnt areas, this research seeks to generate field-based evidence on how fire influences regeneration patterns and soil conditions, thereby contributing to improved understanding and management of fire-affected forest ecosystems.

MATERIALS AND METHODS

Study Area

District Buner is in the Malakand Division of Khyber Pakhtunkhwa (KPK), Pakistan. It spans an area of approximately 1,865 square kilometers and is situated between 34°25′N to 34°50′N latitude and 72°30′E to 72°50′E longitude. Elevation ranges from approximately 366 m to 2,970 m above sea level, with the lowest elevations occurring in the southern plains around Totalai and the highest elevations reaching Dosara Peak in the northern mountainous region. Climate, rainfall, temperature, slope, aspect, soil properties, vegetation cover, forest fires, and anthropogenic activities, all play significant roles in shaping ecosystem dynamics and post-fire recovery processes. The dominant soil type in the study area was sandy loam, characterized by slightly acidic conditions and low to moderate organic matter content. The area represents subtropical to temperate climate, annual rainfall of approximately 1,000–1,650 mm, variable temperature regimes, mountainous topography, and pine forest vegetation, all of which influence soil properties and post-fire ecosystem dynamics. Buner lies within a transitional zone between subtropical and temperate climates, with moderate to heavy rainfall, especially during the monsoon season. The district supports a range of vegetation types, including subtropical pine forests dominated by Chir Pine (*Pinus roxburghii*), which are vital for biodiversity conservation and Livelihoods in the region (Ahmed *et al.*, 2006). Buner is bordered by Swat district to the north, Malakand and Mardan to the south, and Shangla to the east. The region is mountainous, with significant forest cover, especially in its northern and eastern parts, making it an ecologically key area in the Hindu Kush foothills (Figure 1). The topography, climatic conditions, and anthropogenic pressures such as deforestation, overgrazing, and frequent forest fires make Buner a suitable site for ecological and regeneration studies.

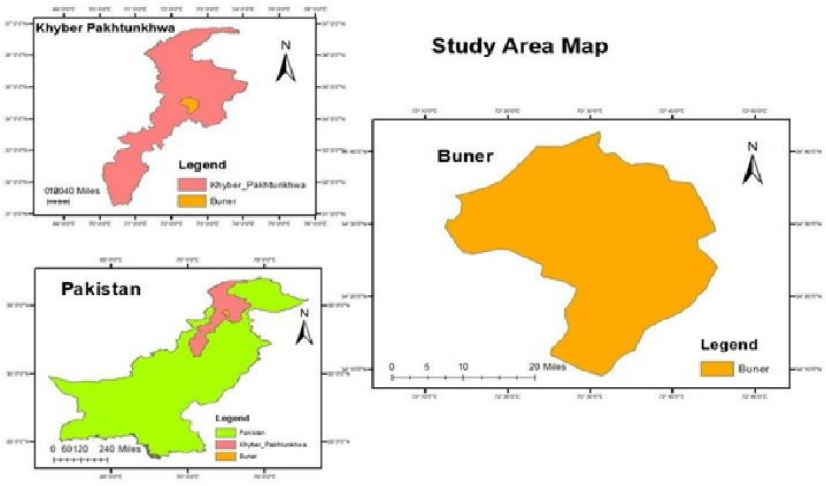

Fig. 1. Geographical location of the study area in Khyber Pakhtunkhwa Province.

Sampling desing

Burnt and unburnt sites were selected based on the verified forest fire records of 2025 and field observations. Four naturally burnt forest patches and four adjacent unburnt patches of *Pinus roxburghii* forests were chosen to ensure comparable environmental conditions. Site selection was carried out to minimize variations in topography and environmental factors, with efforts made to maintain similar slope, aspect, elevation, vegetation type, and soil characteristics across burnt and unburnt sites through visual assessment and field verification. Five representative samples were collected from each patch at 10-20 cm depth. The samples were collected from each patch using auger and were transferred to laboratory for analysis of Potassium (K), Phosphorus (P), Water holding capacity (WHC), Electrical conductivity (EC; 1:2), pH value and organic Nitrogen. Efforts were made to ensure uniform environmental conditions across sampling sites, using visual observations (Verma *et al.*, 2019). Soil organic carbon (SOC) was estimated from soil organic matter (SOM) using the Van Bemmelen conversion factor (SOC = SOM/1.724), which assumes that soil organic matter contains approximately 58% carbon and is widely used in soil science studies (Nelson & Sommers, 1996). Organic nitrogen (ON) was subsequently estimated from SOC using a carbon-to-nitrogen (C:N) ratio of 12, representing the average C:N ratio of forest mineral soils reported in the literature (Brady & Weil, 2017). The calculation used was:

Organic Nitrogen (%) = Soil Organic Carbon (%) / 12

In addition, regeneration status was assessed using 40 circular plots (8.4 m radius), with 20 plots established in burnt sites and 20 in unburnt sites, where all regenerating individuals with a height of ≤4 feet were recorded.

Statistical Analysis

The data of soil samples for all the sites was checked through sample independent T test (**α**=0.05) both for burnt (B1) and unburnt (B0) areas. The results were interpreted based on statistical analysis. Similar T test was applied to regeneration data, and analysis was interpreted statistically for both burnt and unburnt areas. Overall results have been reflected by graphs and tables.

RESULTS

Soil composition

The texture analysis of soil indicated that the unburnt soil comprised 76% sand, 18% silt, and 6% clay, classifying it as sandy loam (Table 1). In contrast, the burnt soil sample contained 80% sand, 14% silt, and 6% clay, corresponding to a loamy sand texture. The overall textural class does not have any significant effect on the nutrients studied because this variation is not significant.

Table 1: Physical composition and textural classification of burnt and unburnt soil samples collected from the study area.

Sample No.	Sample ID	Sand (%)	Silt (%)	Clay (%)	Soil Textural Class
1	B0	76	18	6	Sandy Loam
2	B1	80	14	6	Loamy Sand

Physiochemical properties

Independent samples t-test was employed to assess significant differences between the burnt (B1) and unburnt (B0) groups at a significance level of $\alpha = 0.05$. The null hypothesis, assuming no difference between the two groups, was tested across multiple parameters (Table 2). Results indicate that parameters with p-values less than 0.05 exhibit statistically significant differences between the groups, whereas values greater than 0.05 indicate no significant difference. Phosphorus was the only parameter showing no significant difference between the groups.

Table 2: Statistical comparison of selected soil physicochemical properties between burnt and unburnt soil samples (n = 40)

Parameters	Sig (α=0.05)	B0 Mean	B1 Mean	Remarks
Potassium (K)	0.000	15.75	20.54	Significant variations exist between B0 and B1
Phosphorus (P)	0.145	2.8	2.837	No statistical variation between B0 and B1
Water holding capacity (WHC)	0.000	25.4695	12.621	Significant variations exist between B0 and B1
Electrical conductivity (EC)	0.001	0.458	0.05955	Significant variations exist between B0 and B2
pH	0.002	6.351	6.4865	Significant variations exist between B0 and B3
Organic carbon	0.000	0.58525	0.256	Significant variations exist between B0 and B5

Effect of Forest Fire on Soil Potassium and Phosphorus Concentration

Analysis of soil samples revealed different responses of potassium and phosphorus to forest fire. Potassium concentrations were significantly higher in burnt soils (20.54 mg/kg) compared to unburnt soils (15.75 mg/kg) (Figure 2), indicating a substantial increase following fire and suggesting that forest fire markedly influences soil potassium dynamics. In contrast, phosphorus levels showed only a slight difference between burnt (2.84 mg/kg) and unburnt soils (2.80 mg/kg) (Figure 3), and this variation was not statistically significant. These findings indicated that while forest fire can significantly enhance potassium availability, phosphorus concentrations remain relatively stable under the conditions of this study.

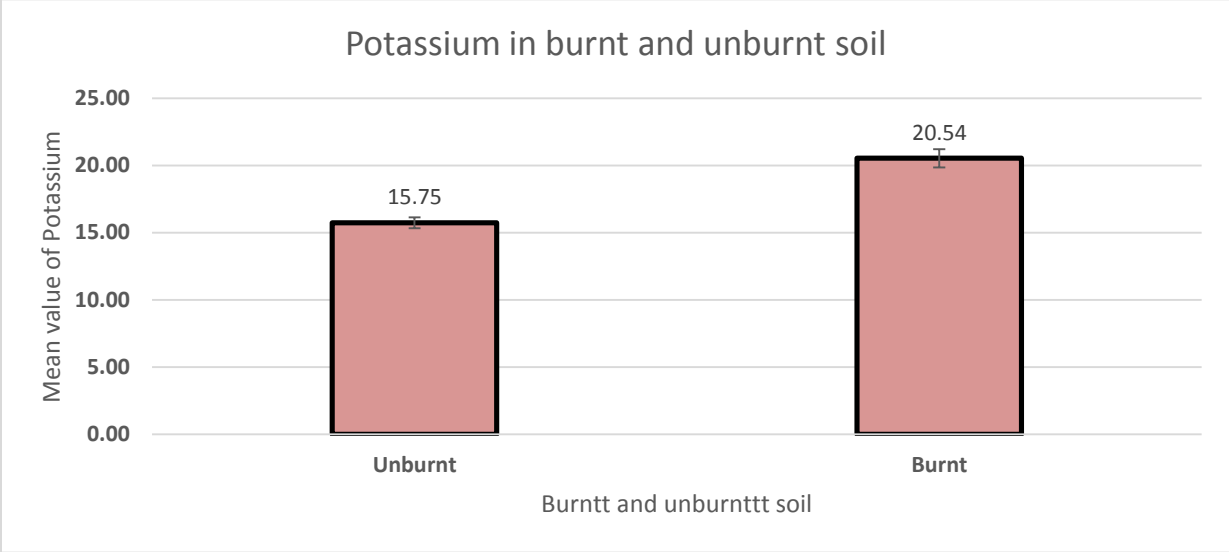

Figure 2: Variation in potassium (K) concentration between burnt and unburnt soil samples.

Effect of Forest Fire on Soil Phosphorus Concentration

The Two independent sample T test indicates the non-significant variation of phosphorus between burnt and unburnt soil sample. The unburnt soil sample reflects a mean value of 2.80 mg/kg is not much lesser than mean value of 2.84 mg/kg for burnt soil samples (Figure 3). Our null hypothesis of no difference between burnt and unburnt soil samples for phosphorus is therefore accepted at (α=0.05) and alternatively we concluded that there is no significant variation between these two treatments of burnt and unburnt soil samples. From the statistical analysis, we concluded that Phosphorus does not change significantly with forest fire and is not affected by forest fire.

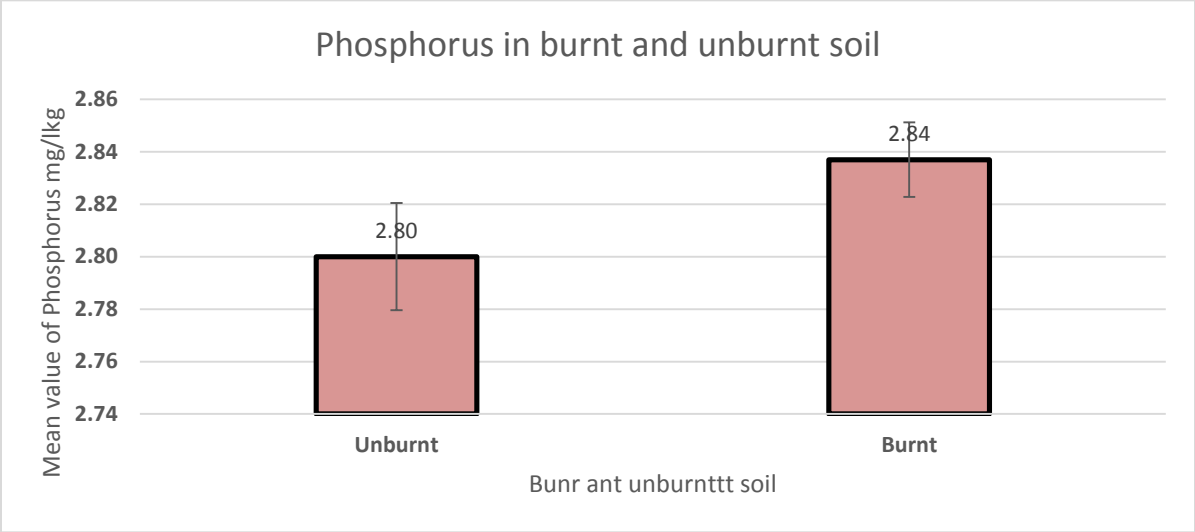

Figure 3: Comparison of phosphorus (P) concentrations in burnt and unburnt soil samples.

Comparison of water holding capacity

The unburnt soil sample reflects mean value of 25.74% much lesser than mean value of 12.62% for burnt soil samples (Figure 4). The Two independent sample T test indicates the significant variation of water holding capacity between burnt and unburnt soil samples. Our null hypothesis of no difference between burnt and unburnt soil samples for water holding capacity is therefore rejected at (α=0.05) and alternatively we concluded that there are significant variations between these two treatments of burnt and unburnt soil samples. From the statistical analysis, we concluded that water holding capacity does not remain constant and is affected by forest fire in contradiction to our null hypothesis.

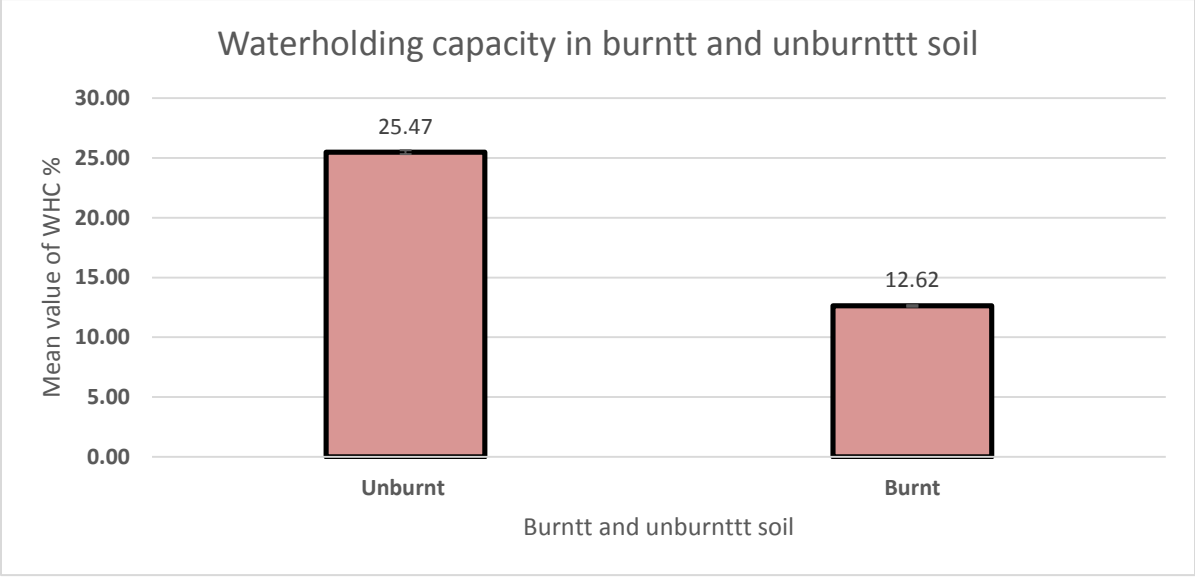

Fig. 4. Comparison of water holding capacity (%) between burnt and unburnt soil samples.

Comparison of Electrical conductivity

Our null hypothesis of no difference between burnt and unburnt soil samples for electrical conductivity is rejected at (α=0.05) and alternatively we concluded that there are significant variations between these two treatments of burnt and unburnt soil samples. The unburnt soil sample reflects mean electrical conductivity value of 0.46% much lesser than mean value of 0.06% for burnt soil samples (Figure 5). The Two independent sample T test indicates the significant variation of electrical conductivity between burnt and unburnt soil samples. From the statistical analysis, we concluded that electrical conductivity does not remain constant and is affected by forest fire in contradiction to our null hypothesis.

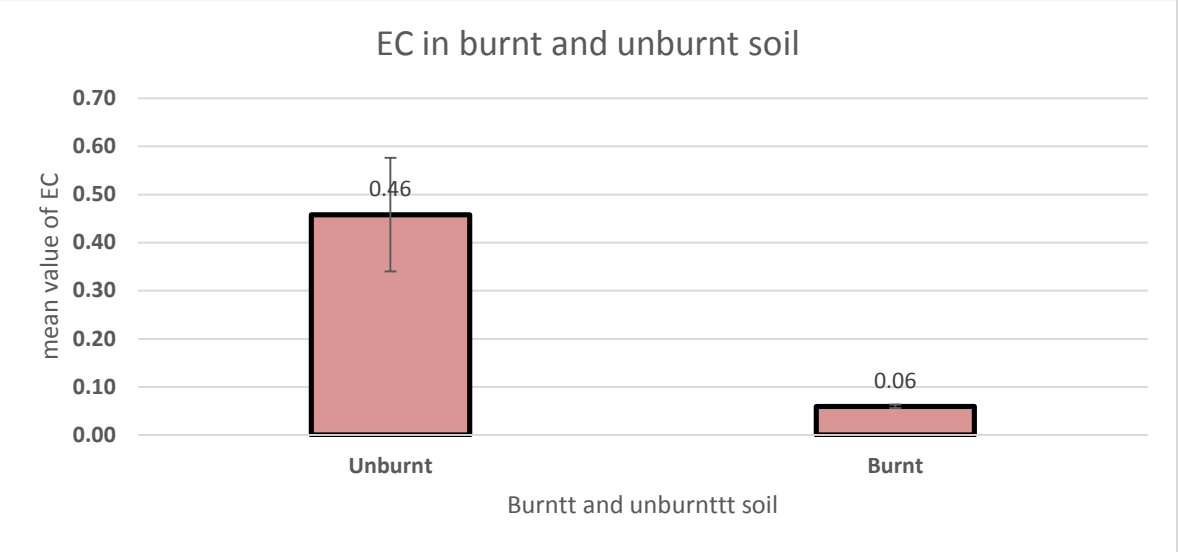

Fig. 5. Comparison of electrical conductivity (EC) between burnt and unburnt soil samples.

Comparison of Ph value

Acidity of soil is represented by its (pH) value. A lower pH value indicates higher acidity while a higher pH value indicates lower acidity. Our null hypothesis of no difference between burnt and unburnt soil samples for (pH)is therefore rejected at (α=0.05) and alternatively we concluded that there are significant variations between these two treatments of burnt and unburnt soil samples. The Two independent sample T test indicates the significant variation of (pH) between burnt and unburnt soil sample. The unburnt soil sample reflects mean value of 6.35 much lesser than mean value of 6.49 for burnt soil samples (Figure 6). From the statistical analysis, we concluded that (pH) does not remain constant and is affected by forest fire in contradiction to our null hypothesis.

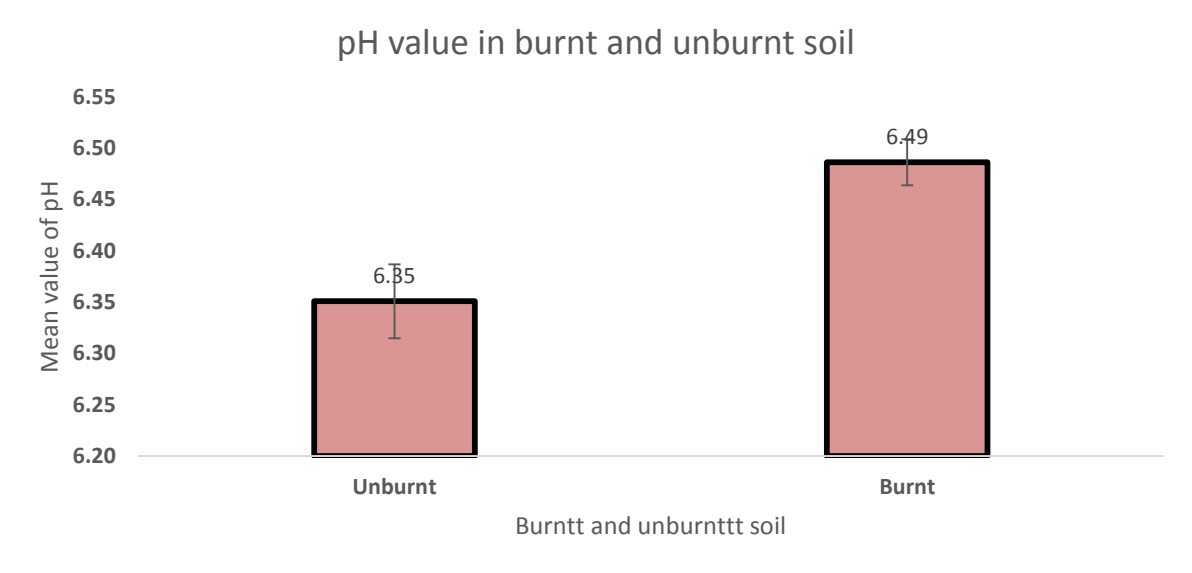

Fig. 6. Comparison of pH values between burnt and unburnt soil samples

Comparison of organic Nitrogen

The Two independent sample T test indicates the significant variation of organic nitrogen between burnt and unburnt soil samples. The unburnt soil sample reflects mean value of 15.75 much lesser than mean value of 20.54 for burnt soil samples (Figure 7). Our null hypothesis of no difference between burnt and unburnt soil samples for organic nitrogen is therefore rejected at (α=0.05) and alternatively we concluded that there are significant variations between these two treatments of burnt and unburnt soil samples. From the statistical analysis, we concluded that organic nitrogen does not remain constant and is affected by forest fire in contradiction to our null hypothesis.

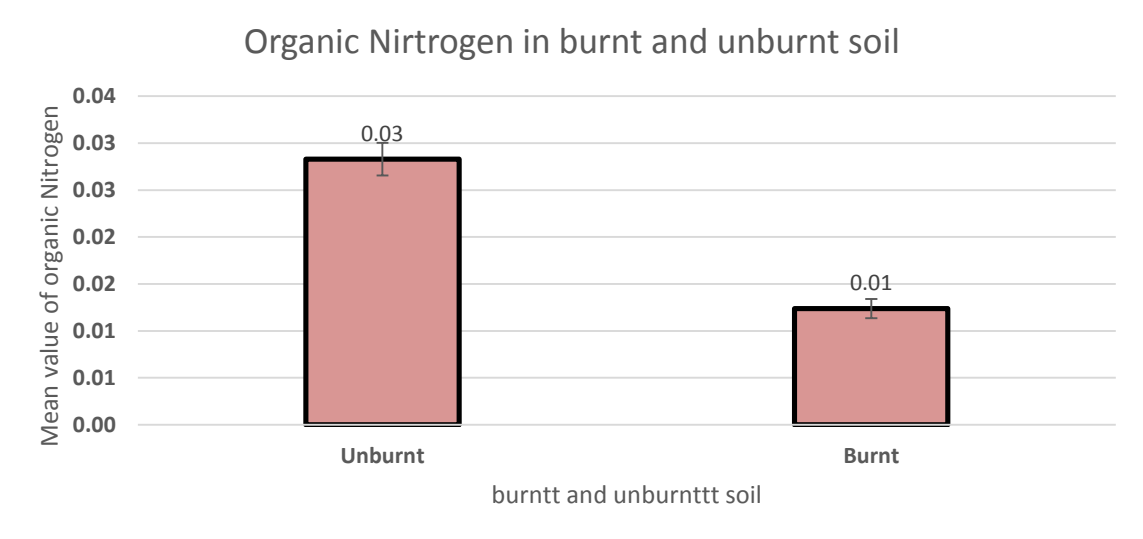

Fig. 7. Comparison of organic nitrogen content between burnt and unburnt soil samples.

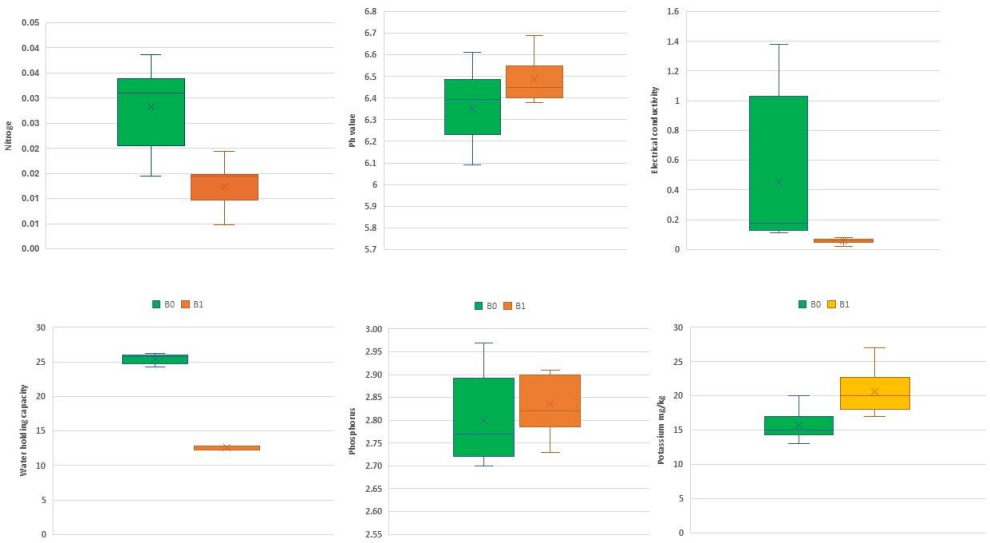

Fig. 8. Quartile distribution of the soil physicochemical parameters.

Effect of forest fire on regeneration

The change caused by fire is important, so forest managers or scientists can use this information to plan restoration, recovery, or conservation efforts in the burnt areas. It shows that fire has a real impact on the environment. The Two independent sample T test indicates the significant variation of regeneration in burnt and unburnt soil samples. The unburnt soil sample reflects mean value of 4 samples larger than mean value of 34.6 for burnt soil samples (Figure 9).

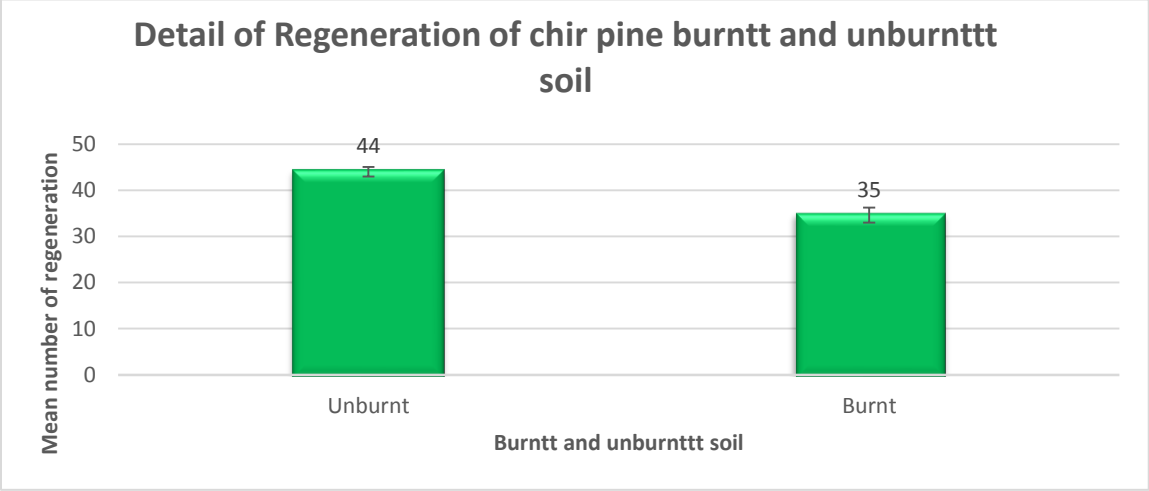

Fig. 9. Comparison of regeneration characteristics between burnt and unburnt soil samples.

Our null hypothesis of no difference between burnt and unburnt soil samples for regeneration is therefore rejected at (α=0.05) and alternatively we concluded that there are significant variations between these two treatments of burnt and unburnt soil samples (Table 4.3).

Table 3: Statistical comparison of regeneration parameters between burnt and unburnt sites using an independent samples t-test.

Data	Unburnt	Burnt
Mean	44	34.6
Variance	20.63157895	53.2
Observations	20	20
Pooled Variance	36.91578947	
Hypothesized Mean Difference	0	
Df	38	
t Stat	4.892397925	
P(T<=t) one-tail	9.28501E-06	
t Critical one-tail	1.68595446	
P(T<=t) two-tail	.000	Significant
t Critical two-tail	2.024394164	

From the statistical analysis, we concluded that regeneration does not remain constant and is affected by forest fire in contradiction to our null hypothesis (Figure 10).

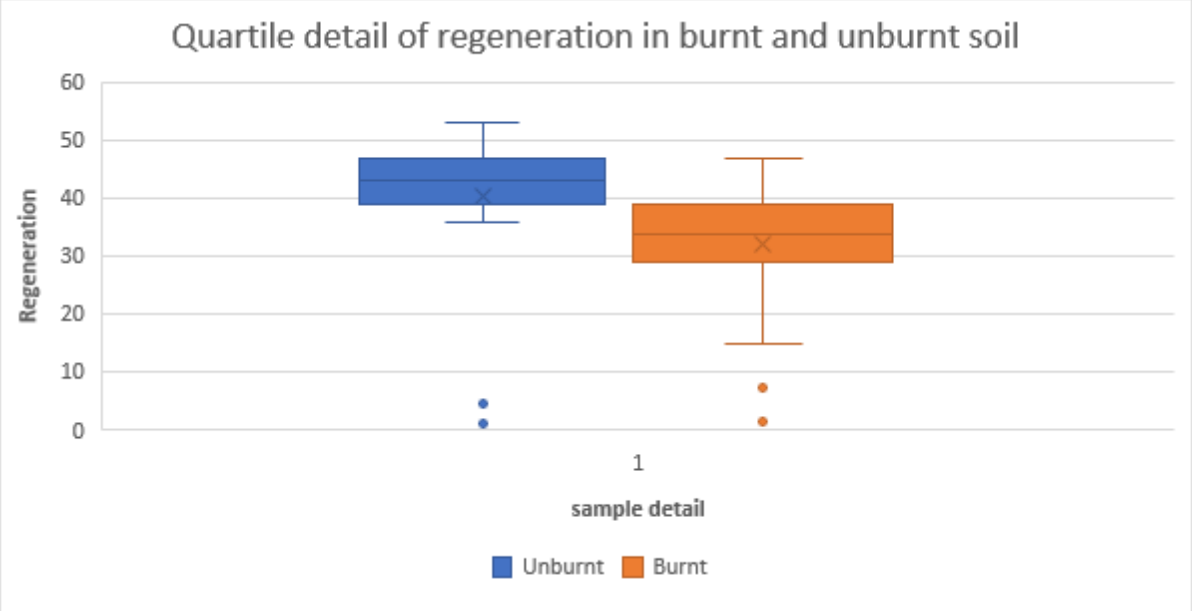

Fig. 10. Quartile analysis of regeneration data collected from burnt and unburnt sites.

DISCUSSION

The aim of the study was to provide site-specific data from District Buner, Pakistan to address a critical knowledge gap of post-fire scenario of vegetation regeneration and soil properties in Himalayan foothill ecosystems. The study was carried out in the scenario of increasing frequency and intensity of forest fires which is largely attributed to anthropogenic activities and current trends of global warming and climate change. Subtropical pine forests dominated by *Pinus roxburghii* in northern Pakistan are increasingly vulnerable to forest fires, posing serious ecological and management challenges (Muhammad *et al*., 2024). This increase frequency has led to ecological disturbance rather than natural regulators specifically in fire prone ecosystem of subtropical Chir pine (*Pinus roxburghii*) forests (Kelly *et al.,* 2020; Phillips *et al.,* 2022). The result revealed that most soil physicochemical parameters reflect statistical variations between burnt and unburnt sites other than phosphorus. Although these findings indicated that forest fire induces modification in soil properties, the magnitude and direction of change vary among nutrients and are influenced by post-fire environmental conditions (Certini, 2005). Phosphorus, which is often reported to increase immediately after fire due to ash deposition (Elakiya *et al.,* 2023), did not show a significant increase in this study and can be explained by the high rainfall in the study area, which likely facilitated rapid leaching of soluble potassium from the soil. Such site-specific climatic influences emphasize the importance of local environmental conditions in determining post-fire nutrient dynamics. Phosphorus reflected non-significant results but increasing trend in burnt soils, coherent with studies suggesting that fire can temporarily enhance available phosphorus through the mineralization of organic matter (Moret-Ferguson *et al.,* 2024). However, the lack of statistical non significance indicates that this effect may be temporary or spatially variable. Indeed, phosphorus dynamics are highly dependent on fire intensity, soil type, and post-fire erosion processes (Certini, 2005). On the other hand, soil physical properties reflected more consistent responses to forest fire. The reduction in water holding capacity (WHC) recorded in burnt areas aligns with the findings that fire decreases soil porosity and increases hydrophobicity due to the combustion of organic matter. Soil pH was also found to increase in burnt areas, indicating the incorporation of ash residues into the soil. This short-term alkalization is a well-documented phenomenon and can influence nutrient availability and microbial activity (Certini, 2005). The increase in soil potassium concentration and reduction in water holding capacity in burnt sites is consistent with findings from other studies conducted in Chir pine and subtropical forest ecosystems. Previous studies from the Himalayan region of India have reported increased potassium concentrations following fire due to the deposition of ash and the mineralization of organic matter (Sharma *et al*., 2023; Verma *et al*., 2025). Similarly, studies conducted by González-Pérez *et al*., (2004) indicated that forest fires can significantly alter soil physicochemical properties by increasing the availability of certain nutrients while reducing soil moisture retention and organic matter content. Increase in soil pH observed in the present study is also in agreement with studies from Mediterranean and temperate forest ecosystems, where post fire ash deposition temporarily increased soil alkalinity (Alcañiz *et al*., 2018). Both national and international studies recorded significant reduction in natural regeneration in burnt areas. High-intensity fires negatively affect seedling establishment and sapling survival, thereby reducing regeneration potential Research conducted in subtropical Chir pine forests of the western Himalayas (Bargali *et al*., 2023; Sharma *et al*., 2023). Similarly, studies from

other fire-prone ecosystems at global level have reported that severe fires can impair vegetation recovery by altering soil properties, reducing seed banks, and creating unfavorable conditions for seedling establishment (Keeley & Pausas, 2022). These findings suggest that the effects of forest fire on soil properties and regeneration dynamics are strongly influenced by fire intensity, post-fire environmental conditions, and ecosystem characteristics. The loss of these nutrients may therefore hinder long-term ecosystem recovery and productivity. The study also documented significant changes in regeneration in burnt areas as compared to unburnt areas. This finding aligns with previous studies indicating that low-intensity fires may promote seed germination while high-intensity fires can suppress sapling survival and overall regeneration capacity (Sharma *et al.,* 2023; Bargali *et al.,* 2023). The reduction in regeneration in burnt areas indicates that fire intensity exceeded the threshold thereby limiting recovery potential. This has important implications for forest management, as regeneration dynamics directly influence long-term forest structure and biodiversity conservation.

Overall, the findings of this study reinforce the effects on both regeneration and soil are intensity-dependent (Kutiel & Shaviv, 1989). Although sub-tropical forests of *Pinus roxburghii* (chir pine) provide various ecosystem services and act as watershed for low lying regions. However, this species is prone to human induced fire primarily due to local communities' dependence on various resources exacerbated by the current dry conditions (Muhammad *et al*., 2024). Subtropical Chir pine forests in District Buner, frequent anthropogenic fires combined with the highly flammable nature of pine needle litter create conditions that can significantly alter both soil properties and vegetation regeneration patterns (Hussain *et al.,* 2021; Kumar *et al.,* 2013). Despite its contributions, this study has certain limitations that should be acknowledged. The spatial scale and number of sampling sites may not fully capture the heterogeneity of the landscape, and the short-term nature of the study limits the ability to assess long-term recovery processes. Moreover, the focus on selected parameters does not account for other important factors such as soil microbial communities, faunal interactions, and detailed vegetation dynamics, which also play critical roles in post-fire ecosystem recovery. Therefore, future research should prioritize long-term monitoring and incorporate a broader range of ecological indicators. Remote sensing and GIS approaches could further enhance understanding of fire regimes and support the development of predictive models for ecosystem recovery (Attri *et al.,* 2020). Such comprehensive approaches are essential for designing effective forest management strategies, including controlled burning, restoration interventions, and community-based fire management, to ensure the sustainability of these ecologically and socioeconomically important forest systems.

CONCLUSION

Soil physicochemical properties and regeneration dynamics are significantly influenced by forest fire in subtropical Chir pine (*Pinus roxburghii*) forests of District Buner, Pakistan. The analysis of burnt sites exhibited increased potassium concentration and soil pH, whereas water holding capacity, electrical conductivity, and organic carbon content declined significantly. However, phosphorus concentration remained relatively unaffected. Regeneration was also significantly decreased in burnt areas, indicating that fire significantly affecting forest ecosystem recovery and long-term forest sustainability. The findings further demonstrate that forest fire can substantially modify soil nutrient dynamics and affect natural regeneration processes in subtropical pine ecosystems. These changes have important implications for forest productivity, biodiversity conservation,

and ecosystem resilience. In the light of above findings, it is therefore recommended that effective fire management strategies, including fire prevention measures, fuel load reduction, soil conservation practices, and community-based fire management programs, should be prioritized to mitigate fire impacts and promote sustainable forest restoration and management in fire-prone landscapes.

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Research Paper **Open Access**

Post-wildfire Ecological Recovery in Hazarganji Chiltan National Park Balochistan

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SUMMARY

Hazarganji Chiltan National Park is the second largest National Park of Balochistan, situated in the Mastung and Quetta districts, which encompasses approximately 27,421 hectares of mountain. In 2025, the national park experienced a significant wildfire event that burned through extensive areas of temperate vegetation, altering the surface of soil and the existing native community of vegetation. Post-fire monitoring following above-average precipitation in 2026 revealed an unexpected ecological response, with burned areas exhibiting significantly higher greenness, a higher density of seedlings, and significant floral diversity compared to nearby unburned sites, contradicting the expected negative outcomes for ecological balance. This study documents vegetation recovery following wildfire and examines the edaphic and ecological mechanisms associated with regeneration in burnt areas, and examines the relationship between soil modification and ecological transition caused by wildfires, precipitation that follows, and natural regeneration patterns. By using phytosociological approaches and assessments of soil physicochemical properties in paired burned and unburned plots, we demonstrated that remarkable natural regeneration after the fire was facilitated by the release of nutrients from fire, a reduction in competition from established vegetation, and the retention of improved soil moisture. These findings contribute to fire ecology management within the national park and suggest that short-term ecological responses to wildfire may vary under favorable climatic conditions.

Keywords: Edaphic factors; Hazarganji Chiltan National Park; Natural regeneration; Phytosociology; Pyrogeography; Soil nutrients; Vegetation recovery; Wildfire.

INTRODUCTION

The Hazarganji Chiltan National Park is the second largest national park in Balochistan, established in 1980 under the provisions of the Balochistan Wildlife Act – 1974 (Act XIX of 1974), Government of Balochistan Forest and Wildlife Department. It is located about 20 km west of Quetta and falls within Mastung and Quetta districts (latitude 30°01'–30°09'N, longitude 66°44'–66°55'E). The total area covers 27,421 hectares of the Sulaiman mountain range at elevations ranging from approximately 5,900 ft to 10,990 ft above sea level (Anonymous, 1998; Anjum et al., 2019). The primary aim of declaring the national park was to protect and conserve the endemic critically endangered Chiltan

wild goat (*Capra aegagrus chialtanensis*). The park supports approximately 88 vascular plant species and diverse faunal assemblages across piedmont plains, foothills, dry stream beds, stream edges, mobile gravel scree, rock scree, rocky terraces, steep rocky cliffs, and subalpine cushion shrubland, as well as an arid-to-semi-arid montane ecosystem (WWF, 1998). In 1998, the Management Plan of HCNP was prepared by WWF-Pakistan and through this documents, the management framework of the national park was established. The Forest and Wildlife Department identified main endemic fauna and and native vegetation as a main objective and recorded the principal habitats (WWF, 1998 ;Mehmood et al., 2024)).

During the summer, on the 12th of June, 2025, the Chiltan side was severely affected by a forest fire (Figure 1). The exact cause of the wildfire remains uncertain, although prolonged drought and extreme summer temperatures may have contributed to fire susceptibility. It destroyed a significant portion of the vegetation mosaic in the park on Chiltan, including standing trees, shrub cover, the herbaceous layer, and accumulated surface litter. The burnt portion displayed the anticipated post-fire landscape immediately after the fire with nearly complete removal of ground and standing vegetation, and soil surface minerals were denuded. The naturalists, conservationist and agencies, expressed grave concern about the long-term ecological effects of the wildfire on biological diversity, water retention, and soil stability.

Fig. 1. The Chiltan area experienced severe wildfire disturbance, leading to extensive loss of trees, shrubs, and associated vegetation.

In April and May 2026, more than normal precipitation events exceeded the annual average rainfall, which led to an unexpected significant increase in diversity. Both the snowfall in late winter and increased rainfall in spring provided significant moisture input over the Chiltan highlands, resulting in an– exceptionally wet period. This higher precipitation affected the early stages of recovery of ecological functions and completely changed the disturbance caused by the wildfire in June 2025. Repeat field assessments by the Deputy Conservator and his team, including individuals from academia (University of Balochistan), conducted in late spring and early summer 2026, revealed a notable ecological response. The patches that had burned in 2025 due to the forest fire were largely greener, more heavily vegetated, and floristically richer (Figure 2) than nearby

areas that had not been influenced or affected by the wildfire. Contrary to the common perception that fire is universally detrimental, this fire-driven floral response is consistent with a larger body of ecological research that shows the function of fire as an engineer of ecosystems in Semi-arid and Mediterranean-type environments worldwide. (Wali et al., 2022; Zamin et al., 2023).

Fig. 2. The area burned in mid-summer 2025 is now characterized by high floral diversity, including species such as *Allium lamondiae*, *Pistacia atlantica* subsp. *cabulica*, *Arnebia fimbriata*, *Salvia macrosiphon*, *Papaver rhoeas*, *Rhamnus persica*, *Ferula oopoda*, *F. ovina*, *F. costata*, and *Fraxinus xanthoxyloides* (WWF, 1998).

In arid and semi-arid environments, a variety of biological processes support vegetation regrowth after wildfire. Both organic and inorganic compounds are released by fire, which are trapped in the standing plants in the form of biomass, and the accumulated litter is converted into ash. This process provides the availability of essential micro- and macronutrients on a temporary basis. These minerals and nutrients includes Phosphorus, Potassium, base cations, etc., and improves the nutrition in seedling otherwise the survival rate of these young plants is 20-30% in nutrient-poor soils (Ahmed et al., 2024). Moreover, the fire reduces competition, dense canopy, removing the suppression established by dominant plants for seedlings recruitment. Solar energy is effectively absorbed by blackened soil and help in melting of snow which prolongs soil moisture. This moisture is more crucial in early season of sprouting. When the fire enhances the edaphic and soil conditions coincide with the significant rainfall than average which is recorded in 2026, there can be a remarkable increase in recruitment of seeds from soil seed bank and plant resprouting (Ullah et al., 2024a). This leads in a floral response that is substantially greater and more striking superior to that of neighboring unburned areas.

This study evaluates and records the post-fire vegetation recovery process on the western slope (chiltan side) of national park following June 2025 fire incident and the winter 2026 precipitation events. This analysis uses phytosociological procedures along with physicochemical analysis of soil to achieve several main objectives such as comparing vegetation diversisty, species richness, and natural regeneration indices between burned (disturbed) and unburned (undisturbed) regions across various sites at western slope and elevation zones characterizing edaphic changes, noting the key native species that drive recovery after wildfire and deriving implications for preservation, protection, management, and conservation in fire ecology planning within the national park. This

study contributes to understanding wildfire as a natural ecological process influencing vegetation dynamics in arid, semi-arid, and dry temperate habitats and further provides a measurable basis for future adaptive fire management policies (Khan et al., 2023; Hussain, 2019).

MATERIALS AND METHODS

Study Area and Fire Context

The research was conducted between 1st April to 10th of May 2026 on the western slope (Chiltan massif) of Hazarganji-Chiltan National Park (HCNP), Balochistan, Pakistan (Figure 3 A), circling affected sites and adjacent unburned reference areas. Normalized Difference Vegetation Index (NDVI) differencing from Sentinel-2 multispectral data (Bands 4 and 8; spatial resolution 10 m) was used to locate and identify the spoiled burnt sites, which were estimated and calculated to be between 1,500 and 2,000 hectares. The zones under investigation span elevations of 1,800 m to 3,100 m (5,906–10,171 ft), centered with coordinates: 30°02'15.33"N, 66°52'52.22"E (Figure 4). The forest fire ignited during the peak of dry season in the month of June 2025, which is characterized by short relative humidity, high air temperatures, and prevailing easterly winds (Figure 3 B), which expanded wildfire into the montane forest cover.

The park is situated within an arid-to-semi-arid montane climate, with a mean annual precipitation of 150–244 mm, received primarily as winter snowfall and spring rainfall (Dawood et al., 2021). Meteorological records from the Pakistan Meteorological Department (2026) indicate that cumulative precipitation during the 2026 growing season exceeded the long-term mean by approximately 30–50% across the study area, which is considered to have provided the primary hydrological impetus for accelerated post-fire vegetative recovery.

Fig. 3. A: The area affected area of chiltan during summer, 2025, **B**: Expanding pattern of wildfire in area under investigation.

Sampling design

A stratified, paired plot design was employed to quantify vegetation recovery responses across the burned perimeter and into unburned reference areas. The study area was stratified into three elevation zones: lower (1,800–2,300 m), middle (2,300–2,800 m), and upper (2,800–3,100 m).

Fig. 4. A: Hybrid/layer view of HCNP**, B:** The satellite image of the study area, **C:** The main area burnt in summer 2025, **D:** The map of Balochistan and HCNP.

Within each zone, sampling plots were established in both burned (B) and unburned (UB) sites, yielding a total of 60 plots (30 burned; 30 unburned), with 10 paired B/UB plots per elevation zone (Figure 5).

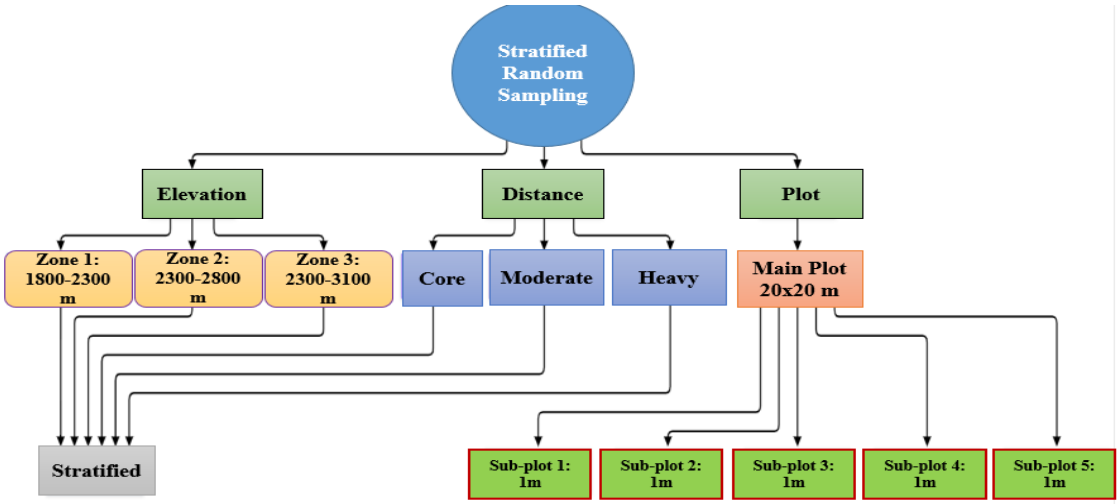

Fig. 5. The area is divided into Zone 1 to 3 with different elevations and main plots with 20x20 m with sub-plots.

Each main plot measured 20 m × 20 m and was used for vegetation structural analysis. Five nested 1 m × 1 m sub-plots were systematically positioned within each main plot (one at each corner and one at the center) for assessment of herbaceous cover, seedling density, and soil sampling (Figure 6). Burned and unburned plots were matched by elevation (±50 m), aspect, and slope angle to minimise the influence of confounding abiotic variables. Plot corners were permanently marked and georeferenced using a Garmin eTrex 10 differential GPS unit (positional accuracy ±0.3 m).

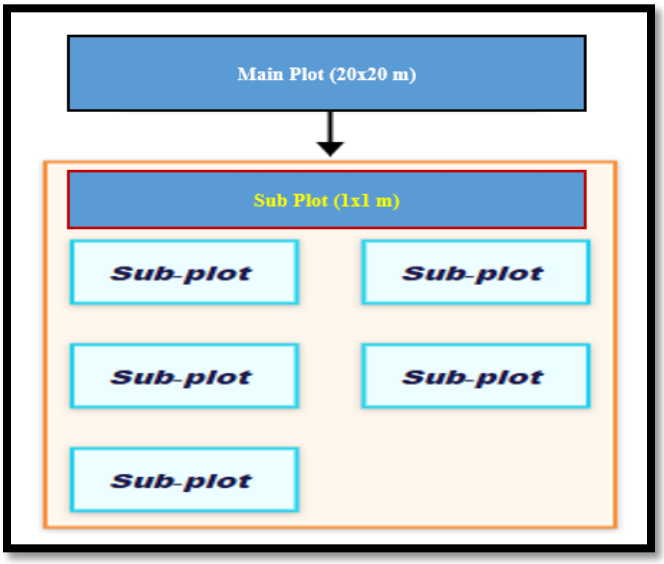

Fig. 6. The main plot is further divided in five sub-plot with size 1x1m.

Vegetation Assessment

Within each main plot, all vascular plant species were identified to species level using Flora of Pakistan (Nasir & Ali, 1970–onward), the Hazarganji-Chiltan National Park Management Plan (WWF, 1998), and Plants of the World Online (POWO, 2024; Royal Botanic Gardens, Kew). Taxonomic identifications were verified against herbarium voucher specimens held at the University of Balochistan Herbarium and confirmed by a regional plant taxonomist.

For each species in each plot, frequency of occurrence, density (individuals m^{-2}), and canopy cover were recorded. Cover was estimated using the Braun-Blanquet phytosociological cover-abundance scale (Kent, 2012). Species importance was quantified using the Importance Value Index (IVI), calculated as the sum of relative frequency, relative density, and relative cover, expressed on a scale of 0–300 (Kent, 2012; Mueller-Dombois & Ellenberg 2016)

Natural regeneration was assessed by recording all seedlings and saplings with height < 1 m (woody species) and all individuals < 1-year-old (herbaceous species) within each 1 m × 1 m sub-plot, expressed as individuals per square meter (ind. m^{-2}). Canopy greenness was quantified using digital hemispherical photography captured with a Nikon D3500 camera fitted with an 8 mm fisheye lens, positioned 1.3 m above ground at plot meter. HemiView v2.1 (Delta-T Devices, 2019) was used to analyzed the images to derive canopy cover fraction (%), making it feasible to compare vegetative regeneration between disturbed and undisturbed locations objectively and quantitatively.

Soil Sampling and Analysis

Soil samples were collected from each plot at two depth increments: 0–15 cm (topsoil) and 15–30 cm (subsoil). At each depth, three randomly located sub-samples were collected within the main plot and composited to yield one composite sample per depth per plot, producing a total of 360 soil samples. All samples were air-dried, ground, and sieved through a 2 mm stainless-steel mesh prior to laboratory analysis.

The following physicochemical parameters were determined for each sample using standard procedures (Sparks et al. 2020), soil particle size distribution by the hydrometer method; soil pH in a 1:5 (w/v) soil:distilled water suspension using a calibrated pH meter; electrical conductivity (EC, dS m^{-1}) in the same 1:5 suspension using a conductivity meter; soil organic matter (SOM, %) by wet oxidation (Walkley-Black procedure); total nitrogen (%) by macro-Kjeldahl digestion; available phosphorus (mg kg^{-1}) by the Olsen bicarbonate-extraction method; and exchangeable potassium (mg kg^{-1}) by flame photometry following ammonium acetate extraction (pH 7.0). Gravimetric soil moisture content (%) was determined by oven-drying at 105 °C for 24 h (Reynolds, 2010). Soil water repellency was assessed using the Water Drop Penetration Time (WDPT) test with a mini-disk infiltrometer, following the classification scheme (Bisdom et al. 1993; Doerr et al. 2000). In burned plots, ash content and charcoal residue were quantified gravimetrically by loss-on-ignition at 375 °C and 550 °C, respectively (Heiri et al. 2001), supplemented by visual characterisation. All laboratory analyses were conducted at the Soil Science Laboratory, Department of Microbiology, University of Balochistan, Quetta.

Statistical Analysis

All statistical analyses were performed in R v4.3.0 (R Core Team, 2023) and IBM SPSS Statistics v26.0 (IBM Corp., 2019). The significance threshold was set at $\alpha = 0.05$ throughout.

Species diversity within each plot was characterised using the Shannon-Wiener diversity index (H′), Simpson's evenness index (E_1/D), and Sørensen's similarity coefficient for pairwise comparison of burned and unburned plots, (Magurran & McGil, 2011).

Differences in vegetation recovery variables (IVI, seedling density, green cover fraction) and soil physicochemical parameters between burned and unburned plots were evaluated using paired t-tests where data satisfied normality (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variance (Levene's test), or the Wilcoxon signed-rank test for non-normally distributed variables (Field et al., 2013). The effect of fire treatment on seedling density, after accounting for elevation as a continuous covariate, was assessed using analysis of covariance (ANCOVA), following the standard procedures (Quinn & Keough 2023).

Relationships between soil physicochemical properties and vegetation recovery indices (IVI, H′, seedling density) were quantified using Pearson product-moment correlation and multiple linear regression with stepwise variable selection based on the Akaike Information Criterion (AIC), as implemented in R v4.3.0 (R Core Team, 2023). Plant community compositional structure was visualised using Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarity, and the significance of environmental gradients (elevation, SOM, available phosphorus, soil moisture content) in structuring community composition was assessed by Redundancy Analysis (RDA) following Hellinger transformation of species abundance data, with significance of individual environmental vectors evaluated by permutation testing (Borcard et al. 2018; Legendre & Legendre 2012).

RESULTS

Post-Fire Green Canopy Cover

Mean green canopy cover, derived from digital hemispherical photography analysed in HemiView v2.1, was significantly greater in burned plots than in unburned plots across all three elevation zones (Figure 7). Overall, burned plots recorded a mean green canopy cover of 47.3 ± 8.6%, compared with 29.1 ± 6.4% in unburned plots (paired t-test: t_{29} = 12.84, $p < 0.001$), representing an 18.2 percentage point difference (Table 1; Figure 8). The greatest difference was observed in the middle elevation zone (2,300–2,800 m), where burned plots recorded 51.8 ± 9.1% cover versus 31.4 ± 7.0% in unburned plots (Δ = +20.4%; $p < 0.001$).

Table 1: The green canopy cover by elevation zone and burn treatment (2026).

Elevation Zone	Burned Mean ± SD (%)	Unburned Mean ± SD (%)	Difference (%)	p-value
1,800–2,300 m	43.1 ± 7.2	27.6 ± 5.8	+15.5	< 0.001
2,300–2,800 m	51.8 ± 9.1	31.4 ± 7.0	+20.4	< 0.001
2,800–3,100 m	46.9 ± 8.4	28.3 ± 6.2	+18.6	< 0.001
All zones combined	47.3 ± 8.6	29.1 ± 6.4	+18.2	< 0.001

The Green cover estimated from digital hemispherical photography analyzed in HemiView. n = 30 burned plots, 30 unburned plots. Paired t-test values by zone wise; combined row from overall paired t-test i.e. t = 12.84, df = 29)

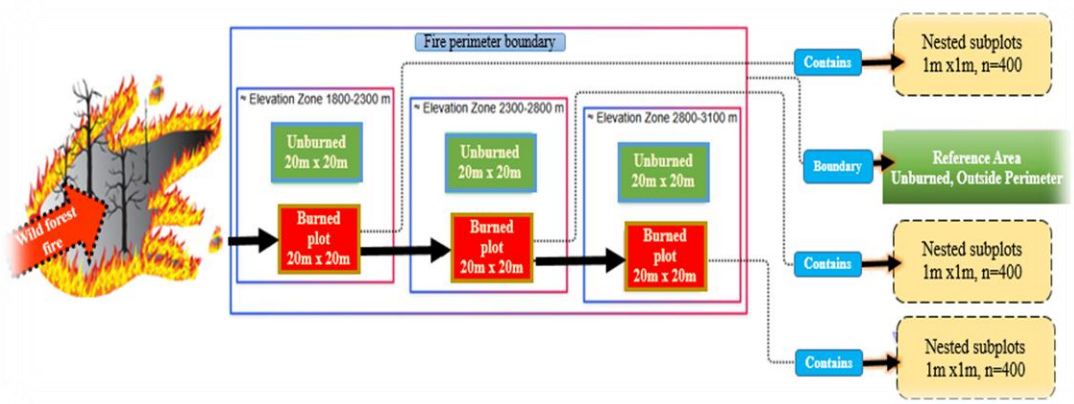

Fig. 7. The eastern direction of the wildfire and comparing the sub-plots of main plots of burned and unburned regions.

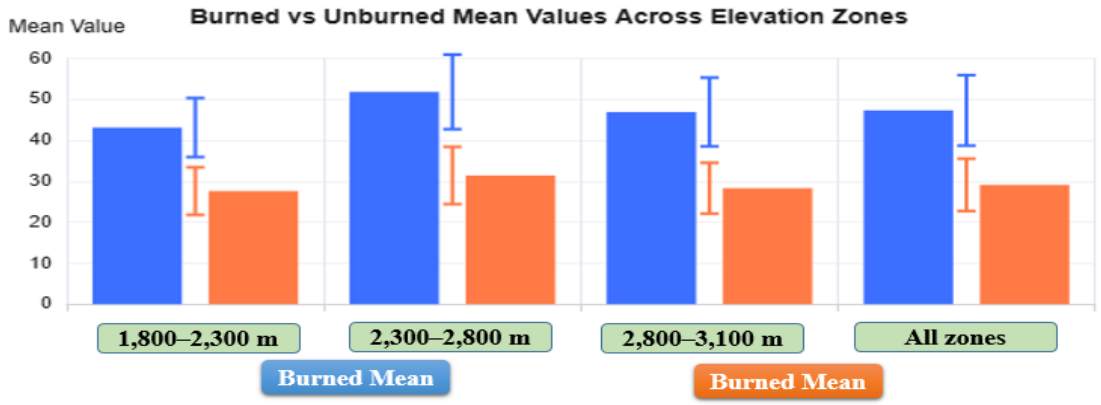

Fig. 8. Green canopy cover (%) across elevation zones and burn conditions.

Floral composition and richness

A total of 73 vascular plant species belonging to 42 families and 64 genera were recorded across all sampling plots. Of these, 58 species were recorded exclusively in burned plots, 67 exclusively in unburned plots, and 6 species were common to both treatment types. Eleven species were recorded solely in burned plots; these were predominantly early-successional taxa including fire-following annuals and geophytes.

Mean species richness per plot was significantly greater in burned plots ($\bar{S}$ = 21.8 ± 4.2) than in unburned plots ($\bar{S}$ = 16.3 ± 3.1; Mann–Whitney U = 188.5, $p < 0.001$). Shannon-Wiener diversity was also significantly higher in burned plots ($H' = 2.91 \pm 0.27$) relative to unburned plots ($H' = 2.43 \pm 0.31$; Mann–Whitney U-test, $p < 0.001$ at all zones; Table 2). The Mann–Whitney U test was selected for these comparisons as species richness and diversity indices did not satisfy the assumption of normality (Shapiro-Wilk test, $p < 0.05$). Among families, Papilionaceae showed a higher relative representation in burned plots compared with unburned plots across all elevation zones. Anacardiaceae, Alliaceae, Compositae, Oleaceae, and Labiatae were other numerically most prominent plant families.

Table 2: Species richness & Shannon-Wiener diversity (H') by Altitude and burn treatment.

Elevation Zone	Burned S	Burned H′	Unburned S	Unburned H′	U-statistic	p-value
Lower (1,800–2,300 m)	19.4 ± 3.8	2.71 ± 0.29	15.1 ± 2.9	2.31 ± 0.28	212.0	< 0.001
Middle (2,300–2,800 m)	24.6 ± 4.7	3.14 ± 0.24	18.9 ± 3.4	2.61 ± 0.30	178.5	< 0.001
Upper (2,800–3,100 m)	21.4 ± 3.9	2.88 ± 0.31	14.9 ± 2.7	2.37 ± 0.33	194.0	< 0.001
Overall	21.8 ± 4.2	2.91 ± 0.27	16.3 ± 3.1	2.43 ± 0.31	188.5	< 0.001

where S = mean species richness per plot; H' = Shannon-Wiener diversity index. U-stat = Mann-Whitney U statistic. n = 10 burned + 10 unburned plots per elevation zone (30 + 30 overall).

Patterns of natural regeneration

Mean seedling density was significantly greater in burned plots (17.6 ± 5.3 ind. m^{-2}) than in unburned plots (7.9 ± 3.1 ind. m^{-2}) across all elevation zones (ANCOVA controlling for elevation: $F = 89.3$, $p < 0.001$; Table 3). The ANCOVA approach was applied here specifically to isolate the fire treatment effect on seedling density while accounting for the continuous effect of elevation as a covariate. Seedling density was greatest in the middle elevation zone (2,300–2,800 m) in burned plots (20.4 ± 5.8 ind. m^{-2}), with significantly lower values in unburned plots at the same zone (11.2 ± 3.4 ind. m^{-2}; Figure 9).

Among dominant regenerating species in burned plots, Pistacia khinjuk / P. atlantica and Berberis calliobotrys / B. baluchistanica recorded the highest IVI values (62.3 and 54.7, respectively) and the greatest seedling densities (4.8 and 4.1 ind. m^{-2}, respectively; Table 4). Cousinia stocksii showed a recruiter response in burned plots (IVI = 38.4; 2.7 ind. m^{-2}). In contrast, Artemisia maritima and Peganum harmala recorded higher IVI values and seedling densities in unburned plots, indicating suppressed regeneration in burned conditions. Herbaceous species including Cousinia and Papaver spp. were recorded with greater frequency in burned sub-plots, consistent with recruitment from the soil seed bank.

Table 3: Indices of Natural regeneration (seedling density, ind. m^{-2}) by zone and burn treatment, where Seedling density expressed as individuals per m^2. Differences tested by Mann-Whitney U (by zone) and ANCOVA controlling for elevation (overall). Overall mean across all plots = 12.75 ± 6.4 ind. m^{-2}.

Elevation Zone	Burned Mean ± SD	Max	Unburned Mean ± SD	Max	Difference	p-value
Lower	15.2 ± 4.6	26.3	7.3 ± 2.9	13.1	+7.9	< 0.001
Middle	20.4 ± 5.8	34.1	11.2 ± 3.4	18.4	+9.2	< 0.001
Upper	14.3 ± 4.2	23.8	5.6 ± 2.1	10.2	+8.7	< 0.001
Overall	17.6 ± 5.3	34.1	7.9 ± 3.1	18.4	+9.7	< 0.001

Table 4: Dominant regenerating species in burned vs. unburned plots: Importance Value Indices (IVI) and regeneration density, where IVI = Relative Frequency + Relative Density + Relative Cover (maximum 300).

Species	Burned IVI	Burned Seedling (ind. m^{-2})	Unburned IVI	Unburned Seedling (ind. m^{-2})	Fire Response
Pistacia khinjuk / P. atlantica	62.3	4.8	38.1	1.9	Resprouter
Berberis calliobotrys/ B. baluchistanica	54.7	4.1	29.4	1.4	Resprouter
Cousinia stocksii	38.4	2.7	22.6	1.1	Recruiter
Artemisia maritima	22.1	1.4	38.4	2.6	Suppressed
Peganum harmala	11.3	0.8	16.8	1.3	Suppressed

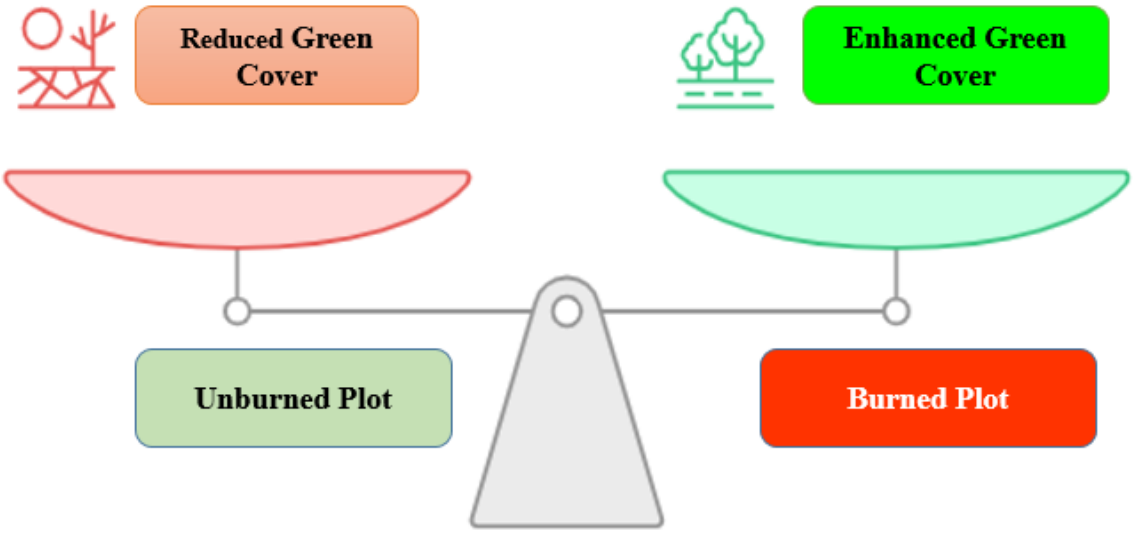

Fig. 9. The sites burned due to wildfire in June, 2025 exhibited more regeneration than the unburned or partially burned sites.

Comparison of soil physicochemical properties between burned and unburned plots

Significant differences between burned and unburned plots were observed for several soil physicochemical parameters at 0–15 cm depth (Table 5). All comparisons were made using paired t-tests, which were appropriate for these variables given that normality (Shapiro-Wilk, $p > 0.05$) and homogeneity of variance (Levene's test, $p > 0.05$) assumptions were satisfied.

Soil pH was significantly higher in burned plots (8.2 ± 0.4) than in unburned plots (7.9 ± 0.3; $t = 2.84$, $p = 0.007$). Soil organic matter (SOM) was significantly lower in burned plots (1.47 ± 0.38%) than in unburned plots (2.11 ± 0.42%; $t = -5.46$, $p < 0.001$). Gravimetric soil moisture content was significantly greater in burned plots (14.2 ± 2.8%) than in unburned plots (9.8 ± 2.1%; $t = 5.91$, $p < 0.001$). Available phosphorus was approximately twice as high in burned plots (18.4 ± 4.2 mg kg^{-1}) relative to unburned plots (8.9 ± 2.7 mg kg^{-1}; $t = 9.18$, $p < 0.001$). Available potassium was also significantly greater in burned plots (187.3 ± 41.2 mg kg^{-1}) than in unburned plots (94.6 ± 22.8 mg kg^{-1}; $t = 9.03$, $p < 0.001$). Electrical conductivity ($t = 1.42$, $p = 0.162$) and available nitrogen ($t = -0.81$, $p = 0.423$) did not differ significantly between treatments.

Table 5: The mean soil physicochemical properties between 30 burned & 30 unburned plots (0–15 cm depth).

Parameter	Units	Burned Mean ± SD	Unburned Mean ± SD	t / U statistic	p-value
pH	-	8.2 ± 0.4	7.9 ± 0.3	t = 2.84	0.007
Organic matter	(%)	1.47 ± 0.38	2.11 ± 0.42	t = −5.46	< 0.001
Moisture	(%)	14.2 ± 2.8	9.8 ± 2.1	t = 5.91	< 0.001
Electrical conductivity	(dS m^{-1})	0.71 ± 0.18	0.64 ± 0.15	t = 1.42	0.162

Available N	(mg kg⁻¹)	68.4 ± 16.2	72.1 ± 14.9	t = −0.81	0.423
Available P	(mg kg⁻¹)	18.4 ± 4.2	8.9 ± 2.7	t = 9.18	< 0.001
Available K	(mg kg⁻¹)	187.3 ± 41.2	94.6 ± 22.8	t = 9.03	< 0.001

Relation between plants and soil in post-wildfire recovery

Within burned plots, Pearson product-moment correlation analysis indicated significant positive associations between seedling density and available phosphorus ($r = 0.81$, $p < 0.001$), soil moisture ($r = 0.77$, $p < 0.001$), available potassium ($r = 0.72$, $p < 0.001$), and soil organic matter ($r = 0.58$, $p < 0.001$). Soil pH was negatively correlated with seedling density ($r = -0.41$, $p = 0.024$; Table 6). A multiple linear regression model incorporating available phosphorus, soil moisture, available potassium, and SOM as predictor variables explained 83.4% of the variance in seedling density within burned plots ($R^2 = 0.834$, $F_{4,25} = 31.6$, $p < 0.001$; Figure 12).

Table 6: Relationship between soil parameters and seedling density within burned plots where n = 30 (Pearson correlations).

Soil Parameter	r	p-value	Direction	Ecological Mechanism
Available P (mg kg⁻ ¹)	0.81	< 0.001	Positive	Ash-released P; seedling nutrition
Moisture (%)	0.77	< 0.001	Positive	Reduced evapotranspiration; soil water retention
Available K (mg kg⁻ ¹)	0.72	< 0.001	Positive	Ash-released K; osmotic regulation
Organic matter (%)	0.58	< 0.001	Positive	Residual SOM; microbial activity
pH	−0.41	0.024	Negative	Ash alkalinity; micronutrient availability

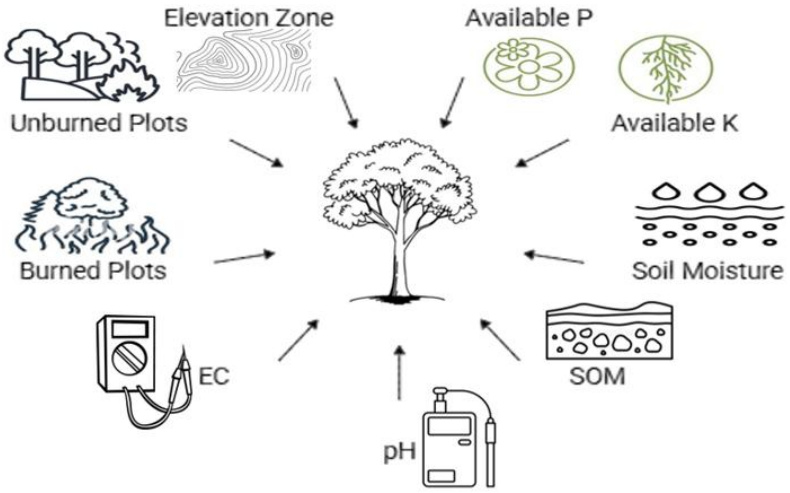

Fig. 12. The ecological vectors influencing the vegetation patterns.

DISCUSSION

Ecological phenomenon of post-wildfire landscape recovery

The significantly greater green canopy cover, species richness, and seedling density recorded in burned plots relative to unburned plots in 2026 indicate a positive short-term vegetation response following the 2025 wildfire at Hazarganji-Chiltan National Park. This pattern is consistent with observations from other arid and semi-arid montane systems in which fire acts as a periodic disturbance to which indigenous flora has developed adaptive responses, rather than functioning as a uniformly degrading agent (Ullah et al., 2024a; Zamin et al., 2023).

Three post-fire edaphic and structural mechanisms appear to underlie the observed vegetation response. First, the approximately twofold increase in available phosphorus and potassium in burned plots is consistent with ash-mediated nutrient release, which has been documented as a short-term fertilisation effect in multiple fire ecology studies (Ahmed et al., 2024; Hussain, 2019). This temporary nutrient enrichment is likely to have improved seedling establishment in the nutrient-limited, shallow soils characteristic of the park. Second, removal of the shrub canopy by fire reduced competitive suppression of seedlings by established adult plants, releasing resources including light, moisture, and nutrients to understory recruits. This mechanism is particularly relevant in shrubland communities approaching climax, where canopy closure constrains regeneration (Haq & Badshah, 2021). Third, the significantly greater soil moisture content in burned plots (14.2 ± 2.8% versus 9.8 ± 2.1% in unburned plots) likely reflects a reduction in evapotranspiration following canopy removal, which allowed a greater proportion of the 2026 precipitation to enter the soil profile rather than being intercepted and transpired by established vegetation.

It is crucial significant to note that during the spring season 2026, the area received approximately 30-50 percent more precipitation than average (Pakistan Meteorological Department, 2026). The rise in quantity of precipitation and interaction between fire-mediated edaphic factors/conditions is likely to amplified the observed vegetation response. The forest fire prepared soil by incrasing nutrient available, minimising evapotranspiration, and enhancing soil seed bank recruits, while the heavy precipitation than the average provided hydrological trigger for germination and early seedling survival establishment. The post-fire recovery advantage may have been considerably attenuated under average (Mehmood et al., 2024; Ahmed, 2019). This dependence shows how crucial it is to include inter-annual climate variability in studies of post-fire recovery in semi-arid ecosystems.

Species composition and successional implications

Between burned and unburned plots, the differential species composition provides insight into the successional dynamics of flora in the national park. The eleven species recorded exclusively in burned plots were predominantly early-successional taxa, including fire-following annuals and resprouting geophytes. This pattern suggests that the soil seed bank of the park contains a component of disturbance-dependent taxa whose germination is inhibited under intact canopy and whose recruitment is triggered by the combination of canopy removal and the nutrient-enriched seedbed provided by ash deposition. Analogous seed bank dynamics have been reported in semi-arid and arid systems across central and western Asia, where fire is recognised as a significant driver of species

turnover in shrubland communities (Wali et al., 2022; Khan et al., 2023).

The higher relative representation of Papilionaceae in burned plots is consistent with the early-successional role of nitrogen-fixing legumes following fire, where reduced competition and elevated phosphorus and potassium availability provide establishment opportunities for this group (Ahmed et al., 2024). The strong resprouting response of Pistacia khinjuk / P. atlantica and Berberis calliobotrys / B. baluchistanica in burned plots reflects lignotuber-based and root-crown regeneration, morphological traits associated with fire adaptation in these taxa (Anjum et al., 2020). The elevated IVI and seedling density of Pistacia in the middle elevation zone (2,300–2,800 m) is of particular conservation significance, as Pistacia-dominated montane woodlands represent a ecologically complex habitat type in which natural regeneration is typically slow and episodic under undisturbed conditions (WWF, 1998; Ali et al., 2018). Conversely, the reduced IVI of Artemisia maritima and Peganum harmala in burned plots relative to unburned plots suggests competitive suppression of these taxa under post-fire conditions, consistent with their characterisation as disturbance-tolerant but not fire-adapted species.

Impact of rainfall

The significant decrease in soil organic matter in burned plots relative to unburned plots is consistent with consumption of the surface organic horizon and soil litter layer during combustion, a well-documented short-term post-fire soil response (Sparks et al., 2020). Despite this reduction, the positive correlation between residual SOM and seedling density within burned plots ($r = 0.58$, $p < 0.001$) indicates that even reduced post-fire organic matter levels contributed to seedling establishment, likely through support of microbial activity and soil structural properties relevant to water retention.

The modest but significant elevation of soil pH in burned plots (8.2 ± 0.4 versus 7.9 ± 0.3) is attributable to alkaline ash deposition, consistent with the chemistry of wood ash from woody shrub combustion (Doerr et al., 2000). The negative correlation between pH and seedling density ($r = -0.41$, $p = 0.024$) suggests that pH elevation at the upper range may have modestly constrained establishment of some species through reduced micronutrient availability, though the pH values recorded remain within the tolerance range of most plant species characteristic of arid montane soils in the region.

The absence of significant differences in available nitrogen and electrical conductivity suggests that wildfire may have exerted limited short-term influence on these parameters portrayal. Although the fire can enhance soil Nitrogen availability in ash deposition through organic matter mineralization, nitrogen losses resulting from volatilization during combustion may counterbalance these gains which leads to no net change in available nitrogen. The interplay between fire-induced nutrient release and nutrient loss processes is due to this neutral response. Same patterns have been documented in fire ecology studies, where the response of Nitrogen changes considerably according to fire intensity, severity, fuel characteristics, slope, biomass combustion etc. Consequently, the lack of significant differences in available Nitrogen and electrical conductivity may show that the wild forest fire exerted only a limited influence on these soil properties under the prevailing site conditions (Ahmed et al., 2024).

The available Phosphorus (P), moisture of soil, available Potassium (K), and SOM collectively account for a significant portion of the variance in young seedling density within burnt plots, according to the multiple regression model ($R^2 = 0.834$), showing that

the soil nutrient and moisture environment developed by post-fire conditions was the main determinant of regeneration intensity.

Implications of preservation, conservation, protection and fire management

The outcomes of this work have implications for the management of forest fires in government policies in HCNP and protected areas (PAs) in Balochistan. The data show that wildfire of moderate intensity, under favorable rainfall, can produce short-term vegetation responses that differ from those at near unburned sites, in the form of canopy cover, greenness, species richness, and natural regeneration density. The data and results are in line with the increasing amount of data in other fire ecological studies and show that wildfire does not always harm vegetation that has acclimated to it and that the effects of fire in different ecosystems or habitat types vary depending on the situation (Ullah et al., 2024a; Zamin et al., 2023).

These results recommend several management measures. First, in order to differentiate between short-term disaster and trajectories of longer-term recovery, vegetation monitoring after wildfire must be methodically included into park management procedures in fire-prone areas. Second, the potential role of prescribed burning as a management tool for accumulated fuel load reduction and biodiversity maintenance should be conducted which may be helpful for fire-stimulated recruitment. Third, post-fire restoration interventions should be timed to coincide with years of above-average anticipated precipitation to maximise the probability of successful establishment of seedlings. Fourth, the existing Management Plan (1998) should be revised to include fire ecology knowledge, given the time elapsed since its formulation and the evidence presented here of non-uniformly negative fire effects on the Chiltan flora.

The findings of this study should be regarded with caution as they relate to the ecological circumstances seen in Hazarganji Chiltan National Park (HCNP) in 2025–2026. As recorded and shown in other ecosystems, high-intensity, high-frequency, or geographically widespread wildfires and disturbance may result in fundamentally different way and conclusions, which may include long-term destruction of plant structure, soil characteristics, loss of reptiles, birds, endogeic/anecic fauna etc. (Zamin et al., 2023). If the observed regeneration response is sustained and to clarify the pace and direction of community recovery in later years long-term evaluation and monitoring that goes beyond a single post-fire growing season (2026) is crucial to assess and clarify.

CONCLUSION

The findings demonstrate considerable ecological resilience and natural regeneration capacity following the 2025 wildfire under favorable post-fire climatic conditions. The research revealed a remarkable ecological outcome in the form of higher canopy (18%) cover, which was triggered by exceptional precipitation events in 2026, species richness (21.8 vs 16.3 species per plot), Shannon-Wiener Diversity (H' = 2.91 vs. 2.43), and natural regeneration density (17.6 vs. 7.9 individual m^2) when compared to near-reference sites (unburned). The study of edaphic factors confirmed that the recovery and abundance were driven mainly by interconnected processes, i.e., nutrient pulse mediated by ash, reduction in competition among established vegetation, and higher soil moisture. These factors engaged synergistically with higher precipitation events in 2026 than average rainfall/snowfall to produce an extra seedling recruitments. The highly noted plant species achieving recovery after disturbance include Pistacia khinjuk, Berberis

calliobotrys, Fraxinus xanthozyloides, Pistacia atlantica subsp. cabulica, and Papaver rhoeas, which are local species in the mosaic of the park. The recorded and observed post-wildfire recovery shows that, when the area is supported by adaptive management, the vegetation of the national park possesses considerable ecological resilience, underscoring its capacity for natural regeneration following the 2025 wildfire and highlighting promising preservation and conservation outcomes. These findings indicate that moderate wildfire may contribute to positive short-term vegetation responses under favorable climatic conditions. and recommends developing a scientific post-wildfire vegetation monitoring transect, updating the management plan (1998), an appraisal of the ecological suitability of controlled burning as a management tool for biodiversity conservation, and classifying the soil seed bank of the region to better understand the fire-responsive potential of flora.

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Review Article **Open Access**

Functional Diversity and Tropical Forest Ecosystem Dynamics and Productivity: A Review

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SUMMARY

Tropical forests are being reshaped by land-use change, climate stress, altered disturbance regimes, and biotic change. However, the ecological consequences of biodiversity loss cannot be fully understood from species richness alone. This narrative review examines how functional diversity—the range, distribution, and relative abundance of organismal traits—influences forest productivity, carbon storage, resource use, stability, resilience, and ecosystem multifunctionality. The review synthesizes a carefully selected body of literature published between 2016 and 2026, excluding duplicate records, retracted publications, studies published before 2016, and research that did not directly address biodiversity–ecosystem functioning relationships in forests. The evidence shows that functional diversity generally enhances biomass production and carbon storage by promoting resource-use complementarity. However, these effects are rarely independent of other factors, including functional identity, stand structure, phylogenetic diversity, soil conditions, climate, and disturbance history. Positive diversity–productivity relationships are most evident where trait differences improve access to limiting resources, although the influence of dominant species and tree-size variation can exceed that of trait diversity in some forest systems. The relationship between functional diversity and resilience is more conditional. Hydraulic and response diversity can improve resistance and recovery following disturbance, yet recent syntheses does not support a universally positive effect of functional diversity on ecosystem recovery. Likewise, mixed-species stands do not necessarily maximize ecosystem multifunctionality because different ecosystem functions respond to distinct biotic and abiotic drivers. The review therefore calls for a multiscale framework that is context dependent and integrates functional effect traits, response traits, vertical structure, belowground interactions, and environmental constraints. This approach could improve tropical forest conservation, restoration and monitoring by shifting management from maximizing species numbers to maintaining complementary strategies, key functional identities, response diversity and spatial heterogeneity.

Keywords: Climate change; ecosystem functioning; forest ecosystem dynamics; forest productivity; functional diversity; functional traits; tropical regions

INTRODUCTION

Tropical forests harbor an excessively large portion of the world's terrestrial biodiversity while playing a vital role in regulating carbon, water, and nutrient cycles across local, regional, and global scales. However, their functional integrity is increasingly threatened by land-use change, forest degradation, climate-induced drying, and interacting disturbance regimes. These pressures alter species composition, forest structure, and trait distributions rather than simply reducing species richness (Aguirre-Gutiérrez et al., 2020; Sovann et al., 2025). Such changes are ecologically significant because shifts in community composition influence biomass accumulation, resource acquisition, regeneration, and the capacity of forests to resist and recover from disturbance (Mori et al., 2017; Matsuo et al., 2026b). As a result, biodiversity conservation can no longer focus solely on preserving species numbers. It must also consider the ecological strategies represented by species and the ecosystem functions they support (Mammola et al., 2021; Bonilla-Valencia et al., 2022).

Functional diversity provides a mechanistic link between biodiversity and ecosystem functioning by describing the range, distribution, and relative abundance of functional traits within ecological communities. These traits determine how species acquire resources, interact with one another, and respond to environmental change. Unlike species richness, which treats all species as functionally equivalent, trait-based approaches distinguish communities composed of ecologically similar species from those containing diverse functional strategies (Mammola et al., 2021). This distinction is particularly important in species-rich tropical forests, where high taxonomic diversity may coincide with functional convergence under strong environmental filtering, whereas forests with relatively fewer species may exhibit considerable functional differentiation through niche partitioning (Aguirre-Gutiérrez et al., 2020; Cheng et al., 2020). Consequently, functional diversity can reveal changes in ecosystem capacity that remain undetected when assessments rely solely on taxonomic measures (Bonilla-Valencia et al., 2022).

Recent research, however, does not support the simple assumption that increasing functional diversity always enhances ecosystem functioning. For example, in a tropical cloud forest, functional diversity explained substantially more variation in aboveground biomass than either species or phylogenetic diversity, suggesting that trait differences mediated the influence of soil phosphorus on resource acquisition and biomass accumulation (Cheng et al., 2020). In contrast, a study in subtropical forests found that phylogenetic diversity was a stronger predictor of aboveground biomass across tree strata, although community-weighted trait values remained influential (Li et al., 2026). Other studies have reported that tree-size diversity, dominant trait values, or the identity of key species can explain ecosystem functioning more effectively than trait dispersion alone (Mahaut et al., 2020; Noulèkoun et al., 2023). Collectively, these findings indicate that functional diversity should not be viewed as a universal replacement for taxonomic, phylogenetic, or structural diversity. Rather, it represents one component of a multidimensional framework for understanding forest ecosystem functioning.

Another limitation of previous reviews is the tendency to treat productivity, stability, resilience, and ecosystem multifunctionality as interchangeable concepts. In reality, these outcomes represent distinct ecological properties. Productivity refers to the rate or amount of biomass production, stability describes the consistency of ecosystem

functioning over time, and resilience reflects the ability of ecosystems to resist, recover from, or adapt to disturbance. Ecosystem multifunctionality extends this perspective by evaluating the simultaneous maintenance of multiple ecological processes, including biomass production, decomposition, nutrient cycling, carbon storage, and water regulation (Gladstone-Gallagher et al., 2019; Damptey et al., 2022). A particular trait composition may enhance one ecosystem function while constraining another, and the dominant drivers often vary across ecological processes, spatial scales, and stages of forest succession (Li et al., 2024; Matsuo et al., 2026a). Distinguishing these outcomes is therefore essential for accurately evaluating the ecological role of functional diversity. Against this background, this review pursues four main objectives. First, it examines the conceptual basis and measurement of functional diversity in forest ecosystems. Second, it synthesizes current evidence on functional diversity and ecosystem productivity dynamics. Third, it evaluates how environmental factors, including climate, soil conditions, disturbance regimes, successional stage, and spatial scale, modify functional diversity–ecosystem and functioning relationships. Finally, it identifies areas of consensus, unresolved inconsistencies, key research gaps, and practical implications for tropical forest conservation and management implications. The review argues that functional diversity provides the greatest explanatory value when interpreted alongside functional identity, response diversity, forest structure, and environmental context, rather than as an isolated measure of biodiversity.

MATERIALS AND METHODS

Scope and eligibility

This article adopts a narrative review design to integrate conceptually diverse evidence from experiments, observational inventories, meta-analyses, restoration chronosequences, and methodological reviews. The evidence base comprised a collected quantity of literature published between 2016 and 2026. Studies were eligible when they examined functional traits or functional diversity in relation to forest productivity, biomass or carbon storage, nutrient and water dynamics, stability, resilience, multi functionality, disturbance, succession, restoration, or conservation. Tropical and subtropical forest studies formed the core of the synthesis, while selected temperate, boreal, savanna, and cross-biome studies were retained when they tested mechanisms or concepts directly relevant to forest biodiversity–ecosystem functioning relationships.

The screening process removed publications dated before 2016, duplicate records, retracted publications, and sources whose relevance was limited to unrelated taxa or ecosystems. Studies were also excluded when bibliographic information or reported findings were insufficient for faithful interpretation. The final synthesis used 51 sources and prioritized peer-reviewed journal articles, reviews, meta-analyses, and recent empirical studies. Because the included studies differed substantially in design, spatial extent, trait selection, response variables, and statistical methods, no pooled effect size was calculated. Instead, the review used thematic comparison to distinguish recurring mechanisms, context-dependent effects, and unresolved contradictions.

Literature Analysis and Synthesis Approach

The evidence was organized in five analytical stages. The first stage examined definitions, indices, and scale-related measurement issues. The second compared mechanisms linking functional diversity to productivity and carbon storage, with

particular attention to complementarity, selection, functional identity, vertical structure, and belowground interactions. The third assessed environmental and dynamic controls, including drought, soil fertility, land-cover change, succession, and landscape configuration. The fourth separated stability, resilience, and multi functionality to avoid conceptual conflation. The final stage translated the synthesis into conservation, restoration, monitoring, and research priorities. Within each stage, studies reporting similar results were integrated into shared claims, whereas contradictory findings were retained and interpreted in relation to differences in biome, forest layer, resource limitation, disturbance type, and temporal scale.

This approach is appropriate for the present purpose because the literature does not examine one standardized exposure and outcome. Functional diversity may be represented by functional richness, dispersion, divergence, evenness, community-weighted means, response diversity, or functional redundancy, while ecosystem outcomes range from annual increment and aboveground biomass to soil carbon, habitat quality, resistance, recovery, and multifunctionality (Magneville et al., 2022; Mammola et al., 2021). A narrative synthesis can therefore expose conceptual mismatches that would be obscured by treating all indices and outcomes as equivalent. It also enables explicit attention to the conceptual transitions between ecological mechanism, environmental context, and management application.

RESULTS AND DISCUSSION

Concept of Functional Diversity and Measurement in Forest Ecosystems

Community Composition and Ecological Diversity

Functional diversity describes the variation in biological traits that determine how organisms acquire resources, tolerate environmental conditions, interact with other species, and contribute to ecosystem processes. Its value lies in providing a mechanistic link between community composition and ecosystem functioning, rather than assuming that all species contribute equally to ecological processes (Mammola et al., 2021). In forest ecosystems, commonly assessed functional traits include maximum tree height, wood density, specific leaf area, seed size, leaf nutrient concentrations, hydraulic vulnerability, and stem or leaf dry matter content. These traits reflect key ecological trade-offs associated with growth, resource acquisition, persistence, and stress tolerance (Lammerant et al., 2023; Amissah et al., 2021). However, the ecological significance of any trait depends on the ecosystem process being examined. A trait that effectively predicts growth, for example, may not reliably explain drought recovery, decomposition, or other ecosystem functions.

A clear distinction between functional effect traits and response traits is therefore essential. Functional effect traits directly influence ecosystem processes such as productivity, carbon storage, nutrient cycling, and decomposition. In contrast, response traits determine how species respond to environmental stresses and disturbances, including drought, fire, logging, grazing, and pathogen outbreaks. Although a single trait may serve both functions, these roles should not be considered interchangeable (Gladstone-Gallagher et al., 2019). For instance, a community may contain several species that perform similar ecological functions but differ in their responses to disturbance, thereby maintaining both functional redundancy and response diversity. Conversely, high variation in traits associated with growth may provide limited

ecological insurance if species share similar vulnerability to the dominant disturbance (Correia et al., 2018; Dendoncker et al., 2023).

Functional diversity should also be distinguished from functional identity. Functional diversity metrics quantify the distribution and variation of trait values within a community, whereas community-weighted mean traits represent the characteristics of the most abundant species and are commonly used to evaluate mass-ratio or dominance effects. This distinction helps explain why communities with comparable levels of functional diversity may differ markedly in biomass or ecosystem functioning if one community is dominated by tall, dense-wooded, or otherwise highly productive species (Mensah et al., 2016; Mensah et al., 2021). Increasing evidence suggests that complementarity and dominance are not competing mechanisms but often operate simultaneously. Consequently, the key ecological question is not whether functional diversity or functional identity is more important, but which trait combinations and dominant functional strategies best support ecosystem functioning under specific environmental conditions and disturbance regimes.

Components of Functional Diversity

Functional richness measures the amount of trait space occupied by a community and is commonly interpreted as the breadth of ecological strategies available for resource use. Functional evenness describes how regularly species abundances fill that trait space, while functional divergence assesses whether abundant species occur toward the edges rather than the center of the trait distribution. Functional dispersion measures the average distance of species from a community trait centroid and is less directly dependent on species richness than some other indices (Mammola et al., 2021; Magneville et al., 2022). These indices are not interchangeable because each represents a different ecological hypothesis. A positive relationship between functional richness and biomass may indicate broader niche occupation, whereas a functional divergence effect may indicate that abundant species use contrasting resources or occupy different strata.

Community-weighted means and multivariate diversity indices should consequently be analyzed together whenever possible. In tropical semi-arid forests and savannas, functional identity and divergence contributed differently to aboveground carbon, demonstrating that dominant strategies and niche differentiation can have distinct effects within the same system (Mensah et al., 2021). In temperate forests, aboveground biomass relationships also varied among functional diversity measures, reinforcing the need to avoid interpreting a single index as a complete description of community function (Vargas-Larreta et al., 2021). Methodological tools that calculate multiple facets of functional diversity improve comparability, but reliable interpretation still depends on transparent trait selection, abundance weighting, distance calculation, and treatment of missing data (Magneville et al., 2022).

Trait choice is especially consequential in tropical forests because plant height, wood density, leaf economics, seed traits, and hydraulic traits operate at different biological scales and respond differently to environmental filtering. Height diversity may capture vertical partitioning of light, while wood density may reflect growth–survival trade-offs and hydraulic safety. Specific leaf area can indicate acquisitive versus conservative resource strategies, yet its relationship with biomass can reverse between canopy and understory layers (Li et al., 2026). Multitrait indices may therefore conceal compensating

or opposing single-trait effects. Strong review and empirical designs should report both aggregate indices and trait-specific results so that the ecological mechanism remains visible.

Measurement of Functional diversity

Functional diversity is scale dependent because local communities are embedded within heterogeneous landscapes. Alpha diversity describes trait variation within plots, beta diversity captures turnover among plots, and gamma diversity represents the total functional space across a landscape. Relationships observed at plot level cannot automatically be extended to landscapes because spatial homogenization may reduce complementary functions even when local richness remains stable (Gonzalez et al., 2020; Suárez-Castro et al., 2022). Landscape experiments and frameworks increasingly emphasize that between-patch structural heterogeneity can support biodiversity and multifunctionality across scales by creating contrasting microhabitats and disturbance refugia (Müller et al., 2023).

Vertical scale is equally important. Canopy, subcanopy, and understory layers experience different light, moisture, and competitive environments, and the traits that promote biomass in one layer may not have the same effect in another. In the subtropical forest studied by Li et al. (2026), acquisitive trait means and phylogenetic diversity were associated with overstory biomass, whereas conservative strategies, phylogenetic structure, and functional richness contributed more strongly to understory biomass. This layer-specific result helps reconcile conflicting studies by showing that whole-stand averages can combine distinct mechanisms. Future forest analyses should therefore align trait measurement, diversity calculation, and ecosystem response at compatible spatial and vertical scales.

The transition from conceptual definition to empirical testing depends on this measurement discipline. Functional diversity indices are valuable not because they are intrinsically superior to species richness, but because they encode specific assumptions about niche breadth, dominance, redundancy, or response variation. The following sections evaluate whether those assumptions are supported when the outcome is forest productivity and carbon storage, and then examine how the apparent effects change under environmental stress and disturbance.

Functional Diversity and Ecosystem Productivity Dynamics

Effect of Species diversity on Functional Diversity

Two complementary mechanisms are widely used to explain positive biodiversity–productivity relationships are; niche complementarity and selection (or dominance) effects. Niche complementarity occurs when species differ in resource acquisition, phenology, rooting depth, crown architecture, or stress tolerance, enabling mixed communities to use resources more efficiently than functionally uniform stands. In contrast, selection effects arise when diverse communities are more likely to contain highly productive species that disproportionately contribute to stand-level biomass (Forrester & Bauhus, 2016; Mensah et al., 2016). Rather than operating independently, these mechanisms often act together. Dominant species may establish the baseline level of productivity, while functionally contrasting neighbours enhance resource-use efficiency and reduce competition.

Experimental studies demonstrate that functional and phylogenetic diversity contribute differently to the overall biodiversity effect. In paired biodiversity experiments, both forms of diversity were positively associated with complementarity but negatively associated with selection effects, indicating that greater differences among species reduce competitive inequality while strengthening niche partitioning (M. Huang et al., 2020). Tree height variation was particularly influential. Selection effects were strongest in communities dominated by species of similar height, whereas complementarity increased with greater phylogenetic divergence. These findings suggest that increasing functional diversity does not necessarily increase productivity directly; instead, it alters the relative contributions of complementarity and dominance to ecosystem functioning.

Species identity can also have a stronger influence on productivity than overall trait dissimilarity. In a controlled biodiversity experiment, biomass production was explained more effectively by the presence and average trait values of a key species than by functional dissimilarity alone. Moreover, the outcome depended on whether species mixtures reduced or intensified interspecific competition (Mahaut et al., 2020). Although this study was not conducted in a forest ecosystem, its underlying mechanism is highly relevant to forests, where long-lived dominant trees shape canopy structure, light availability, soil conditions, and regeneration over extended periods. These findings caution against interpreting positive biodiversity effects as evidence of complementarity without also considering species dominance and competitive interactions.

Evidence from forest ecosystems further shows that complementarity varies with environmental conditions and resource availability. Mixed-species stands often outperform monocultures when species interactions improve access to limiting resources or increase resource-use efficiency. However, the strength and direction of these effects depend on species composition, stand structure, and environmental conditions (Forrester & Bauhus, 2016). For example, in water-limited forests of Italy, both mass-ratio and complementarity effects contributed to annual stand growth, although complementarity played a greater role under Mediterranean than temperate climatic conditions (Lammerant et al., 2023). These findings indicate that functional diversity should be viewed as a potential mechanism for resource complementarity, with its ecological benefits largely determined by environmental context.

Effect of Forest Identity and Structure on Functional Diversity

Recent evidence consistently shows that functional diversity complements, rather than replaces, functional identity in explaining forest productivity and carbon storage. In a tropical cloud forest, functional diversity accounted for 53% of the variation in aboveground biomass, compared with 17% explained by species diversity and only 6% by phylogenetic diversity. Furthermore, the influence of soil phosphorus on biomass was mediated primarily through its effects on functional diversity (Cheng et al., 2020). Similarly, in moist Afromontane forests, species diversity enhanced aboveground carbon storage through both functional diversity and functional dominance, demonstrating the simultaneous operation of complementarity and selection effects (Jeldu et al., 2024). Earlier studies in African forests reported comparable patterns, showing that species diversity promoted carbon accumulation through interactions between functional diversity and dominant traits, with maximum tree height highlighting the importance of vertical canopy partitioning (Mensah et al., 2016).

Nevertheless, the importance of functional identity may exceed that of functional diversity in some ecosystems. In tropical semi-arid forests and savannas, aboveground carbon storage responded differently to dominant trait values and functional divergence, indicating that the characteristics of abundant species can determine biomass even where complementary functional strategies exist (Mensah et al., 2021). Likewise, in Himalayan temperate forests, dominant stem and leaf traits were the primary drivers of soil organic carbon, whereas functional divergence made only a modest additional contribution (Pandey et al., 2026). Together, these findings support a hierarchical interpretation in which dominant species largely determine the magnitude of ecosystem functions, while functional diversity enhances resource partitioning and improves the efficiency and stability of ecosystem processes.

Stand structural diversity provides an additional dimension that is not fully captured by taxonomic or functional diversity. In dryland agroforestry parklands, variation in tree size was the strongest predictor of aboveground carbon, exceeding the direct effects of both species richness and functional diversity. Community-weighted maximum tree height also contributed substantially to carbon storage (Noulèkoun et al., 2023). Structural diversity enhances canopy packing, vertical light interception, and the coexistence of multiple tree size classes. However, it may also reflect differences in stand age, disturbance history, or management practices rather than biodiversity alone. Consistent with this interpretation, studies from West African semi-arid ecosystems found that both species richness and variation in tree diameter and height were positively associated with biomass, particularly within shrub layers, demonstrating that taxonomic and structural diversity often reinforce one another (Dimobe et al., 2025).

The contribution of phylogenetic diversity further illustrates that measured functional traits do not always capture all ecologically relevant differences among species. Li et al. (2026) reported that phylogenetic diversity predicted aboveground biomass more effectively than functional diversity across multiple forest strata, contrasting with studies in which measured functional traits provided greater explanatory power (Cheng et al., 2020). One possible explanation is that phylogenetic relationships incorporate unmeasured traits and long-term evolutionary strategies that are not represented by the selected functional traits. Alternatively, the measured traits may not correspond to the primary ecological constraints operating within a particular forest. Rather than representing conflicting conclusions, these contrasting findings highlight the context dependence of biodiversity–ecosystem functioning relationships. The predictive value of functional or phylogenetic diversity depends on the traits measured, resource limitations, forest structure, and the evolutionary history of the species assemblage.

Belowground mediation and soil carbon

Aboveground diversity–productivity relationships are partly generated belowground. Tree richness and functional diversity altered soil fungal community composition, and fungal composition directly increased productivity and strengthened net diversity effects under contrasting water availability (Fahey et al., 2023). The mediation was associated more with community composition than with total fungal richness, indicating that interaction networks and functional guilds may matter more than simple microbial counts. This finding expands complementarity beyond direct plant–plant interactions by showing that diverse tree communities can reorganize mycorrhizal, pathogenic, and other fungal relationships that influence nutrient acquisition and plant performance.

Plant diversity also influences longer-term soil carbon and nitrogen dynamics. A global meta-analysis found that plant diversity generally increased soil carbon across ecosystems, although effect sizes depended on ecosystem and experimental context (Chen et al., 2020). Decadal evidence from forest diversity experiments subsequently showed that tree diversity increased soil carbon and nitrogen accrual, supporting the proposition that biodiversity effects can strengthen through time as litter inputs, root turnover, and microbial feedbacks accumulate (Chen et al., 2023). These temporal dynamics are important because short-term biomass responses may underestimate the contribution of diversity to ecosystem nutrient capital.

Yet soil carbon is not determined by functional diversity alone. In temperate deciduous forests, soil nutrients promoted soil organic carbon, whereas a small number of functionally acquisitive, large trees constrained storage, showing that dominant strategies and edaphic conditions can override a simple positive diversity effect (Larsary et al., 2023). In Himalayan forests, community-weighted stem and leaf traits were more influential than functional divergence for soil organic carbon, although diversity retained an additional role (Pandey et al., 2026). Together, these studies suggest that soil carbon emerges from the interaction of litter quality, root inputs, microbial processing, stand structure, and environmental constraints rather than from trait dispersion in isolation.

The productivity synthesis therefore yields a qualified agreement. Functional diversity frequently enhances biomass and carbon where trait differences expand resource use or mediate access to limiting nutrients, but its effects are inseparable from dominant trait values, tree size, vertical structure, phylogenetic history, and soil biota. The next analytical step is to ask when these mechanisms persist, weaken, or reverse under climatic stress, disturbance, and succession.

Environmental Drivers of Functional Diversity

Functional Diversity and Resource Dynamics

Water availability is one of the strongest modifiers of functional diversity–productivity relationships because it changes both community assembly and the value of particular traits. Along water-limitation gradients, forest communities shift toward more conservative strategies, and the relative contributions of mass-ratio and complementarity effects vary among traits and climatic regions (Lammerant et al., 2023). In Ghana, drought survival and species distributions were structured by plant traits along a rainfall gradient, demonstrating that environmental filtering selects combinations of hydraulic and resource-use strategies rather than random sets of species (Amissah et al., 2021). These shifts can alter productivity even when species richness changes little.

Long-term drought can also erode the functional differences on which complementarity depends. Across drier tropical forests, prolonged moisture deficits were associated with increasing functional, taxonomic, and phylogenetic homogeneity, indicating convergence toward a narrower set of drought-tolerant strategies (Aguirre-Gutiérrez et al., 2020). Such convergence may maintain short-term persistence but reduce response options under future climatic extremes. The result therefore connects community assembly to resilience: environmental filtering can favor locally successful traits while simultaneously shrinking the range of strategies available for coping with novel conditions.

Climatic sensitivity is also evident in productivity analyses. The relationship between woody-plant functional diversity and productivity varies with climate, implying that the same level of trait diversity can generate different outcomes under contrasting temperature and precipitation regimes (Yan et al., 2024). Global analyses of biodiversity and ecosystem stability similarly show that climate mediates the strength and direction of diversity effects rather than functioning as a background variable (García-Palacios et al., 2018). These findings argue against transferring coefficients from one region to another without testing whether the relevant limiting resource and disturbance regime are comparable.

Importantly, diversity does not always buffer an acute drought event. In a young tree diversity experiment exposed to the 2018 European drought, species richness did not reduce plot-level declines in remotely sensed vegetation condition, and highly diverse mixtures sometimes showed lower index values than less diverse stands (Hajek et al., 2024). This result may reflect antagonistic interactions, stand age, species composition, or the mismatch between species richness and hydraulic response diversity. It reinforces the need to measure the traits that control drought resistance and recovery rather than using richness as a proxy for insurance.

Responses of Functional Diversity to Land-Use Change and Disturbance

Land-cover change modifies functional diversity by replacing structurally complex forests with simplified regrowth or production systems. Field observations from Cambodian tropical landscapes showed that conversion among evergreen forest, regrowth forest, and cashew plantation altered stand structure, species diversity, leaf functional traits, and soil conditions together (Sovann et al., 2025). Because these changes are coupled, attributing ecosystem outcomes to one biodiversity dimension without considering land-use history can be misleading. Converted systems may retain some species or biomass while losing trait combinations, vertical complexity, and soil conditions needed for long-term function.

Peri-urban and culturally protected forests illustrate how land-use context affects both biodiversity and carbon. Peri-urban forests in Burkina Faso contained higher woody diversity, denser stands, and greater biomass than adjacent agroforestry systems, underscoring the value of remnant forests within rapidly changing urban landscapes (Balima et al., 2023). Sacred groves in southwestern Nigeria also retained substantial tree diversity and structural conservation value despite not being established through formal biodiversity policy (Onyekwelu et al., 2022). These cases show that conservation effectiveness depends not only on legal categories but also on land-use intensity, stand structure, and social institutions that maintain habitat continuity.

Biotic disturbances add further complexity because diversity can facilitate or inhibit invasion depending on host composition and propagule pressure. Across non-native forest insects and pathogens in the United States, host biomass and richness sometimes increased invasion, while the effects of non-host diversity varied in direction and magnitude among pests (Ward et al., 2022). This variability limits broad claims that diverse forests are automatically more invasion resistant. Functional interpretations should distinguish diversity that increases host availability from diversity that dilutes hosts, disrupts transmission, or supports natural enemies.

Landscape configuration also affects functional diversity independently of total habitat amount. A meta-analysis of tropical mammal communities found that composition and configuration metrics produced context-dependent effects on functional diversity, with habitat heterogeneity emerging as important in highly transformed hotspots (Chacón-Pacheco et al., 2026). Although this evidence concerns mammals, it is relevant to forest ecosystem functioning because seed dispersal, herbivory, and trophic interactions depend on the persistence of functionally distinct animal communities. Forest conservation should therefore connect plant trait diversity with the functional diversity of interacting taxa rather than treating vegetation as a closed system.

Functional Diversity in Succession, Restoration, and Ecosystem Recovery

Secondary succession provides a natural test of whether taxonomic, functional, and structural recovery proceed together. Across young secondary forests in Australia, Ghana, and Mexico, recovery was multidimensional and varied among landscapes and forest attributes, showing that one indicator cannot represent the full process of forest regeneration (Matsuo et al., 2026b). Species richness, functional composition, structural development, and biomass may recover at different rates because they are constrained by prior land use, climate, dispersal, and local species pools. Restoration assessments based only on canopy cover or tree number may therefore overestimate the return of ecological function.

Early secondary forests also reveal that environment and vegetation quantity can initially dominate biodiversity effects. In young Ghanaian forests, climatic wetness and soil conditions influenced more ecosystem functions than diversity or functional composition, while carbon, water, and nutrient cycles responded to different sets of drivers (Matsuo et al., 2026a). This does not imply that biodiversity is unimportant; rather, its influence may strengthen as stands develop and biotic interactions become more complex. The finding helps explain why strong biodiversity–function relationships in mature forests may not appear during the first years after abandonment.

Restoration after rubber clearance shows a related temporal pattern. Ecosystem functions and multifunctionality increased across a chronosequence, but structural modeling indicated that plant diversity and soil pH were more influential than soil microbial diversity in explaining recovery (Lu et al., 2025). The result highlights the importance of aboveground community reassembly while also showing that abiotic recovery can constrain biological effects. Restoration strategies should therefore combine diverse plant establishment with interventions that address degraded soils and unfavorable site conditions. The reassembly of ecological interactions is another emerging dimension of recovery. Seed-dispersal networks can influence which functional strategies reach and establish in regenerating forests, and their structure may change from highly connected early-successional networks toward more modular configurations later in succession (Lussier et al., 2025). This network perspective extends functional diversity beyond static trait inventories by emphasizing the processes that rebuild communities. It also identifies dispersers and plant species that connect successional stages as potential management priorities.

These dynamic studies provide a necessary transition from productivity to resilience. A forest can regain biomass rapidly through a few dominant pioneers while remaining functionally narrow, structurally simplified, or dependent on unstable interactions.

Evaluating whether recovery is durable therefore requires explicit measures of resistance, response diversity, redundancy, and the simultaneous maintenance of multiple ecosystem functions.

Functional Diversity, Stability, Resilience, and Multifunctionality

Functional Diversity, Stability, and Resilience: Evidence and Mechanisms

Functional diversity is often expected to stabilize ecosystem functioning through asynchronous responses among species. When species differ in sensitivity to drought, temperature, pests, or other disturbances, declines in one population may be compensated by stable or increasing performance in another. This insurance mechanism depends more directly on response diversity than on the total spread of effect traits (Correia et al., 2018; Gladstone-Gallagher et al., 2019). Functional redundancy can further protect a function when several species contribute similarly, but redundancy provides insurance only when those species do not share the same vulnerability. Empirical studies support parts of this framework while also revealing sign reversals. In post-logging forests, deciduous response diversity was positively associated with productivity, whereas coniferous response diversity showed a negative relationship over the observed recovery period, probably because species identity and regeneration speed altered the short-term outcome (Correia et al., 2018). In Sahelian savannas, functional redundancy and response diversity declined for most examined functional groups over several decades of drought and grazing, indicating erosion of the capacity to maintain primary production and biogeochemical functions (Dendoncker et al., 2023). These studies show that response diversity is process- and disturbance-specific rather than a universally positive community property.

Meta-analytic evidence provides the strongest caution against generalization. A synthesis of terrestrial plant communities found no overall positive relationship between functional diversity and resilience, and trait variety was negatively related to resilience in woody ecosystems, while the direction of effects varied with biome, disturbance, time scale, study design, and resilience metric (Lipoma et al., 2024). A broader review of functional redundancy similarly found that stability and resilience effects depended on how redundancy and ecosystem response were defined and measured (Biggs et al., 2020). The implication is not that functional diversity is irrelevant, but that resilience claims require an explicit disturbance, response variable, and temporal window. Remote sensing is increasingly capable of addressing these temporal and spatial limitations. Satellite-derived essential biodiversity variables can integrate vegetation structure, productivity, and disturbance response across tropical forests, providing repeated observations that field plots alone cannot deliver (Aguirre-Gutiérrez et al., 2026). Remote sensing has also been used to relate post-disturbance productivity to pre-disturbance response diversity and to detect early changes in drought condition (Correia et al., 2018; Hajek et al., 2024). However, spectral or structural indicators should be linked to field-measured traits and demographic processes so that observed change can be interpreted mechanistically.

At regional scale, diversity may influence the risk of large ecosystem transitions. Modelled tree diversity at multiple spatial scales was associated with lower likelihood of Amazon tipping behavior, indicating that local richness, spatial turnover, and regional diversity can contribute to forest stability in different ways (Van Passel et al., 2026). This emerging evidence aligns with the argument that resilience is generated across levels of

organization rather than within plots alone. It also suggests that conserving beta diversity and landscape heterogeneity may be as important as maximizing local functional richness.

Ecosystem multifunctionality

Multifunctionality changes the biodiversity question from whether diversity increases one process to whether it maintains several functions at the same time. This distinction matters because a community that maximizes wood production may not maximize soil carbon, decomposition, water regulation, nutrient retention, or habitat quality. In Ghanaian natural, restored, and agroforestry systems, functional richness and site conditions were positively related to ecosystem-service proxies and multifunctionality, showing that functional structure and environmental quality jointly shaped performance (Damptey et al., 2022). The result supports functional diversity but also warns that differences among ecosystem types were strongly influenced by soil properties.

Secondary forest evidence reinforces this interaction. In Pinus yunnanensis secondary forests, tree-species diversity and stand attributes affected individual functions and multifunctionality differently, and climate altered these relationships (Wang et al., 2022). In forests of the eastern Tibetan Plateau, soil moisture, functional diversity, and diameter variation positively influenced multifunctionality, whereas species richness was not a significant predictor (Ni et al., 2026). These studies agree that functional and structural dimensions can be more informative than richness, but they also show that abiotic controls remain central.

Mixed-species management does not guarantee higher multifunctionality. Comparisons among monoculture, mixed, and secondary plantation types found that mixed plantations did not necessarily provide greater multifunctionality because the response depended on stand transformation pathway and the functions included in the index (Li et al., 2024). This contradiction is important for restoration policy: increasing species number without selecting complementary traits, appropriate stand structure, and site-compatible species may produce limited gains. Multifunctionality should be treated as a design problem involving trade-offs among functions rather than as an automatic consequence of mixture.

Riparian forests provide a clear example of multiple biodiversity dimensions contributing to different functions. Species richness was positively related to habitat quality and aboveground carbon across environmental gradients, maximum tree height was a strong predictor, and phylogenetic diversity mediated some richness effects depending on proximity to streams and conservation status (Kinnoumè et al., 2024). The findings show that taxonomic, functional, structural, and phylogenetic dimensions can be complementary rather than redundant. They also demonstrate that conservation status and local environmental position can change the pathway through which diversity affects function. Across the reviewed evidence, multifunctionality is therefore more consistently associated with combinations of functional diversity, dominant traits, structural variation, and favorable soil or moisture conditions than with species richness alone. Yet the particular combination differs among carbon, nutrient, water, and production functions. Reviews should avoid collapsing these functions into one index without reporting which components drive the result, because an unchanged multifunctionality score can conceal gains in one function and losses in another. Management decisions require both the aggregate pattern and the function-specific trade-offs.

Conservation and Management Implications

The principal management implication is that conserving species number is necessary but insufficient. Forest plans should protect the breadth of functional strategies, the dominant species that sustain major stocks or fluxes, and the response diversity that allows functions to persist under disturbance. This requires identifying which traits support local objectives such as carbon storage, drought tolerance, water regulation, habitat quality, or post-disturbance recovery rather than applying a universal trait list (Mori et al., 2017; Bonilla-Valencia et al., 2022). Because different functions respond to different traits, multifunctional management should retain complementary combinations instead of optimizing a single high-performing strategy. For carbon-focused management, the evidence supports maintaining large trees, vertical and diameter variation, and functionally complementary species, while recognizing that dominant identities may account for much of the immediate stock. Functional richness and dispersion were associated with greater aboveground carbon in several forests, but structural diversity and height-related identity were sometimes stronger predictors (Jeldu et al., 2024; Noulèkoun et al., 2023; Majeed et al., 2026). Protecting only a few high-carbon species may maximize short-term storage yet reduce response diversity and future adaptive capacity. A balanced strategy should conserve high-performing dominant species together with less abundant species that occupy distinct trait space or provide insurance under changing conditions.

Drought adaptation requires particular attention to hydraulic and conservative strategies. Long-term drying can homogenize tropical forest communities, while water limitation changes the balance between complementarity and dominance (Aguirre-Gutiérrez et al., 2020; Lammerant et al., 2023). Assisted regeneration or enrichment planting should therefore avoid assembling species that are taxonomically diverse but hydraulically similar. Trait-based planning should combine drought-resistant strategies with sufficient response variation to prevent one climatic threshold from affecting all species simultaneously. Restoration should be evaluated as multidimensional recovery. Young secondary forests may regain vegetation quantity before they recover functional composition, structure, interaction networks, and multiple ecosystem processes (Matsuo et al., 2026a, 2026b). Chronosequence evidence after rubber clearance indicates that plant diversity and soil recovery jointly shape multifunctionality, while seed-dispersal networks influence the reassembly of future plant communities (Lu et al., 2025; Lussier et al., 2025). Practical monitoring should therefore combine survival and canopy measures with trait composition, structural complexity, soil condition, dispersal processes, and function-specific indicators.

Conservation also needs a landscape perspective. Local plot diversity cannot compensate fully for the loss of beta diversity, structural heterogeneity, riparian connectivity, and habitat configuration across landscapes (Suárez-Castro et al., 2022; Müller et al., 2023). Riparian buffers, sacred groves, peri-urban remnants, regrowth forests, and production stands can contribute different functional strategies and services when managed as connected components rather than isolated patches (Kinnoumè et al., 2024; Onyekwelu et al., 2022; Balima et al., 2023). Maintaining heterogeneity across patches may enhance regional response diversity and provide sources for recolonization after disturbance. Monitoring systems should integrate field inventories with remote sensing. Field data remain essential for measuring traits, species identity, regeneration, and belowground

interactions, whereas repeated satellite observations can detect changes in productivity, canopy structure, disturbance, and recovery across large and inaccessible tropical regions (Aguirre-Gutiérrez et al., 2026). Linking these data within essential biodiversity variable frameworks can make functional change more visible to conservation reporting and management. The critical requirement is calibration: remotely sensed indicators should be connected to ecological traits and processes rather than used as unexamined proxies.

Research Gaps and Emerging Directions

The first major gap is inconsistency in trait selection and metric interpretation. Studies frequently use the same label, functional diversity, for different combinations of traits and indices, making apparent contradictions difficult to evaluate. A study emphasizing leaf economics may conclude that functional diversity is weak, while another including height, wood density, roots, and hydraulic traits may detect a strong effect because it measures different ecological axes (Mammola et al., 2021; Magneville et al., 2022). Future research should justify traits through explicit process hypotheses, report single-trait and multitrait results, and conduct sensitivity analyses showing how conclusions change with trait sets and distance metrics.

Second, intraspecific trait variation remains underrepresented. Tropical tree traits vary with ontogeny, canopy position, soil fertility, water availability, and local adaptation, yet many analyses assign one species-level mean to every individual. This practice can underestimate niche overlap or create false differences among communities. Layer-specific findings and climate-sensitive relationships indicate that trait expression is context dependent, and future work should incorporate within-species variation where it is likely to influence competition or stress response (Li et al., 2026; Yan et al., 2024).

Third, the literature needs more causal and long-term evidence. Many tropical studies are cross-sectional forest inventories that identify associations but cannot determine whether diversity increases function, productive sites support more diversity, or both respond to a third environmental driver. Long-term experiments and repeated measurements show that biodiversity effects can accumulate through soil feedbacks and change over succession, while short observation windows can reverse the apparent effect of response diversity (Chen et al., 2023; Correia et al., 2018). Permanent plots, restoration experiments, and disturbance-based before–after designs are therefore essential.

Fourth, belowground and multitrophic mechanisms are insufficiently integrated. Soil fungi can mediate diversity–productivity relationships, and animal functional diversity influences seed dispersal, herbivory, and regeneration, but most forest studies analyze tree traits without measuring these interaction pathways (Fahey et al., 2023; Lussier et al., 2025; Chacón-Pacheco et al., 2026). Future research should connect plant effect traits with microbial guilds, disperser networks, herbivores, and pathogens. Such integration is likely to explain why similar tree diversity produces different outcomes across sites.

Fifth, resilience research requires standardized definitions without erasing ecological specificity. Resistance, recovery rate, temporal invariability, persistence, and adaptation are distinct properties, and functional redundancy is not equivalent to response diversity. The absence of a general positive functional diversity–resilience relationship may reflect true ecological context dependence as well as inconsistent response variables and observation periods (Biggs et al., 2020; Lipoma et al., 2024). Studies should define the

disturbance, reference state, temporal window, function, and organizational scale before testing resilience hypotheses.

Sixth, the relationship between local diversity and landscape resilience remains poorly resolved. Multiscale frameworks predict that beta diversity and structural heterogeneity can stabilize regional functioning, but empirical forest evidence is still limited relative to plot-scale research (Gonzalez et al., 2020; Müller et al., 2023). Emerging satellite time series and spatial biodiversity models create opportunities to test whether functionally heterogeneous landscapes recover faster or avoid tipping behavior (Aguirre-Gutiérrez et al., 2026; Van Passel et al., 2026). These analyses should be paired with field validation to distinguish compositional change from spectral or structural change.

Finally, research must engage more directly with management trade-offs. A stand designed for rapid timber production, maximum carbon stock, drought resilience, biodiversity conservation, or multifunctionality may require different species and trait combinations. Mixed plantations do not automatically improve all functions, and early secondary forests may remain controlled by site conditions even when diversity increases (Li et al., 2024; Matsuo et al., 2026a). Management experiments should therefore compare alternative trait-based assemblages across several functions, disturbances, and time horizons rather than evaluating success through one endpoint.

CONCLUSION

Recent evidence confirms that functional diversity is a powerful but conditional framework for understanding tropical forest ecosystem dynamics and productivity. Trait differences can enhance biomass production, carbon storage, nutrient acquisition, and multifunctionality through complementarity, while dominant trait values, key species, tree-size variation, and phylogenetic history often determine the magnitude and direction of these effects. Soil fertility, fungal communities, water limitation, land-use history, succession, and spatial configuration further modify the relationship, explaining why results differ among forests. The strongest agreement is that species richness alone rarely captures the full ecological basis of forest functioning. The strongest contradiction is that functional diversity does not consistently outperform other biodiversity dimensions and does not universally increase resilience or multifunctionality. These conclusions are compatible rather than opposing: functional diversity is most useful when the selected traits correspond to the limiting process, the metric represents a clear mechanism, and environmental and structural context are modeled explicitly. A high-quality global review must therefore move beyond the linear proposition that more functional diversity always produces more function. The more defensible proposition is that forests maintain functions when they contain appropriate combinations of effect traits, complementary resource-use strategies, high-performing identities, structural heterogeneity, and response diversity distributed across layers and landscapes. Conservation and restoration strategies based on this multidimensional view are better positioned to maintain tropical forest productivity and ecosystem services under accelerating environmental change.

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Research Paper **Open Access**

Evaluation of anti-oxidant, anti-microbial and cytotoxic activities of selected medicinal plants from arid region of Pakistan

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SUMMARY

In current study methanolic extract of aerial parts of Chrozophora tinctoria, Euphorbia milii, Euphorbia royleana, Heliotropium calcareum, Tamarix dioica and Verbena tenuisecta was tested for antioxidant, antimicrobial and cytotoxic potential. Folin-Ciocalteu and aluminium chloride colorimetric assays, DPPH, phosphomolybdate and reducing power assays were used to estimate total phenolic, flavonoid content and antioxidant potential respectively. Antimicrobial assay was performed by disc diffusion method and cytotoxic potential was evaluated by brine shrimp lethality (BSL) assay. The results revealed that all the selected extracts were found with significant amount of total phenolic and flavonoid contents and have good antioxidant potential at maximum applied dose (100µg/mL). All the extracts were found active against Bacillus subtilis strain while, no extract was active against tested fungal strains. In BSL assay, E. milii, H. calcareum and V. tenuisecta exhibited significant cytotoxicity with % lethality value ranges from 25±0.80-91±0.76 at 200 µg/mL concentration. On the basis of these results, we can conclude that these plants may have potential for antioxidant, anti-microbial and antitumor lead compounds. Therefore, it is suggested to explore these plants for in vivo and in vitro discovery of new drugs.

Keywords: Brine shrimp lethality assay; *Chrozophora tinctoria*; DPPH assay; *Euphorbia milii*; *Heliotropium calcareum*; *Tamarix dioica*

INTRODUCTION

Medicinal herbs have been used in different healthcare systems for the treatment of human diseases and these days, plants are still play an important role for the identification of new and novel drugs (Alarcon *et al*., 2015). Recently, it has been reported that in developing countries, about 80% of the population rely on medicinal plants derived products (Ahmad *et al*., 2025). Medicinal plants contain biologically active molecules that can be used for therapeutic purposes and large number of plants has been used in traditional system for many years (Ahmad *et al*., 2012a). For the investigation of these biologically active constituents, it is important to have necessary tools like chemical

screening methods and suitable biological assays.

The search for new anti-microbial constituents is necessary due to microbial resistance to available drugs and the appearance of fetal opportunistic infections (Fatima *et al*., 2009). Almost all the available anti-fungal and anti-bacterial agents are expensive and have serious side effects (Morens *et al*., 2004). In recent decades, natural anti-oxidants and colorants present in foods have attracted interest because of their safety and potential nutritional and therapeutic effect (Espin *et al*., 2000). Several synthetic dyes have been banned due to allergic symptoms and carcinogenesis. Furthermore, colorants derived from natural products are also used in cosmetic industry because of lesser side effects, UV protection and anti-aging effects (Chengaiah *et al*., 2010). Different bioassay used for assessing the anti-tumor activity of plant extracts have varied over years. These assays have produced important anti-tumor drugs such as vinca alkaloids (vincristine and vinblastine), podophyllotoxin derivatives, Taxol and 10-hydroxy-campothecin. Initial screening for anti-tumor potential using *Artemia salina* (brine shrimps) can be used as fairly inexpensive, rapid and reliable pre-screening tool for anti-tumor agents because it presents good correlation between brine shrimp lethality and human carcinoma (Yee *et al*., 2017).

In Pakistan, about 5,700-6,000 species of plants are present (Ahmed and Murtaza, 2014) and of these, 400-600 are used for the medicinal purposes (Malik et al., 2005). *Chrozophora tinctoria* is an annual plant of the family Euphorbiaceae that exists in Africa, Europe and tropical parts of Asia including Pakistan and India. It is used as a coloring agent and gives luminescence to dyes and pigments. *Chrozophora tinctoria* is used as an anti-microbial, as an anti-nociceptive, and for wound healing in diabetics (Sher et al., 2018; Iqbal et al., 2022). Plants of *Chrozophora* are used traditionally as laxative, cathartics and emetics, for mouth ulcers, warts, skin burns, menstrual problems, GIT complains, joint pain, jaundice and migraine. The plant is rich in alkaloids, flavonoids, phenylpropanoid glycosides, and coumarins (Iqbal et al., 2022).

E. milii is generally known as Christ of Thorns and it has green leaves and flowers ranging from colors such as red, white, pink, and yellow. The plant is also found in other countries, like Brazil and Pakistan. *E. milii* is used to treat warts, hay fever, digestive problems, and breathing complaints like asthma and bronchitis, and cancer. The bulbs of *E. milii* are widely used to treat indigestion, constipation, stomach aches, diarrhea, and nausea. Phytochemical studies of *E. milii* showed that flavonoids, triterpenes, saponins, phenols, and tannins can be found in various parts of the plant. *E. royleana* Boiss. belongs to family Euphorbiaceae that is originated from China, Pakistan and India (Şafak Odabaşı, 2024). *E. royleana* is used to treat ear pain, diarrhea, paralysis, skin disorders and body aches, asthma, constipation, jaundice, anemia and cough (Adil et al., 2024; Al-Ansi et al., 2024; Akram et al., 2020). Alkaloid, flavonoids, saponins, tannins, steroids, phyto-sterols and phenols are present in crude extract (Naheed et al., 2024).

Heliotropium calcareum belongs to family Boraginaceae known as khashafa, and mamani in Pashto and Indian heliotrope in English. Members of the genus *Heliotropium* have perceptible pharmacological significance and have been used medicinally for various diseases such as GIT complaints, snakebite and scorpion sting, fever and cough, rheumatoid arthritis, jaundice, wound healing and blood purification (Khurm et al., 2016; Saeed et al., 2017).

Tamarix dioica. Roxb. ex Roth (family Tamaricaceae) commonly known as Lai that grows as small tree or shrub. It is found abundantly in Pakistan, Nepal, India (Bharat), Afghanistan, Bhutan, Iran, Kashmir, Bangladesh and Myanmar. The leaves of *T. dioica* are applicable for enlarged spleen, liver disease, diuretic, astringent and carminative (Samejo et al., 2023). *Verbena tenuisecta* belongs to Verbenaceae and it has traditionally been used in treatment of inflammation, diarrhea, fever, gastrointestinal disorders, and certain sexually transmitted diseases. Phytochemical investigations of *Verbena* species have verified many bioactive constituents, including sterols, volatile oils, triterpenoids, iridoids, phenolic compounds, flavonoids, dihydrochalcones, anthocyanidins, and phenylethanoids (Azeem et al., 2025).

In the current study, plants from different genera (*Chrozophora*, *Euphorbia*, *Heliotropium*, *Verbena* and *Tamarix*) were selected for initial screening.

MATERIALS AND METHODS

Collection and identification of plants

The selected plants were collected (aerial parts) from different areas of Khyber Pakhtunkhwa and Punjab. The aerial parts were selected because they are traditionally used in folk medicine and are reported to contain high concentrations of biologically active secondary metabolites, including phenolics, flavonoids, alkaloids, terpenoids, and glycosides. These metabolites play important roles in exhibiting diverse pharmacological activities in humans (Isah, 2019). Furthermore, harvesting aerial parts is more sustainable than uprooting the entire plant, thereby supporting conservation of natural populations. The collected plants were identified by comparing with available literature and were authenticated by Dr. Muhammad Abid, Lecturer, Institute of Forest Sciences, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur. Voucher specimen of each plant was deposited to the said institute. The list of the selected plants is shown in Table 1.

Table 1: The list of selected plants used in the present study.

No	Plant	Voucher number
1.	*Chrozophora tinctoria*	ISF-Her-102
2.	*Euphorbia milii*	ISF-Her-103
3.	*Euphorbia royleana*	ISF-Her-104
4.	*Heliotropium calcareum*	ISF-Her-105
5.	*Tamarix dioica*	ISF-Her-106
6.	*Verbena tenuisecta*	ISF-Her-107

Crude extract preparation

Each specimen was washed with distilled water and dried in shade and ground to make powder. Each plant powder weighing 100 g was soaked in 70% methanol (400 mL) and was kept at room temperature for 7 days with occasional shaking. The plant extracts were passed through muslin cloth, followed by filtration through Whatman (Grade 1) filter paper. This process was repeated three times for each plant and filtrates of each plant were pooled together and concentrated by rotary evaporation. The extracts were stored in closed vials and kept in a cool and dry place for further analysis (Saeed *et al*., 2017). Percent extract recovery was calculated by using following formula.

$$\% \text{ Extract recovery} = \frac{a}{b} * 100$$

Where;

a= weight of dried crude extract

b= weight of powdered material of each plant

Total phenolic and flavonoid contents

Folin-Ciocalteu assay with a slight modification was used (Marinova *et al*., 2005). Sample extract or standard solution (1 mL) was added to deionized water (1.5 mL) follwed by Folin-Ciocalteu's reagent. Solution was shaken and incubated for 5 min. The Na_2CO_3 solution (6%) in was added (4 mL) to the mixture to makeup the total volume of 25 mL. The mixture was kept at room temperature for 90 min and reading was taken at 630 nm using microplate reader (T80+ PG Instruments, UK). The result were expressed as µg gallic acid equivalent per g dry weight (µg GAE/g DW) (Marinova *et al*., 2005).

Aluminium chloride calorimetric method described by Bag *et al*. (2015) with little modifications was used for the estimation of total flavonoid. Distilled water was used to prepare aluminum chloride (10%) and 1 M potassium acetate solutions while sample and standard solutions were prepared in DMSO. Briefly, 20 µL of each plant extract was added to 10 µL of both aluminum chloride and potassium acetate followed by 160 µL of distilled water. The mixture was incubated at room temperature for 30 min, reading was taken at 415 nm wavelength using microplate reader (T80+ PG Instruments, UK). Results were measured in µg equivalents of quercetin per gram of plant dry weight (µg QE/g DW) (Bag *et al*., 2015).

Determination of antioxidant activity

DPPH assay

About 10 µL from each sample prepared in DMSO were mixed with 190 µL of DPPH solution prepared in methanol. Incubation of the reaction mixture was done for 1 h at 37°C in dark. By using a microplate reader (T80+ PG Instruments, UK), optical density was measured at 517 nm. By using the following formula, percent scavenging activity was calculated (Haq *et al*., 2011).

$$\% \text{ Scavenging activity} = \frac{1-Abs}{Abc} \times 100$$

Where; Abs = Absorbance of DPPH solution with sample

Abc = Absorbance of negative control (containing the reagent except the sample)

Total antioxidant capacity

Phosphomolybdate assay was used to measure total antioxidant capacity of extract as described by Fatima *et al*. (2015). Sample solution (0.1 mL) of each extract was mixed with 1 mL of phosphomolybdate reagent solution in eppendorf tube. These encapped tubes were incubated for 90 min at 95°C in water bath. The absorbance was taken at 695 nm after cooling samples at room temperature. Blank was prepared by taking 1 mL of reagent solution and appropriate amount of solvent. The blank was incubated under similar conditions. Ascorbic acid was used as standard (positive control) (Fatima *et al*., 2015). Total antioxidant capacity was estimated by formula;

$$\text{Antioxidant effect (\%)} = [\frac{Abc - Abs}{Abc}] \times 100$$

Where; Abs= absorbance of sample,

Abc= absorbance of control

Potassium ferricyanide colorimetric assay

Reducing power of samples was determined by using Potassium ferricyanide colorimetric assay described by Fatima *et al*. (2015). The mixture containing 2 mL plant extract, 2 mL of 0.2 M phosphate buffer and 2 mL potassium ferricyanide (10 mg/mL) was incubated at 50°C for 20 min. To the above mixture, 2 mL of 10% trichloroacetic acid was added and mixture was centrifuged at 3000 rpm for 10 min, upper layer of solution was decanted. About 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride was added to (2 mL) supernatant and incubated for 10 min. The absorbance was measured at 700 nm. An increase in absorbance indicates the reducing power of reaction mixture which was expressed as µg ascorbic acid equivalent per gram sample dry weight (µg AAE/g DW). Ascorbic acid was used as positive control (Fatima *et al*., 2015).

Anti-microbial assay

Anti-bacterial activity

The anti-bacterial activity of selected extracts was investigated using 96 well microplate reader (T80+ PG Instruments, UK) with some modifications in the reported protocol (Razmavar *et al*., 2014). Briefly, the stock solution of each extract was separately prepared by dissolving 0.1 g of extract with 100 mL of solvent (methanol) to produce a final concentration of 100 mg/mL. Then, 20 µL of each extract solution was individually impregnated into sterile, blank discs on both sides. Methanol-loaded discs were used as negative control. The anti-bacterial effect was evaluated by measuring the diameter of inhibition zone around each disc. The experiment was carried out thrice. Five bacterial strains including *B. subtilis, P. aeruginosa, S. aureus, E. coli* and *K. pneumonia* were used in the current study.

Anti-fungal activity

The anti-fungal activity of selected extracts was determined by the disc diffusion assay (Razmavar *et al*., 2014; Fatima *et al*., 2015). Five fungal strains (*A. niger, A. fumigatus, Mucor, F. Solani* and *A. flavus*) were used in this study. An aliquot (100 µL) of each fungal strain was mopped on sterile Sabouraud Dextrose agar containing plate. The sterile filter paper disc wetted with each extract (5 µL) was placed on plate. The diameter of zone of

growth inhibition (mm) around each sample and control treated disc was measured after 24-48 h incubation at 28°C.

Brine shrimp lethality assay for cytotoxic activity

In this assay, stock solution of each extract was prepared by dissolving 60 mg of each sample in 6 mL of methanol. *Artemia salina* (brine shrimp) eggs were hatched in a bi-partitioned tank filled with artificial sea water (3.8% sea salt solution supplemented with 6 mg dried yeast, pH 7). After 24-48 h of incubation, phototropic nauplii were harvested by using micropipette and transferred (10 nauplii) to vials against 200, 100, 50, 25 and 12.5 ppm concentrations of each sample. Each dilution was added in triplicate and solvent was allowed to evaporate. Vials were kept for 3 h in a high vacuum to make sure that solvent was completely evaporated. Sufficient volume of sea water was added to make volume 5 mL in each vial. The vials were kept under illumination at 25-28°C. After 24 h, living nauplii were counted with the help of magnifying glass. For negative control, respective solvent was taken in vial and evaporated, following addition of 10 nauplii and a volume of artificial sea water to make up 5 mL. The % death was calculated by Abbott's formula;

% Death= [(Test-Control)/Survivors of control] x 100

LD_{50} was determined accordingly by using Probit analysis ("FIN" program) (Haq *et al*., 2012).

RESULTS AND DISCUSSION

Extract yield

Highest yield (%) of extracts was calculated for *C. tinctoria* (18), followed by *V. tenuisecta* (16.7), *H. calcareum* (13), *T. dioica* (11.3), *E. milii* (11) and *E. royleana* (7.5). Metabolite diversity and extraction yield depends on the habitat, edaphic factors and climatic conditions (Rasheed *et al*., 2016). Besides, nature of extraction solvent can change the extraction efficiency and biological activities. The maceration along with the combination of different solvents can be a better option for the extraction of secondary metabolites from medicinal plants (Kanwal *et al*., 2016).

Total phenolic and flavonoid contents

The phenolic and flavonoids are the known anti-oxidant present in plants and have considerable effects on human health (Kanwal *et al*., 2016). Selected plants *C. tinctoria*, *E. milii*, *E. royleana*, *H. calcareum*, *T. dioica* and *V. tenuisecta* showed remarkable extent of phenolics and flavonoids as shown in Figure 1.

The highest concentration of total phenolic was found in *E. milii (45.26*±0.27 µg GAE/mg) followed by *T. dioica (42.96*±0.4 GAE/mg) while the least concentration was detected in *H. calcareum (5.51*±0.31 GAE/mg). The flavonoid concentration was found highest in *H. calcareum* crude extract (43.41±0.31 µg QE/mg) and lowest in (8.55±0.34 µg QE/mg) C. *tinctoria.* The relatively higher flavonoid content may be attributed to the predominance of flavonoid compounds within *Heliotropium* species (Fayed et al., 2021). Moreover, total phenolic and total flavonoid contents were quantified using different colorimetric assays and calibration standards (gallic acid and quercetin, respectively), which may produce different numerical values because of differences in molar absorptivity and assay sensitivity. The phenolic and polyphenolic constituents like flavonoids and tannins play a major role in pharmacological effects against human diseases including cancer,

inflammation, skin, neurodegenerative and cardiovascular disorders due to their hydrogen donating ability (Iqbal *et al*., 2017). Phenolics and flavonoids are secondary metabolites produced in various parts of plants in response to environmental conditions and protect plants from harmful effects of environment (Valentine et al., 2003). Pennycooke et al. (2005) reported that chilling stress is led to elevated total phenolic content and antioxidant capacity in petunia. Antioxidants are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals (reactive oxygen species, ROS). An imbalance between antioxidants and free radicals results in oxidative stress, will/may lead to cellular damage (Kukic et al., 2006).

Oxidative stress is considered to be substantial, if not crucial, in the initiation and development of many current conditions and diseases, including: inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, arteriosclerosis and aging (Lukyanova et al., 2007). Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process (Zima et al., 2001; Astley, 2003). This theory is supported by increasing evidence suggesting/indicating that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower the risk of disease (Atoui et al., 2005).

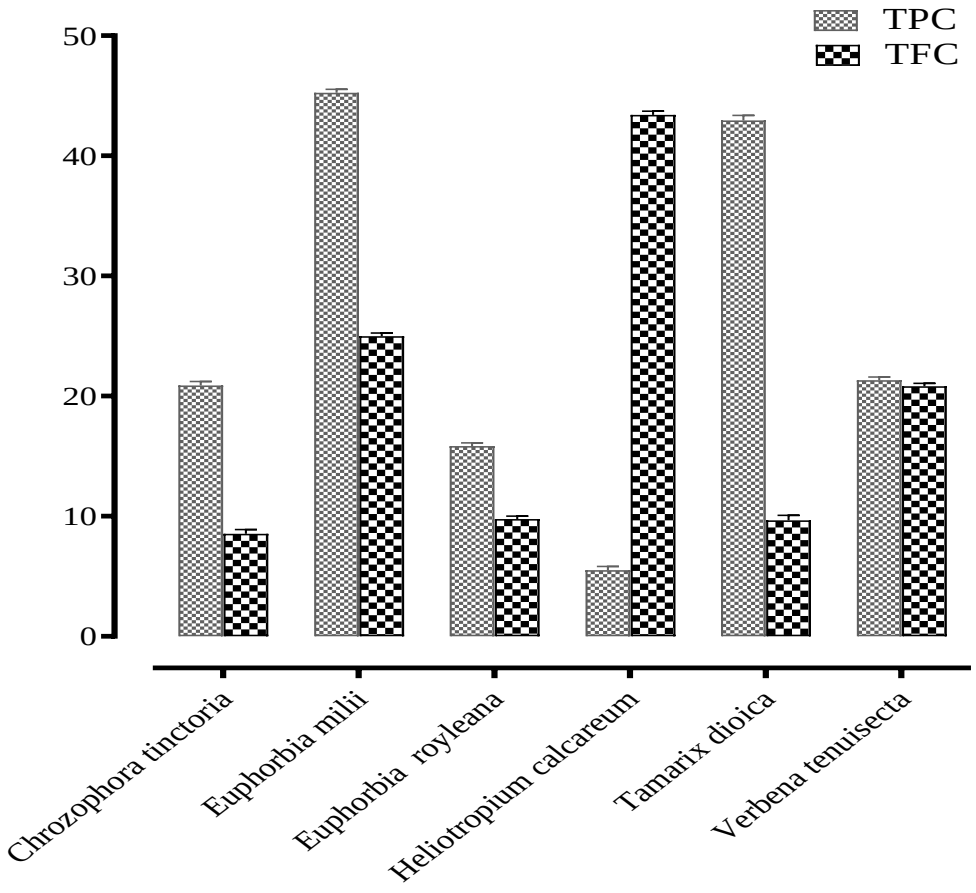

Fig. 1. Total phenolic and flavonoid contents of selected crude extracts. Y-axis represents µg GAE/mg of extract and µg QE/mg of extract for total phenolic and flavonoid contents, respectively.

Anti-oxidant activity

Free radical scavenging (DPPH % scavenging) effect of the extracts was determined by taking vitamin C as standard. The total of 6 samples, C. tinctoria, E. milii, E. royleana, H. calcareum, T. dioica and V. tenuisecta presented significant anti-oxidant potential at 100 µg/mL with % scavenging values of 70.00±0.34, 91.72±0.27, 57.23±0.25, 38.72±0.34, 92.19±0.41 and 91.66±0.25, respectively (Table 2).

The phosphomolybdate assay was used to determine the antioxidant capacity of the extracts. The tested samples (C. tinctoria, E. milii, E. royleana, H. calcareum, T. dioica and V. tenuisecta) showed % antioxidant effect with the values of 27.42±0.34, 32.67±0.27, 26.53±0.25, 10.00±0.31, 42.27±0.41 and 78.66±0.25 µg AAE/mg, respectively (Table 2). Ascorbic acid (vitamin C) was used as standard.

In reducing power assay, reducing power of the extracts (C. tinctoria, E. milii, E. royleana, H. calcareum, T. dioica and V. tenuisecta) was measured by using vitamin C as a standard. All the extracts exhibited remarkable reducing power with the values of 78.06±0.34, 175.87±0.27, 52.16±0.25, 23.90±0.31, 177.38±0.41 and 134.64±0.25 µg AAE/mg, respectively (Table 2). It has been reported in the literature that the phenolic and flavonoid constituents are the major contributor to the anti-oxidant activities of the plants (Huang et al., 2010). On the basis of quantitative results, it may be suggested that the DPPH scavenging effect, total anti-oxidant capacity and reducing power of the selected extracts might be due to the presence of considerable amount of total phenolic and flavonoid content (Ataie et al., 2016). It is also reported that the interplay between free radicals and anti-oxidants is important in maintaining health and preventing the onset and progression of several pathologies like cardiovascular diseases and cancer (Huang et al., 2010). Hence, the consumption of these selected plants (as food) may be valuable in the management of oxidative stress produced in degenerative disorders including cancer.

Table 2: Anti-oxidant activities of selected plant extracts.

Samples	**DPPH Assay** (% Scavenging)	**Total Antioxidant Capacity** (µg AAE/mg)	**Reducing Power Assay** (µg AAE/mg)
Chrozophora tinctoria	70.00 ±0.34	27.42±0.34	78.06±0.34
Euphorbia milii	91.72 ±0.27	32.67±0.27	175.87±0.27
Euphorbia royleana	57.23 ±0.25	26.53±0.25	52.16±0.25
Heliotropium calcareum	38.72 ±0.34	10.00±0.31	23.90±0.31
Tamarix dioica	92.19 ±0.41	42.27±0.41	177.38±0.41
Verbena tenuisecta	91.66 ±0.25	78.66±0.25	134.64±0.25

Anti-microbial assay

In an anti-bacterial assay, results were expressed in % inhibition of each bacterial strain. Results showed that total of six extracts (*C. tinctoria*, *E. milii*, *E. royleana*, *H. calcareum*, *T. dioica* and *V. tenuisecta*), all the extracts were not significantly active against *B. subtilis* specie with the zone inhibition of 6±0.35, 6±0.32, 6±0.35, 7±0.34, 6±0.41 and 6±0.41, respectively (Table 3). *C. tinctoria* was active against *P. aeruginosa* and *K. pneumoniae*

species with respective zone inhibition of 10±0.41 and 6±0.32. *V. tenuisecta* was active (7±0.36) against *P. aeruginosa* strain. No extract was active against *S. aureus* and *E. coli* strains of bacteria. However, none of the extract was found active against any of the tested fungal strain. The emergence of multidrug resistant to various microbes is posing the extreme threat to manhood (Marshall and Levy, 2011). The remarkable activity shown by *C. tinctoria* extract against *B. subtilis*, *P. aeruginosa* and *K. pneumoniae* makes us infer that it could be an important alternative to fight this nightmare. Though all the extracts exhibited considerable anti-bacterial activity against *B. subtilis* but not against all tested organisms in contrast to expectation. A limited anti-bacterial effects of all plant extracts suggest that there is no complete promise between the folkloric uses of medicinal plants in the crude form for the remedy of infectious ailments (Manandhar *et al*., 2019). However, further study is still needed to explore the effectiveness of these plants in inhibiting the growth of microorganisms. Moreover, no anti-fungal activity was exhibited by all selected crude extracts, which may be due to the extraction (cold maceration) method and the use of crude extracts. Therefore, is suggested to isolate the bioactive compounds from different fractions of selected crude extracts.

Table 3: Anti-bacterial activity of selected plant extracts.

Samples	Antibacterial Activity (Zone of inhibition 50 µg/disc (mm))				
	Bacillus subtilis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*E. coli*	*Klebsiella pneumoniae*
Chrozophora tinctoria	6± 0.35	10± 0.41	0	0	6± 0.32
Euphorbia milii	6± 0.32	0.0	0	0	0
Euphorbia royleana	6± 0.35	0	0	0	0
Heliotropium calcareum	7± 0.34	0	0	0	0
Tamarix dioica	6± 0.41	0	0	0	0
Verbena tenuisecta	6± 0.41	7± 0.36	0	0	0

Brine shrimp lethality assay

Brine shrimp assay is a quick and comprehensive cytotoxic assay for bioactive compounds of natural and synthetic origin. Cytotoxic potential of all the tested extracts (*C. tinctoria*, *E. milii*, *E. royleana*, *H. calcareum*, *T. dioica* and *V. tenuisecta*) was assessed against brine shrimps (*Artemia salina*) larvae. It was observed that only *E. milii*, *H. calcareum* and *V. tenuisecta* presented significant effect at 200 µg/mL with respective

lethality potential (%) of 91±0.76, 80±0.55 and 25±0.80 (Figure 2). The correlation between brine shrimp lethality assay and growth inhibition of human tumor cells (*in-vitro*) has been established as a significant preliminary tool for anti-cancer research (Yee *et al*., 2017). Keeping in view the above results, our study demonstrated that *E. milii, H. calcareum* and *V. tenuisecta* extracts have potential for secondary metabolites with anti-cancer effects.

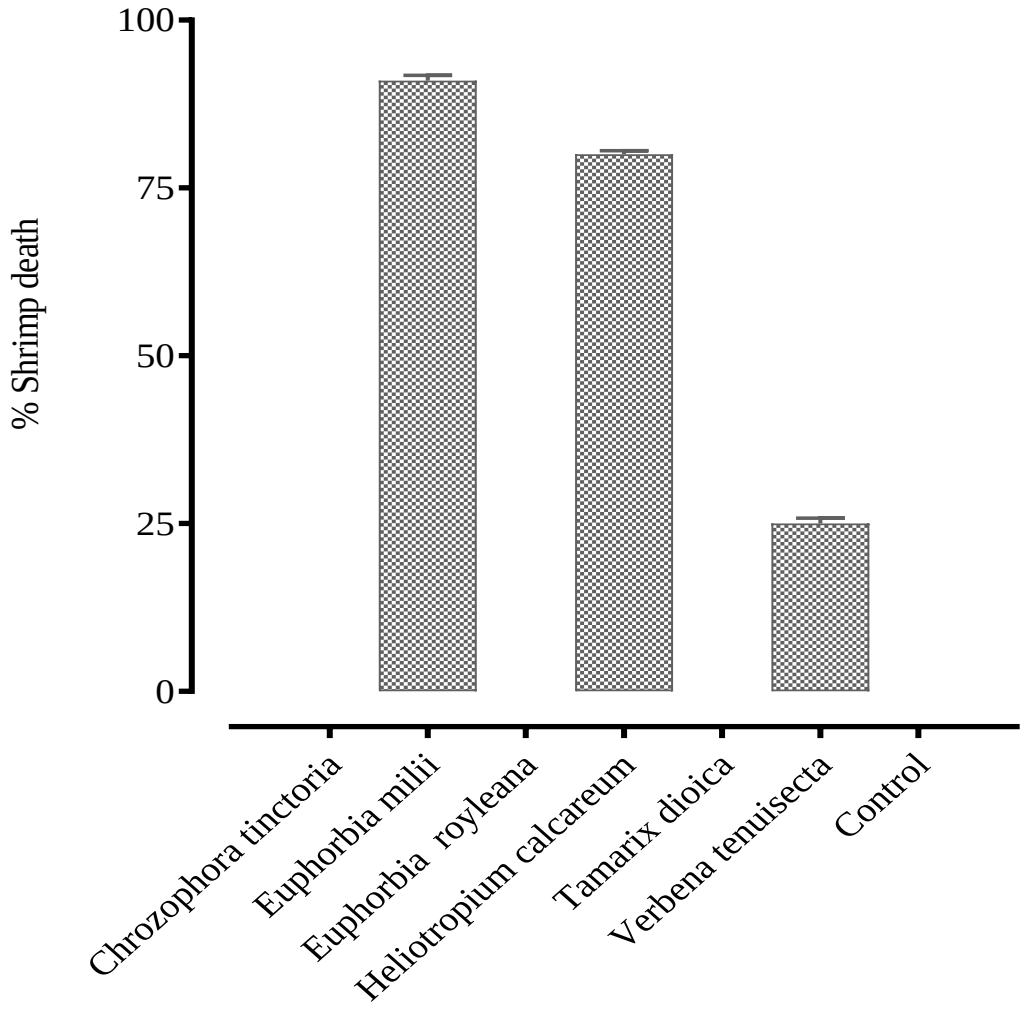

Fig. 2. Graphical presentation of brine shrimp lethality of selected plant extracts (200 µg/mL) against *Artemia salina*

DISCUSSION

The aim of the study was to provide site-specific data from District Buner, Pakistan to address a critical knowledge gap of post-fire scenario of vegetation regeneration and soil properties in Himalayan foothill ecosystems. The study was carried out in the scenario of increasing frequency and intensity of forest fires which is largely attributed to anthropogenic activities and current trends of global warming and climate change. Subtropical pine forests dominated by *Pinus roxburghii* in northern Pakistan are increasingly vulnerable to forest fires, posing serious ecological and management challenges (Muhammad *et al*., 2024). This increase frequency has led to ecological disturbance rather than natural regulators specifically in fire prone ecosystem of subtropical Chir pine (*Pinus roxburghii*) forests (Kelly *et al.,* 2020; Phillips *et al.,* 2022). The result revealed that most soil physicochemical parameters reflect statistical variations between burnt and unburnt sites other than phosphorus. Although these findings indicated that forest fire induces modification in soil properties, the magnitude and direction of change vary among nutrients and are influenced by post-fire environmental conditions (Certini, 2005). Phosphorus, which is often reported to increase immediately after fire due to ash deposition (Elakiya *et al.,* 2023), did not show a significant increase in this study and can be explained by the high rainfall in the study area, which likely facilitated rapid leaching of soluble potassium from the soil. Such site-specific climatic influences emphasize the importance of local environmental conditions in determining post-fire nutrient dynamics. Phosphorus reflected non-significant results but increasing trend in burnt soils, coherent with studies suggesting that fire can temporarily enhance available phosphorus through the mineralization of organic matter (Moret-Ferguson *et al.,* 2024). However, the lack of statistical non significance indicates that this effect may be temporary or spatially variable. Indeed, phosphorus dynamics are highly dependent on fire intensity, soil type, and post-fire erosion processes (Certini, 2005). On the other hand, soil physical properties reflected more consistent responses to forest fire. The reduction in water holding capacity (WHC) recorded in burnt areas aligns with the findings that fire decreases soil porosity and increases hydrophobicity due to the combustion of organic matter. Soil pH was also found to increase in burnt areas, indicating the incorporation of ash residues into the soil. This short-term alkalization is a well-documented phenomenon and can influence nutrient availability and microbial activity (Certini, 2005). The increase in soil potassium concentration and reduction in water holding capacity in burnt sites is consistent with findings from other studies conducted in Chir pine and subtropical forest ecosystems. Previous studies from the Himalayan region of India have reported increased potassium concentrations following fire due to the deposition of ash and the mineralization of organic matter (Sharma *et al*., 2023; Verma *et al*., 2025). Similarly, studies conducted by González-Pérez *et al*., (2004) indicated that forest fires can significantly alter soil physicochemical properties by increasing the availability of certain nutrients while reducing soil moisture retention and organic matter content. Increase in soil pH observed in the present study is also in agreement with studies from Mediterranean and temperate forest ecosystems, where post fire ash deposition temporarily increased soil alkalinity (Alcañiz *et al*., 2018). Both national and international studies recorded significant reduction in natural regeneration in burnt areas. High-intensity fires negatively affect seedling establishment and sapling survival, thereby reducing regeneration potential Research conducted in subtropical Chir pine forests of the western Himalayas (Bargali *et al*., 2023; Sharma *et al*., 2023). Similarly, studies from

other fire-prone ecosystems at global level have reported that severe fires can impair vegetation recovery by altering soil properties, reducing seed banks, and creating unfavorable conditions for seedling establishment (Keeley & Pausas, 2022). These findings suggest that the effects of forest fire on soil properties and regeneration dynamics are strongly influenced by fire intensity, post-fire environmental conditions, and ecosystem characteristics. The loss of these nutrients may therefore hinder long-term ecosystem recovery and productivity. The study also documented significant changes in regeneration in burnt areas as compared to unburnt areas. This finding aligns with previous studies indicating that low-intensity fires may promote seed germination while high-intensity fires can suppress sapling survival and overall regeneration capacity (Sharma *et al.,* 2023; Bargali *et al.,* 2023). The reduction in regeneration in burnt areas indicates that fire intensity exceeded the threshold thereby limiting recovery potential. This has important implications for forest management, as regeneration dynamics directly influence long-term forest structure and biodiversity conservation.

Overall, the findings of this study reinforce the effects on both regeneration and soil are intensity-dependent (Kutiel & Shaviv, 1989). Although sub-tropical forests of *Pinus roxburghii* (chir pine) provide various ecosystem services and act as watershed for low lying regions. However, this species is prone to human induced fire primarily due to local communities' dependence on various resources exacerbated by the current dry conditions (Muhammad *et al*., 2024). Subtropical Chir pine forests in District Buner, frequent anthropogenic fires combined with the highly flammable nature of pine needle litter create conditions that can significantly alter both soil properties and vegetation regeneration patterns (Hussain *et al.,* 2021; Kumar *et al.,* 2013). Despite its contributions, this study has certain limitations that should be acknowledged. The spatial scale and number of sampling sites may not fully capture the heterogeneity of the landscape, and the short-term nature of the study limits the ability to assess long-term recovery processes. Moreover, the focus on selected parameters does not account for other important factors such as soil microbial communities, faunal interactions, and detailed vegetation dynamics, which also play critical roles in post-fire ecosystem recovery. Therefore, future research should prioritize long-term monitoring and incorporate a broader range of ecological indicators. Remote sensing and GIS approaches could further enhance understanding of fire regimes and support the development of predictive models for ecosystem recovery (Attri *et al.,* 2020). Such comprehensive approaches are essential for designing effective forest management strategies, including controlled burning, restoration interventions, and community-based fire management, to ensure the sustainability of these ecologically and socioeconomically important forest systems.

CONCLUSION

The current study revealed the presence of remarkable amount of total phenolic and flavonoid contents. Moreover, the anti-oxidant, anti-microbial and cytotoxic activities of selected plant extracts may explain the medicinal importance of these plants. Therefore, current study suggests the further investigation on the isolation of bioactive compounds responsible for these activities. Furthermore, selected plants should be investigated in future against various cancer cell lines to attain their comprehensive spectrum of cytotoxicity potential.

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Research Paper **Open Access**

Determining relationship between different parameters of Jand (*Prosopis cineraria*) under annual lopping at Rakh Goharwala (Thal)

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SUMMARY

This study assessed the relationship between stem diameter and growth/biomass parameters in *Prosopis cineraria* (Jand) trees subjected to annual lopping in Rakh Goharwala, Punjab, using data from 2004-2008. The research aimed to create dependable correlations between diameter at breast height (DBH) and tree attributes such as leaf/twig biomass and branch biomass. Regression analysis showed quadratic models offered the best fit for most relationships. A strong correlation was observed between DBH and leaf/twig biomass, and branch biomass, indicating DBH's reliability in predicting fodder production. Crown area also displayed a strong dependence on DBH, while stem height and crown radius exhibited weaker associations. The findings confirmed DBH as a useful, non-destructive predictor for biomass estimation, valuable for rangeland managers and foresters, particularly in arid and semi-arid environments where P. cineraria is a significant fodder source for local livestock and communities.

Keywords: Agroforestry; allometric modeling; arid rangelands; *Prosopis cineraria*; tree biomass

INTRODUCTION

Prosopis cineraria (L.) belongs to Family Fabaceae, which is an extraordinary versatile tree of Cholistan Desert. *P. cineraria* is locally known as “Jhand”, which holds an important place in rural area of Southern Punjab, Pakistan (Rafay et al., 2018). Jand (*P. cineraria*), a multipurpose tree species of immense ecological and socio-economic importance, thrives across the arid and semi-arid regions of Pakistan (Kord et al., 2024). It is a medium-sized tree with compound leaves, small dark-green leaflets, and a relatively narrow crown that casts only light shade. In the harsh landscapes of the Thal desert and adjoining rangelands, this tree serves as a cornerstone species, supporting both human livelihoods and ecological stability. Its leaves and twigs provide a dependable source of fodder, particularly for goats and camels, while its branches are used as fuelwood and fencing material (Khan et al., 2023). Moreover, its nitrogen-fixing ability

improves soil fertility, thereby enhancing the productivity of otherwise marginal lands. *P. cineraria* are one of the highly valued plant in the native system of medicine (Kord et al., 2024). It is also known to possess antibacterial, anthelmintic antifungal, anticancer antiviral and many other pharmacological qualities. Moreover, it has been reported in the literature that the seeds and leaves of *P. cineraria* are the rich source of phyto-chemicals such as flavonoids, quinones, alkaloids, tannins, and phenols (Sharifi-Rad et al., 2019).

The practice of lopping Jand trees for fodder is centuries old. During the winter season, when green forage is scarce, local communities harvest branches, leaves, and twigs to sustain their livestock (Singh and Bishnoi, 2014). Earlier studies have reported that annual lopping does not negatively affect tree growth, but rather supports higher fodder availability without compromising the long-term health of the species. This resilience has made *P. cineraria* a reliable source of nutrition for livestock during the lean season (Singh and Bishnoi, 2014).

Biomass assessment of trees plays a vital role in forestry and range management (Chaudhry, Muslim, & Mushtaque, 2004; Joshi et al., 2021). Conventionally, biomass is estimated through destructive sampling that involves felling trees and weighing their components. Although accurate, this method is impractical on a large scale, particularly when dealing with species of high ecological and cultural value such as *P. cineraria*. A more feasible approach involves establishing mathematical relationships between easily measurable parameters, such as diameter at breast height (DBH), and more complex traits such as biomass (Singh & Bishnoi, 2014). DBH is widely recognized in forestry as a quick, reliable, and non-destructive measure that correlates strongly with tree volume and biomass (Skladan et al., 2025).

In arid rangelands, where fodder scarcity is a chronic problem, developing predictive models for biomass estimation becomes even more critical. By linking DBH with parameters like leaf/twig biomass, branch biomass, and crown dimensions, forest managers can estimate fodder production without resorting to destructive methods (Kumar & Tewari, 2000). Such models can serve as valuable tools for planning sustainable lopping regimes, ensuring continued fodder supply while conserving tree populations (Zhou et al., 2019).

Despite the ecological and socio-economic importance of Jand, limited research has been undertaken in Pakistan to establish DBH-based predictive relationships for its biomass production. This gap highlights the need for scientific studies that combine traditional management practices with modern forest measurement techniques.

The present study was therefore designed to investigate the effect of DBH on leaf/twig and branch biomass of Jand trees, and to examine the relationship of DBH with other parameters. By addressing these objectives, the study aims to contribute to the development of reliable biomass estimation tools for Jand trees under annual lopping practices, thereby supporting sustainable rangeland management in the Thal region.

MATERIALS AND METHODS

Study Area

The study was conducted at Rakh Goharwala, Thal (District Bhakkar, Punjab, Pakistan). The area is characterized by sandy soils, low rainfall, and hot summers typical of arid zones. Vegetation is sparse and dominated by hardy species, with P. cineraria occupying

a central role in the ecological system and as a fodder source for livestock.

Study Duration

Fieldwork spanned four consecutive years (2004–2005 and 2007–2008), coinciding with routine forest management activities that included lopping and auctioning of trees, usually between November and January.

Sampling Procedure

Lists of trees scheduled for lopping were obtained from the office of the Divisional Forest Officer (DFO), Range Management, Bhakkar. The DBH of each tree was measured, and trees were grouped into diameter classes to ensure balanced sampling across size categories. From each class, a 10% sample was selected, comprising 60 trees per class.

Data Collection

For each sampled tree, the following data were recorded:

- Before lopping: DBH, stem height, crown height, crown radius
- After lopping: Leaf and twig biomass (twigs ≤ 6 inches) and branch biomass

Leaf and twig material was carefully separated from lopped branches, weighed, and recorded. Branch biomass was also measured to assess brushwood production.

Data Analysis

Regression analysis was performed to establish relationships between DBH and different growth and biomass parameters. A quadratic regression equation was found to provide the best fit for most parameters. The relationships were illustrated with tables and figures to highlight trends across diameter classes.

RESULTS

Diameter Class Distribution

The dispersal of *P. cineraria* trees among diameter classes and their corresponding branch biomass (average) has been presented in Table 1. A total of 360 annually lopped trees were evaluated, with 60 trees representing each diameter class. Most sampled trees were mature to over-mature, with estimated ages exceeding 100 years, while a few individuals were reported by local communities to be approximately 300 years old.

The average stem diameter ranged from 25.23 cm in the smallest diameter class (≤11 inches circumference equivalent) to 50.07 cm in the largest class (>19 inches). Average branch biomass increased progressively from 38.70 kg tree^{-1} in the smallest diameter class to a maximum of 77.09 kg tree^{-1} in the 17–19 diameter class before declining to 71.79 kg tree^{-1} in the largest diameter class.

Table 1: Number of Prosopis cineraria trees and branch biomass in different dia. Classes.

Diameter class	**No. of trees lopped**	**Average diameter (cm)**	**Average branch biomass (Kg tree^{-1})**
Upto 11	60	25.23	38.70
11-13	60	28.95	42.11

13-15	60	34.03	49.81
15-17	60	39.10	69.99
17-19	60	43.97	77.09
>19	60	50.07	71.79

Branch biomass

The weight of branches from each tree is very important for brush wood estimation. In this study the branch biomass displayed a diverse quadratic relationship with stem diameter as shown in Figure 1. From the results, it is evident that biomass has increased steadily with the increasing DBH from the smallest diameter class upto 17–19 cm class. This indicated the enhanced biomass growth in young and middle-aged trees. However, beyond this stage, branch biomass declined despite continued increases in stem diameter.

The observed reduction in branch biomass among the largest diameter classes likely reflects the reduced physiological activity and slower growth rates characteristic of mature and over-mature Jhand (P. cineraria) trees. Similar growth behaviour was observed for leaf and twig biomass, suggesting that canopy productivity reaches its optimum in intermediate-sized trees before declining with advancing age.

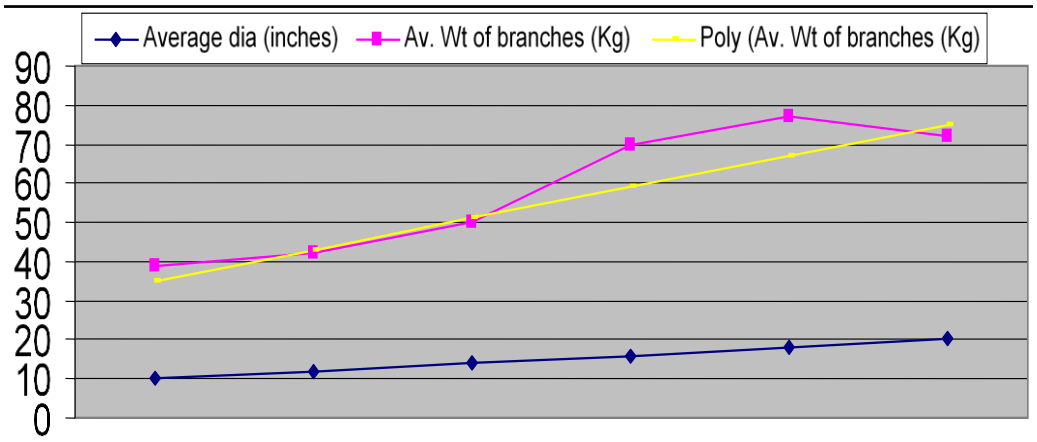

Fig. 1. Relationship between diameter and wt. of branches *Prosopis cineraria*.

DISCUSSION

This study demonstrated that stem diameter (DBH) is a strong predictor of branch biomass in annually lopped P. cineraria. The branch biomass increased with increasing diameter until intermediate size classes (15–17 cm), after which productivity declined in the largest trees. This indicated that biomass accumulation is not linear throughout the life cycle of the species but follows a quadratic growth pattern, reflecting age-related changes in physiological efficiency.

DBH has consistently been identified as the most reliable single predictor of above-ground biomass in woody species because it integrates cumulative radial growth and resource acquisition over time (Picard et al., 2012). Recent studies have further confirmed that allometric models based on DBH provide highly accurate estimates of tree biomass across diverse ecosystems, including dryland forests and agroforestry systems

(Chave et al., 2014; Kuyah et al., 2022). The strong relationship observed in the present study therefore supports the continued use of DBH as a rapid and non-destructive estimator of fodder biomass in arid rangelands.

The increase in branch biomass with increasing diameter up to intermediate size classes agrees with the biological growth strategy of P. cineraria. Young and middle-aged trees allocate substantial resources to canopy expansion, resulting in greater production of branches and foliage. However, as trees become over-mature, metabolic activity declines and a larger proportion of assimilates is allocated to structural maintenance rather than new biomass production (Ryan et al., 2021). Consequently, branch production decreases despite continued increases in stem diameter.

The decline in biomass observed in the largest diameter class is consistent with previous studies on P. cineraria and other dryland tree species. Earlier work by Mahmood et al. (2001) reported maximum fodder production in medium-sized Jand trees, followed by reduced productivity in older individuals because of declining crown vigour. Similarly, Kumar et al. (2021) reported that mature Prosopis trees exhibited lower annual biomass increment than younger trees owing to reduced photosynthetic efficiency and increased maintenance respiration. Comparable findings have also been reported for other multipurpose dryland species including Acacia nilotica and Ziziphus mauritiana, where biomass production peaks during middle age before declining in senescent trees (Singh et al., 2020).

Annual lopping appears to maintain productive canopy growth without adversely affecting long-term tree performance. Controlled pruning removes older branches and stimulates the production of new shoots, which are generally more nutritious and digestible for livestock. Similar responses have been reported by Orwa et al. (2020), who observed increased shoot regeneration following moderate pruning in several agroforestry tree species. Likewise, studies in arid regions of India and Pakistan have shown that sustainable lopping of P. cineraria enhances fodder availability while maintaining tree survival and ecological stability (Yadav et al., 2019; Kumar et al., 2023).

The predominance of mature and over-mature trees within the study area also has important management implications. Although older trees contribute significantly to ecosystem services such as soil stabilization, carbon storage and biodiversity conservation, they produce comparatively lower quantities of edible biomass. In contrast, intermediate-sized trees provide maximum fodder yield. Maintaining a balanced age structure through natural regeneration and protection of young trees would therefore improve long-term productivity while preserving ecological functions. Similar recommendations have been proposed for sustainable management of arid silvopastoral systems across South Asia (FAO, 2022).

Overall, the results confirmed that DBH-based allometric relationships provide an effective tool for estimating branch biomass without destructive harvesting. Such models are particularly valuable in arid ecosystems where repeated destructive sampling is impractical and conservation of mature trees remains a priority.

CONCLUSION

The present study established that stem diameter at breast height (DBH) is a reliable and practical predictor of biomass in P. cineraria under annual lopping regimes. Leaf/twig and branch biomass showed strong correlations with DBH. Biomass production was found to increase with DBH up to mid-size classes (15–17 cm), after which productivity declined in over-mature trees, indicating maximum productivity in young to middle-aged individuals. These results have important implications for rangeland management. By using DBH as a simple field measurement, managers can estimate biomass yield without destructive sampling, thereby improving planning for sustainable fodder supply. Maintaining a balanced population of young and middle-aged trees can help maximize fodder production while conserving older trees for ecological stability. In the arid rangelands of Thal, where livestock heavily depend on Jand during critical periods, such approaches can significantly enhance both ecological resilience and rural livelihoods.

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