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# Soil Biological Properties under the Crown of *Pistacia atlantica*, *Quercus brantii*, and *Quercus infectoria* in a Sacred Forest Stand

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**Abstract**

This study presents a novel and meaningful investigation of the distinct soil biological properties beneath

crown trees included *Pistacia atlantica*, *Quercus brantii*, and *Quercus infectoria*. The research is particularly significant as it was conducted within the sacred forest stand, an ecosystem protected from human disturbance for centuries. Soil samples were collected beneath and beyond the crown of mature trees from each. The results revealed that the interaction between tree species and crown position creates a fine-scale heterogeneity of soil microbial activity. The soil beneath tree crowns, particularly those of *Pistacia atlantica*, supported significantly higher microbial respiration (BR, SIR), biomass carbon (MBC), and dissolved organic carbon (DOC) than the open areas between crowns. MBC exhibited a high positive correlation with BR, SIR and DOC. MBC differences among species (*P. atlantica* > *Q. infectoria* > *Q. brantii*) reflect variations in crown architecture and litter production The enhanced microbial functioning was most pronounced under *P. atlantica*, which improved microclimatic conditions and resource inputs. The stability of the microbial metabolic quotient ($qCO_2$) across the landscape suggests a consistently efficient and non-stressed microbial community. Our results highlight that tree identity, especially keystone species like *P. atlantica*, is a critical driver of belowground heterogeneity.

**Keywords:** Tree Crown Microclimate; *Pistacia atlantica*; Fertility Islands; Soil Microbial Ecology; Zagros Oak Forest

## Introduction

Fine-scale heterogeneity is an inherent property of forest ecosystems, arising from distinct biogeochemical interactions between individual tree species and their immediate soil environment. In protected landscapes like the sacred groves forest stands of Iran's Zagros Mountains, these species-specific feedback cycles are particularly pronounced. Here, Soil operates not only as a growing medium, but also as a dynamic record of biological activity, shaped by the litter chemistry,

root exudates, and microclimates of dominant trees [1, 2]. It is widely recognized that tree species transfer distinct "biological and chemical signatures" on the soil through their differing functional traits [3–5]. These signatures, reflected in microbial community structure and activity, are fundamental to understanding ecosystem resilience and carbon dynamics [6–8]. By studying these interactions in the centuries-old, minimally disturbed context of a sacred forest, we can establish a critical baseline for natural soil biological variation.

The Zagros sacred groves, with their unique assemblage of *P. atlantica*, *Q. brantii*, and *Q. infectoria*, represent more than just cultural relics; they are living laboratories of plant-soil coevolution [9, 10]. However, a surprising gap exists in quantifying the soil biological quality beneath these specific crowns. Key indicator such as basal respiration (BR), microbial biomass carbon (MBC), and metabolic quotients ($qCO_2$) remain poorly documented for these species within a less disturbed system. This study directly addresses by asking a simple but crucial question: how do these three foundation species differentially influence the biological properties of the soil directly under their crowns? The answer provides a necessary benchmark for soil quality, especially in semi-arid regions like the Zagros, where individual trees create vital islands of fertility, modulating soil moisture and organic matter dynamics in an otherwise demanding environment [11, 12, 9, 4].

Methodologically, we contend that detecting these species-level effects requires a sampling design capable of isolating tree influence from inherent microsite variation. The sharp gradients in soil properties from under crown to the open areas are well-documented [13, 14, 4]. Therefore, our approach paired under- crown and outside-crown sampling for each individual tree, using a replicated design to statistically separate the signal of species identity from background variability. By linking biological soil measurements such as substrate-induced respiration (SIR) to this well-

defined spatial framework, we aim to build a more causal understanding of how tree identity controls microbial performance, creating a model transferable to other semi-arid forest ecosystems [15, 16, 14, 17].

This study was designed to quantify the distinct legacy effects of *Pistacia atlantica*, *Quercus brantii*, and *Quercus infectoria* on key soil biological properties within a minimally disturbed Zagros sacred forest. Through this comparative assessment, it further evaluates the reliability of these biological parameters as sensitive indicators for ecosystem condition. Furthermore, by establishing baseline soil health characteristics in this preserved forest ecosystem, we hypothesized that interspecific differences in crown architecture shape distinct fertility islands, leading to significant variation in soil microbial activity and carbon cycling beneath tree crowns.

## Results

### Soil Microbial Respiration and Carbon Pools

Results from the analysis of variance revealed that tree species, sampling position (under vs. outside crown), and their interaction significantly affected ($p<0.01$) basal respiration (BR) (Table 1). ANOVAs, followed by Duncan's Post-hoc test showed that while BR outside the crown of *Pistacia atlantica* did not differ significantly from the other species, the highest BR value (1.67 mg $CO_2$ $g^{-1}$ dry soil $day^{-1}$) was recorded under its crown (Figure 1-a). Substrate-induced respiration (SIR) was significantly influenced by sampling position ($p < 0.05$) and as well as by the species×position interaction ($p < 0.01$). Substrate-induced respiration (SIR) beneath the crown of *Pistacia atlantica* was significantly higher than measured in the other treatments ($p < 0.01$) (Figure 1-b).

Similarly, microbial biomass carbon (MBC) was significantly affected by species, position, and their interaction ($p < 0.01$). Beneath the crown, MBC values decreased in the following order: *Pistacia atlantica* (1394 mg $kg^{-1}$) > *Q. infectoria* (1027 mg $kg^{-1}$) > *Q. brantii* (816 mg $kg^{-1}$). The maximum recorded MBC (1980 mg $kg^{-1}$) was found under the crown of *Pistacia atlantica* (Figure 1-c). Dissolved organic carbon (DOC) in the soil also varied significantly by both species and position ($p < 0.01$), with a significant interaction between these factors ($p < 0.05$). The highest DOC concentrations were measured beneath crown of *Pistacia atlantica* and *Q. infectoria* 1329 mg $kg^{-1}$ and 1061 mg $kg^{-1}$ respectively (Figure 1-d).

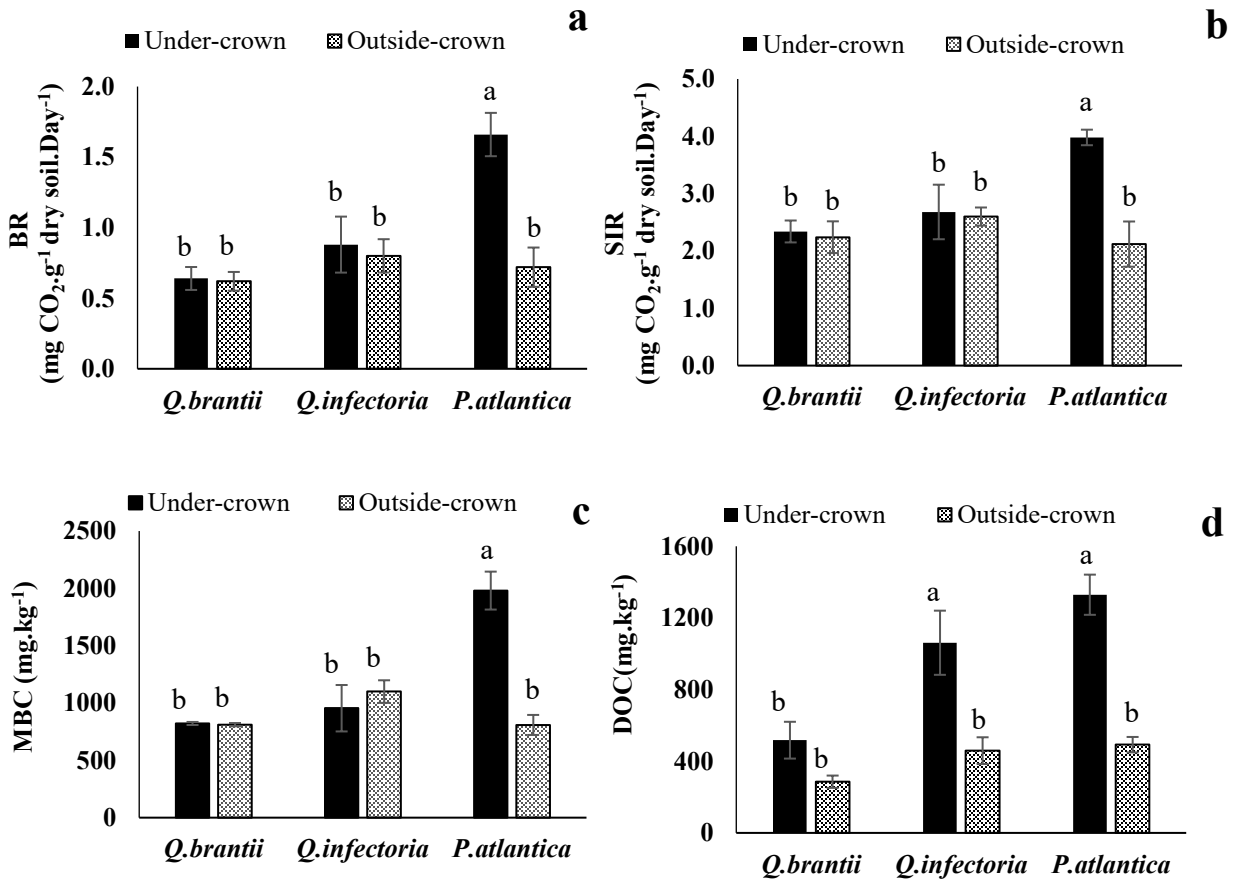
**Table 1:** ANOVA results for the effects of the studied treatments on selected soil properties in the forest sacred grove.

| Source of Variation | | df | Variables Examined | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **BR** | **SIR** | **MBC** | **DOC** | **MBN** | **DON** | **$qCO_2$** | **qmic** | **$C/N_{mic}$** | **pH** | **humidity** |
| Species | F-Value | | $8.94^{**}$ | $3.23^{ns}$ | $11.94^{**}$ | $12.87^{**}$ | $2.85^{ns}$ | $3.21^{ns}$ | $0.89^{ns}$ | $3.51^{*}$ | $1.67^{ns}$ | $6.59^{**}$ | $7.47^{**}$ |
| | Mean Squares | 2 | 0.8 | 1.45 | 856802.7 | 6884843.6 | 2968.05 | 8200.31 | 2963.23 | 0.00041 | 49.6 | 0.82 | 6.49 |
| Sample | F-Value | 1 | $10.07^{**}$ | $7.73^{*}$ | $12.49^{**}$ | $43.77^{**}$ | $0.441^{ns}$ | $4.8^{*}$ | $0.86^{ns}$ | $1.26^{ns}$ | $2.8^{ns}$ | $56.43^{**}$ | $31.08^{**}$ |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position (P) | Mean Squares | | 0.9 | 3.47 | 896486.5 | 2328538.8 | 460.21 | 12253.32 | 28706.13 | 0.000015 | 83.33 | 0.705 | 27.02 |
| | F-Value | | 7.4** | 5.82** | 18.04** | 4.37* | 1.07ns | 2.86ns | 1.26ns | 11.01** | 3.51* | 0.987ns | 1.77ns |
| S × P | Mean Squares | 2 | 0.662 | 2.61 | 1294645 | 232747.6 | 1114.45 | 7294.35 | 41922.23 | 0.000128 | 104.53 | 0.012 | 1.54 |
| | F-Value | | | | | | | | | | | | |
| Error | Mean Squares | 24 | 0.09 | 0.45 | 71749.53 | 53204.7 | 1042.61 | 2553.84 | 33216.85 | 0.000012 | 29.77 | 0.012 | 0.87 |
| CV | - | - | 34.7 | 24.9 | 23 | 27.5 | 36.1 | 52.6 | 22.7 | 27.3 | 25 | 15 | 18.4 |

106 **p < 0.01, *p < 0.05, ns = not significant

107




108

109

**Figure 1.** Interaction effect of species and sampling position on a) soil basal respiration (BR), b) substrate-induced respiration (SIR), c) microbial biomass carbon (MBC) and d) dissolved organic carbon (DOC). Different letters show significant differences (mean ± SE).

## Nitrogen Pools and Microbial Metabolic Indicators

In contrast to the carbon-related variables, microbial biomass nitrogen (MBN) was not significantly influenced by any of the experimental factors (Table 1). The highest and lowest values (115.6 mg $kg^{-1}$ under *Pistacia atlantica* and 65.9 mg $kg^{-1}$ outside *Q. brantii* tree crown, respectively) were not statistically different (Figure 2-a). Dissolved organic nitrogen (DON), however, was significantly higher in the under crown position (114.6 mg $kg^{-1}$) compared to the outside-crown position (74.2 mg $kg^{-1}$) ($p < 0.05$), with the maximum value (177 mg $kg^{-1}$) found under tree crown of *P. atlantica* (Figure 2-b). Dissolved organic nitrogen (DON) was not significantly affected by tree species or their interactions.

The metabolic quotient ($qCO_2$) was not significantly affected by the treatments, with its highest value (251.27 µg $CO_2$ $mg^{-1}$ MBC $day^{-1}$) observed under-crown *Q. infectoria* (Figure 2-c). Conversely, the microbial quotient (qMic) differed significantly among species ($p < 0.05$), with a significant species-position interaction ($p < 0.01$). The highest qMic (0.02) was found outside the crown of *Q. infectoria*, while the lowest (0.01) was under tree crown of *Q. brantii* (Figure 2-d).

**a**

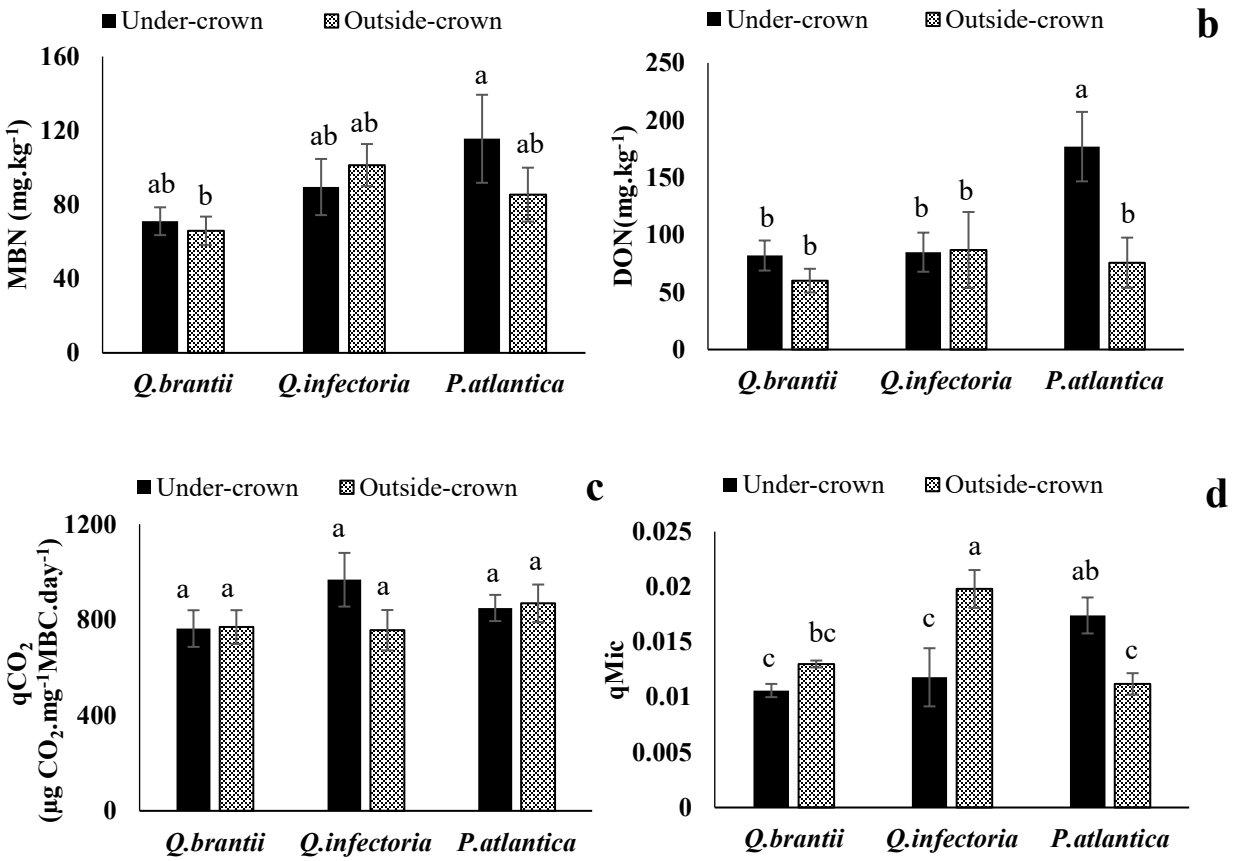



**Figure 2.** Interaction effect of species and sampling position on a) microbial biomass nitrogen (MBN), b) dissolved organic nitrogen (DON), c) metabolic quotient ($qCO_2$) and d) microbial quotient (qMic). Different lowercase letters show significant differences among treatments (mean ± SE).

## Microbial Stoichiometry and Soil Properties

The microbial C/N ratio (C/Nmic) was not significantly affected by species or position alone, but their interaction was significant ($p < 0.05$). The *P. atlantica* beneath crown combination resulted in a significantly higher C/Nmic (20.8) than other treatments (Figure 3-a). Soil pH was significantly influenced by both species and position ($p < 0.01$), with the highest value (7.48) recorded under *P. atlantica* tree crown (Figure 3-b). Soil moisture content was also significantly

affected by species and position ($p < 0.01$), being highest beneath the crown of *P. atlantica* (5.1%) (Figure 3-c). Soil pH and moisture showed no significant interactions.

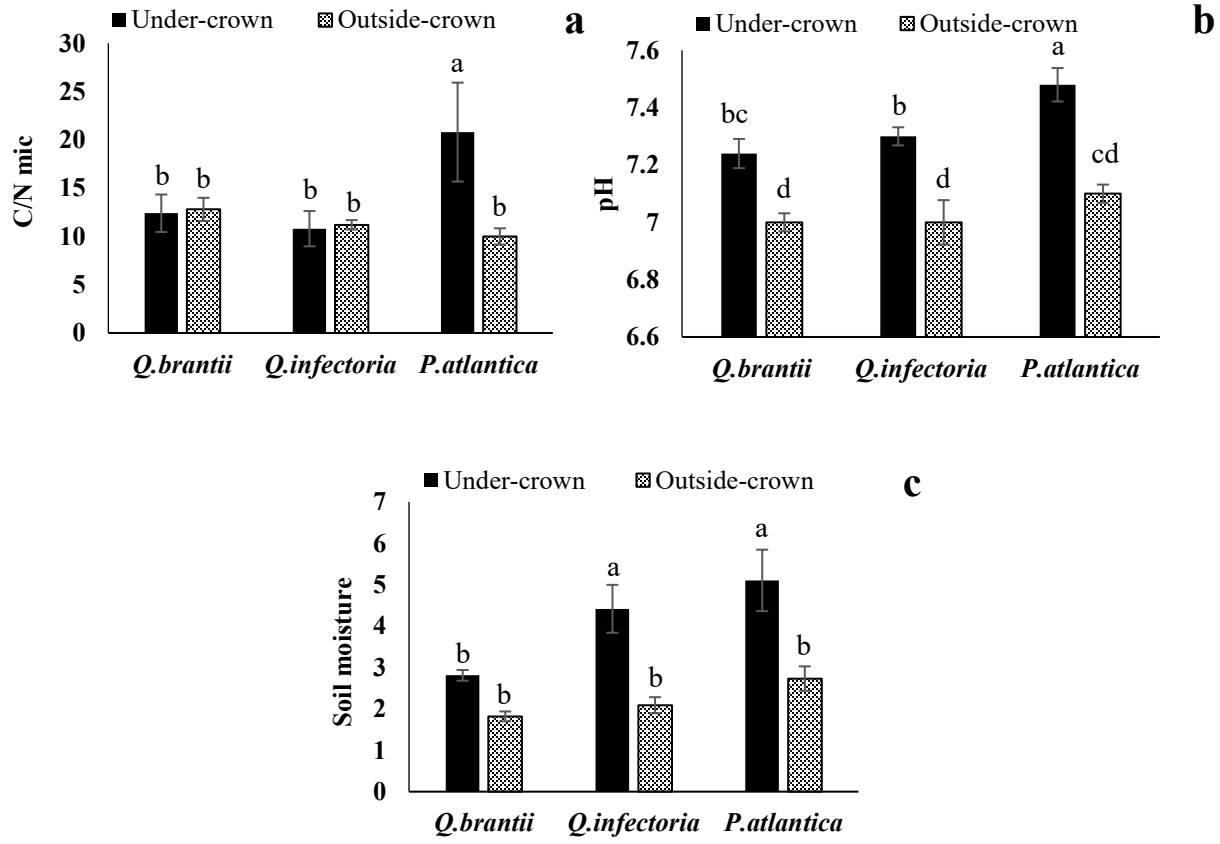



**Figure 3.** Interaction effect of species and sampling position on a) microbial C/N ratio, b) soil pH and c) soil moisture percentage. Different letters show significant differences (mean ± SE).

## Interrelationships among soil properties

Pearson correlation analysis revealed a strong, positive interrelationship among key microbial activity indicators (Table 2). Basal respiration (BR), substrate-induced respiration (SIR), and microbial biomass carbon (MBC) were all highly correlated ($r \geq 0.797$, $p < 0.01$), with the strongest correlation observed between BR and MBC ($r = 0.905$, $p < 0.01$) (Table 2). Dissolved organic

carbon (DOC) was highly correlated with soil humidity ($r = 0.829$, $p < 0.01$) and with both BR ($r = 0.793$, $p < 0.01$) and MBC ($r = 0.738$, $p < 0.01$). The metabolic quotient ($qCO_2$) was largely independent of other parameters, weakly positively correlated with BR ($r = 0.378$, $p < 0.05$). In contrast, the microbial quotient (qMic) was positively associated with MBC, SIR, BR, and MBN ($r = 0.473–0.707$, $p < 0.01$). Positive correlations were observed between the microbial C/N ratio and several carbon-related parameters, such as BR and MBC. Soil pH was significantly correlated with both DOC ($r = 0.661$, $p < 0.01$) and soil moisture ($r = 0.642$, $p < 0.01$).

**Table 2:** Pearson Correlations Among Studied Soil Properties

| Parameter | BR | SIR | MBC | DOC | MBN | DON | qco2 | qmic | $C/N_{mic}$ | pH | Humidity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BR** | 1 | | | | | | | | | | |
| **SIR** | 0.840** | 1 | | | | | | | | | |
| **MBC** | 0.905** | 0.797** | 1 | | | | | | | | |
| **DOC** | 0.793** | 0.690** | 0.738** | 1 | | | | | | | |
| **MBN** | 0.455* | 0.479** | 0.568** | 0.497** | 1 | | | | | | |
| **DON** | 0.659** | 0.580** | 0.671** | 0.586** | 0.162 | 1 | | | | | |
| **qco2** | 0.378* | 0.266 | -0.003 | 0.294 | -0.026 | 0.109 | 1 | | | | |
| **qmic** | 0.601** | 0.579** | 0.707** | 0.290 | 0.473** | 0.411* | -0.119 | 1 | | | |
| **$C/N_{mic}$** | 0.619** | 0.460* | 0.570** | 0.357 | -0.319 | 0.598** | 0.089 | 0.353 | 1 | | |
| **pH** | 0.526** | 0.384* | 0.538** | 0.661** | 0.150 | 0.388* | 0.134 | -0.037 | 0.468** | 1 | |
| **Humidity** | 0.669** | 0.582** | 0.632** | 0.829** | 0.501** | 0.426* | 0.248 | 0.191 | 0.179 | 0.642** | 1 |

Significance levels (two-tailed): *p < 0.05, **p < 0.01

## Discussion

Our findings demonstrate that both treatments including crown position and tree species identity strongly influence soil microbial activity within the sacred grove ecosystem. The substantially

166 higher basal respiration (BR) beneath tree crowns particularly under *P. atlantica* is most probably
167 linked to increased organic matter inputs from litterfall, which enrich the upper soil layers with
168 nutrients and readily available carbon substrates [18]. The tree crown drives a richer soil microbial
169 community, enhancing both its abundance and diversity. This effect is largely driven by the
170 increased input of litter and root exudates, which provide a steady supply of food resources for soil
171 microorganisms [19]. The tree crown also plays a key role, supporting soil physical and chemical
172 stability. It buffers against temperature extremes and conserves moisture, collectively creating far
173 more favorable conditions for microbial activity [18].
174 The extensive crown of *P. atlantica* likely enhances this effect by moderating temperature
175 extremes and conserving soil moisture [4]. Its deep root system may further sustain microbial
176 processes during drier periods by drawing upon water and nutrients from deeper horizons [20].
177 The very strong correlation observed between BR and microbial biomass carbon (MBC) ($r = 0.905$,
178 $p < 0.01$) emphasizes the close relationship between microbial biomass and metabolic activity in
179 this relatively undisturbed forest stand.
180 The raised substrate-induced respiration (SIR) under crowns, and especially under P. atlantica,
181 signals the presence of a larger, more metabolically responsive microbial community capable of
182 rapidly exploiting additional carbon sources [21]. This pattern reflects the resource-rich microsites
183 created by tree crowns, where higher quantities of litter, root exudates, and nutrients support a
184 more abundant and diverse microbial assemblage [19]. These species-specific SIR responses
185 correspond with previous research indicating that *P. atlantica* significantly enhances soil
186 biological quality in semi-arid Iranian forests [22].
187 Microbial biomass carbon (MBC) was substantially higher beneath tree crowns, particularly under
188 P. atlantica. This pattern highlights the combined impact of increased organic matter inputs and

189 improved microclimatic conditions. The beneath tree crowns receives substantial annual litterfall,
190 which, combined with enhanced root exudation, that supports a more abundant and diverse soil
191 microbial community. As a result, this promotes a greater standing microbial biomass carbon pool
192 [19]. The stability of this microbial system is further reinforced by the unique crown-generated
193 microclimate, which moderates temperature variations and sustains higher and more stable soil
194 moisture levels conditions essential for sustaining microbial processes. The pattern of MBC
195 between species *P. atlantica* > *Q. infectoria* > *Q. brantii* reflects known differences in crown
196 architecture and litter production potential, where more extensive and denser crowns typically
197 contribute higher organic inputs [23, 24]. The strong correlations among BR, SIR, and MBC ($r \geq$
198 0.797, $p < 0.01$) further indicate an integrated microbial system in which biomass, carbon
199 availability, and metabolic activity are closely interlinked.
200 Soil dissolved organic carbon (DOC) exhibited a distribution similar to MBC, with the highest
201 concentrations under *P. atlantica* and *Q. infectoria*. The strong relationship between DOC and soil
202 moisture ($r = 0.829$, $p < 0.01$) emphasizes the critical role of water availability in regulating the
203 size and persistence of labile carbon pools. Positive correlations between DOC and both BR ($r =$
204 0.793) and MBC ($r = 0.738$) reinforce the function of DOC as a central substrate supporting
205 microbial metabolism and biomass accumulation.
206 Microbial biomass nitrogen (MBN), in contrast, showed no significant effects, despite the highest
207 values occurred under P. atlantica. Dissolved organic nitrogen (DON), however, responded
208 strongly to crown influence, with elevated levels of DON under tree crowns. These patterns
209 indicate that while nitrogen cycling reflects carbon-related spatial patterns to some extent, species-
210 specific differences are less pronounced. This may the inherently greater mobility of nitrogenous
211 compounds in soil.

The lack of significant species effects on MBN is also consistent with the strong stoichiometric homeostasis commonly observed in soil microbial communities [25]. Microorganisms tend to tightly regulate their internal C:N ratios even when external carbon inputs undergo substantial variation, resulting in relatively stable nitrogen assimilation across environmental gradients. This stability has been widely documented in both forested and agricultural soils and reflects a fundamental characteristic of microbial elemental regulation [26–28].

The absence of significant differences in metabolic quotient ($qCO_2$) across species and crown positions suggests broadly comparable microbial metabolic efficiency within this minimally disturbed ecosystem. Such stability supports with the conceptual framework of Anderson and Domsch [29], who noted that $qCO_2$ tends to remain low and relatively constant under non-stressful environmental conditions, where microorganisms minimize maintenance energy demands., The slightly increased $qCO_2$ beneath Q. infectoria, despite the lack of significantly difference, may reflect minor differences in microbial community composition or substrate use efficiency between species.

The relative stability in $qCO_2$ across treatments also is consistent with extensive evidence that this index is most responsive under disturbance, nutrient limitation, or conditions requiring elevated maintenance respiration. In undisturbed forest soils, $qCO_2$ typically shows little variation, reflecting microbial communities functioning near optimal efficiency with minimal physiological stress [30, 31].

In contrast, species and their interactions significantly affect qMic, showing variation in microbial carbon-use efficiency. The notably higher qMic outside the crown of *Q. infectoria* suggests that this species may enhance microbial carbon-use efficiency in open areas, possibly due to species-specific litter chemistry or distinct crown-driven microbial assemblages [17].

Such raised qMic values in open microsites may also be associated with the typically more recalcitrant chemical composition of *Q. infectoria* litter, which contains higher concentrations of phenolic and other structurally resistant compounds. These compounds can stimulate oxidative microbial pathways while constraining biomass accumulation, thereby increasing qMic [32, 33]. The significant interaction effect on microbial C/N ratio (C/Nmic), with maximum values under *P. atlantica*, highlights species-level influences on microbial community composition. The elevated C/Nmic beneath crown of *P. atlantica* may reflect the unique chemical composition of its litter and exudates, including phenolic and terpene compounds known to influence microbial community dynamics [34]. Despite this, the relatively narrow range of C/Nmic across treatments suggests an overall stable microbial stoichiometry typical of undisturbed forest soils [29].

The moisture and pH of soil beneath tree crown were significantly higher than outside of crown which demonstrates the important role of trees in influencing local edaphic conditions. The crown-related increase in pH may stem from alkaline litter inputs and species-specific root exudates, especially in Pistacia species [34, 35]. Soil pH showed strong correlation with DOC (r=0.661) and moisture (r=0.642), indicating influence of chemical and physical soil properties on microbial processes.

Soil pH plays an important role in regulating microbial community composition and function as a powerful predictor, even moderate species-driven shifts can alter bacterial–fungal balances, enzymatic capacities, and nutrient cycling rates. Numerous studies have shown that pH exerts a dominant influence on microbial diversity and carbon cycling dynamics [36, 37], supporting our interpretation that pH differences among tree species likely contributed to observed patterns in BR, SIR, and MBC.

Soil moisture is generally higher under crowns likely reflects reduced surface evaporation and improved water retention resulting from litter accumulation and shading [38, 39]. The species gradient in soil moisture (*P. atlantica* > *Q. infectoria* > *Q. brantii*) reflects crown structure and litter production, supporting the widely recognized concept of trees acting as "fertility islands" in forest ecosystems [11, 40, 4].

The consistently superior performance of *P. atlantica* in enhancing soil biological properties underscores its ecological importance within the Zagros forest landscape. Its wide crown, substantial litter input, and deep root system collectively create favorable microhabitats that support high microbial abundance and activity. The strong correlations among microbial parameters highlight the close linkage between carbon availability, microbial biomass, and metabolic activity.

Sacred groves such as Nehmehsher serve as important ecological hot spot [41, 42] for these species-specific soil processes, providing valuable benchmarks for understanding natural forest dynamics. Minimal anthropogenic influence in these areas allows tree–soil feedbacks to operate without disruption, promoting high microbial diversity, efficient nutrient cycling, and overall ecosystem resilience.

Collectively, our results emphasize the value of preserving tree species diversity in forest conservation and management strategies. Given that species differ markedly in their impacts on soil biological functioning, shifts in species composition whether through land-use change, disturbance, livestock grazing or climate-driven alterations may have far-reaching consequences for belowground processes and ecosystem stability in the Zagros forests.

## Conclusion

This study demonstrates that tree species within the Zagros sacred groves impart a specific biogeochemical legacy on soil microbial processes *Pistacia atlantica* functioned as a keystone species, its distinct crown architecture and litter quality creating a superior microsite for microbial communities, as evidenced by significantly heightened levels of microbial respiration, biomass carbon, dissolved organic carbon, and moisture. These results underscore that plant-soil feedbacks are strongly species-specific, with individual trees forming distinct "fertility islands" that govern carbon dynamics at a fine spatial scale.

A tightly coupled, carbon-driven belowground system was revealed, where microbial activity responded sensitively to labile carbon availability. In contrast, nitrogen cycling parameters showed more uniform distributions, indicating a broader, less species-dependent regulatory mechanism. The stability of microbial metabolic efficiency across all species further reflects the functional resilience of these undisturbed soils.

In practical terms, these findings affirm that the conservation of native tree diversity, and particularly of functionally pivotal species like *P. atlantica*, is critical for maintaining the heterogeneous soil processes that underpin ecosystem fertility. Preserving these sacred groves safeguards essential microbial-mediated functions, ensuring long-term forest health and resilience amidst environmental change.

## Materials and methods

### Study site, soil sampling

This study was conducted on a set of soils collected in the Namasher Sacred Grove, a 3.8-hectare forest patch in Baneh County, Kurdistan Province, Iran (36°07′46″ N, 45°43′54″ E). Local

traditions and field evidence indicate the grove has been strictly protected from human exploitation for over 400 years, making it an ideal reference site [43, 10, 44]. The terrain is characterized by a south-southwest aspect with gentle slopes of 5–10%, at an elevation of 1350 m a.s.l. The overstory is dominated by *Quercus brantii* (comprising >70% of the canopy cover), with *Pistacia atlantica* as a common associate. *Quercus infectoria* is present only as scattered individuals.

The regional climate features cold, extended winters and moderate summers, influenced by both Siberian and Mediterranean air masses with mean annual precipitation is 650 mm, and average annual temperature is 14°C [45]. Soils at the site are classified as Calcisols (Inceptisols and Entisols), with a brown, calcareous profile and a texture ranging from clay loam to clay.

Soil was sampled in late September 2025 from a sacred grove. We selected five mature, healthy individuals of each species (*P. atlantica, Q. brantii, Q. infectoria*) with similar crown sizes. Per tree, soil was collected from two microsites: under tree crown (1 m from the trunk) and beyond the drip line. At each microsite, four subsamples from the top 10 cm of soil were composited. This yielded 30 composite samples (3 species × 5 trees × 2 positions). Samples were sieved to 2 mm, stored at 4°C, and soil moisture was determined immediately.

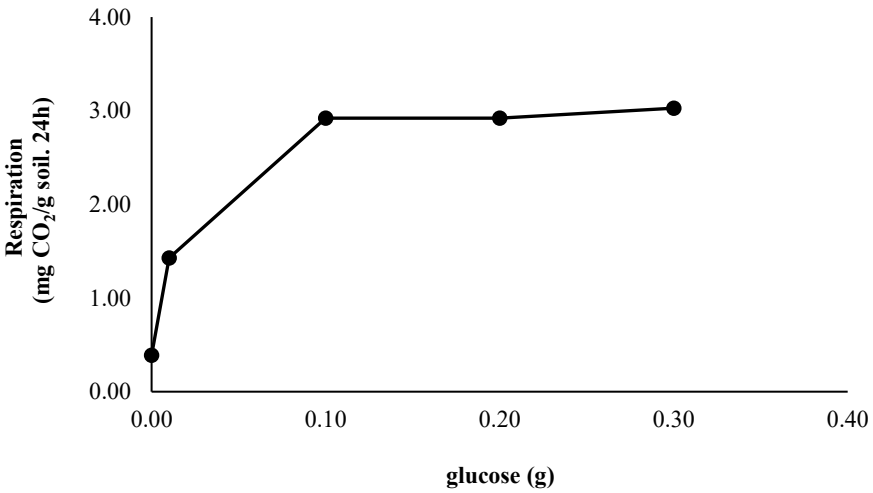
**Soil physicochemical and biological analyses**

Water content (WC) was measured using the gravimetric method [46] after oven-drying at 105°C for 24 hours. Soil pH was measured in distilled water suspension (1:5) at 20 °C through the potentiometric method [47]. Field capacity (70% water-holding capacity) was established using a pressure plate apparatus set at 0.3 bar [48].

Soil gravimetric moisture content was determined by oven-drying soil samples at 105°C for 24 h. Soil pH was determined in a 1:5 soil-to-water extract using potentiometric method [47]. In addition, Field capacity (FC) was determined using a pressure plate apparatus [48].

Basal respiration (BR) was determined by incubating 25 g of soil, adjusted to 70% field capacity, in airtight glass jars for 24 h.Carbon dioxide evolved during incubation was trapped in 0.1 N NaOH placed inside each jar. Following incubation, the absorbed $CO_2$ was quantified by back-titration with 0.1 N HCl, according to the method described by Anderson [21]. Basal respiration rates were expressed as mg $CO_2$ $g^{-1}$ dry soil $day^{-1}$. Incubation was conducted at room temperature to ensure uniform experimental conditions. Substrate-induced respiration (SIR) was measured by stimulating microbial activity through glucose addition. Based on a preliminary optimization experiment, 0.1 g of glucose was added to each soil sample. The $CO_2$ evolved during incubation was trapped in 0.5 N NaOH and quantified by titration with 0.5 N HCl, following the same methodological approach [21].

To determine the optimal glucose concentration, an experiment was conducted under the same conditions as the SIR measurement. Glucose was added at 0, 0.01, 0.1, 0.2, and 0.3 g to soil samples adjusted to 70% of field capacity, with each treatment performed in triplicate. At the end of the incubation, respiratory activity was measured for each glucose level, and the concentration that produced the highest respiratory response was selected as the optimal concentration for SIR measurements (Figure 4).




342

343 **Figure 4.** Optimizing the glucose level for the SIR

344

345 Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform
346 fumigation-extraction technique following [49]. Both Fumigated and non-fumigated soils samples
347 were extracted with 0.5 M $K_2SO_4$. The extracts obtained from fumigation were analyzed for
348 organic carbon and nitrogen using the Walkley–Black and Kjeldahl methods, respectively [50].
349 Microbial biomass C and N were calculated as the difference between fumigated and non-
350 fumigated. Extracts from non-fumigated soils were used to determine dissolved organic carbon
351 (DOC) and nitrogen (DON) [51].

352 To further evaluate microbial activity and efficiency, two indices were calculated. The metabolic
353 quotient ($qCO_2$) was calculated as the ratio of BR to MBC [52]. The microbial quotient (qMic) was
354 calculated as the ratio of MBC to soil organic carbon (SOC) [53].

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## Statistical analyses

Data were tested for normality and homoscedasticity by Kolmogorov-Smirnov and Levene's tests before comparisons between groups. A two-way ANOVA was use to analyzing the effects of crown position and species on soil properties. Following the results of ANOVAs, Duncan's test was applied for post hoc pairwise comparison. ($p < 0.05$), Pearson correlation coefficients were calculated to determine the relationship between parameters. All statistical analyses were performed in SPSS (v.26), and figures were prepared in MS Excel.

## Author Contributions

A.A. performed field sampling, data analyses and wrote the manuscript. V.H. conceived the study, designed the experiment, and wrote the manuscript. M.M.V. conducted the experiment. K.M.S. assisted with field sampling, data interpretation and wrote the manuscript. All authors (A.A., V.H., M.M.V. and K.M.S.) reviewed and edited the manuscript.

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## Ethics declarations

## Competing interests

The authors declare no competing interests.

## Ethics approval

Soil samples were collected from the sacred forest stand at the Namasher with permission from the Baneh County Natural Resources Office. All fieldwork was conducted in compliance with relevant local regulations and guidelines.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**References**


1. Brussaard, L., de Ruiter, P. C. & Brown, G. G. Soil biodiversity for agricultural sustainability. *Agric. Ecosyst. Environ.* **121**, 233–244 (2007). https://doi.org/10.1016/j.agee.2006.12.013

2. Prescott, C. E. The influence of the forest canopy on nutrient cycling. *Tree Physiol.* **22**, 1193–1200 (2002). https://doi.org/10.1093/treephys/22.15-16.1193

3. Hertel, D. Tree roots in canopy soils of old European beech trees an ecological reassessment of a forgotten phenomenon. *Pedobiologia* **54**, 119–125 (2011). https://doi.org/10.1016/j.pedobi.2010.11.003

4. Rostamizad, P., Hosseini, V. & Mohammadi-Samani, K. Effects of Persian turpentine tree litter and slope aspect on soil chemical properties in a Zagros forest, *Iran. J. For. Res.* **31**, 1583–1588 (2020). https://doi.org/10.1007/s11676-019-00950-9

5. Wilson, B. Influence of scattered paddock trees on surface soil properties: a study of the Northern Tablelands of NSW. *Ecol. Manag. Restor.* **3**, 211–219 (2002). https://doi.org/10.1046/j.1442-8903.2002.00115.x

6. Doran, J. W. & Zeiss, M. R. Soil health and sustainability: managing the biotic component of soil quality. *Appl. Soil Ecol.* **15**, 3–11 (2000). https://doi.org/10.1016/S0929-1393(00)00067-6

7. Jansson, J. K. & Hofmockel, K. S. The soil microbiome from metagenomics to metaphenomics. *Curr. Opin. Microbiol.* **43**, 162–168 (2018). https://doi.org/10.1016/j.mib.2018.01.013

8. Staddon, W. J., Duchesne, L. C. & Trevors, J. T. The role of microbial indicators of soil quality in ecological forest management. *For. Chron.* **75**, 81–86 (1999). https://doi.org/10.5558/tfc75081-1

9. Mohammadi-Samani, K., Hosseini, V. & Rostami, H. Physical and chemical properties of soil in sacred groves and surrounding oak woodlands in Baneh County. *J. For. Wood Prod.* **74**, 383–394 (2022) (in Persian). https://doi.org/10.22059/jfwp.2021.317825.1154

10. Plieninger, T., Quintas-Soriano, C., Torralba, M., Mohammadi-Samani, K. & Shakeri, Z. Social dynamics of values, taboos and perceived threats around sacred groves in Kurdistan, Iran. *People Nat.* **2**, 1237–1250 (2020). https://doi.org/10.1002/pan3.10158

11. Aponte, C., García, L., Pérez-Ramos, I., Gutiérrez, E. & Marañón, T. Oak trees and soil interactions in Mediterranean forests: a positive feedback model. *J. Veg. Sci.* **22**, 856–867 (2011). https://doi.org/10.1111/j.1654-1103.2011.01298.x

12. Cesarz, S. et al. Tree diversity effects on soil microbial biomass and respiration are context dependent across forest diversity experiments. Glob. *Ecol. Biogeogr.* **31**, 872–885 (2022). https://doi.org/10.1111/geb.13461

13. Gurmesa, G. A., Schmidt, I. K., Gundersen, P. & Vesterdal, L. Soil carbon accumulation and nitrogen retention traits of four tree species grown in common gardens. *For. Ecol. Manag.* **309**, 47–57 (2013). https://doi.org/10.1016/j.foreco.2013.02.015

14. Han, M., Tang, M., Shi, B. & Jin, G. Effect of canopy gap size on soil respiration in a mixed broadleaved-Korean pine forest: evidence from biotic and abiotic factors. *Eur. J. Soil Biol.* **99**, 103194 (2020). https://doi.org/10.1016/j.ejsobi.2020.103194

15. Bardgett, R. D. & van der Putten, W. H. Belowground biodiversity and ecosystem functioning. *Nature* **515**, 505–511 (2014). https://doi.org/10.1038/nature13855

16. Devi, N. B., Singh, L. I., Yadava, P. S. & Khan, M. R. Shift in microbial biomass, soil and microbial stoichiometry in different land uses of Northeast India. *The Microbe* **3**, 100085 (2024). https://doi.org/10.1016/j.microb.2024.100085

17. Prescott, C. E. & Grayston, S. J. Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *For. Ecol. Manag.* **309**, 19–27 (2013). https://doi.org/10.1016/j.foreco.2013.02.034

18. Sardans, J. & Peñuelas, J. Plant-soil interactions in Mediterranean forest and shrublands: impacts of climatic change. *Plant Soil* **365**, 1–33 (2013). https://doi.org/10.1007/s11104-013-1591-6

19. Anderson, J. P. E. & Domsch, K. H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **10**, 215–221 (1978). https://doi.org/10.1016/0038-0717(78)90098-6

20. Limane, A., Smail-Saadoun, N., Belkebir-Boukais, A. & Kissoum-Hamdini, K. Root architecture adaptation of *Pistacia atlantica* subsp. *atlantica* according to an increasing climatic and edaphic gradient: case of a north-south transect in Algeria. *Turk. J. Bot.* **38**, 536–549 (2014). https://doi.org/10.3906/bot-1308-9

21. Anderson, J. P. E. Soil respiration. In Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties (ed. Page, A. L. et al.) 837–871 (American Society of Agronomy, Madison, 1982).

22. Zarafshar, M., Rousta, M., Matinizadeh, M., Talebi, K., Bordbar, S., Alizadeh, T., Nouri, E. & Bader, M. Scattered wild pistachio trees profoundly modify soil quality in semi-arid woodlands. *CATENA* **224**, 106983 (2023). https://doi.org/10.1016/j.catena.2023.106983

23. Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B. & Matzner, E. Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Sci.* **165**, 277–304 (2000). https://doi.org/10.1097/00010694-200004000-00001

24. McElhinny, C., Lowson, C., Schneemann, B. & Pachón, C. Variation in litter under individual tree crowns: implications for scattered tree ecosystems. *Austral Ecol.* **35**, 87–95 (2010). https://doi.org/10.1111/j.1442-9993.2009.02016.x

25. Zechmeister-Boltenstern, S., Keiblinger, K. M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J. & Wanek, W. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. *Ecol. Monogr.* **85**, 133–155 (2015). https://doi.org/10.1890/14-0777.1

26. Manzoni, S., Trofymow, J. A., Jackson, R. B. & Porporato, A. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecol. Monogr.* **80**, 89–106 (2010). https://doi.org/10.1890/09-0179.1

27. Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S. & Richter, A. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Front. Microbiol.* **5**, 22 (2014). https://doi.org/10.3389/fmicb.2014.00022

28. Sinsabaugh, R. L. et al. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **11**, 1252–1264 (2008). https://doi.org/10.1111/j.1461-0248.2008.01245.x

29. Anderson, T.-H. & Domsch, K. H. The metabolic quotient for $CO_2$ ($qCO_2$) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biol. Biochem.* **25**, 393–395 (1993). https://doi.org/10.1016/0038-0717(93)90140-7

30. Anderson, T.-H. & Domsch, K. H. Application of eco-physiological quotients ($qCO_2$ and qD) on microbial biomasses from soils of different cropping histories. *Soil Biol. Biochem.* **22**, 251–255 (1990). https://doi.org/10.1016/0038-0717(90)90094-G

31. Wardle, D. A. & Ghani, A. A critique of the microbial metabolic quotient ($qCO_2$) as a bioindicator of disturbance and ecosystem development. *Soil Biol. Biochem.* **27**, 1601–1610 (1995). https://doi.org/10.1016/0038-0717(95)00093-T

32. Fioretto, A., Papa, S. & Fuggi, A. Litter-fall and litter decomposition in a low Mediterranean shrubland. *Biol. Fertil. Soils* **39**, 37–44 (2003). https://doi.org/10.1007/s00374-003-0675-5

33. Sinsabaugh, R. L., Carreiro, M. M. & Repert, D. A. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* **60**, 1–24 (2002). https://doi.org/10.1023/A:1016541114786

34. Ait Said, S., Fernandez, C., Greff, S., Derridj, A., Gauquelin, T. & Mevy, J.-P. Inter-population variability of leaf morpho-anatomical and terpenoid patterns of *Pistacia atlantica* Desf. ssp. *atlantica* growing along an aridity gradient in Algeria. *Flora* **206**, 397–405 (2011). https://doi.org/10.1016/j.flora.2010.08.002

35. Hosseini, V., Akhavan, R. & Tahmasebi, M. Effect of pistachio (Pistacia atlantica) canopy on the spatial distribution of soil chemical characteristics (case study: Sarvabad, Kurdistan). *Iran. J. For.* **4**, 13–24 (2012) (in Persian).

36. Fierer, N. & Jackson, R. B. The diversity and biogeography of soil bacterial communities. Proc. Natl. *Acad. Sci. USA* **103**, 626–631 (2006). https://doi.org/10.1073/pnas.0507535103

37. Rousk, J., Brookes, P. & Bååth, E. The microbial PLFA composition as affected by pH in an arable soil. *Soil Biol. Biochem.* **42**, 516–520 (2010). https://doi.org/10.1016/j.soilbio.2009.11.026

38. Caldeira, M. C., Ibáñez, I., Nogueira, C., Bugalho, M. N., Lecomte, X., Moreira, A. & Pereira, J. S. Direct and indirect effects of tree canopy facilitation in the recruitment of Mediterranean oaks. *J. Appl. Ecol.* **51**, 349–358 (2014). https://doi.org/10.1111/1365-2664.12189

39. Silva, I. Jr., Dubeux, M., Santos, M., Mello, A., Cunha, M., Apolinário, V. & Freitas, E. Tree canopy management affects dynamics of herbaceous vegetation and soil moisture in silvopasture systems using arboreal legumes. *Agronomy* **11**, 1509 (2021). https://doi.org/10.3390/agronomy11081509

40. Christel, H. et al. The spatial distribution of tree-tree interaction effects on soil microbial biomass and respiration. *Ecol. Evol.* **14**, e11530 (2024). https://doi.org/10.1002/ece3.11530

41. Mohammadi-Samani, K., Pordel, N., Hosseini, V. & Shakeri, Z. Effect of land-use changes on chemical and physical properties of soil in western Iran (Zagros oak forests). *J. For. Res.* **31**, 637–647 (2020). https://doi.org/10.1007/s11676-018-0799-y

42. Shakeri, Z., Mohammadi-Samani, K., Maarofi, H., Khoonsiavashan, K. & Sharifi, K. Species diversity, life form, and chorotypes of plant species in sacred groves and surrounding silvopastoral woodlands of Northern Zagros, Iran. *Iran. J. For. Poplar Res.* **29**, 101–113 (2021) (in Persian). https://doi.org/10.22092/IJFPR.2021.354366.1998

43. Koosha, N., Mohammadi-Samani, K. & Hosseini, V. Carbon stock and some chemical properties of soil in sacred groves and pollarded forest stands of Northern Zagros forests. *Water Soil* **36**, 579–591 (2022) (in Persian). https://doi.org/20.1001.1.20084757.1401.36.5.4.4

44. Shakeri, Z., Mohammadi-Samani, K., Bergmeier, E. & Plieninger, T. Spiritual values shape taxonomic diversity, vegetation composition, and conservation status in woodlands of the Northern Zagros, *Iran. Ecol. Soc.* **26**, 30 (2021). https://doi.org/10.5751/ES-12290-260130

45. Anonymous. Statistical information of the Meteorological Department of Kurdistan Province, Baneh County (in Persian) (2023).

46. Allen, S. E., Grimshaw, H. M., Parkinson, J. A. & Quarmby, C. Chemical Analysis of Ecological Materials. 565 (Blackwell Scientific Publications, Oxford and London, 1974).

47. McLean, E. O. Soil pH and lime requirement. In Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties (ed. Page, A. L. et al.) 199–224 (American Society of Agronomy, Madison, 1982).

48. Jury, W. A. & Horton, R. Soil Physics. 384 (John Wiley & Sons, Hoboken, 2004).

49. Schinner, F., Öhlinger, R., Kandeler, E. & Margesin, R. Methods in Soil Biology. 426 (Springer, Berlin and Heidelberg, 2012). https://doi.org/10.1007/978-3-642-60966-4

50. Nelson, D. W. & Sommers, L. E. Total carbon, organic carbon, and organic matter. In Methods of Soil Analysis, Part 3: Chemical Methods (ed. Sparks, D. L.) 961–1010 (Soil Science Society of America & American Society of Agronomy, Madison, 1996). https://doi.org/10.2136/sssabookser5.3.c34

51. Jones, D. L. & Willett, V. B. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biol. Biochem*. **38**, 991–999 (2006). https://doi.org/10.1016/j.soilbio.2005.08.012

52. Insam, H. & Haselwandter, K. Metabolic quotient of the soil microflora in relation to plant succession. *Oecologia* **79**, 174–178 (1989). https://doi.org/10.1007/BF00388474

53. Lepcha, N. & Devi, N. Effect of land use, season, and soil depth on soil microbial biomass carbon of Eastern Himalayas. *Ecol. Process*. **9**, 1–4 (2020). https://doi.org/10.1186/s13717-020-00269-y