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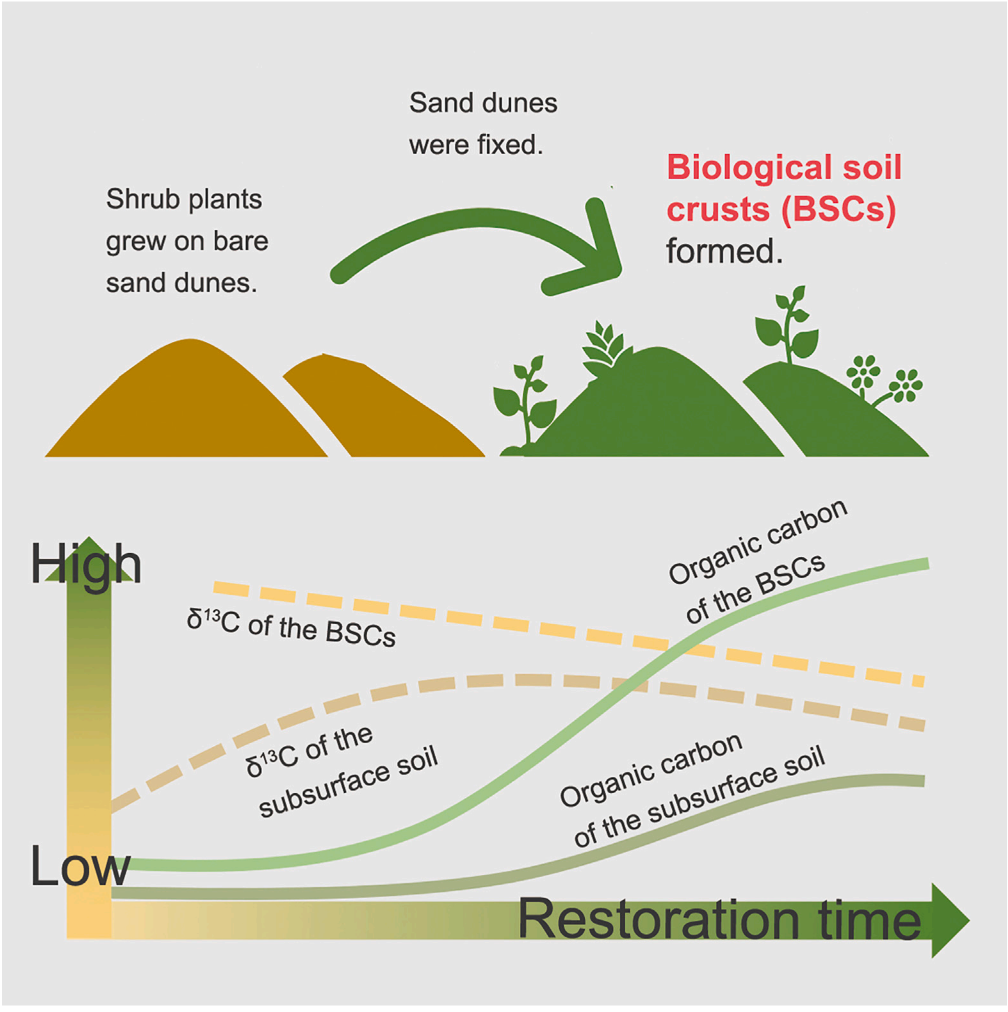

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Highlights

Sand dune fixation increased the SOC content and facilitated the BSC formation

Substantial fractionation of stable carbon isotopes during early formation of BSCs

Rapid increase in $\delta^{13}C$ values of BSCs possibly linked to microbial activities

Microbial succession may influence the initial turnover of soil carbon

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Significant carbon isotopic fractionation during early formation of biological soil crusts with indications for dryland carbon cycling

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SUMMARY

Clarifying the accumulation and decomposition of soil organic carbon (SOC) is crucial for comprehending carbon cycling in terrestrial ecosystems. SOC enrichment and decomposition lead to the fractionation of stable carbon isotopes, a complex process influenced by various factors, including microbes. However, this fractionation process during early soil formation and the role of microorganisms remain poorly explored. This study investigated the relative composition of stable carbon isotopes ($\delta^{13}C$) of recently formed biological soil crusts (BSCs) on stabilized sand dunes in the Tengger Desert, Northern China. A notable increase in $\delta^{13}C$ was observed during early BSC development, likely driven by cyanobacteria's direct fixation of CO_2. Yet, $\delta^{13}C$ values of BSCs gradually declined, approaching those of soils under native vegetation, probably linked to microbial succession within the BSCs. This finding highlights the potential microbial influence on early soil carbon turnover and underscores the effectiveness of isotope tracers for studying this process.

INTRODUCTION

Soil organic carbon (SOC) is a critical component of the global carbon cycle, containing at least three times more carbon than the atmosphere or terrestrial vegetation.[1] Previous studies suggest that microbes play a significant role in SOC accumulation, decomposition efficiency of plant residues, and stability of organic matter.[2–4] However, how microbes influence carbon turnover during the initial stages of soil formation remains poorly understood. Addressing this question could help clarify long-standing controversial issues related to carbon cycling, especially in demanding settings where newly weathered materials are frequently exposed and soils are in their early stages of development. Such environments include regions with challenging conditions like deserts[5,6] and glacier melting fronts,[7] where the intricate dynamics of soil carbon uptake and storage are yet to be comprehensively elucidated.

The process of SOC enrichment and decomposition also leads to fractionation of its stable carbon isotopes, which is a complex process influenced by various factors, including microbes.[8,9] However, the impact of microbial activities on carbon isotopic fractionation has been studied less extensively. Some studies consider that fractionation of carbon isotopes during SOC decomposition is much less than during photosynthesis.[10,11] However, a recent study proposes strong fractionation effects during the decomposition of soil organic matter.[12] Therefore, the fractionation of stable carbon isotopes and their relative composition ($\delta^{13}C$) in soil organic matter may help to trace the processes of carbon cycling and underscore the role of microbes in carbon accumulation especially during early stages of soil formation.[13] For example, previous studies have effectively employed ^{13}C labeling to investigate the allocation of photosynthetic carbon within plant-soil system.[14,15]

Deserts are characterized by sparse vegetation and infertile soils with low content of organic matter. Despite this, biological soil crusts (BSCs) that thrive on the stabilized ground surfaces are widely distributed in desert regions.[16] They are different from the subsurface soil that mainly consists of mineral grains, but instead, BSCs are formed by a composite layer of bacteria, fungi, lichens, and mosses that interact with mineral particles. Gradually, BSCs evolve into soils under the influence of soil-forming factors, such as vegetation, small-sized soil fauna, microorganisms, climate and their parent material.[17] Therefore, in the arid deserts, BSCs not only represent the stabilization of ground surface but also signify the initial phase of soil formation. Furthermore, they play a vital role in maintaining ecosystem stability by improving the microhabitat.[18–20]

Previous studies have demonstrated that carbon accumulation in soils is influenced by organic residues such as plant litter, along with their decomposition products.[21] The microbial communities in BSCs may also have significant impacts on carbon turnover during early soil formation,[22] although this aspect has received less attention. A recent study revealed that the carbon source of organic matter may change during different stages of BSC formation which is closely related to microbial succession.[23] However, few studies have investigated how the stable

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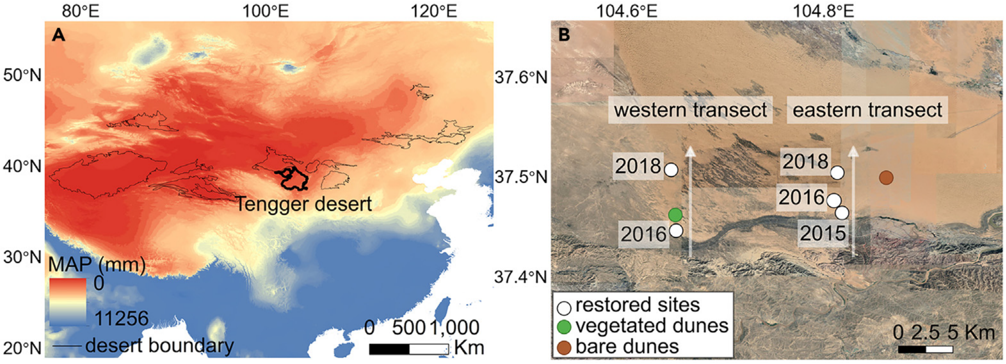

Figure 1. Geographic location of Tengger Desert and sampling sites

(A) Geographic location and annual precipitation of Tengger Desert in East Asia.

(B) Sampling locations along two transects in the southeastern part of the Tengger Desert. The sites were restored in different years (2015, 2016, and 2018), as indicated in the figure. At the time of sampling in 2022, the sites have been restored for 7, 6, and 4 years for 2015, 2016, and 2018 sites, respectively.

carbon isotopic composition ($\delta^{13}C$) of organic matter is affected by microorganisms during BSC formation, which is an essential aspect of the soil carbon cycling. This study will try to fill this research gap by investigating the stable carbon isotopic fractionation during initial formation of BSCs in the desert regions.

Tengger Desert, a typical arid desert located in north-central China, has a continental monsoonal climate with an annual mean temperature of ~10°C and precipitation of 180 mm, decreasing toward the Northwest. In recent years, sand dunes in the southern part of Tengger Desert have undergone stabilization through large-scale sand fixation and vegetation restoration (Figures 1 and S1). Initially, the active dunes were maintained in a bare soil condition, and restoration efforts, including the use of straw checkerboards and planting, contributed to stabilizing the sands. Subsequently, plants gradually grew and recovered, leading to an increase in vegetation cover. This, in turn, facilitated the establishment and growth of vegetation, progressing toward stabilized dunes.[24] Consequently, the shrub ecosystem gradually recovered with higher vegetation cover and increased species richness, leading to the gradual formation of BSCs on the stabilized dune surface.[16,24,25] These BSCs formed at different times, depending on when the sand dunes were stabilized, providing an ideal opportunity to investigate the content and isotopic fractionation of SOC during the formation of BSCs. The results will help to illustrate the carbon turnover in challenging environments characterized by the exposure of newly weathered materials, gradual vegetation recovery, and early soil development. The analysis of BSC chronosequences on sand dunes in the Tengger Desert will also advance our understanding of the potential contribution of microbial activities to early soil carbon accumulation and dryland carbon cycling.

RESULTS

SOC content of subsurface soils and BSCs

A systematic collection of BSC, subsurface soil (0–2 cm layer of sand below the BSC, Figure S2) and plant litterfall was conducted at the restoration sites along two transects in the southeastern part of the Tengger Desert (Figure 1). BSCs were formed on the surface of sand dunes at these sites that were restored in different years (2015, 2016, and 2018). At the time of sampling (in the year 2022), the dunes at the sites along the eastern transect have been fixed for 4, 6, and 7 years, respectively, while the dunes at the sites along the western transect have been fixed for 4 and 6 years, respectively. The successional stages (algae, algae-lichen, lichen, lichen-moss, moss) and morphological characteristics of BSCs have been well described by Lan et al.[26] Based on these criteria and in combination with field and microscopic observations (Figure S3), the sampled BSCs from the southeastern Tengger Desert were identified and classified. In essence, based on their different developmental stages, the collected BSCs were categorized into two types in this study. Along the eastern transect, algae BSCs composed mainly of cyanobacteria were found at the sites restored for 4, 6, and 7 years. In the western transect, algae BSCs were sampled at the sites restored for 6 years. Likely due to drier conditions, BSCs at the sites in the western transect that were restored for 4 years exhibited very weak development with a thin, fragile layer, and thus they were not sampled. No BSCs were observed in non-restored, bare mobile dunes, while mature lichen-moss BSCs were present on the surface of naturally vegetated dunes.

Soil samples (including BSCs and subsurface soils) were collected from the restoration sites, non-restored bare dunes, and naturally vegetated dunes, and were analyzed in the lab. The results show that the mean SOC content of the BSCs is 0.35% ($n = 21$), ranging from 0.07% to 1.19%; while the mean SOC content of subsurface soils has a lower value of 0.06% ($n = 40$), with the highest value of 0.55% in the naturally vegetated dunes and the lowest value of 0.004% in the bare dunes (Table S1). As illustrated in Figure 2, the SOC contents of both BSCs and subsurface soils increased with the years of restoration, and the increase in BSCs was more rapid. For example, the SOC contents of BSCs collected from the dunes in the eastern transect increased by 0.18% over the restoration period, while that of subsurface soils increased by only 0.02%. Moreover, the naturally vegetated dunes with relatively high vegetation coverage have the highest SOC content in both subsurface soils and BSCs.

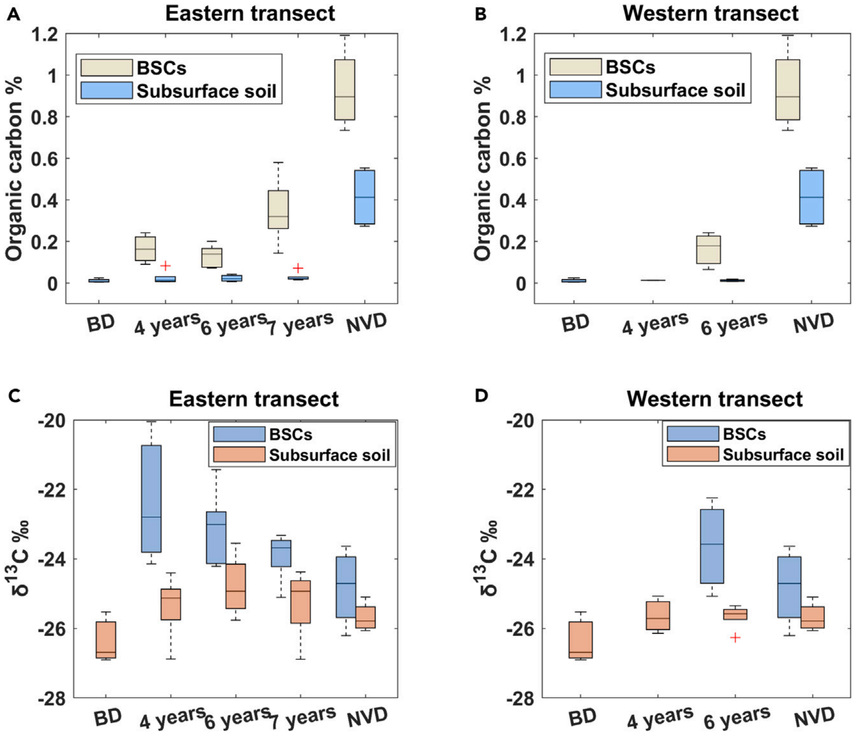

Figure 2. Boxplots of SOC content and $\delta^{13}C$ values in BSCs and subsurface soils collected from dunes restored in different years from the two transects

(A) SOC content of BSCs and subsurface soils from the eastern transect.

(B) SOC content of BSCs and subsurface soils from the western transect.

(C) $\delta^{13}C$ values of BSCs and subsurface soils from the eastern transect.

(D) $\delta^{13}C$ values of BSCs and subsurface soils from the western transect. The samples taken from naturally vegetated dunes (NVD) serve as a reference for the equilibrium state under current climate conditions, while the samples taken from non-restored bare dunes (BD) serve as a reference for the initial state of dunes before restoration.

$\delta^{13}C$ value of subsurface soils and BSCs

The mean $\delta^{13}C$ value of BSCs is −23.58‰ (n = 21), ranging from −26.21‰ to −20.05‰, with the highest found in the site restored in 2018 (equivalent to 4-year restoration) in the eastern transect, and the least value found in naturally vegetated dunes (Table S1). Meanwhile, the $\delta^{13}C$ values of subsurface soils are more negative and show a limited increase after restoration. The mean $\delta^{13}C$ value of subsurface soils in the naturally vegetated dunes is −25.68‰ (ranging from −26.06‰ to −25.10‰), which is slightly higher than that of bare dunes with an average value of −26.37‰ (ranging from −26.90‰ to −25.52‰). More importantly, the $\delta^{13}C$ values of BSCs increased significantly right after the 4-year restoration in the eastern transect (and 6-year restoration in the western transect) and then gradually decreased with the development of BSCs over time, until approached toward the naturally vegetated dunes where the lichen-moss BSCs are maturely developed. However, the $\delta^{13}C$ values of BSCs are still slightly higher than that of subsurface soils in the naturally vegetated dunes.

$\delta^{13}C$ value of litterfall and soils under different plant species

Plant litterfall samples were collected from the study sites and their $\delta^{13}C$ values were examined (Table S2). The shrub species at these sites are mostly C3 plants, including *Hedysarum scoparium*, *Artemisia ordosica*, *Caragana korshinskii*, and *Ceratoides latens*. These plant species are commonly found in the restoration sites and natural vegetated dunes, and the development of BSCs occurred beneath these shrubs. The results reveal that the $\delta^{13}C$ values of plant litterfall typically exhibit more negative values in contrast to those of BSCs and subsurface soils (Figure 3). More interestingly, although the BSCs in the restoration sites were found under different plant species (*Hedysarum scoparium*, *Artemisia ordosica*, and *Caragana korshinskii*), the $\delta^{13}C$ values of BSCs were higher than those of subsurface soils consistently among them (Figure 3). However, the difference between the $\delta^{13}C$ value of BSCs and subsurface soils is minor in naturally vegetated dunes dominated by native plant of *Ceratoides latens*. Additionally, the variance of the $\delta^{13}C$ values of BSCs was larger than that of subsurface soils in all sites, regardless of the plant species.

DISCUSSION

The gradual increase in SOC content of both BSCs and subsurface soils over time indicates effective carbon accumulation in these sites (Figure 2). According to previous studies, the increase in SOC can be attributed to more input from plant litterfall as well as increased

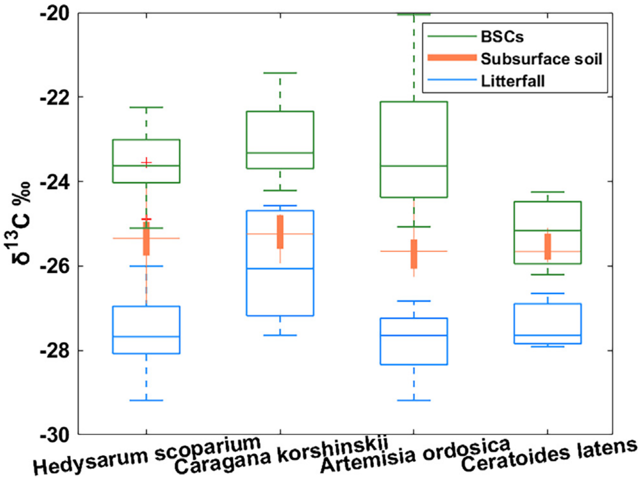

Figure 3. Boxplot of δ^{13}C values of litterfall, BSCs, and subsurface soils collected from sites dominated by different plant species

microbe-derived carbon addition during restoration.[27] Additionally, SOC acts as a binder in the formation of aggregates, which enhances soil erosion resistance and reduces the loss of organic matter, forming a positive feedback loop that accelerates carbon accumulation.[28] Note that after 6–7 years of restoration in the study area, the SOC content of both BSCs and subsurface soils had not yet reached the level of naturally vegetated dunes.

The δ^{13}C values of subsurface soils changed slightly over time (Figure 2), and were higher than those of litterfall from various plant species (Figure 3). Previous studies have also reported positive δ^{13}C deviation in subsurface soils relative to litterfall, ranging from 0.05‰ to 2‰.[29,30] The positive δ^{13}C deviation in subsurface soils is likely caused by microbial activity.[31] Microorganisms preferentially use lighter carbon (^{12}C) during decomposition of plant residues, resulting in ^{13}C enrichment in subsurface soils.[32] Recently, Klink et al. have pointed out that δ^{13}C of microbe-derived carbon is more positive than that of the litterfall and plant-derived carbon.[31] Our findings were consistent with these studies, indicating a similar positive δ^{13}C deviation in subsurface soils relative to litterfall.

More significantly, we found a much higher δ^{13}C value in BSCs compared to litterfall, with a positive deviation of upto 2‰–4‰ (Figure 3). Some newly formed BSCs even had δ^{13}C values as high as −20.05‰ (Figure 2C). Given that the isotopic deviation caused by its decomposition remains within 2‰, and the biomass of plant litterfall is limited, this significant δ^{13}C deviation in BSCs cannot be solely attributed to microbial decomposition of plant residues. However, it may indicate another mechanism of SOC accumulation in BSCs. BSCs, consisting of cyanobacteria and other photosynthetic organisms,[23] rely heavily on atmospheric CO_2 fixation,[33] while subsurface soils may receive carbon from both plant litter inputs and root exudates.[34] The isotopic signatures of these carbon sources are very likely to be different,[31] resulting in the observed difference in δ^{13}C values between BSCs and subsurface soils. When utilizing atmospheric CO_2 for photosynthesis, cyanobacteria may preferentially assimilate ^{12}C,[35] resulting in organic carbon depleted in ^{13}C relative to atmospheric CO_2. However, previous studies have shown that algae, including cyanobacteria, have relatively positive δ^{13}C values despite isotopic fractionation during carbon fixation.[36] We propose that cyanobacteria communities, which are prevalent particularly in the newly developed BSCs in the Tengger Desert,[33] may explain the anomalous δ^{13}C deviation. This is supported by observations of a large difference in δ^{13}C values between surface and subsurface cyanobacteria in a lake environment, indicative of direct CO_2 fixation.[37] Previous studies have also suggested that the desert crusts may absorb CO_2 from the atmosphere,[38,39] making the desert a potential carbon sink.[5,40] Similarly, Elbert et al. and Jassey et al. have pointed out that carbon sequestration by soil algae plays an indispensable role in the global carbon cycle and our study provides direct isotopic evidence supporting this perspective.[3,41]

In addition, the highest δ^{13}C values are typically observed in the newly formed, immature algae crusts when cyanobacteria dominate, while the mature lichen-moss BSCs prevalent in naturally vegetated dunes have different isotopic signatures. Generally, the δ^{13}C values of BSCs decrease consistently over time (Figure 2), in accordance with the shift in BSC composition from algae to lichen-moss as BSCs mature.[23] This trend is not observed in subsurface soils, though. We propose two possible explanations for this phenomenon. One explanation is linked to changes in the microbial communities of the BSCs. Zhang et al. have noted that cyanobacteria dominate the early stage of BSCs, constituting 46.9% of the total microbial community abundance, while their abundance decreases to less than 5% in the moss crusts.[42] Additionally, the diversity of nitrogen-fixing bacteria and fungi increases as the BSCs develop.[43,44] The alternative explanation is likely associated with variations in the available carbon source. In the initial stage of crust formation, the cyanobacteria may directly utilize atmospheric CO_2 for photosynthesis. However, different microbial communities in the lichen-moss crusts could potentially utilize respired CO_2 released from subsurface soils,[45] blending the isotopic signature of the two components. The isotopic signal of mature BSCs approaches that of subsurface soils, which may be resulted from the change of the carbon source from microbe-derived carbon at the initial stage of BSCs to a combination of plant-derived carbon. Regardless, more research is needed to test these hypotheses.

Furthermore, the $\delta^{13}C$ values of BSCs have large variance, which may be related to the variability in the types of photosynthetic organisms and their metabolic pathways in different BSCs.[43] In comparison, the $\delta^{13}C$ values of subsurface soils exhibited no significant variation across different plant species (Figure 3) or restoration years (Figure 2). Although microbial decomposition of plant substances affects the isotopic fractionation of plant-derived carbon, the $\delta^{13}C$ values of both BSCs and subsurface soils in the restoration sites tend to become consistent and gradually approach those of naturally vegetated dunes over time. This suggests that after a few years of development, the $\delta^{13}C$ values of BSCs and subsurface soils likely shift toward a plant-soil equilibrium determined by climatic conditions, rather than by specific plant species. These findings provide direct evidence for using soil organic $\delta^{13}C$ as an indicator of vegetation succession and environmental change,[8,13] as it reflects long-term consequences of climate-plant-soil interactions.

In conclusion, this study examined the carbon dynamics during the initial formation of soils on stabilized dunes in the arid Tengger Desert, focusing on SOC content and $\delta^{13}C$ value of BSCs, subsurface soils and litterfalls. The results revealed that with increasing restoration time, the SOC content of both BSCs and subsurface soils gradually increased. Notably, the $\delta^{13}C$ values of BSCs exhibited a rapid initial increase, likely due to cyanobacteria directly utilizing atmospheric CO_2 for photosynthesis. However, over time, the $\delta^{13}C$ values gradually declined and approached that of soils under naturally vegetated dunes, probably linked to microbial succession within BSCs. The findings reveal the anomalous isotopic signature of microorganisms, possibly cyanobacteria, in carbon sequestration during early soil formation in desert ecosystems. They also suggest the potential contribution of microorganisms to organic matter accumulation in early soils of other harsh environments, although further research is needed to determine the extent of their contribution. The study highlights the application of carbon isotope technology in investigating microbial impacts on soils and its potential implications for understanding early life evolution on Earth and exoplanets.

Limitations of the study

In this study, the samples were collected from various sites that had undergone restoration at different years, and these samples were analyzed as part of a chronosequence. Continuous sampling from a single location is absent. The observed variations between different sites could partially be attributed to local factors such as diverse topography, slight differences in soil physical and chemical properties, and the impact of human activities, all of which may influence the experimental outcomes. Additionally, this study employed SOC $\delta^{13}C$ values to indicate microbial carbon sequestration. We did not conduct direct sequencing of microbial communities in the BSCs due to different sampling treatments.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Data and code availability
- METHOD DETAILS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109114.

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AUTHOR CONTRIBUTIONS

Conceptualization, X.Z., investigation, X.Z. and Z.L.-X., methodology, Z.L.-X. and Z.B., formal analysis, X.Z., Z.L.-X., and Z.C., resources, Z.B. and X.Z., writing – original draft, Z.L.-X., writing – review and editing, X.Z., Z.B., S.B., Z.C., and J.M.A., supervision, X.Z., funding acquisition, X.Z.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
hydrochloric acid	repository	N/A
Software and algorithms		
MATLAB (R2019b)	Mathworks, USA	https://ww2.mathworks.cn/academia/tah-portal/nanjing-university-31425309.html
IBM SPSS Statistics 25	SPSS, USA	https://www.ibm.com/cn-zh/products/spss-statistics
QGIS Desktop 3.28.2	QGIS.ORG, Switzerland	https://qgis.org/en/site/forusers/download.html
ArcMap 10.4.1	Environment System Research Institute, USA	https://www.esri.com/en-us/arcgis/products/arcgis-desktop/resources

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Zhiwei Xu (zhiweixu@nju.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request

METHOD DETAILS

A systematic collection of plant litterfall, BSC, and subsurface soil (0–2 cm layer below the BSC) was conducted at the restoration sites along two transects in the southeastern part of the desert (104°23′E, 37°31′N). These restoration sites were originally covered by bare dunes, but they were stabilized after the implementation of straw checkerboard and vegetation restoration practices in different years (2015, 2016 and 2018). At the time of sampling (in the year 2022), the dunes at the sites along the eastern transect have been fixed for 4, 6 and 7 years, while the dunes at the sites along the western transect have been fixed for 4 and 6 years, respectively. The shrub species at these sites are mostly C3 plants, including *Hedysarum scoparium*, *Caragana korshinskii*, *Artemisia ordosica*, and *Ceratoides latens*.[24] Algae BSCs were formed on the stabilized dunes at the sites along the eastern transect. They were in the developmental stage, ranging from immature to relatively mature, and mainly composed of cyanobacteria. Probably due to different environmental conditions, immature algae BSCs were only observed at the sites restored for 6 years in the western transect, but not at the sites restored for 4 years. Soil samples were also taken from the non-restored, active bare dunes and naturally vegetated dunes. BSCs were not found in bare dunes, while the mature lichen-moss BSCs were found in the naturally vegetated dunes. The identification and classification of BSCs were conducted based on field and microscopic observations of the BSC's morphological characteristics, following the criteria proposed by Lan et al.[26]

The BSC samples under the dominant plant species, as well as subsurface soils, were carefully collected using a knife, with three duplicate samples taken in each sampling plot. In total, 61 soil samples (including BSCs and subsurface soils) were collected from the restoration sites, non-restored bare dunes, and naturally vegetated dunes. 25 plant litterfall samples were collected from the restoration sites and naturally vegetated dunes.

The soil sample pretreatment process involves three steps: (1) crushing the samples, (2) removing inorganic carbon from the sample, and (3) drying. To perform these steps, the samples are crushed into powder, and 2–3 g of each sample are placed into a 50 mL centrifuge tube. Subsequently, 30–35 mL of 10% hydrochloric acid is added to each tube, and the samples are allowed to react for 72 h. During this period, the tubes are shaken 2–3 times a day, and the centrifuge tube is opened to release CO_2. After the acid reaction, the sample is centrifuged for 5 min (at 500 revolutions per minute), and the supernatant is decanted. The samples are then rinsed with ultra-pure water until the supernatant becomes neutral. Following this, the samples are dried in an oven at 40°C until a constant weight is achieved. Subsequently, the ^{13}C abundance and the content of soil organic carbon are determined using a Finnigan MAT 253 mass spectrometer in the Stable Isotope Lab, School of Earth Sciences and Engineering in Nanjing University.