https://doi.org/10.17221/106/2024-PSE

Rhizosphere and non-rhizosphere soil organic carbon and its labile fractions in alpine desertified grassland affected by vegetation restoration

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Citation: Zhou C.Y., Qian H.Y., Liu A.Y., Hu Y.F., Wang W., Chen G., Li Z. (2024): Rhizosphere and non-rhizosphere soil organic carbon and its labile fractions in alpine desertified grassland affected by vegetation restoration. Plant Soil Environ., 70: 523–533.

Abstract: Grasslands are the predominant land use type in China, which is currently encountering significant desertification issues. Consequently, restoring grassland vegetation has important implications for terrestrial carbon (C) levels and, consequently, the global C balance. This study focused on *Salix cupularis*, the primary plant used for desert control on the eastern edge of the Qinghai-Tibet Plateau. We analysed the rhizosphere and non-rhizosphere soil up to the depth of 60 cm after *Salix cupularis* growth for 0–24 years, examining soil total organic carbon (TOC) and its labile fractions. Following restoration, there was a gradual increase in TOC and its labile fractions, with the most significant changes observed in the rhizosphere soil at a depth of 0–20 cm. After 24 years of restoration, the TOC content in both rhizosphere and non-rhizosphere soil had increased by 141.74% and 39.44%, respectively. Labile organic C in the rhizosphere soil increased more rapidly and pronouncedly compared with the TOC. Specifically, dissolved organic C and easily oxidised organic C in the rhizosphere soil saw substantial increases of 211.03% and 217.65%, respectively. Meanwhile, compared with the 4 years of restoration, soil C pool management index of the 8–24 years soils increased, ranging from 15.70% to 132.21%. Therefore, long-term vegetation restoration on the eastern margin of the Qinghai-Tibet Plateau can significantly enhance TOC and its labile fractions, as well as improve soil C sink capacity and quality.

Keywords: ecological restoration; ecosystem; soil labile carbon; carbon pool management index; soil depth

Soil total organic carbon (TOC) is a critical factor in assessing soil fertility, which serves as a key indicator of ecosystem service levels (Bationo et al. 2007). Labile organic carbon (LOC) primarily originates from the decomposition of animals and plant remains by microorganisms, secretions from crop roots, and simple organic acids (Panchal et al. 2022). LOC reflects the rate of organic matter (OM) mineralisation, decomposition, and microbial activity in the soil, which has the characteristics of short turnover time and rapid turnover rate. It is more responsive to soil environmental changes compared with TOC (Wu et al. 2020). The carbon pool management index (CPMI), which considers both the total amount and activity of the soil carbon (C) pool, is commonly used to characterise the soil C pool under varying environmental conditions (Jiang et al. 2021). Consequently, the use of LOC and CPMI to investigate the impact of environmental changes on the soil C pool has emerged as a prominent research focus on soil and environmental science (Bationo et al. 2007, Jiang et al. 2021).

Supported by the National Natural Science Foundation of China, Project No. 42077046; by the Sichuan Science and Technology, Project No. 2022YFS0469, and by the Sichuan Agricultural University Research Interest Program, Project No. 20241397.

https://doi.org/10.17221/106/2024-PSE

The rhizosphere, identified as the microenvironment where plants exert the most significant on soil impact, serves as a crucial site for material and energy exchange with the surrounding environment (Zhao et al. 2022a). Through processes such as root secretions, rhizosphere deposits and nutrient cycles, plants modify rhizosphere soil properties and microbial community structures, consequently influencing TOC fractions (Zhao et al. 2022b). Research has shown that the rhizosphere effect plays a pivotal role in regulating the soil C cycle (Brzostek et al. 2013). It has been observed that OM mineralisation can either be suppressed by 50% or stimulated by 380%, thereby impacting the TOC sequestration (Cui et al. 2023). However, the precise mechanisms governing the sequestration of TOC in the rhizosphere, influenced by plant C input and OM mineralisation, remain unclear. Therefore, investigating the characteristics of nutrients and TOC fractions in both rhizosphere and non-rhizosphere soils is crucial for understanding the interactions between vegetation, microbes and soil environments, as well as for studying the C sequestration effects during vegetation growth (Li et al. 2020, Cui et al. 2023).

The alpine grasslands in northwestern Sichuan, located on the eastern margin of the Tibetan Plateau, play a crucial role in protecting water resources and ecological security (Ma et al. 2020). Desertification of grasslands has seriously threatened the ecological environments, biodiversity, and livestock economic development of the Northwestern Plateau in Sichuan, China (Ma et al. 2020). The *Salix cupularis*, which is a deciduous small shrub belonging to the *Salix* genus of the Willow family, stands out for its cold and drought-resistant characteristics (Li et al. 2021). In recent years, the *Salix cupularis* Rehder has been used as a sand-fixing plant and is widely applied in ecological restoration on alpine sandy regions in Northwestern Sichuan, China, with notable results (Li et al. 2021, Hu et al. 2022). However, existing research mostly focuses on the impact and ecological environmental effects of the ecological restoration, while studies on the *Salix cupularis* adaptability to cold sandy soil and its impact on LOC fraction changes are relatively limited. This study investigated the changes in the *Salix cupularis* rhizosphere and non-rhizosphere TOC and its labile fractions at different depths of 0–60 cm, as affected by the long-term restoration after 4, 8, 16 and 24 years on alpine grasslands at the eastern edge of the Qinghai-Tibet Plateau, China.

MATERIAL AND METHODS

Study site and land history. The study area is situated on the Restoration Demonstration region of the degraded grassland in Hongyuan County (33°1'N, 102°37'E), China, at the eastern margin of the Tibetan Plateau (Figure 1). The average elevation in this region exceeds 3 400 m a.s.l. Annual precipitation averages 791.95 mm, with approximately 60–75% occurring from May to September. The mean annual temperature is 1.1 °C, with the coldest and warmest monthly averages of –10.3 °C and 10.9 °C, respectively. The soil is classified as cambic aerosol (FAO 2006), characterised by sandy texture, loose structure, and low nutrient content. The restoration demonstration area features gently undulating moving, semi-moving, and semi-fixed dunes, covering 35.56 ha. The dominant shrub vegetation species in the recovery area are mainly *Salix cupularis*. There were hardly any plants before treatment. In this restoration region, the cuttings of *Salix cupularis*

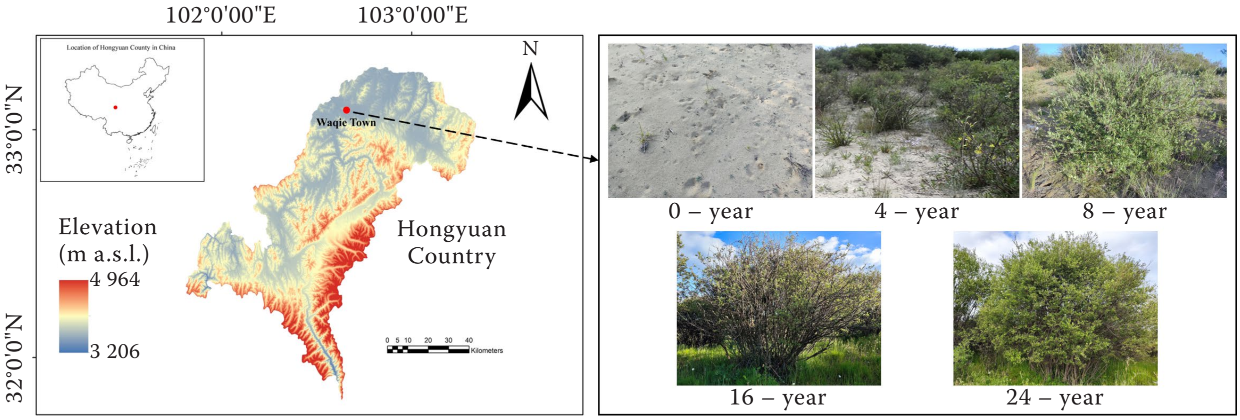

Figure 1. Location of the study area and images of different age of *Salix cupularis*

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Table 1. Sampling point information of each sample and growth of *Salix cupularis*

Years	Serial number	Elevation (m a.s.l.)	Slop (°)	Height (cm)	Crown breat (cm)
0	CK	3 420	< 5°	–	–
4	R/NR	3 416	< 5°	69–74	73–78
8	R/NR	3 422	< 5°	111–118	113–122
16	R/NR	3 418	< 5°	178–183	165–178
24	R/NR	3 417	< 5°	227–235	220–229

CK – control; R – rhizosphere; NR – non-rhizosphere

were planted in holes along rows at intervals of 2.5 m. The rows were spaced 1–2 m apart for each *Salix cupularis* cutting. The fence + banning grazing was established to reduce the transportation of sand and fix it in place, and grasses (e.g., *Elymus nutans* Griseb. and *Avena sativa* L.) were planted in a mixed arrangement in the intervals between *Salix cupularis* in the initial stage of recovery. After plants had been successfully established, the sandy lands were left alone to restore naturally, without human management or disturbance. Basic information on *Salix cupularis* in different planting years is presented in Table 1. From 1996 to 2020, the average annual temperature and rainfall in the study area showed an increasing trend year by year (Figure 2).

Soil sampling and parameters analysis. A time-for-space substitution approach was used. The *Salix cupularis* trees (4, 8, 16 and 24 years), growing naturally under enclosed grazing prohibition conditions with roughly uniform environmental conditions, were selected as subjects of research (Figure 1). Randomly, a 20 m × 20 m plot was set up in the *Salix cupularis* growth area for each restoration year, ensuring a distance greater than 50 m between the plots. Within each plot, three *Salix cupularis* trees, similar in height and crown width, were chosen for experiment replication, ensuring a distance greater than 5 m between them. Simultaneously, soil samples from unfenced, unrestored bare sand land served as the control (CK). As no *Salix cupularis* rhizosphere existed in the unrestored land, CK can also be served as both rhizosphere and non-rhizosphere soil samples. In June 2020, rhizosphere soil was collected by using the shaking-off method (Singh and Dubey 2012). The specific method is consisted of selecting areas where the *Salix cupularis* root system was densely distributed, digging a 60 cm soil profile along the base of the *Salix cupularis*, and dividing the soil layers into 0–20, 20–40, and 40–60 cm. Areas within the tree crown projection range where the roots of the *Salix cupularis* were densely distributed were dug out for each soil layer. Large soil

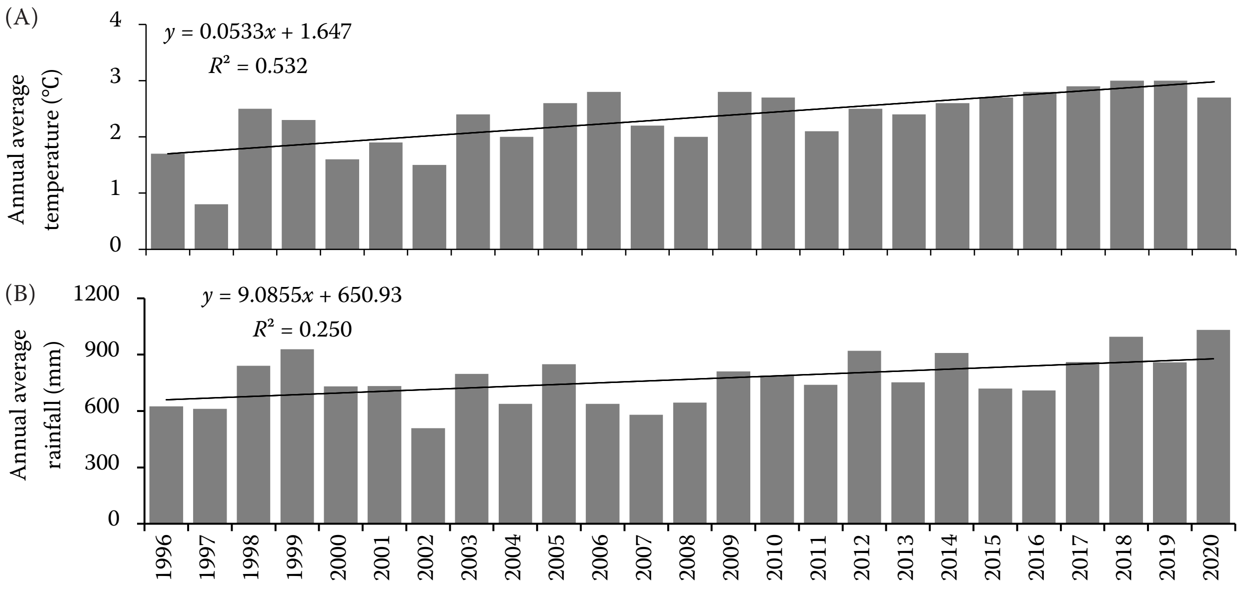

Figure 2. Variation characteristics of annual average (A) temperature and (B) rainfall from 1996 to 2020

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chunks without roots were carefully shaken off, and small roots with a diameter of less than 2 mm were picked out. A sterile brush was used to swiftly brush the remaining soil on the small root surfaces into a sealable bag, representing the rhizosphere soil. Rhizosphere and non-rhizosphere soils were each sampled in two portions one portion dried and sieved through a 0.15 mm sieve for measuring TOC, dissolved organic carbon (DOC), easily oxidisable organic carbon (EOC), particulate organic carbon (POC), total nitrogen (TN), total phosphorus (TP) and available phosphorus (AP), another portion preserved on dry ice in a 4 °C thermostatic carrier for measuring pH, soil water content (SWC), and microbial biomass carbon (MBC).

The moist soil sample was oven-dried at 105 °C for 24 h to measure the SWC. The soil pH (soil:water; 1:2.5) was determined by a combination electrode. The TOC was measured using the potassium dichromate external heating method. The soil TN was measured using the semi-micro Kjeldahl method. The soil TP was measured by the sulfuric acid-perchloric acid digestion method, and AP was measured by using the molybdenum-antimony colourimetric method (Lu 2000). The POC was obtained by dispersing soil samples in 5 g/L $(NaPO_3)_6$ solution for 18 h and then passing them through a 0.053 mm sieve using a flow of distilled water (Cambardella and Elliott 1992). The DOC was obtained by successively extracting soil samples with distilled water (Liang et al. 1998). The EOC was determined by using the $KMnO_4$ (333 mmol/L) oxidation procedure (Lefroy et al. 1993).

Calculations and statistical analysis. The CPMI is calculated using the methodology used by Blair et al. (1995). We selected CK as the reference.

$$\mathrm{CPI} = \frac{\text{TOC in the treatment}}{\text{TOC in the CK}} \quad (1)$$

$$\mathrm{CPA} = \frac{\text{EOC}}{(\text{TOC} - \text{EOC})} \quad (2)$$

$$\mathrm{CPAI} = \frac{\text{CPA in the treatment}}{\text{CPA in the CK}} \quad (3)$$

$$\mathrm{CPMI} = \mathrm{CPI} \times \mathrm{CPAI} \times 100 \quad (4)$$

In the calculation formula of CPMI, CPA, CPI, and CPAI are all index parameters reflecting the carbon turnover rate, while CPMI reflects the stability of the soil carbon pool. The higher the value of CPMI, the stronger the stability of the carbon pool and the higher the soil quality.

Statistical analysis was done with SPSS 20.0 (Chicago, USA). A one-way ANOVA following Fisher's *LSD* (least significant difference) test was applied to compare differences among treatment means at $P < 0.05$. Pearson's method was used to analyse the correlation between the data.

RESULTS

Changes in TOC content and profile distribution occur during different restoration years. Experimental results indicate that as the years of restoration increase, the TOC content in the rhizosphere soil shows an upward trend across different soil layers (0–20, 20–40, and 40–60 cm). The most significant increase is observed in the 0–20 cm soil layer, with TOC content reaching 1.83, 2.19, and 2.78 g/kg after 8, 16, and 24 years of restoration and representing a substantial increase compared with the control group (CK), respectively. Similar trends are observed in the 20–40 cm and 40–60 cm soil layers. Furthermore, significant differences in TOC content are observed between rhizosphere and non-rhizosphere soils across all layers, with rhizosphere TOC consistently higher after restoration periods. These findings highlight the positive impact of *Salix cupularis* restoration on increasing TOC content in rhizosphere soil (Figure 3A).

Changes in DOC content and profile distribution occur during different restoration years. The results of the experiment indicate that with years of restoration increasing, DOC content in the rhizosphere soil shows a consistent increase across different soil layers (Figure 3B). Particularly in the 0–20 cm soil layer, the DOC content significantly rises after 4, 8, 16, and 24 years of restoration, reaching 9.09, 10.73, 15.79, and 21.71 mg/kg and representing a substantial increase compared with the control group (CK), respectively. Similar trends are observed in the 20–40 cm and 40–60 cm soil layers, with notable increases in DOC content with increasing restoration years. The study also highlights significant differences in DOC content between rhizosphere and non-rhizosphere soil layers, particularly in the 0–20 cm and 20–40 cm layers. These findings suggest that the restoration of *Salix cupularis* significantly enhances the DOC content in the rhizosphere soil.

Changes in EOC content and profile distribution occur during different restoration years. The experimental results indicate that with restoration years increased, the EOC content in rhizosphere soil exhibits an upward trend across different soil layers (0–20, 20–40, and 40–60 cm). Particularly in the 0–20 cm

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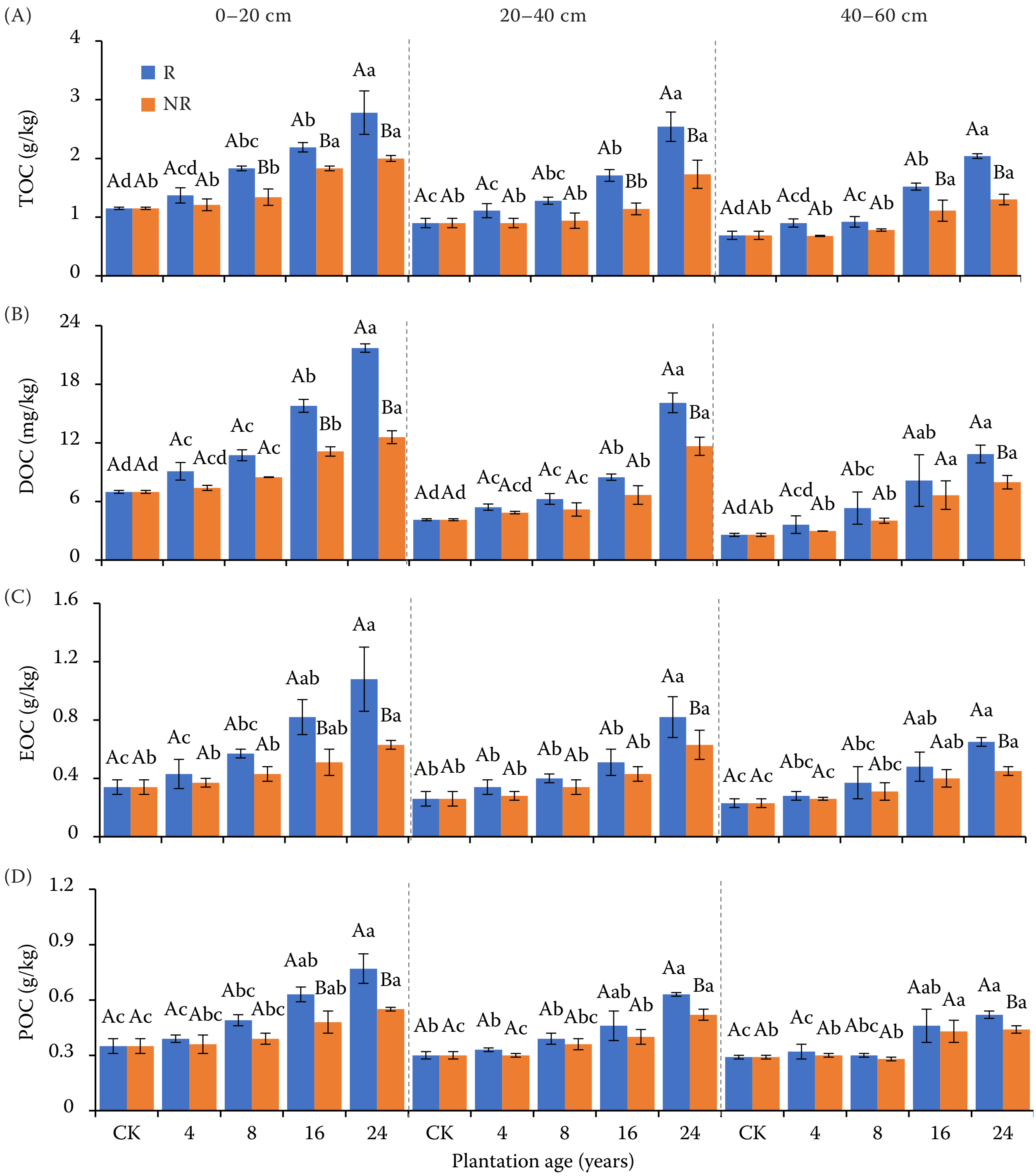

Figure 3. (A) Total organic carbon (TOC); (B) dissolved organic carbon (DOC); (C) easily oxidisable organic carbon (EOC) and (D) particulate organic carbon (POC) of rhizosphere and non-rhizosphere soil to different age of *Salix cupularis*. Different lowercase letters indicate significant differences between years ($P < 0.05$). Different capital letters indicate significant differences between the rhizosphere (R) and non-rhizosphere (NR) ($P < 0.05$); CK – control

soil layer, the EOC content significantly increases after 16 and 24 years of restoration, measuring 0.82 g/kg and 1.08 g/kg and representing a 141.18% and 217.65% increase compared to the CK ($P < 0.05$), respectively. These findings suggest that the restoration of *Salix cupularis* significantly enhances the EOC content in rhizosphere soil. Additionally, as the soil layer depth increases, the EOC content in

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rhizosphere soil decreases, with a widening difference between superficial and deeper layers, highlighting the pronounced impact of *Salix cupularis* restoration on enhancing EOC content in the superficial soil rhizosphere. Specifically, in the 0–20 cm soil layer, the rhizosphere EOC is significantly higher than the non-rhizosphere after 16 and 24 years of restoration, showing increases of 61.15% and 72.72%, respectively. In the 20–40 cm and 40–60 cm soil layer, the difference between rhizosphere and non-rhizosphere EOC is only significant after 24 years of restoration, with rhizosphere EOC exceeding non-rhizosphere by 31.80% and 43.74%, respectively ($P < 0.05$) (Figure 3C).

Changes in POC content and profile distribution occur during different restoration years. As shown in Figure 3D, with the increase in restoration years, the POC content in rhizosphere soil shows a notable upward trend across different soil layers. Particularly in the 0–20 cm layer, the POC content significantly surpasses the control group after 16 and 24 years of restoration, reaching 0.63 g/kg and 0.77 g/kg, respectively ($P < 0.05$). These results indicate that the restoration of *Salix cupularis* leads to a substantial enhancement in POC content in rhizosphere soil. Furthermore, the difference in POC content between rhizosphere and non-rhizosphere soils is significant, particularly in the 0–20 cm layer after 16 and 24 years of restoration, with the rhizosphere POC exceeding non-rhizosphere by 30.44% and 40.34%, respectively ($P < 0.05$). In the deeper soil layers (20–40 cm and 40–60 cm), the rhizosphere POC content also shows significant increases compared with non-rhizosphere after 24 years of restoration, indicating the positive impact of *Salix cupularis* restoration on POC levels.

Changes in MBC content and profile distribution occurred during different restoration years. The results presented in Figure 4 demonstrate a consistent increase in MBC content in the rhizosphere soil across different soil layers with increasing restoration years. Particularly, the MBC content in the 0–20 cm soil layer shows a substantial rise compared with the CK after 4, 8, 16 and 24 years of restoration, reaching values of 118.64, 151.91, 163.16, and 232.09 mg/kg, respectively. These represent significant increases of 31.79, 68.75, 81.25, and 157.82% compared with the control ($P < 0.05$). These findings indicate that the restoration of *Salix cupularis* significantly enhances the MBC content in the rhizosphere soil. Furthermore, a significant difference in MBC content between rhizosphere and non-rhizosphere soils is observed in the 0–20 cm and 20–40 cm soil layers, with rhizosphere MBC consistently higher in both cases ($P < 0.05$). Specifically, in the 0–20 cm layer, rhizosphere MBC is significantly greater than non-rhizosphere after 16 and 24 years of restoration, exceeding 36.70% and 56.95%, respectively. Similarly, in the 20–40 cm layer, rhizosphere MBC is significantly higher than non-rhizosphere after 24 years of restoration, surpassing 29.84% ($P < 0.05$). However, no significant difference in MBC is observed between rhizosphere and non-rhizosphere soils in the 40–60 cm layer.

Changes in CPMI at different restoration years. As shown in Figure 5, with CK as a reference, there is no significant trend in the change of CPA and CPAI in both rhizosphere and non-rhizosphere soils with the increase of restoration years. Both CPI and CPMI in rhizosphere and non-rhizosphere soils increase gradually with more years of restoration, reaching significance ($P < 0.05$) after 16 years of cultivation. Compared with 4 years, the rhizosphere soil's CPI and CPMI for 8 to 24 years increased by 16.55–120.15% and 24.79–132.21%, which were both

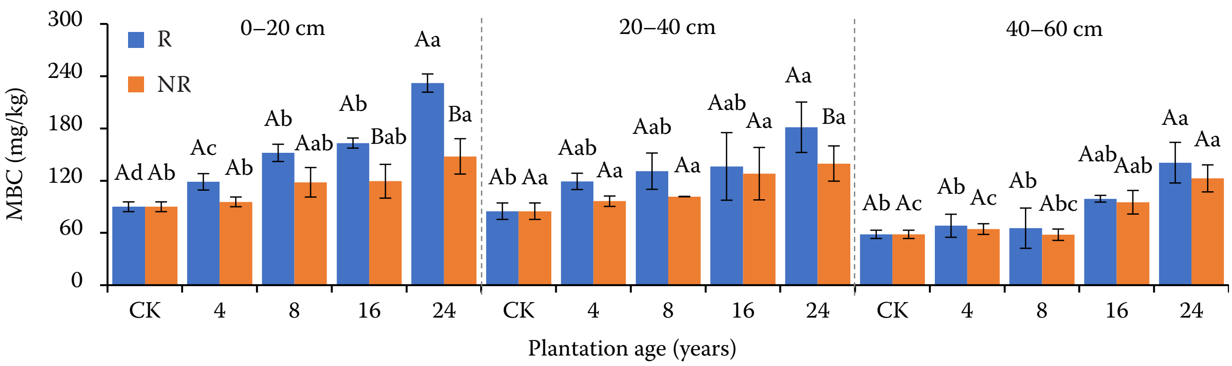

Figure 4. Microbial biomass carbon (MBC) of rhizosphere and non-rhizosphere soil to different age of *Salix cupularis*. Different lowercase letters indicate significant differences between years ($P < 0.05$). Different capital letters indicate significant differences between the rhizosphere (R) and non-rhizosphere (NR) ($P < 0.05$); CK – control

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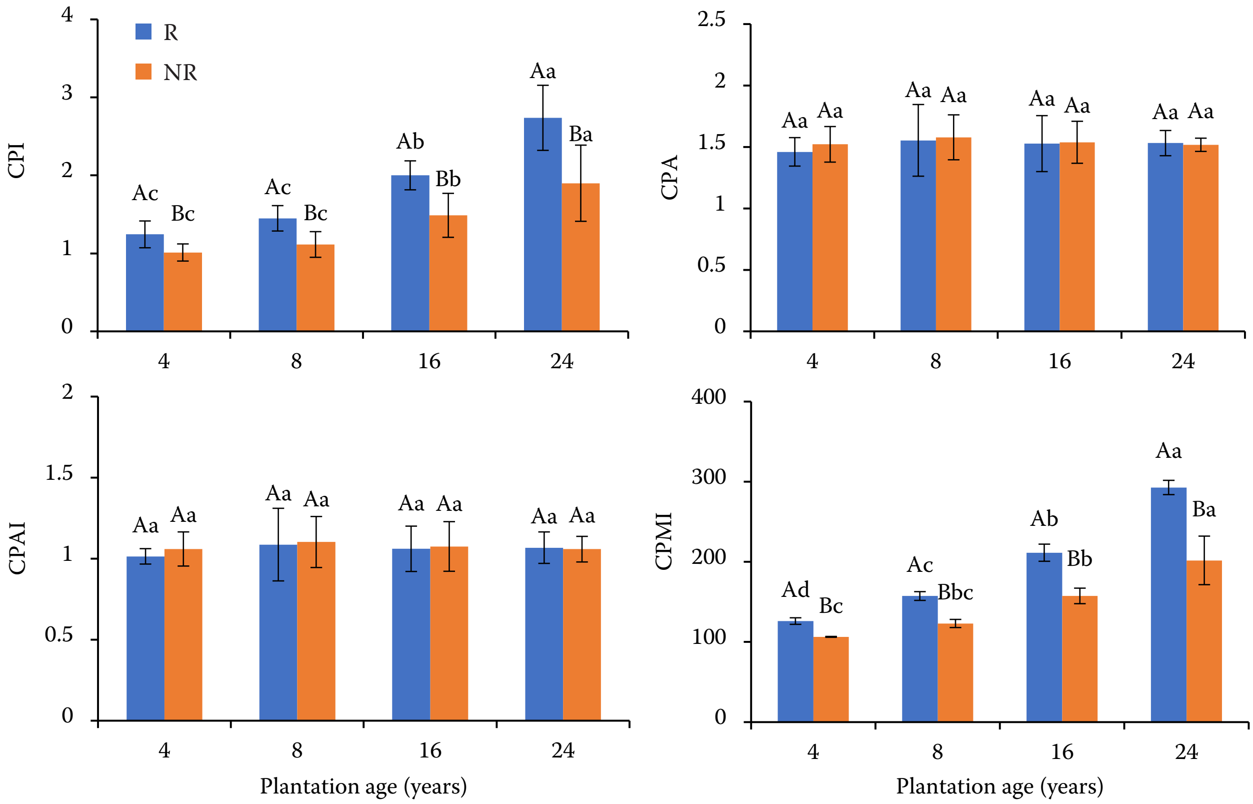

Figure 5. Carbon pool management index (CPMI) of rhizosphere and non-rhizosphere soil to different age of *Salix cupularis*. Different lowercase letters indicate significant differences between years ($P < 0.05$). Different capital letters indicate significant differences between the rhizosphere (R) and non-rhizosphere (NR) ($P < 0.05$). CPI – carbon pool index; CPA – carbon pool activity; CPAI – carbon pool activity index

significantly higher than those in non-rhizosphere soils, respectively.

Correlation of soil total carbon and its labile fractions with other factors. It can be seen from Table 3 that soil TOC and its labile fractions are significantly positively correlated with SWC, TN, TP and AP, and negatively correlated with pH, temperature and rainfall (except TOC and temperature). SWC, TN, TP, AP, pH, temperature and rainfall have a higher influence on labile carbon fractions than TOC.

DISCUSSION

The TOC main sources of input include the decomposition of surface litter with the participation of microorganisms, the amount of carbon-containing OM secreted by the root system and the number of fallen objects during plant growth and development (Finzi et al. 2015, Pausch and Kuzyakov 2018). The output process mainly involves soil respiration dissipation and loss of carbon-containing substances due to surface runoff and infiltration after precipitation, with the participation of soil microorganisms, soil animals, and plant root systems (Finzi et al. 2015, Pausch and Kuzyakov 2018). In this study, with the increase of restoration years, the TOC content in rhizosphere and non-rhizosphere soils showed a significant upward trend in the 0–20 cm soil layer, and the TOC content in rhizosphere soil was significantly higher than in non-rhizosphere. Chen et al. (2023) found in a study of grasslands in the Inner Mongolia Plateau in China that long-term ecological restoration measures can increase the TOC content of semi-arid grasslands in Northern China. He et al. (2022) found in a study on karst in Southwest China that long-term vegetation restoration improves the accumulation of TOC, which is consistent with the changing trend in this study. This may be due to the increase of soil nutrient content (SWC, TN, TP and AP) with the increase of restoration years (Tables 2–3), promoting plant growth, increasing accumulation of plant litter, and increasing in soil animals and microorganisms under the forest, which in combination lead to the input of OM higher than the loss of

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Table 3. Pearson's correlation coefficients for soil total carbon and its labile fractions with other parameters of *Salix cupularis*

Index	SWC	pH	TN	TP	AP	Temperature	Rainfall
TOC	0.415**	–0.076	0.211*	0.248*	0.223*	–0.069	–0.359**
DOC	0.751**	–0.673**	0.628**	0.765**	0.840**	–0.639**	–0.631**
EOC	0.652**	–0.729**	0.500**	0.682**	0.791**	–0.628**	–0.645**
POC	0.692**	–0.702**	0.533**	0.687**	0.791**	–0.661**	–0.665**
MBC	0.587**	–0.565**	0.446**	0.714**	0.726**	–0.535**	–0.571**

*P < 0.05; **P < 0.01; SWC – soil water content; TN – total nitrogen; TP – total phosphorus; AP – available phosphorus; TOC – total organic carbon; DOC – dissolved organic carbon; EOC – easily oxidisable organic carbon; POC – particulate organic carbon; MBC – microbial biomass carbon

Table 2. Soil physicochemical properties in the different reclamation years

Location	Year	Depth (cm)	SWC (%)	pH	TN (g/kg)	TP (g/kg)	AP (mg/kg)
R/NR	0	0–20	7.68 ± 0.50^{c}	7.31 ± 0.04^{a}	0.21 ± 0.01^{d}	146.63 ± 2.79^{d}	7.94 ± 0.14^{d}
		20–40	6.45 ± 0.22^{b}	7.20 ± 0.04^{a}	0.20 ± 0.01^{a}	143.66 ± 5.30^{b}	7.79 ± 0.45^{c}
		40–60	5.60 ± 0.56^{b}	7.13 ± 0.05^{a}	0.19 ± 0.00^{a}	141.84 ± 4.43^{b}	7.84 ± 0.37^{b}
R	4	0–20	10.63 ± 0.52^{b}	6.99 ± 0.04^{b}	0.24 ± 0.00^{c}	174.94 ± 4.60^{c}	8.77 ± 0.18^{d}
		20–40	7.76 ± 0.73^{ab}	6.95 ± 0.07^{b}	0.20 ± 0.00^{a}	163.18 ± 3.50^{ab}	8.38 ± 0.62^{c}
		40–60	6.85 ± 0.44^{ab}	6.90 ± 0.05^{b}	0.19 ± 0.01^{a}	145.96 ± 2.04^{ab}	7.76 ± 0.36^{b}
	8	0–20	11.60 ± 0.84^{b}	6.86 ± 0.08^{b}	0.25 ± 0.00^{bc}	185.12 ± 11.83^{bc}	10.75 ± 0.61^{c}
		20–40	8.02 ± 0.88^{ab}	6.91 ± 0.05^{b}	0.21 ± 0.02^{a}	174.32 ± 1.59^{a}	9.42 ± 0.66b^{c}
		40–60	7.10 ± 0.46^{ab}	6.86 ± 0.06^{bc}	0.18 ± 0.01^{a}	151.09 ± 2.24^{ab}	8.74 ± 0.70^{b}
	16	0–20	11.80 ± 0.75^{b}	6.64 ± 0.00^{c}	0.28 ± 0.00^{ab}	209.93 ± 11.63^{ab}	13.54 ± 0.59^{b}
		20–40	9.32 ± 0.66^{ab}	6.70 ± 0.09^{c}	0.20 ± 0.02^{a}	178.18 ± 6.34^{a}	11.28 ± 1.31^{ab}
		40–60	8.19 ± 0.59^{a}	6.69 ± 0.07^{c}	0.18 ± 0.00^{a}	155.69 ± 9.30^{ab}	10.29 ± 1.06^{ab}
	24	0–20	14.29 ± 0.89^{a}	6.46 ± 0.08^{c}	0.30 ± 0.00^{a}	231.27 ± 5.64^{a}	16.71 ± 0.76^{a}
		20–40	11.51 ± 2.74^{a}	6.61 ± 0.04^{c}	0.19 ± 0.01^{a}	181.21 ± 12.54^{a}	13.52 ± 0.59^{a}
		40–60	8.10 ± 0.65^{a}	6.67 ± 0.05^{c}	0.19 ± 0.01^{a}	159.18 ± 2.98^{a}	12.23 ± 1.23^{a}
NR	4	0–20	9.43 ± 1.56^{a}	7.03 ± 0.05^{b}	0.21 ± 0.01^{b}	158.39 ± 4.40^{b}	8.12 ± 0.43^{c}
		20–40	7.39 ± 0.59^{a}	7.02 ± 0.10^{ab}	0.18 ± 0.01^{a}	148.84 ± 6.26^{a}	7.76 ± 0.34^{c}
		40–60	6.56 ± 0.05^{b}	7.04 ± 0.10^{a}	0.17 ± 0.01^{a}	140.25 ± 4.34^{a}	7.38 ± 0.22^{cd}
	8	0–20	10.91 ± 2.35^{a}	6.98 ± 0.02^{ab}	0.22 ± 0.01^{ab}	176.60 ± 15.53^{ab}	9.75 ± 1.16^{bc}
		20–40	7.06 ± 0.27^{a}	6.99 ± 0.05^{ab}	0.19 ± 0.02^{a}	152.30 ± 12.71^{a}	8.74 ± 0.37^{bc}
		40–60	6.53 ± 0.13^{b}	7.03 ± 0.12^{a}	0.17 ± 0.01^{a}	139.83 ± 8.54^{a}	8.74 ± 0.50^{bc}
	16	0–20	11.24 ± 0.52^{a}	6.94 ± 0.06^{ab}	0.24 ± 0.01^{ab}	193.25 ± 4.53^{a}	11.51 ± 0.39^{ab}
		20–40	8.94 ± 0.89^{a}	6.91 ± 0.06^{c}	0.19 ± 0.01^{a}	154.50 ± 17.35^{a}	10.19 ± 0.58^{b}
		40–60	7.75 ± 0.44^{a}	6.92 ± 0.03^{a}	0.17 ± 0.01^{a}	135.71 ± 7.56^{a}	9.63 ± 0.32^{b}
	24	0–20	12.21 ± 0.65^{a}	6.88 ± 0.03^{c}	0.24 ± 0.00^{a}	190.77 ± 13.65^{a}	13.70 ± 0.90^{a}
		20–40	9.72 ± 2.05^{a}	6.89 ± 0.02^{c}	0.18 ± 0.01^{a}	163.83 ± 6.26^{a}	12.05 ± 0.92^{a}
		40–60	7.76 ± 0.22^{a}	6.95 ± 0.02^{a}	0.17 ± 0.01^{a}	141.80 ± 4.83^{a}	11.01 ± 0.53^{a}

SWC – soil water content; TN – total nitrogen; TP – total phosphorus; AP – available phosphorus. Values represent mean ± standard deviation; different lowercase letters indicate significant differences among the restoration years at P < 0.05. R – rhizosphere; NR – non-rhizosphere

https://doi.org/10.17221/106/2024-PSE

mineralisation and decomposition, resulting in the increase of TOC content (He et al. 2022). At the same time, the *Salix cupularis* has a large root system, and its root secretions, fallen root hairs, and root epidermal cells are important factors influencing the TOC in its rhizosphere soil. The rhizodeposits and carbon-containing organic substances in the root secretions of the *Salix cupularis* supplement the TOC in its rhizosphere soil, which is likely the main reason why its rhizosphere TOC content is higher than that in non-rhizosphere soil (Huang et al. 2022).

The LOC accounts for only a small proportion of TOC, which is easy to oxidise and mineralise and turns over quickly, mainly including DOC, EOC, POC, and MBC (Cai et al. 2023). Results show that the vegetation restoration promotes the increase of DOC, EOC, POC and MBC content in rhizosphere and non-rhizosphere soils of *Salix cupularis*, which is consistent with the overall trend of TOC change. This is in line with the research results of Wang et al. (2023) on long-term afforestation restoration in a rocky desertification area. Furthermore, it aligns with the findings of Ding et al. (2019) who researched the TOC fractions in the rhizosphere and non-rhizosphere soils of four tree species in a mixed forest in the north of Daxing'anling. The dynamic changes in the content of labile carbon fractions in the rhizosphere soil of *Larix gmelinii*, *Pinus sylvestris* var. *mongolica* Litv., *Betula platyphylla*, and *Populus davidiana* are similar and significantly higher than those in non-rhizosphere soil, with obvious rhizosphere effect, which is similar to the results of this research. The reasons for this can be looked at from three aspects. Firstly, soil LOC comes from plant residues and litter, and ecological restoration increases vegetation coverage and biomass (Shu et al. 2023), increases litter return, improves soil nutrients and enhances soil microbial activity (He et al. 2022), and the increase in soil moisture content (Table 2), contributes to the accumulation of soil LOC (Table 3) (Li et al. 2016). Furthermore, afforestation restoration promotes the formation of soil macro-aggregates, and it also benefits the accumulation of LOC when the contact between soil OM and soil microorganisms and air decreases (Zhang et al. 2023). Additionally, since *Salix cupularis* has the function of wind protection and sand fixation, it can effectively prevent the loss of soil, water and litter in the alpine desert, and it can also prevent exogenous sand from entering the restored plot. This creates a suitable soil environment for plant growth and development. Well-grown plants can then input more organic matter into the soil, promoting an increase in TOC and its labile fractions, thus creating a cycle that restores the alpine desert ecological environment (FAO 2006, Hu et al. 2022).

The present result also showed that the contents of TOC and its labile fractions decreased approximately with the increase of the soil layer. This result should be due to the fact that topsoil has more humus than subsoil. The input of OM is conducive to the growth and reproduction of soil microorganisms and promotes the accumulation of TOC and its labile fractions (Wang et al. 2023).

Furthermore, the research revealed a notable rise in both the average annual temperature and average annual rainfall within the study area from 1996 to 2020. Correlation analysis indicated a negative relationship between TOC, labile carbon fractions and the aforementioned climate variables (Figure 2, Table 3). Despite this negative correlation, the TOC and its fractions content continued to exhibit an upward trend (Figures 3–4). These findings suggest that the restoration of alpine desertified grassland through the planting of *Salix cupularis* in Northwest Sichuan, China could potentially mitigate the escalating levels of carbon dioxide attributed to global warming.

Compared with TOC content, CPMI is a comprehensive measure of the quantity and quality of TOC. It reflects the changes in the C dynamics of the soil system and can more comprehensively reflect soil fertility and quality. The higher the CPMI value, the better the relative soil quality (Zhao et al. 2014). The results of this study show that as the restoration years increased, the soil CPMI also gradually increased (Figure 5). This is broadly consistent with the research results of Pang et al. (2019) on the impact of vegetation restoration on CPMI. Therefore, fencing off the area from grazing and planting *Salix cupularis* is a way that can significantly enhance the "sink" function of soil C and increase soil quality.

In conclusion, after afforestation of the 0–60 cm alpine desert grassland in the eastern Qinghai-Tibet Plateau, the TOC and its labile fractions exhibited an upward trend over 24 years, leading to gradual soil quality improvement. The most significant soil changes were observed in the surface layer (0–20 cm). Additionally, the TOC and its labile fractions in the rhizosphere soil of the 0–60 cm layer were progressively increased compared with non-rhizosphere soil as the years of restoration advanced. This indicates

https://doi.org/10.17221/106/2024-PSE

that DOC, EOC, POC and MBC in surface rhizosphere soil layers are more sensitive indicators than TOC. After 16 years of *Salix cupularis* planting, the soil CPMI of both rhizosphere and non-rhizosphere soil rises significantly, which enhances the soil C sink capacity and overall soil quality. In conclusion, this study sheds light on the impact of vegetation restoration on TOC and its labile fractions in alpine desert grasslands, holding significant implications for the long-term maintenance of global carbon balance.

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Received: March 9, 2024
Accepted: June 18, 2024
Published online: July 22, 2024