Opuntia ficus-indica chronosequence affects soil carbon mineralization rates

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Abstract

***Opuntia ficus-indica*, known as cactus pear, is exceptionally well-suited for arid climates, thriving through its crassulacean acid metabolism (CAM) which enhances water use efficiency and enables photosynthesis under drought conditions. This adaptive feature supports both fruit production and use as forage in drought-prone regions such as Africa and South America. This study investigates the impact of orchard age on soil organic carbon (SOC) mineralization across orchards aged 1 to 30 years in Roccapalumba and San Cono, Sicily (Italy). Soil samples from 0- to 15-, 15- to 30-, and 30- to 60-cm layers were incubated at 25°C to measure CO_2 emission as a proxy of microbial activity and the breakdown of organic matter. The results demonstrated that SOC mineralization rates, expressed in mg CO_2-C kg^{-1} soil d^{-1}, tended to increase with the age of the orchard. This enhanced mineralization process is the result of soil C accumulation over time, and it is critical for sustaining soil fertility and promoting sustainable agricultural practices in ecosystems where CAM plants are prevalent. The study highlights the importance of understanding SOC dynamics to optimize soil management practices, aiming to maintain productivity and ecological balance in arid agricultural systems. These findings offer a valuable foundation for designing targeted strategies to improve carbon cycling and soil health, ultimately contributing to sustainable land use in regions characterized by water scarcity.**

Keywords: carbon mineralization, chronosequence, Mediterranean agroecosystems, microbial activity, *Opuntia ficus-indica,* soil organic carbon

INTRODUCTION

Opuntia ficus-indica (L.) Mill. (*OFI*), commonly known as cactus pear, is a strategic crop for agricultural production and ecosystem resilience in arid and semi-arid regions. As a crassulacean acid metabolism (CAM) species, *OFI* exhibits exceptional water-use efficiency and drought tolerance, making it a strategic crop for sustainable agriculture in water-limited environments (Neupane et al., 2021; Quiroz et al., 2021). Thanks to its ability to produce substantial biomass with minimal water input, it serves not only as a valuable food and forage crop but also as a promising option for land restoration and climate change mitigation (Acharya et al., 2019; De Wit et al., 2019).

Beyond its agricultural benefits, *OFI* significantly enhances soil health and fertility. The species contributes to soil organic carbon (SOC) accumulation through root turnover and litter inputs, improving soil structure and increasing nutrient availability in degraded soils (Bariagabre et al., 2016). SOC represents the largest carbon reservoir in terrestrial ecosystems and plays a key role in sustaining soil fertility, enhancing ecosystem resilience, and mitigating climate change (Cotrufo and Lavallee, 2022). Preserving and enhancing SOC stocks is therefore fundamental across various ecosystems to maintain soil productivity and reduce atmospheric CO_2 concentrations. The persistence of SOC is controlled by the balance between organic carbon inputs and losses, with carbon mineralization being a primary process responsible for SOC losses; this changes also in relation to the soil volume available to the *OFI*

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root system (Hassan et al., 2020). SOC mineralization involves both microbial and non-cellular pathways that convert organic matter into CO_2 (Kéraval et al., 2018). This process is regulated by multiple factors, including soil texture, C:N ratio, pH, moisture, and SOC content (Riffaldi et al., 1996), as well as the availability of dissolved organic carbon, which stimulates non-cellular CO_2 release. The chemical composition of organic inputs also affects decomposition with high C:N ratios and elevated lignin or cellulose contents tending to reduce mineralization, while soluble carbon compounds typically enhance decomposition rates (Marzi et al., 2020). Soil mineralogy, particularly the presence of reactive iron oxides, and microbial community activity further regulate whether carbon is lost or stabilized in the soil matrix (Wilhelm et al., 2022).

In arid ecosystems, revegetation has been shown to enhance both SOC mineralization rates and their temperature sensitivity (Q10), with soil phosphorus availability, temperature, and C:N ratio identified as key drivers (Yang et al., 2022). *Q_{10} is an index expressing the temperature sensitivity of SOC mineralization. It indicates how much the rate of carbon mineralization increases with a 10°C rise in temperature. Soil organic carbon exists in two main functional pools including particulate organic matter (POM), which is physically unprotected and rapidly decomposed, and mineral-associated organic matter (MAOM), which forms through microbial processing and binding of soluble compounds to mineral surfaces. While POM is directly subject to mineralization, its decomposition also contributes to the formation of MAOM via microbial transformation and sorption processes. This balance determines the long-term fate of organic matter in soil (Cotrufo et al., 2022; Zhong et al., 2023; Manzoni and Cotrufo, 2024). Although *OFI* is widely acknowledged for its ecological and agronomic benefits, its long-term effects on SOC mineralization in Mediterranean drylands remain poorly understood. In particular, no studies have systematically evaluated the influence of plantation age, soil volume, soil depth, and site-specific factors on carbon turnover under *Opuntia*-based systems (Hassan et al., 2020). This study addresses this gap by investigating SOC mineralization along a chronosequence (1 to 30 years) of *OFI* orchards located in two of the most important cactus pear cultivation areas in Sicily(Italy) – Roccapalumba (Palermo) and San Cono (Catania) – which represent distinct pedoclimatic conditions. To capture vertical variability in carbon dynamics, SOC mineralization was assessed at three soil depths: 0-15, 15-30, and 30-60 cm.

MATERIALS AND METHODS

Study sites and orchard management

The study was conducted in two representative cactus pear cultivation areas in Sicily, Italy: Roccapalumba (Palermo) and San Cono (Catania), which differ in climate, soil characteristics, and orchard management. Roccapalumba features clay-rich soils developed over marly-calcareous substrates, a dry upper meso-Mediterranean climate (561 mm annual rainfall; 15.7°C mean annual temperature) (Bueno et al., 2024), and elevations ranging from 400 to 600 m. San Cono is characterized by well-drained, volcanic-derived soils with comparatively higher fertility, a semi-arid Mediterranean climate (650 mm, 16.5°C; Bellumori et al., 2023), and similar elevation.

Based on the Soil Map of Italy (Costantini et al., 2012), Roccapalumba (unit 35) is mainly composed of Luvisols, Vertisols, Calcisols, Cambisols, Regosols and Phaeozems, while San Cono (unit 36) is dominated by Cambisols, Calcisols, Regosols, Phaeozems, Kastanozems, and Luvisols with varying subtypes. Orchards ranged from 1 to 30 years old. Planting density differed across sites and orchard ages: Roccapalumba ranged from 1000 plants ha^{-1} in young orchards to 333 plants ha^{-1} in older ones, while San Cono had densities from 333 to 2000 plants ha^{-1} depending on age. Minimum tillage was adopted at both sites, except in San Cono's older orchards (20-30 years), where a no-tillage system was used.

Soil sampling and preparation

Soil sampling was conducted in 24 *OFI* orchards, equally divided between Roccapalumba and San Cono (12 orchards per site). Two orchards were selected for each age class (1, 5, 10, 15, 20, and 30 years), following a chronosequence approach to infer temporal changes in soil properties. A stratified sampling design was adopted to account for the known vertical and temporal variability in soil carbon dynamics, following recommendations from previous studies showing improved estimation accuracy and reduced sampling error in SOC-related measurements when using stratification (Rodeghiero and Cescatti, 2008; Potash et al., 2023).

Soil samples were extracted using a gas-powered auger (Blackstone PD530). For each depth interval (0-15, 15-30, and 30-60 cm), three replicate cores were collected and subsequently homogenized to produce a single composite sample per layer.

All samples were placed in sealed polyethylene bags immediately after collection and shipped to the North Florida Research and Education Center (NFREC), University of Florida, for laboratory analysis.

Incubation of samples and CO_2-C mineralized (CMIN) measurement

The incubation experiment followed the soil-test biological activity method described by Franzluebbers (2020) and Franzluebbers et al. (2018), with minor adaptations. For each sample, 50 g of air-dried soil sieved to <2 mm were placed in 50-mL beakers inside 1-L sealed glass jars. Moisture was adjusted to 50% water-filled pore space (WFPS), calculated using the following formula:

$$\text{Water (mL)} = 0.5 \times \text{Porosity (P)} \times \text{Soil Volume (mL)} \quad (1)$$

where P = 1-(bulk density/particle density) and particle density was assumed to be 2.65 Mg m^{-3}.

CO_2 was captured using 10 mL of 1 mol L^{-1} NaOH solution placed in a vial inside each incubation jar. An additional vial with 10 mL of distilled water was included to maintain internal humidity. Jars were incubated at 25±0.5°C for 24 days.

NaOH traps were replaced at days 3, 10, and 24 of incubation. After each interval, the solutions were sealed and titrated with 1 mol L^{-1} HCl after addition of 1.5 mol L^{-1} $BaCl_2$ to precipitate carbonate as $BaCO_3$. Phenolphthalein was used as a pH indicator. The amount of mineralized carbon (CO_2-C) was calculated using the following equation:

$$\text{C-day (mg C kg}^{-1}\text{ soil)} = [(\text{Blank-Sample}) \times \text{mol L}^{-1}\text{ HCl} \times 6\times1000]/\text{soil weight (g)} \quad (2)$$

CO_2 emissions were measured on days 3, 10, and 24 of incubation, with NaOH traps replaced at each interval. Cumulative CO_2 emission over the 24-day incubation period was calculated by summing the values from each interval. To determine the average daily mineralization rate, the total cumulative emission was divided by the duration of the incubation (24 days). The final unit of measurement for the mineralization rate is expressed as mg C kg^{-1} soil day^{-1}. Alkali blanks (without soil) were included at a ratio of 1 per 25 jars.

Statistical analysis

All statistical analyses were performed in R version 4.4.3. Carbon mineralization data (CMIN) were log-transformed [log(CMIN + 1)] to meet assumptions of normality and homoscedasticity. An initial linear mixed-effects model was fitted using the lmer function from the *lme4* package, with site, orchard age, and soil depth as fixed effects, and replicate as a random intercept. Due to a significant site effect ($p<0.001$), separate models were subsequently developed for Roccapalumba and San Cono.

For each site, linear mixed-effect models were constructed using the nlme package, including age, depth, and their interaction as fixed effects, and replicate as a random effect. To account for heteroscedasticity among depth layers, a variance structure was added using the varIdent function. Model residuals were assessed for normality using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Model performance was quantified using marginal and conditional R^2 values from the *MuMIn* package. Table 1 summarizes the statistical models, detailing structure, residual diagnostics, and variance explained.

Table 1. Summary of the linear mixed-effects models used to analyze soil carbon mineralization.

Model	Fixed effects	Random effect	Variance structure	Shapiro-Wilk (p)	Levene's test (p)	R^2 (marginal/ conditional)
Global model	Site × age × depth	1/replicate	VarIdent	0.0589	HC	0.771/0.771
Roccapalumba	Age × depth	1/replicate	VarIdent	0.0584	HC	0.587/0.704
San Cono	Age × depth	1/replicate	VarIdent	0.03199	HC	0.854/0.895

varIdent was applied to Depth; HC = heteroscedasticity corrected.

Pairwise post-hoc comparisons were conducted using Tukey's HSD method to assess differences among orchard ages, soil depths, and age × depth combinations within each site. Post-hoc tests were performed separately for each depth layer to identify within-layer differences across ages.

RESULTS AND DISCUSSION

A global mixed-effects model including site, orchard age, and soil depth as fixed effects revealed a highly significant site effect ($p<0.001$) and a significant effect of age ($p<0.05$), indicating that SOC mineralization varied significantly with orchard age and between sites, while soil depth had no significant effect. No significant interaction terms were detected, but due to the strong site effect, site-specific models were developed to better assess the influence of age and depth within each location.

Depth-wise differences in carbon mineralization

As a first step, post-hoc comparisons were performed to evaluate differences in carbon mineralization among depths within each site. In Roccapalumba, mineralization at 0-15 cm was significantly higher than at both 15-30 and 30-60 cm ($p<0.05$), while no significant differences were observed between the two deeper layers. This is consistent with the typical enrichment of labile carbon substrates and microbial biomass in surface soils, which stimulates microbial respiration (Cotrufo et al., 2022; Wilhelm et al., 2022).

In San Cono, the same pattern was observed, with significantly higher values in the surface layer compared to 15-30 and 30-60 cm ($p<0.05$), and no difference between the other layers.

In addition, mineralization rates were consistently higher in Roccapalumba than in San Cono at all depths. The statistical comparison among all six sites × depth combinations revealed a highly significant effect (* $p<0.001$) (Figure 1), supporting the hypothesis that clay-rich soils, such as those in Roccapalumba, promote greater microbial processing of organic matter likely due to the higher abundance of reactive mineral surfaces and enhanced organo-mineral associations (Wilhelm et al., 2022).

The marked and consistent differences in carbon mineralization across depths further justify the use of site-specific models for Roccapalumba and San Cono. In-depth comparisons of soil layers within each orchard age revealed site-specific stratification patterns.

In Roccapalumba, significant differences among depths were detected only in the 30-year-old orchard, where surface mineralization (0-15 cm) reached 65.00±7.07 mg C kg^{-1} day^{-1}, nearly 1.7 times higher than at 30-60 cm (41.75±6.72 mg C kg^{-1} day^{-1}).

In San Cono, significant vertical differentiation was observed starting from the 15-year-old orchards. At 15 years, carbon mineralization was already higher at 0-15 cm (17.46±0.00 mg C kg^{-1} day^{-1}) compared to 30-60 cm (12.83±0.53 mg C kg^{-1} day^{-1}). This stratification became more evident in the 20-year-old orchard, with surface mineralization nearly doubling that of the subsoil (25.15±3.09 vs. 12.52±2.39 mg C kg^{-1} day^{-1}). These results indicate that, despite the overall lower mineralization rates, San Cono exhibits earlier vertical segregation of labile carbon, likely due to limited downward movement and faster organic matter turnover near the surface. All depth-wise values and statistical groupings are summarized in Table 2.

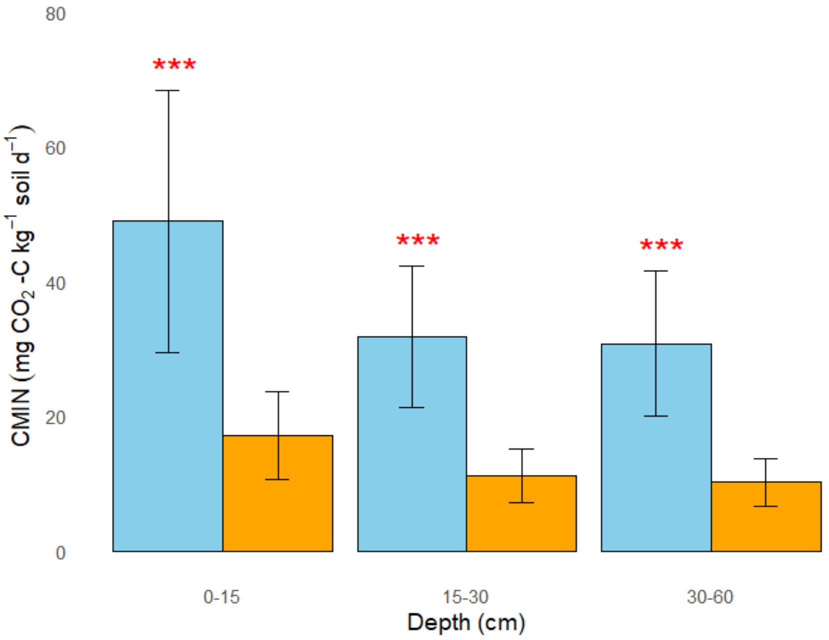

Figure 1. Comparison of mean daily carbon mineralization (± SD) across soil depths (0-15, 15-30, 30-60 cm) in Roccapalumba(light blue) and San Cono (orange). Asterisks indicate overall significance across sites and depths (*** $p<0.001$).

Table 2. Mean daily carbon mineralization (± SD) across soil depths and orchard ages in Roccapalumba and San Cono.

Age	Depth/layer	CMIN – Roccapalumba	CMIN – San Cono
1 year	0-15	37.12±1.24 a	7.65±1.68 a
	15-30	26.12±11.49 a	7.33±0.88 a
	30-60	25.38±9.37 a	7.15±1.68 a
5 years	0-15	24.12±3.36 a	19.21±4.42 a
	15-30	20.88±0.88 a	10.08±1.59 a
	30-60	18.25±0 a	9.02±1.5 a
10 years	0-15	43.25±2.12 a	11.46±0.53 b
	15-30	48.75±4.24 a	7.58±0.35 ab
	30-60	40.5±12.02 a	5.52±1.5 a
15 years	0-15	60.38±26.69 a	17.46±0 c
	15-30	26.25±2.47 a	9.77±0.8 a
	30-60	28.12±8.31 a	12.83±0.53 b
20 years	0-15	63.75±25.1 a	25.15±3.09 b
	15-30	30±1.77 a	16.77±2.39 ab
	30-60	30.38±10.78 a	12.52±2.39 b
30 years	0-15	65±7.07 a	21.96±2.3 a
	15-30	38.88±0.18 b	15.08±3.18 a
	30-60	41.75±6.72 b	14.15±2.21 a

Different letters indicate significant differences among depths within the same orchard age (Tukey's HSD, $p<0.05$).

Model results and comparisons in Roccapalumba

Although the overall mixed-effects model for Roccapalumba did not reveal a significant main effect of orchard age ($p=0.31$), post-hoc comparisons within the 0-15 cm layer indicated significant age-related differences among orchard ages, indicating a progressive increase in carbon mineralization with stand age in surface soils.

At 0-15 cm depth in Roccapalumba, carbon mineralization significantly varied with orchard age (Tukey's HSD). The lowest value was in the 5-year-old orchard (24.12±3.36 mg C kg^{-1} day^{-1}), while the 30-year-old orchard showed the highest (65.00±7.07 mg C kg^{-1} day^{-1}), indicating a clear accumulation of labile carbon in mature stands, likely due to increased biomass input and microbial activity (Figure 2). This trend suggests a progressive enrichment of labile organic carbon in surface soils as plantations mature, likely driven by increased biomass inputs, rhizosphere activity, and microbial turnover over time. This pattern aligns with findings from other perennial or grassland systems, where progressive restoration or stand development over decades leads to increases in labile carbon inputs and microbial activity, particularly in surface soils (Liu et al., 2017; Johansson et al., 2024; Wang et al., 2009).

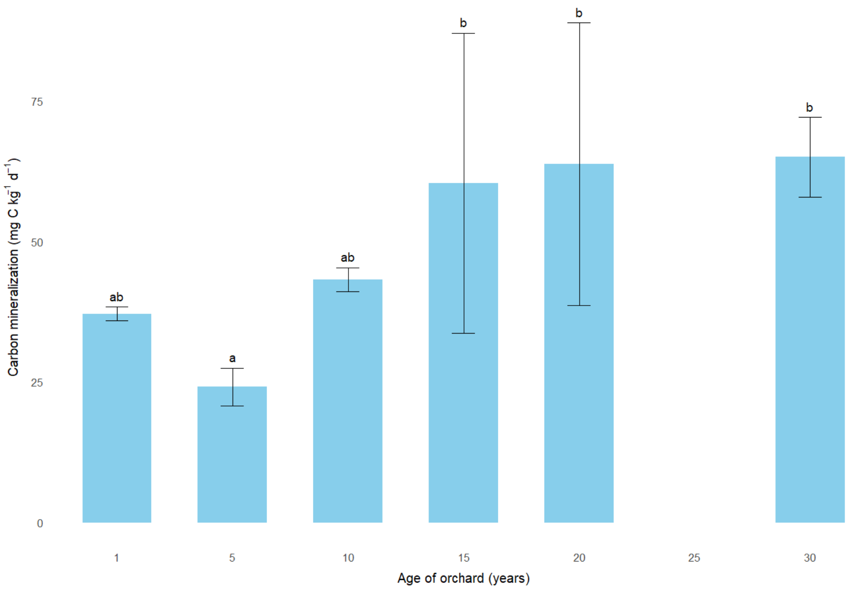

Figure 2. Mean daily carbon mineralization (± SD) at 0-15 cm depth in Roccapalumba across orchard ages. Different letters indicate significant differences among orchard ages within this depth (Tukey's HSD, p<0.05).

At 15-30 cm, significant age-related differences were also observed. Mineralization ranged from 20.88±0.88 mg C kg^{-1} day^{-1} (5 years) to 48.75±4.24 (10 years), with older orchards showing intermediate values. These results suggest a delayed accumulation of labile carbon at intermediate depths, likely driven by progressive root influence and SOC translocation.

At 30-60 cm, no significant differences were detected, but mineralization steadily increased with age – from 18.25 (5 years) to 41.75 mg C kg^{-1} day^{-1} (30 years) – indicating deeper carbon translocation and microbial activity in older stands.

Model results and comparisons in San Cono

The linear mixed-effects model for San Cono revealed significant effects of orchard age (p<0.01) and depth (p<0.001) on SOC mineralization, while the age × depth interaction was not significant (p=0.29). These results indicate that both factors independently contributed to the observed variability in mineralization rates. Pairwise post-hoc comparisons were performed to evaluate differences across orchard ages within each soil depth. At 0-15 cm depth (Figure 3), carbon mineralization rates varied significantly across orchard ages. The lowest value was observed in the 1-year-old orchard (7.65±1.68 mg C kg^{-1} day^{-1}), while the highest occurred in the 20-year-old orchard (25.15±3.09 mg C kg^{-1} day^{-1}). Although the 30-

year-old orchard showed slightly lower mineralization (21.96±2.30 mg C kg^{-1} day^{-1}), values remained substantially higher than in the youngest stands. Similarly, the pattern observed in Roccapalumba was also seen at San Cono, where carbon mineralization rates progressively increased with orchard age, particularly in the 0-15 cm layer.

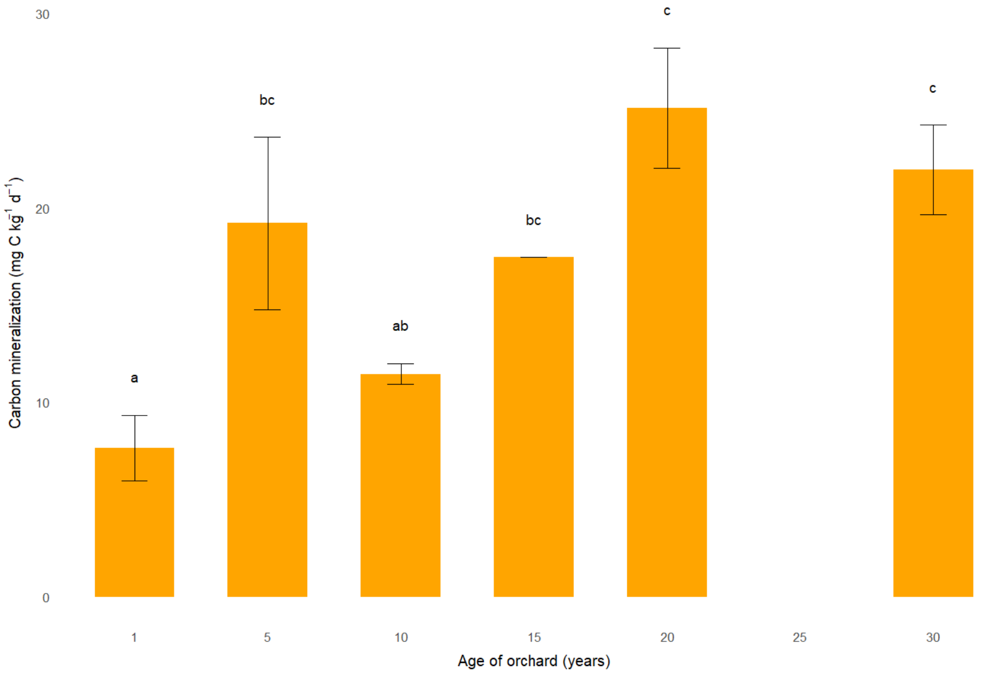

Figure 3. Mean daily carbon mineralization (± SD) at 0-15 cm depth in San Cono across orchard ages. Different letters indicate significant differences among orchard ages within this depth (Tukey's HSD, $p<0.05$).

At 15-30 cm depth, carbon mineralization values ranged from 7.33±0.88 mg C kg^{-1} day^{-1} in the 1-year-old orchard to 16.77±2.39 mg C kg^{-1} day^{-1} in the 20-year-old orchard. Despite some variability, statistical analysis revealed significant differences among orchard ages, with the 20-year-old orchard showing notably higher mineralization than the 1-, 10-, and 15-year-old stands. This suggests a progressive accumulation of labile carbon and microbial activity at intermediate depths, becoming more pronounced after 10 years.

At 30-60 cm depth, carbon mineralization also differed significantly among orchard ages, increasing from 7.15±1.68 mg C kg^{-1} day^{-1} in the 1-year-old orchard to 14.15±2.21 mg C kg^{-1} day^{-1} in the 30-year-old orchard. Post-hoc comparisons indicated higher mineralization in orchards older than 10 years, particularly in the 15-, 20-, and 30-year-old stands, supporting the hypothesis of vertical propagation of labile organic matter over time. These findings confirm the age-related increase in labile carbon and microbial activity, as also observed in Roccapalumba and reported in studies by Liu et al. (2017), Johansson et al. (2024) and Wang et al. (2009)

CONCLUSIONS

This study shows that *Opuntia ficus-indica* cultivation promotes a progressive increase in SOC mineralization with orchard age, particularly in surface soils (0-15 cm), due to cumulative organic inputs and microbial activity. Older orchards (20-30 years) exhibited the highest mineralization rates, especially in Roccapalumba, where turnover extended deeper into the profile. In San Cono, despite overall lower mineralization rates, the age-related increase was consistently evident across all three soil layers, indicating a clear vertical enrichment of labile carbon with time. Unlike other perennial systems – such as citrus orchards – where aging has been linked to SOC loss and reduced soil stability (Cao et al., 2021), *Opuntia*-based systems maintained or enhanced carbon mineralization over time. These findings confirm its potential as a resilient, low-input crop for improving soil quality and carbon cycling in arid environments.

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