

## Artificial cyanobacteria crusts can improve soil fertility and plant growth in a semi-arid area, northern China postprint

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### Abstract

Artificial cyanobacteria crusts are formed by inoculating soil with cyanobacteria. These crusts help prevent soil erosion and restore soil functionality in degraded croplands. However, how fast the artificial cyanobacteria crusts can be formed is a key issue before their practical application. In addition, the effects of artificial cyanobacteria crusts on soil nutrients and plant growth are not fully explored. This study analyzed the effect of inoculation of cyanobacteria from local biological soil crusts on soil nutrients and Pak-choi (*Brassica campestris* L. ssp. *Chinensis* Makino var. *communis* Tsen et Lee; Chinese cabbage) growth in a cropland, northern China through field experiments by comparing with no fertilizer. The results showed that artificial cyanobacteria crusts were formed on the 18th d after inoculation with a coverage of 56.13%, a thickness of 3.74 mm, and biomass of 22.21 g chl<sub>a</sub>/cm<sup>2</sup>. Artificial cyanobacteria crusts significantly improved the soil organic matter (SOM), NO<sub>3</sub>-N, total nitrogen (TN) contents, and the activities of sucrose, alkaline phosphatase, urease, and catalase enzymes of plants on the 50th d after inoculation. Additionally, artificial cyanobacteria crusts led to an increase in plant biomass, improved root morphology, and raised the phosphorus and potassium contents in the plants. Furthermore, the biomass of plant grown with artificial cyanobacteria crusts was comparable with that of grown with chemical fertilizer. The study suggested that, considering plant biomass and soil nutrients, it is feasible to prevent wind erosion in the cropland of arid and semi-arid areas by inoculating cyanobacteria crusts. This study provides new perspectives for the sustainable development and environmental management of cropland in arid and semi-arid areas.

## Full Text

### Preamble

#### Artificial cyanobacteria crusts can improve soil fertility and plant growth in a semi-arid area, northern China

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**Abstract:** Artificial cyanobacteria crusts are formed by inoculating soil with cyanobacteria and help prevent soil erosion while restoring soil functionality in degraded croplands. However, the formation rate of these crusts is a critical factor for practical application, and their effects on soil nutrients and plant growth remain incompletely understood. This study investigated the impact of inoculating local biological soil crust cyanobacteria on soil nutrients and Pak-choi (*Brassica campestris* L. ssp. *Chinensis* Makino var. *communis* Tsen et Lee; Chinese cabbage) growth in northern China cropland through field experiments comparing treatments with and without fertilizer. The results demonstrated that artificial cyanobacteria crusts formed by the 18th day after inoculation, achieving 56.13% coverage, 3.74 mm thickness, and 22.21 g chl<sub>a</sub>/cm<sup>2</sup> biomass. By the 50th day after inoculation, artificial cyanobacteria crusts significantly improved soil organic matter (SOM), NO<sub>3</sub><sup>-</sup>-N, and total nitrogen (TN) contents, while enhancing the activities of sucrase, alkaline phosphatase, urease, and catalase enzymes. Additionally, the crusts increased plant biomass, improved root morphology, and elevated phosphorus and potassium contents in plants. Notably, plant biomass grown with artificial cyanobacteria crusts was comparable to that grown with chemical fertilizer. These findings suggest that inoculating cyanobacteria crusts represents a feasible strategy for preventing wind erosion in croplands of arid and semi-arid regions while simultaneously improving soil nutrients and plant growth, offering new perspectives for sustainable cropland development and environmental management.

**Keywords:** artificial cyanobacteria crusts; wind erosion; soil fertility; plant growth; soil enzyme

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## 1 Introduction

Arid and semi-arid areas constitute approximately 41.00% of Earth' s land surface (FAO, 2019). These regions feature fragile ecosystems where wind erosion represents a major driver of soil degradation. Preventing wind erosion in dryland soils is essential for maintaining soil quality and ensuring sustainable ecosystem development.

Approximately 14.00% of arid and semi-arid areas are cropland (FAO, 2019). Cropland experiences strong wind erodibility due to low vegetation cover and frequent surface disturbance from tillage practices (Zhao et al., 2005), making it more susceptible to wind erosion than grassland or woodland (Guo et al., 2020; Wu et al., 2020). Liu et al. (2023) estimated global average annual dust emissions from cropland at  $1.75 \times 10^9$  g/s from 2017 to 2021, seriously deteriorating air quality and human living environments. Wind erosion reduces fine particle content in surface soil, leading to surface coarsening and nutrient loss that decreases land productivity (Du et al., 2019). The global average annual organic carbon loss from wind erosion in cropland was estimated at  $2.970 \times 10^{12}$  g/a during the same period (Liu et al., 2023). Song et al. (2019) reported annual losses of SOC, TN, and TP of  $0.985 \times 10^{12}$ ,  $0.094 \times 10^{12}$ , and  $0.089 \times 10^{12}$  g/a, respectively, from spring wind erosion in northern China. Simultaneously, wind erosion increases suspended particle content in the atmosphere. Therefore, preventing cropland wind erosion in dry areas is crucial for improving soil quality, increasing food production, and environmental management.

Numerous studies have investigated wind erosion prevention in cropland, identifying three main strategies: (1) conservation tillage, including straw mulching, stubble retention, minimum tillage, and no-tillage (Cong et al., 2016; Li et al., 2020); (2) mulching such as gravel cover and film mulching (Li et al., 2021); and (3) biological measures such as cropland windbreaks or winter planting of oilseed rape, wheat, and camas (Ma et al., 2019; Chang et al., 2021). However, despite their effectiveness, these measures have limitations. Stubble and straw mulching affect economic benefits and seed growth. Mulching is labor-intensive, and materials are easily damaged, potentially causing environmental pollution. Biological measures do not yield immediate results. Therefore, exploring environmentally friendly, cost-effective, and efficient methods to prevent wind erosion in cropland is essential.

Biological soil crusts (BSCs) are complexes formed through the cementation of cyanobacteria, algae, microfungi, lichens, and bryophytes with soil surface particles via pseudoroots, mycelia, and secretions (Weber et al., 2022), and are widely distributed in arid and semi-arid areas. BSCs provide ecological functions such as improving soil texture, increasing soil organic matter content (Belnap and Lange, 2003; Gao et al., 2017), and enhancing soil aggregate stability (Bowker and Belnap, 2008). During the primary developmental stage, cyanobacteria crusts create strong bonds between soil particles through exopolysaccharide exudation and filament networks, increasing sand' s ability to withstand wind

forces (Kheirfam and Asadzadeh, 2020).

Artificial inoculation supplemented with cultivation measures like irrigation can promote cyanobacteria crust formation and development. Artificial cyanobacteria crusts have become a mature biological technique for wind erosion and soil desertification control in drylands. Many studies have developed inoculation and cultivation techniques (Chamizo et al., 2018; Lu et al., 2022; Rossi et al., 2022) and demonstrated feasibility in desertification and wind erosion control (Fattahi et al., 2020). For example, Chen et al. (2006) found that artificial cyanobacteria crusts formed after 20 days of inoculation could resist erosion at wind speeds of 7.9 m/s. Kheirfam and Asadzadeh (2020) observed that artificial cyanobacteria crusts with 2.27 mm thickness could reduce sand flow by 96.60% under 20.0 m/s wind speed for 30 minutes. Additionally, wind tunnel tests from our research team showed that artificial cyanobacteria crusts with 50.00% coverage can reduce wind erosion rates by over 90.00% (Huang et al., 2023). However, the formation time required for artificial cyanobacteria crusts with wind erosion protection capacity in cropland remains unclear.

Cyanobacteria crusts influence organic carbon, nitrogen (N), and other nutrient contents in soil, and contribute to plant seed germination and seedling growth (Pushkareva et al., 2015; Sharma et al., 2021). Previous studies showed that cyanobacteria fix  $N_2$  in specialized heterocyst cells, providing fixed N to the host (Adams and Duggan, 2008), and mineralization of their biomass after death is the main mechanism of N transfer to plants (Roger, 2004). Additionally, cyanobacterial photosynthesis drives  $CO_2$  sequestration, elevating soil organic carbon levels (Pushkareva et al., 2015). Concurrent oxygen release enhances soil aeration, creating favorable conditions for aerobic microbial activity and overall soil health (Obana et al., 2007). Elbert et al. (2009) estimated global annual net carbon uptake by biocrusts at approximately  $3.600 \times 10^{15}$  g/a, with nitrogen fixation estimated at about  $45.000 \times 10^{12}$  g/a. Recent studies also found that soil cyanobacteria might inhibit fungal plant pathogens (Eckstien et al., 2024). Thus, artificial cyanobacteria crusts show good application prospects for soil nutrient improvement and crop growth promotion. However, existing studies focus almost exclusively on desert ecosystems, leaving unknown whether similar success can be achieved in cropland through targeted inoculation.

The Loess Plateau of China is among the world's most severely wind-eroded areas, with an annual average wind erosion amount of  $5 \times 10^3 - 10 \times 10^3$  t/km<sup>2</sup> (Tang, 2004). Traditional farming practices require spring plowing and mounding, severely damaging the ground surface and resulting in higher wind erosion intensity (Mendez and Buschiazzo, 2009). Therefore, controlling wind erosion on cropland in this area is necessary. This study selected the wind-water erosion crisscross area of the Loess Plateau as the study area, using Pak-choi (*Brassica campestris* L. ssp. *chinensis* Makino var. *communis* Tsen et Lee; Chinese cabbage) as an indicator plant to address three questions through cyanobacteria crust inoculation: (1) How quickly can cyanobacteria crusts form in cropland soils after inoculation? (2) Do artificial cyanobacteria crusts promote plant

growth? (3) Can artificial cyanobacteria crusts partially replace chemical fertilizer? This study may provide a basis for comprehensive assessment of wind erosion prevention feasibility in cropland through cyanobacteria crust inoculation in arid and semi-arid areas, while offering new ideas for sustainable cropland development and environmental management.

## 2.1 Study Area

The study was conducted in the Liudaogou Watershed in Shenmu City, Shaanxi Province, northern China (38°46' -38°51' N, 110°21' -110°23' E; 1094.0-1273.9 m a.s.l.). The watershed has a semi-arid climate in the middle temperate zone, with topography characterized by typical sandy loess hills (Wang and Takahashi, 1999; Dan et al., 2023). The watershed experiences dramatic climate change, with low precipitation and severe sandstorms in winter and spring, and heavy rainfall in summer (Zha and Tang, 2000). Multi-year average precipitation is 409 mm, concentrated primarily in June–September (Li and Xiao, 2022). Main land use types include cropland, agricultural land, fruit intercropping land, shrubland, woodland, and grassland. Primary crops include corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and sunflower (*Helianthus annuus* L.).

## 2.2 Experimental Design

The experiment was conducted from August 3 to September 22, 2022, in the terraces of Liudaogou Watershed. Daily average temperature during the test period was 9.5°C–27.5°C. The soil type is loessal soil. Field plots were previously cultivated with corn as the preceding crop. Due to experimental time constraints, Pak-choi (*B. campestris*), a common crop in the Loess Plateau, was selected as the indicator crop because of its broad leaf morphology and rapid responsiveness to soil nutrient alterations during short-term cultivation. The Pak-choi variety used was ‘Jinzaosheng’, with a growth period of about 50 days. Excess seedlings were removed when they reached 2 cm height, ensuring one seedling every 10 cm. In addition to different fertilizer treatments, other field measures such as weeding and pest control were identical across all plots. Weeds were removed every 3 days. Pak-choi was harvested on the 50th day after sowing. Rainfall and watering amounts during the plant growth period are shown in Figure 1 [Figure 1: see original paper].

The experiment consisted of three treatments arranged in a randomized complete block design with four replications: (1) control treatment with no fertilizer, (2) nitrogen (15.00% N), phosphorus (P; 15.00% P), and potassium (K; 15.00% K) treatment with 700 kg/hm<sup>2</sup> application rate, and (3) artificial cyanobacteria crusts treatment. A total of 12 plots were established, each measuring 1 m × 1 m.

### 2.3 Cultivation of Artificial Cyanobacteria Crusts

Cyanobacteria crusts used in the experiment were collected from cropland in the Liudaogou Watershed. First, cyanobacteria crust samples were ground and sieved through a 0.1-mm sieve. Sieved soil samples (10 g) were transferred to 150 mL of BG11 culture solution for cyanobacteria and green algae (Rippka et al. 1979) with chemical composition detailed in Tables S1 and S2. The samples were shaken for 24 hours and then placed in an incubator at 25.0°C with light intensity of 1000-2000  $\text{lm}/\text{m}^2$  using a 12-hour light/darkness regime for cultivation (approximately 15 days). After observing cyanobacteria growth, the suspension was transferred to a white plastic bucket containing appropriate amounts of BG11 culture solution and expanded at room temperature for subsequent experiments. The cyanobacteria crusts used in this study were identified as mixed cyanobacteria, with dominant species *Scytonema* sp. and *Nostoc* sp. Chlorophyll concentrations of cyanobacterial suspensions were determined using the method of Wintermans and De Mots (1965).

After Pak-choi sowing, cyanobacteria suspension was sprayed uniformly on the soil surface at an inoculum concentration of 6 g chla/ $\text{cm}^2$ . Following inoculation, soil moisture content was measured with a moisture meter (HH2, Delta-T Devices, Burwell, UK), and water was added when soil moisture content fell below 20.00%, as shown in Figure 1. Cyanobacteria crust biomass was measured every 3 days during the first 18 days after inoculation. Cyanobacteria crust biomass, thickness, and coverage were measured on the 18th day of inoculation and at Pak-choi harvest (the 50th day of inoculation).

### 2.4 Soil Sampling

To clarify the effects of artificial cyanobacteria crusts on cropland nutrients and enzyme activities, soil samples from the 0-2 cm layer were collected on the 30th and 50th days after Pak-choi sowing from each plot using a five-point sampling method. Soil samples from 0-2, 2-5, and 5-10 cm layers were collected on the 50th day of inoculation. Samples were transported to the laboratory and air-dried for determination of soil nutrients and enzyme activities.

### 2.5 Determination of Plant Growth Dynamics and Sampling

To clarify the effects of artificial cyanobacteria crusts on plant growth, when plants reached 20 days of growth, five uniformly growing plants were randomly selected from each replicate every 5 days. Plant height, leaf width, and leaf number were measured and recorded. On the 50th day of plant growth, the portion above the cotyledons was cut as the aboveground portion and weighed to obtain fresh weight. The aboveground portion was then pulverized and mixed for plant nutrient determination. Roots below the cotyledons were placed in plastic bags, carefully cleaned, and dried to determine dry weight.

### 2.6.1 Cyanobacteria Crust Biomass, Thickness, and Coverage

Cyanobacteria crust biomass was expressed as chlorophyll a content per square centimeter of soil. Collected crust samples were ground and placed in 15 mL centrifuge tubes with 8 mL of 95.00% ethanol. Samples were heated in a water bath for 5 minutes, then cooled, shaken for 20 minutes, and centrifuged at 4000 r/min for 10 minutes. After centrifugation, supernatant was transferred to other centrifuge tubes. Absorbance was measured using a UV-vis spectrophotometer detector (UV2300, Techcomp, Shanghai, China) (Ritchie, 2006). Samples were handled in the dark to prevent chlorophyll decomposition. Chlorophyll content was calculated using the following formula:

$$\text{Chl}_a = \frac{11.0935 \times \text{OD}_{665} \times V}{s}$$

where Chl is chlorophyll a content (g/cm<sup>2</sup>); OD<sub>665</sub> is the absorbance value of the extract at 665 nm; V is the volume of 95.00% ethanol (mL); and s is the area of cyanobacteria crusts (cm<sup>2</sup>).

Biocrust thickness was measured with a vernier caliper. Biocrust coverage was investigated using the point-intercept method with a 25 cm × 25 cm gridded quadrat (Belnap et al., 2001).

### 2.6.2 Soil Nutrients and Enzyme Activities

Soil organic matter (SOM) was determined by the dichromate redox titration method (Nelson and Sommers, 1982). Soil total nitrogen (TN) was determined by the Kjeldahl method following H<sub>2</sub>SO<sub>4</sub> catalyst digestion. Soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were extracted by KCl solution and determined by segmented flow analysis. Soil total phosphorus (TP) content was determined by the alkali fusion-Mo-Sb anti-spectrophotometric method. Soil available phosphorus (AP) was determined by the sodium hydrogen carbonate solution-Mo-Sb anti-spectrophotometric method (Carter and Gregorich, 2007).

Soil sucrase activity was determined using the 3,5-dinitrosalicylic acid colorimetric method (Guan, 1986) and expressed as milligrams of glucose consumed in 1 g of soil after 24 hours. Soil alkaline phosphatase activity was determined using the disodium phenyl phosphate colorimetric method (Guan, 1986) and expressed as milligrams of phenol released in 1 g of soil after 24 hours. Soil urease activity was determined using the method of Yang et al. (2007) and expressed as milligrams of NH<sub>3</sub>-N in 1 g of soil after 24 hours. Soil catalase activity was determined using the method of Jin et al. (2009).

### 2.6.3 Plant Nutrients and Root Morphology

Plant N content was determined by phosphoric acid-perchloric acid decoction and Kjeldahl method. Plant P content was determined by molybdenum-antimony antisorbent absorbance photometry. Plant K content was determined by flame atomic absorption spectrophotometry. Plant root morphology indicators, including total root length, total surface area, total volume, and average diameter, were scanned using an Epson Perfection V700 Photo scanner (Epson (China) Co. Ltd., Beijing, China) and analyzed with WinPHIZO software.

## 2.7 Statistical Analysis

Data were organized and analyzed using Excel v.2019 and SPSS v.26.0 software. To clarify the development process of artificial cyanobacteria crusts in cropland and their effects on soil nutrients, enzyme activities, and plant growth, we performed one-way analysis of variance (ANOVA) for cyanobacteria crust biomass, thickness, coverage, soil organic matter, N and P contents, soil enzyme activity, plant biomass, root indices, and plant N, P, and K contents under different treatments. Data were subjected to normal distribution and homoscedasticity tests before ANOVA. Levene' s test was utilized for ANOVA. Multiple comparisons were performed using the least significant difference test ( $\alpha=0.05$ ) when ANOVA assumptions were met and Tamhane' s T2 test when assumptions were violated. Origin v.2023 software was used for graphing.

## 3.1 Artificial Cyanobacteria Crusts

Biomass of artificial cyanobacteria crusts increased sharply after 5 days of inoculation (Fig. 2 [Figure 2: see original paper]), reaching 22.21 g chla/cm<sup>2</sup> on the 18th day—8.0 times higher than the control and 2.7 times higher than the initial inoculum. Similar to the control, no obvious cyanobacteria crust formation was observed in the chemical fertilizer treatment.

Total biocrust coverage reached 59.60% with a thickness of 3.74 mm on the 18th day of inoculation (Table 1 ). Cyanobacteria crust biomass, coverage, and thickness did not change significantly by the 50th day of inoculation (Table 1). Total biocrust coverage was 73.46% with a thickness of 3.80 mm on the 50th day of inoculation (Table 1).

## 3.2 Soil Nutrient Dynamics

Figure 3 [Figure 3: see original paper] shows soil nutrient changes following cyanobacteria crust inoculation. Artificial cyanobacteria crusts showed no significant effect on soil SOM and TN during the first 30 days compared to the control. However, by the 50th day, artificial cyanobacteria crusts significantly increased soil SOM and TN (Fig. 3a and f). The crusts had no significant effect on soil AP and TP throughout the Pak-choi growth period (Fig. 3b and c). Artificial cyanobacteria crusts significantly reduced soil NH<sub>4</sub><sup>+</sup>-N content

compared to the control on the 30th day, though no significant difference was observed by the 50th day (Fig. 3d). Soil  $\text{NO}_3^-$ -N content increased 7.0-fold in artificial cyanobacteria crusts compared to the control on the 30th day, and while  $\text{NO}_3^-$ -N content gradually decreased with Pak-choi growth, it remained significantly higher than the control (Fig. 3e).

### 3.3 Soil Nutrient Distribution in Different Soil Layers

Artificial cyanobacteria crusts increased soil nutrient content primarily in the 0-2 cm layer, with effect extent varying by nutrient type (Fig. 4 [Figure 4: see original paper]). On the 50th day, artificial cyanobacteria crusts significantly increased soil SOM,  $\text{NO}_3^-$ -N, and TN contents in the 0-2 cm layer by 26.44%, 106.46%, and 26.44%, respectively, while showing no significant effect on SOM, TN, and  $\text{NO}_3^-$ -N contents in the 2-10 cm layer or on AP, TP, and  $\text{NH}_4^+$ -N contents in the 0-10 cm layer. Chemical fertilizers significantly increased soil SOM, TP, and TN contents in the 0-2 cm layer and AP contents in the 0-5 cm layer on the 50th day of plant growth.

### 3.4 Soil Enzyme Activities

Artificial cyanobacteria crusts significantly increased soil enzyme activities in the 0-2 cm layer (Fig. 5 [Figure 5: see original paper]). Compared to the control, artificial cyanobacteria crusts increased soil sucrase, alkaline phosphatase, urease, and catalase activities in the 0-2 cm layer by 29.72%, 29.39%, 53.77%, and 21.37%, respectively, and increased soil alkaline phosphatase activity by 62.78% in the 2-5 cm layer. Urease activity in the 0-2 cm layer with chemical fertilizer was significantly increased by 69.70%, and soil sucrase activity in the 2-10 cm layer was also significantly increased.

### 3.5 Plant Growth Dynamics

The promotion of plant growth by artificial cyanobacteria crusts became more evident in later growth stages (after 20 days of inoculation) (Fig. 6 [Figure 6: see original paper]). During the first 20 days, plant height, leaf width, and leaf number in the chemical fertilizer treatment exceeded those in artificial cyanobacteria crusts and control treatments. However, plants treated with artificial cyanobacteria crusts began rapid growth on the 20th day, with height and leaf width gradually surpassing chemical fertilizer treatments by the 40th day. At harvest, artificial cyanobacteria crusts significantly increased plant height, leaf width, and leaf number by 242.86%, 229.03%, and 114.29% compared to the control, respectively. Pak-choi fresh and dry weights in artificial cyanobacteria crusts treatments were 14.0 and 18.0 times higher than the control, respectively. No significant differences were observed between artificial cyanobacteria crusts and chemical fertilizer in plant height, leaf width, plant fresh weight, or dry weight, though leaf number was significantly lower in artificial cyanobacteria crusts (Table 2 ; Fig. 7 [Figure 7: see original paper]).

### 3.6 Nutrient Content in Aboveground Plants

Artificial cyanobacteria crusts significantly increased plant nutrient uptake (Fig. 8 [Figure 8: see original paper]). Compared to the control, P and K contents in aboveground plants increased by 47.64% and 43.41%, respectively, while N content was unaffected. Compared to chemical fertilizer, artificial cyanobacteria crusts increased N content in aboveground plants by 26.22% but decreased P content by 22.59%.

### 3.7 Plant Root Morphology

Artificial cyanobacteria crusts significantly promoted plant root growth (Table 3). Compared to the control, artificial cyanobacteria crusts significantly increased Pak-choi root dry weight, total root length, total surface area, total volume, and average diameter by 700.00%, 258.93%, 356.67%, 503.13%, and 216.67%, respectively. Root dry weight in artificial cyanobacteria crusts was significantly lower than in chemical fertilizer, though no significant differences existed in other root morphology indices.

## 4 Discussion

Severe wind erosion from cropland, particularly in winter and spring in arid and semi-arid areas, threatens sustainable agricultural production and ecological environments (Gomes et al., 2003). As a biological measure that improves soil structure and increases soil stability (Belnap and Lange, 2003; Gao et al., 2017), artificial cyanobacteria crusts offer a promising alternative for sustainable wind erosion management. However, the time required to form cyanobacteria crusts with wind erosion control capacity after inoculation in cropland remains uncertain.

This field experiment demonstrated that cyanobacteria crust coverage, thickness, and biomass on the 18th day of inoculation were 56.13%, 3.74 mm, and 22.21 g chla/cm<sup>2</sup>, respectively. Our previous study showed that crusts with these characteristics can withstand wind erosion at speeds up to 13.0 m/s in controlled tests (Huang et al., 2023), confirming their protective capacity. Moreover, plants achieved certain coverage by the 18th day, providing additional wind erosion protection. Therefore, cyanobacteria crusts formed by the 18th day in this study are likely effective for wind erosion control.

Cyanobacteria crusts demonstrated sustained soil enhancement capabilities through photosynthesis (Zaady et al., 2000; Guan et al., 2020), atmospheric N conversion (Belnap and Lange, 2003), and polysaccharide secretion (Mugnai et al., 2018). In our study, artificial cyanobacteria crusts persisted and increased SOM and N contents by the 50th day while stimulating key enzymatic activities for nutrient cycling. Furthermore, artificial cyanobacteria crusts substantially improved Pak-choi development, with pronounced biomass accumulation in both aboveground and root systems. This growth promotion likely stems

from cyanobacteria crust-mediated modifications to rhizosphere dynamics, particularly through soil nutrient enrichment and optimized plant nutrient acquisition (Godínez-Alvarez et al., 2012). Such rhizospheric improvements create favorable conditions for photosynthetic assimilation and subsequent biomass partitioning (Zaady et al., 2000; Belnap and Lange, 2003).

Growth monitoring revealed that artificial cyanobacteria crusts began enhancing plant development after 20 days of inoculation, aligning with their biological formation period of 15–20 days (Lan et al., 2017; Mugnai et al., 2018). This delay reflects the time required for crust stabilization. As cyanobacteria crusts developed, their benefits—including soil nutrient enrichment and gradual growth promotion—became measurable over time. At harvest, Pak-choi with cyanobacteria crusts produced biomass equivalent to crops receiving 700 kg/hm<sup>2</sup> of standard N-P-K fertilizer, demonstrating that optimized cyanobacteria applications could reduce chemical fertilizer dependency while maintaining yield integrity.

High cropland yields are closely related to chemical fertilizer application, which brings 33.00%–66.00% yield increases (Cassman et al., 1998). However, widespread chemical fertilizer use causes serious problems including reduced soil productivity, environmental pollution, pest resistance development, and reduced food safety (Lin et al., 2020). This study showed that artificial cyanobacteria crusts can promote crop growth in addition to preventing wind erosion. Thus, artificial cyanobacteria crust application provides a new, environmentally friendly approach for chemical fertilizer reduction and agricultural sustainable development in arid and semi-arid areas. However, cyanobacteria crusts at different growth stages may have varying effects on soil nutrients and plant growth (Weber et al., 2022). Our study used Pak-choi, which has a short growth period, as the indicator plant; therefore, whether artificial cyanobacteria crusts exert similar effects on long-term plant growth requires further investigation.

Water is fundamental for cyanobacteria metabolism (Rippin et al., 2017), and water scarcity may affect soil cyanobacteria survival rates (Ayuso et al., 2017). Thus, soil moisture is an important factor influencing artificial cyanobacteria crust formation (Rossi et al., 2022). Notably, this study was conducted in August and September, the monsoon season in the Loess Plateau's wind-water erosion crisscross area, where soil moisture conditions may be more favorable for artificial cyanobacteria crust formation. Temperature significantly affects soil algal cell growth and reproduction (Lu et al., 2022). However, wind erosion generally occurs in winter and spring, when low temperatures can limit artificial cyanobacterial crust formation. Therefore, verification in winter and spring is worthwhile, and we propose exploring cyanobacterial species adapted to cold environments as one solution.

Ecological constraints in arid and semi-arid areas include low soil fertility, frequent droughts, land degradation, biodiversity loss, and declining agricultural productivity (Gaur and Squires, 2018). Artificial cyanobacteria crusts, an effective strategy for wind erosion prevention and soil enhancement, are widely used

in desertified areas (Chen et al., 2006; Kheirfam and Asadzadeh, 2020). However, cropland faces distinct challenges, as wind erosion primarily occurs in winter and spring (Gomes et al., 2003), necessitating rapid crust establishment for timely protection. Our study demonstrates that artificial cyanobacteria crusts can form by the 18th day of inoculation, providing wind erosion prevention while exerting benefits for plant growth. These results will benefit soil quality in arid and semi-arid areas. Integration with modern irrigation infrastructure renders cyanobacteria crusts practically viable for widespread agricultural adoption. Moreover, compared to desertified areas, cropland soil has better nutrient and water status (Zhang et al., 2019), providing more favorable conditions for artificial cyanobacteria crust formation. Additionally, as native soil microorganisms, cyanobacteria inoculants enhance biodiversity without ecological risks (Darby et al., 2007; Lan et al., 2022), differentiating them from invasive bioengineering methods. Therefore, we recommend inoculating artificial cyanobacteria crusts in croplands of arid and semi-arid areas worldwide during fallow periods and at planting time to prevent wind erosion and promote plant growth.

## 5 Conclusions

Wind erosion presents a major threat to agricultural sustainability in semi-arid areas, accelerating soil degradation and contributing to adverse atmospheric effects. Our research showed that surface coverage of artificially inoculated cyanobacteria crusts reached 56.13% by the 18th day, offering a viable solution to mitigate wind erosion in vulnerable agricultural systems.

Cyanobacteria crust introduction substantially enhanced soil nutrient quality, increased plant biomass, and elevated nutrient levels in crops. Compared to the control, inoculation with cyanobacteria crusts increased SOM, TN, and  $\text{NO}_3^-$ -N in surface soils while boosting enzymatic activity. Additionally, cyanobacteria crusts markedly improved Pak-choi growth indices, including fresh weight, plant height, leaf width, and leaf number. Importantly, P and K contents in Pak-choi with cyanobacteria crusts far exceeded those of the control. These results validate the viability of using cyanobacteria crusts to combat wind erosion in arid and semi-arid areas. Furthermore, the biomass-enhancing potential of artificial cyanobacteria crusts offers a novel approach for reducing reliance on chemical fertilizers and promoting sustainable agricultural development in these regions.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Author Contributions

Conceptualization: ZHAO Yunge; Methodology: ZHAO Yunge, ZHOU Nan; Formal analysis: JING Haimeng, ZHOU Nan, TANTAI Yu; Writing - original draft preparation: JING Haimeng; Writing - review and editing: JING Haimeng, ZHAO Yunge; Funding acquisition: ZHAO Yunge; Resources: ZHAO Yunge; Supervision: ZHAO Yunge; Data curation: ZHOU Nan; Investigation: JING Haimeng, ZHOU Nan, TANTAI Yu; Project administration: ZHOU Nan; Validation: JING Haimeng, ZHOU Nan; Visualization: JING Haimeng. All authors approved the manuscript.

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## Appendix

**Table S1** BG11 nutrient solution concentration

Chemical composition	Concentration (g/L)
NaNO <sub>3</sub>	
K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	

Chemical composition	Concentration (g/L)
MgSO <sub>4</sub> · 7H <sub>2</sub> O	
C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub>	
CaCl <sub>2</sub> · 2H <sub>2</sub> O	
C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	
C <sub>6</sub> H <sub>4</sub> O <sub>7</sub> · xFe · yNH <sub>3</sub>	
NaCO <sub>3</sub>	

*Note: The composition of A5 solution concentration is shown in Table S2.*

**Table S2** A5 solution concentration

Chemical composition	Concentration (g/L)
H <sub>3</sub> BO <sub>3</sub>	
MnCl <sub>2</sub> · H <sub>2</sub> O	
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	
CuSO <sub>4</sub> · 5H <sub>2</sub> O	
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	
Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	

*Note: Figure translations are in progress. See original paper for figures.*

*Source: ChinaXiv – Machine translation. Verify with original.*