

Carrier formulation and application concentration synergistically affect soil functions and microbial communities to enhance phosphate-solubilizing bacterial biofertilizer-mediated restoration of degraded alpine meadows

Lu Chi

2215370638@qq.com

Northwest A&F University <https://orcid.org/0009-0002-1184-085X>

Wenquan Yang

Yuze Ning

Zhiting Hao

Enzhou Wang

Pijie Jing

Xinyue Ren

Jiancun Kou

<https://orcid.org/0000-0002-1593-9585>

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1 **Carrier formulation and application concentration synergistically**
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5 Lu Chi ¹ · Wenquan Yang ² · Yuze Ning ¹ · Zhiting Hao ¹ · Enzhou Wang ¹ · Pijie Jing ¹ ·
6 Xinyue Ren ¹ · Jiancun Kou ^{1,*}

7 ¹ College of Grassland Agriculture, Northwest Agriculture and Forestry University, Yangling
8 712100, People's Republic of China

9 ² College of Life Sciences, Northwest Agriculture and Forestry University, Yangling 712100,
10 People's Republic of
11 China

12 *Corresponding author, Email: jiancun02@163.com
13

14 **Abstract**

15 *Background and aims* Degraded alpine meadows on the Tibetan Plateau threaten ecology.
16 Chemical fertilizers risk secondary pollution, yet native P-solubilizing biofertilizer carriers have
17 been scarcely researched so far. This study explores how carrier formulation and concentration
18 synergistically restore meadows for green remediation.

19 *Methods* Four indigenous phosphate-solubilizing bacterial strains were formulated with four
20 carriers (nutrient soil, charcoal, peat-based substrate, and spent mushroom substrate) and applied
21 at three rates (25%, 50%, and 100%) in a one-growing-season field experiment in Qinghai, China.
22 Plant biomass, soil properties, extracellular enzyme activities, and microbial communities were
23 analyzed to evaluate treatment effects and identify drivers.

24 *Results* Biofertilizer concentration and its interaction with carrier formulation significantly
25 affected plant productivity ($P < 0.05$). The 50% concentration level promoted growth the strongest,
26 with F3-50 achieving the highest aboveground biomass ($357.0 \text{ g} \cdot \text{m}^{-2}$), 119.4% higher than the
27 control. Biofertilizers modified soil nutrient availability and enzyme activities, with distinct
28 carrier-dependent responses. The F3-50 treatment showed the highest β -glucosidase activity,
29 whereas phosphorus acquisition enzymes exhibited carrier-specific patterns. Microbial analysis
30 indicated that it had limited effects on bacterial α -diversity, while fungal communities were more
31 responsive. RDA showed that soil organic matter, nitrogen and phosphorus availability, and
32 nutrient-acquisition enzymes explained 37.22% and 60.76% of bacterial and fungal community
33 variation, respectively.

34 *Conclusion* Optimized carrier-concentration combinations improved nutrient cycling,
35 affected microbial communities, and enhanced vegetation productivity. The performance of F3-50
36 highlights its potential for restoring degraded alpine meadows. These findings emphasize that
37 optimizing carrier composition and concentration is critical for maximizing biofertilizer efficacy
38 in nutrient-deficient alpine ecosystems.
39

Keywords alpine meadow; phosphate-solubilizing bacterial biofertilizer; phosphate-solubilizing bacteria; carrier formulation; microbial community; grassland restoration

Introduction

The Qinghai-Tibetan Plateau, known as the “Third Pole of the World”, represents a globally important ecological security barrier (Wang et al. 2022). The alpine meadow ecosystems widely distributed across this region play irreplaceable roles in water conservation, carbon sequestration, and biodiversity maintenance owing to their unique ecological structures and functions (Li et al. 2018; Jiang et al. 2020). However, these ecosystems are highly vulnerable to external disturbances due to severe environmental constraints, including persistently low temperatures and short growing seasons, coupled with intensified anthropogenic pressures from long-term overgrazing and climate change (Wang et al. 2022). Consequently, widespread meadow degradation has become one of the major ecological challenges threatening the Qinghai-Tibetan Plateau, severely limiting the sustainable provision of ecosystem services (Bardgett et al. 2021; Yan et al. 2022). Meadow degradation has triggered evident cascading effects such as vegetation loss, soil nutrient depletion, and declines in ecosystem multifunctionality (Bardgett et al. 2021), posing substantial threats to regional ecological security and the coordinated development of pastoral communities (Sun et al. 2022a), while also potentially endangering ecological stability at broader spatial scales (Guo et al. 2021).

Degraded alpine meadows may be restored using several methods, such as artificial fertilization, one of the most widely adopted management practices (Zong et al. 2021). Its primary mechanism is to alleviate nutrient deficiency by supplementing the needed nutrients, thereby rapidly enhancing plant community productivity and improving soil nutrient availability, ultimately facilitating positive ecosystem succession (Wang et al. 2020). However, long-term application of conventional chemical fertilizers may induce soil acidification, disrupt rhizosphere microbial communities, and deteriorate soil physicochemical properties, potentially compromising the long-term stability of alpine meadow ecosystems (Dai et al. 2019). Therefore, developing environmentally friendly fertilizers with sustained restoration benefits has become a critical research priority for alpine meadow restoration (Gao et al. 2020).

Microbial biofertilizers can improve soil nutrient utilization efficiency and plant growth by regulating microbial-mediated soil processes, modifying rhizosphere environments, and promoting nutrient transformation and plant nutrient acquisition (Pattnaik et al. 2021). Moreover, microbial biofertilizers can enhance plant tolerance to environmental stresses, including low temperature,

drought, and nutrient deficiency, and are therefore considered a promising biotechnological approach for ecological restoration and functional recovery of degraded ecosystems (Coban et al. 2022). However, the field performance of microbial biofertilizers depends not only on the functional microorganisms themselves but also on the properties of carrier materials (Bashan et al. 2014). An ideal carrier should provide a favorable microenvironment for microbial survival and colonization, while possessing sufficient water-retention, nutrient-buffering, and nutrient-supplying capacities to maintain microbial activity and prolong biofertilizer effectiveness (Bolan et al. 2023a; Zhang et al. 2023). Previous studies have demonstrated that physicochemical properties and nutrient availability of carrier materials determine microbial survival conditions and functional efficiency (Bashan et al. 2014). For example, biochar, characterized by abundant pore structures, a high water-holding capacity, and diverse functional groups, can facilitate microbial adsorption, enhance bacterial survival, and promote long-term microbial persistence in soils (Bolan et al. 2023a). In contrast, humus-based or organic fertilizer carriers provide readily available carbon substrates and nutrients, thereby supporting rapid microbial proliferation and functional expression (Vassilev et al. 2022). Thus, the compatibility between carrier materials and functional microorganisms represents a key determinant of biofertilizer performance and ecological benefits (Yang et al. 2024).

Nevertheless, current studies on microbial biofertilizers have mainly focused on microbial strain selection and plant growth-promoting mechanisms (Kour et al. 2020), whereas the effects of different carrier formulations on microbial colonization and overall biofertilizer efficiency remain poorly understood (Bolan et al. 2023a). This knowledge gap is particularly evident in alpine meadow ecosystems, where low temperatures, nutrient scarcity, and strong ultraviolet radiation impose severe constraints on microbial survival and activity. Consequently, systematic evaluation of suitable carrier materials for microbial biofertilizers under extreme alpine conditions is still necessary (Bolan et al. 2023a).

Alpine meadow soils generally exhibit low phosphorus availability because low temperatures and slow weathering processes restrict mineral phosphorus release, resulting in substantial phosphorus accumulation in insoluble or strongly fixed forms (Zhou et al. 2021a). Phosphorus limitation has therefore become a major constraint on plant productivity enhancement and ecological restoration in alpine meadows (Zhang et al. 2025). Accordingly, developing functional microbial biofertilizers based on phosphate-solubilizing bacteria (PSB) represents a promising strategy to alleviate nutrient limitation and promote vegetation recovery by enhancing phosphorus mobilization and availability in degraded ecosystems (Alori et al. 2017).

PSB facilitate the conversion of insoluble phosphorus into plant-available forms through the

secretion of organic acids, phosphatases, and other metabolites (Pan and Cai 2023), thereby improving phosphorus acquisition by plants (Pang et al. 2024a). Consequently, they are considered as the most promising functional microbial groups for degraded grassland restoration (Wu et al. 2024). However, introduced functional microorganisms often experience environmental filtering, competition with indigenous microbial communities, and niche constraints in non-native ecosystems, leading to uncertainty in their long-term colonization and functional expression (Kaminsky et al. 2019). In contrast, indigenous microorganisms that have undergone long-term environmental selection generally exhibit stronger ecological adaptation and stress tolerance. Therefore, developing microbial biofertilizers based on native functional strains may prove more promising for ecosystem-specific applications (Aloo et al. 2022).

In our previous studies (Wu et al. 2024; Wu et al. 2025), four indigenous PSB strains with strong phosphorus-solubilizing capacity were isolated from the rhizosphere of dominant plants in degraded alpine meadows on the Qinghai-Tibetan Plateau. These strains were found to promote plant growth, improve soil physicochemical properties, and affect soil microbial community structures. However, previous research has focused primarily on the growth-promoting effects of these strains, whereas their adaptability to different solid carrier materials and the influence of carrier properties on biofertilizer performance remain unclear.

Therefore, in this study, we used these four PSB strains as functional inoculants and formulated four solid carriers (nutrient soil, charcoal, peat-based substrate, and spent mushroom substrate or SMS). The effects of carrier formulations and concentration on plant growth, soil physicochemical properties, extracellular enzyme activities, and soil microbial communities were systematically evaluated via a field experiment. The objectives of this study were to identify suitable solid carriers for PSB biofertilizers in alpine meadows and elucidate the mechanisms underlying carrier-mediated enhancement of degraded meadow, thereby providing theoretical insights and practical guidance for ecological restoration and microbial fertilizer optimization on the Qinghai-Tibetan Plateau.

Based on the above considerations, we hypothesized that: (1) different carrier formulations and concentration can significantly influence the ecological effects of PSB biofertilizers, and that appropriate carrier concentration combinations can enhance microbial functional expression and promote plant productivity. (2) Carrier-based biofertilizers can facilitate phosphorus mobilization and enhance C, N, and P cycling-related enzyme activities, thereby improving soil nutrient transformation efficiency and promoting vegetation recovery in degraded alpine meadows. (3) Carrier formulations and concentration can affect biofertilizer-mediated ecological functions by altering soil resource availability and microbial community assembly processes, with

combinations of Carrier-based biofertilizers exhibiting optimal resource-supply characteristics showing greater restoration potential.

Materials and methods

Bacterial strains

The bacterial strains used in this study consisted of four indigenous phosphate-solubilizing bacterial (PSB) strains belonging to the genus *Pseudomonas* (phylum Proteobacteria). Previously isolated from the rhizosphere of dominant plants in alpine meadows on the Qinghai-Tibetan Plateau, these strains exhibited strong phosphate-solubilizing capacity, rapid growth, and potential for rhizosphere adaptation (Wu et al. 2024; Wu et al. 2025). All strains grew well on Luria-Bertani (LB) medium containing beef extract (5 g·L⁻¹), peptone (10 g·L⁻¹), NaCl (5 g·L⁻¹), and agar (20 g·L⁻¹) at pH 7.0.

The strains were originally isolated from Menyuan County, Haibei Tibetan Autonomous Prefecture, Qinghai Province, China (100°55'-102°41' E, 37°03'-39°59' N) (Wu et al. 2024). The sampling region is located at an elevation range of 2388-5254 m, with an annual mean temperature of 2.8-7.9 °C and annual precipitation of 360-540 mm, mainly occurring from June to September. This region has a cold and humid alpine semi-arid climate, with natural alpine meadow as the dominant vegetation type. The bacterial strains were isolated from the rhizospheres of dominant plant species, including *Poa annua* and *Kobresia humilis* (Table S1).

Carrier formulations for biofertilizers

Carrier formulations were designed according to previously described methods (Han 2013; Yang et al. 2024). Four carrier materials were selected, including nutrient soil (Pinshi Top Horticulture Co, Ltd, Shanghai, China), commercial charcoal, peat-based substrate (hereafter referred to as peat carrier) (TS1, Klasmann-Deilmann GmbH, Germany; pH 6.0), and spent mushroom substrate (SMS) (Henan Wobao Biotechnology Co, Ltd, China; pH 7.0). Four carrier formulations were prepared based on dry weight ratios, designated as F1, F2, F3, and F4:

F1: 20% nutrient soil + 40% charcoal + 40% peat carrier;

F2: 40% nutrient soil + 40% SMS + 20% peat carrier;

F3: 60% charcoal + 40% peat carrier;

F4: 100% peat carrier.

Preparation of carrier-based biofertilizers and inoculum suspensions

Carrier substrates were weighed and combined according to the prescribed research design (section 1.2), thoroughly mixed, and 150 g of each air-dried substrate was transferred into sterilized bags. The substrates were sterilized twice by autoclaving at 121 °C for 25 min each cycle. After cooling to room temperature under aseptic conditions, the sterilized substrates were transferred into polyethylene bags and adjusted to suitable moisture content using sterile distilled water.

Subsequently, approximately 80 mL of mixed bacterial suspension containing equal volumes of the four PSB strains was added to each bag. The bags were sealed after inoculation, and four randomly distributed holes were punctured on each bag to facilitate gas exchange. Each bag was then placed inside another polyethylene bag, which was similarly perforated and sealed. All procedures were performed under sterile conditions.

The prepared carrier-based biofertilizers were incubated and stored at 25 °C until use. After 45 days of storage, 10 g of each carrier-based biofertilizer was suspended in 100 mL sterile distilled water and shaken at 200 rpm for 1 h to obtain the 100% biofertilizer suspension (defined as 100% concentration). The suspension was subsequently diluted with sterile distilled water to 50% and 25% concentration levels, respectively.

On the day of application, biofertilizer suspensions with different carrier formulations and concentrations were uniformly applied to the corresponding field plots of 4 m × 5 m in size at the same volume (1,000 mL per plot, equivalent to 50 mL·m⁻²) consistently while the control plots (CK) received an equal volume of sterile distilled water for comparison. The optical density of the full-strength bacterial suspension after shaking was approximately 1.0. Each formulation had three concentration levels (25%, 50%, and 100% relative to the full concentration), resulting in twelve treatments from the full factorial combination of four carriers and three concentrations (F1-25, F1-50, F1-100, F2-25, F2-50, F2-100, F3-25, F3-50, F3-100, F4-25, F4-50, F4-100).

Field experiment design

The field experiment was conducted in Henan Mongolian Autonomous County, Huangnan Tibetan Autonomous Prefecture, Qinghai Province, China (34°52' N, 101°32' E), at an altitude of approximately 3590 m. The study site has a typical alpine meadow ecosystem, with an annual mean temperature of approximately 0 °C and annual precipitation of approximately 597.1 mm. The soil type is classified as alpine meadow soil.

In May 2025, three native grass species (*Elymus breviaristatus* 'Tongde', *Festuca sinensis*

‘Qinghai’, and *Poa crymophila* ‘Qinghai’) seeds were sowed at a combined rate of 7.41 g·m⁻² (seed ratio: 2: 1: 1) to establish an artificial grassland in a severely degraded alpine meadow area. Before sowing, the seeds were mixed with a commercial grassland fertilizer (NPK compound fertilizer: total nutrients ≥35%, N 18%, P₂O₅ 12%, and K₂O 5%) at a rate of 450 g·m⁻² manually. The soil surface was subsequently lightly harrowed to incorporate seeds into the upper soil layer. These procedures were consistently applied to all the plots, including the controls, to ensure identical initial nutrient conditions. Subsequently, all plots in the sowed field were uniformly sprayed with the prepared biofertilizers in accordance with the research design, requiring 12 treatments (four carrier formulations × three application concentrations) in total, and each treatment was replicated in triplicate, resulting in 36 experimental plots (plus three control plots), all arranged in a randomized block design. All the plots were covered with nonwoven fabric to maintain temperature and promote seed germination. No further anthropogenic management practices, including fertilization, grazing, or mowing, were conducted throughout the experimental period.

Sample collection and measurements

Soil and plant samples were collected at the end of the growing season in late August 2025 from three 1 m × 1 m quadrats randomly selected in each replicate plot, resulting in 117 quadrats in total. All aboveground plant biomass within each quadrat was harvested at the ground level and oven-dried to a constant weight to determine aboveground biomass. It was aggregated to the plot level by averaging the three quadrat results from the same plot.

Following plant sampling, five soil samples were collected from the 0-20 cm layer within each quadrat randomly using a 5-cm-diameter auger. The five soil cores were then pooled, and cleared of visible roots and stones. The three soil samples from the same plot were thoroughly mixed to ensure homogeneity and considered as one replicate sample. Throughout the sampling process disposable gloves and clean sampling bags were used to minimize cross-contamination. Before further processing, approximately 2 g of fresh soil from each sample was immediately transferred into sterile 1.5 mL cryogenic tubes (three replicates per treatment) and preserved at a low temperature for microbial community sequencing by Shanghai Personal Biotechnology Co., Ltd. The remaining soil was divided into two subsamples: one was used for soil enzyme activity assays, and the other was air-dried under natural conditions, homogenized, and sieved for subsequent physicochemical analyses.

Soil organic matter (SOM) was determined using the potassium dichromate-sulfuric acid oxidation method. Total nitrogen (TN) was measured using the Kjeldahl method. Total phosphorus

(TP) was determined after $\text{HClO}_4\text{-H}_2\text{SO}_4$ digestion using the molybdenum blue colorimetric method. Alkali-hydrolyzable nitrogen (AN) was determined using the alkaline diffusion method. Available phosphorus (AP) was extracted with NaHCO_3 and measured using the colorimetric method. Available potassium (AK) was measured using flame photometry following ammonium acetate extraction. Soil nitrate nitrogen ($\text{NO}_3^- \text{-N}$) and ammonium nitrogen ($\text{NH}_4^+ \text{-N}$) were extracted with $1 \text{ mol}\cdot\text{L}^{-1}$ KCl and quantified using continuous flow injection analysis (Sparks 1996).

Fresh soil samples stored at low temperature were used for soil enzyme activity analyses. β -glucosidase (BG) activity was quantified using p-nitrophenyl- β -D-glucopyranoside as the substrate. Protease (PRO) activity was determined using the ninhydrin colorimetric method. Urease (URE) activity was determined using the indophenol blue colorimetric method. Alkaline phosphatase (AKP) activity was measured using the disodium phenyl phosphate colorimetric method. Leucine aminopeptidase (LAP) and N-acetyl- β -D-glucosaminidase (NAG) activities were determined using substrates producing p-nitroaniline or p-nitrophenol as chromogenic products, respectively (German et al. 2011; Burns et al. 2013).

Microbial community sequencing and bioinformatic analysis

Soil microbial communities were characterized by high-throughput sequencing of bacterial 16S rRNA genes and fungal internal transcribed spacer 2 (ITS2) regions. Amplicons were sequenced on an Illumina NovaSeq platform. Microbial DNA was extracted from approximately 2 g of fresh soil samples. The bacterial 16S rRNA gene V3-V4 region was amplified using primers 338F (5'-ACTCCTACGGGAGGCAG CA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), while the fungal ITS2 region was amplified using primers ITS3F (5'-GCATCGATGAAGAACGCAGC-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3'). Amplicon sequencing was performed by Shanghai Personal Biotechnology Co., Ltd.

Raw sequencing reads were subjected to quality filtering using Trimmomatic v0.33, and primer sequences were removed using Cutadapt v1.9.1 to obtain clean reads. The clean paired-end reads were subsequently processed using the DADA2 pipeline implemented in QIIME2 v2020.6 for denoising, paired-end merging, and chimera removal. The resulting non-chimeric sequences were used to generate amplicon sequence variants (ASVs).

Taxonomic classification of bacterial and fungal ASVs was performed using BLAST and Bayesian classification methods against the SILVA database (release 138) and UNITE database, respectively. Subsequent microbial community analyses included ASV-based community profiling, alpha and beta-diversity analyses, differential abundance analysis, and correlation analyses

between microbial community composition and environmental variables.

Data processing and statistical analysis

Raw data were organized using Microsoft Excel 2021, and statistical analyses were performed using IBM SPSS Statistics 26.0. Two-way analysis of variance (Two-way ANOVA) was conducted to evaluate the effects of carrier formulation, biofertilizer concentration, and their interactions on the measured variables. In light of significant differences among treatments, Duncan's multiple range test was performed for post hoc comparisons at $P < 0.05$.

The Chao1 richness index and Shannon diversity index were calculated using the Majorbio Cloud Platform to evaluate alpha diversity of soil bacterial and fungal communities. Principal component analysis (PCA) based on microbial community abundance data was conducted to visualize differences in microbial community composition among treatments.

Microbial community statistical analyses and visualization were performed using R software (version 4.2.2) with the packages phyloseq, vegan, and ggplot2. The original LDA scores generated by LEfSe were transformed using log10 transformation before visualization and interpretation. Taxa with log10-transformed LDA scores > 0.5 were considered significantly discriminative among treatments.

Pearson correlation analysis was conducted to evaluate relationships between dominant microbial phyla, soil physicochemical properties, soil enzyme activities, and aboveground biomass and to identify the environmental drivers underlying microbial community shifts in different carrier formulation and treatments. Correlation heatmaps were generated to visualize these relationships. Redundancy analysis (RDA) was performed to determine the effects of environmental variables, including soil physicochemical properties and enzyme activities, on microbial community structures. Monte Carlo permutation tests were used to assess the explanatory power and statistical significance of environmental variables ($P < 0.05$). All diagrams were generated using Origin 2021 and the R software.

Results

Effects of carrier-based biofertilizers and concentrations on aboveground biomass

Two-way ANOVA showed that biofertilizer concentration ($F = 270.674$, $P < 0.001$) and its combination with carrier formulation ($F = 33.208$, $P < 0.001$) had significant effects on grassland aboveground biomass, whereas carrier formulation alone did not any significant main effect ($F =$

1.504, $P = 0.237$).

Biofertilizer concentration showed a highly significant effect ($P < 0.001$) on biomass, and all carrier treatments achieved the highest biomass at the 50% concentration, which was significantly higher than that at the 25% and 100% concentrations (Fig. 1). Among all treatments, the F3-50 treatment produced the highest aboveground biomass of $357.0 \text{ g}\cdot\text{m}^{-2}$, 119.4% higher than that of the control ($162.7 \text{ g}\cdot\text{m}^{-2}$). In contrast, at the 100% concentration, aboveground biomass was significantly lower in most carrier treatments than that at the 50% concentration ($P < 0.05$), with the F3-100 treatment showing the lowest biomass ($170.0 \text{ g}\cdot\text{m}^{-2}$), which was close to the control level.

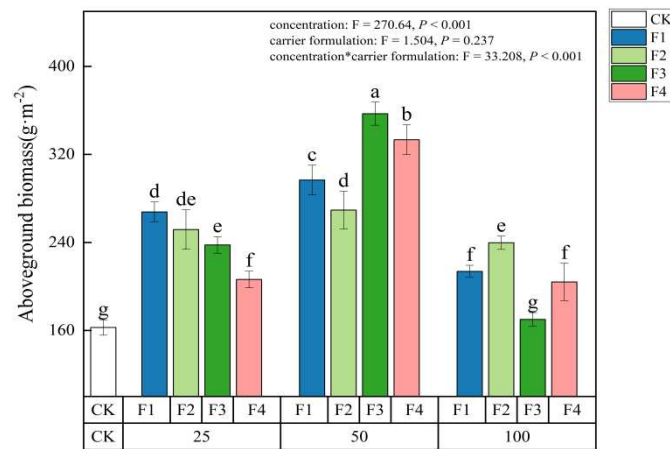


Fig. 1 Two-way ANOVA results showing the effects of carrier formulation and concentration on aboveground biomass. Bars represent means $\pm$ SE ($n = 3$). Different letters indicate significant differences among treatments at $P < 0.05$. 25, 50, and 100: 25%, 50%, and 100% concentrations of the carrier-based biofertilizers, respectively.

Effects of carrier-based biofertilizers and concentrations on soil physicochemical properties

The effects of carrier-based biofertilizers on soil nutrient properties are shown in Fig. 2. SOM content differed significantly among treatments. The F4-50 treatment attained the highest SOM content ($137.07 \text{ g}\cdot\text{kg}^{-1}$), significantly higher than $88.63 \text{ g}\cdot\text{kg}^{-1}$ in the F1-50 treatment and $106.52 \text{ g}\cdot\text{kg}^{-1}$ of the control (CK) ($P < 0.05$) (Fig. 2a).

Ranging from 6.10 to $6.23 \text{ g}\cdot\text{kg}^{-1}$, TN content increased significantly only in the F4-50 and 100% concentration treatments, while the F1-25 and F1-50 treatments showed significantly lower TN contents than the control ($P < 0.05$) (Fig. 2b).

Compared with CK, TP content increased with biofertilizer concentration. It was significantly higher at the 100% concentration than at the 25% concentration ($P < 0.05$) (Fig. 2c).

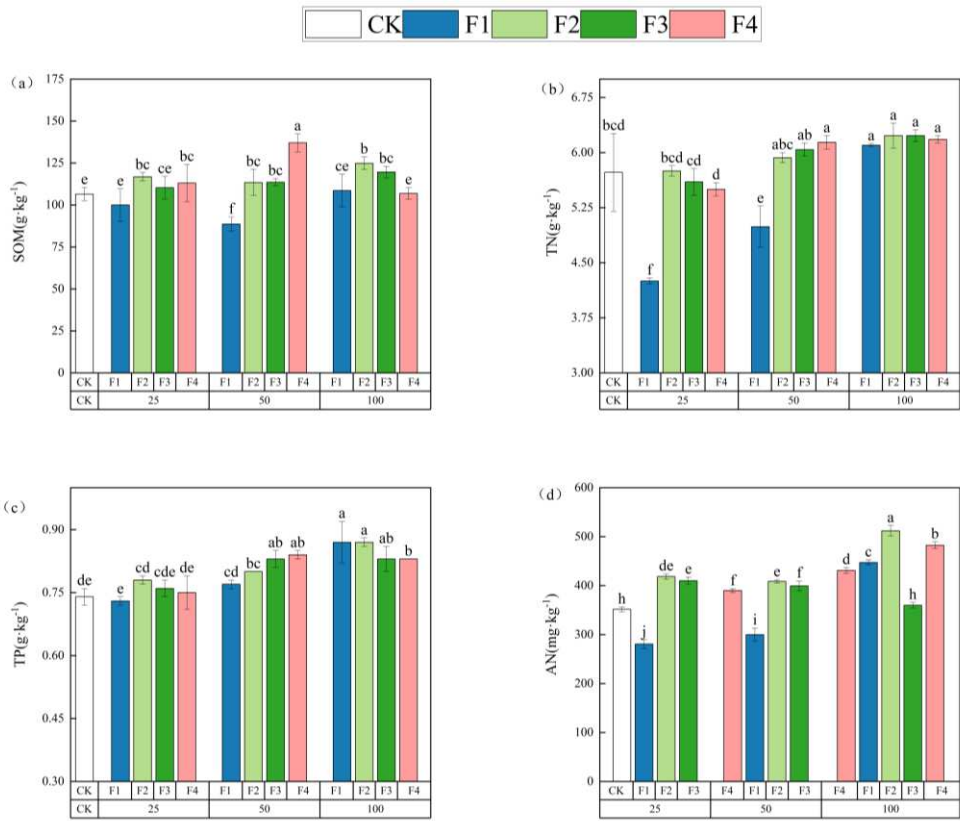
AN content differed significantly among treatments ($P < 0.05$). The F2-100 treatment showed the highest AN content ($512.05 \text{ mg}\cdot\text{kg}^{-1}$), which was significantly higher than all other treatments,

whereas the F1-25 (280.70 mg·kg⁻¹) and F1-50 (299.95 mg·kg⁻¹) treatments showed a significantly lower content than the control (Fig. 2d).

AP content was significantly higher than that of the control only in the F1-25 (8.48 mg·kg⁻¹) and F1-50 (8.79 mg·kg⁻¹) treatments ($P < 0.05$). In contrast, AP content in the 100% treatments was significantly lower than that of the control ($P < 0.05$) (Fig. 2e).

The F4-50 treatment significantly increased AK content compared with the control (199.4 mg·kg⁻¹) ($P < 0.05$), whereas most other treatments showed a significantly lower AK content than the control (Fig. 2f).

Soil NO₃⁻-N contents were generally low across treatments, and all biofertilizer treatments showed significantly lower NO₃⁻-N contents than the control ($P < 0.05$) (Fig. 2g). NH₄⁺-N contents in all treatments except for F1-25 were significantly higher than the control ($P < 0.05$), with the highest value observed in the F3-100 treatment (13.54 mg·kg⁻¹) (Fig. 2h).



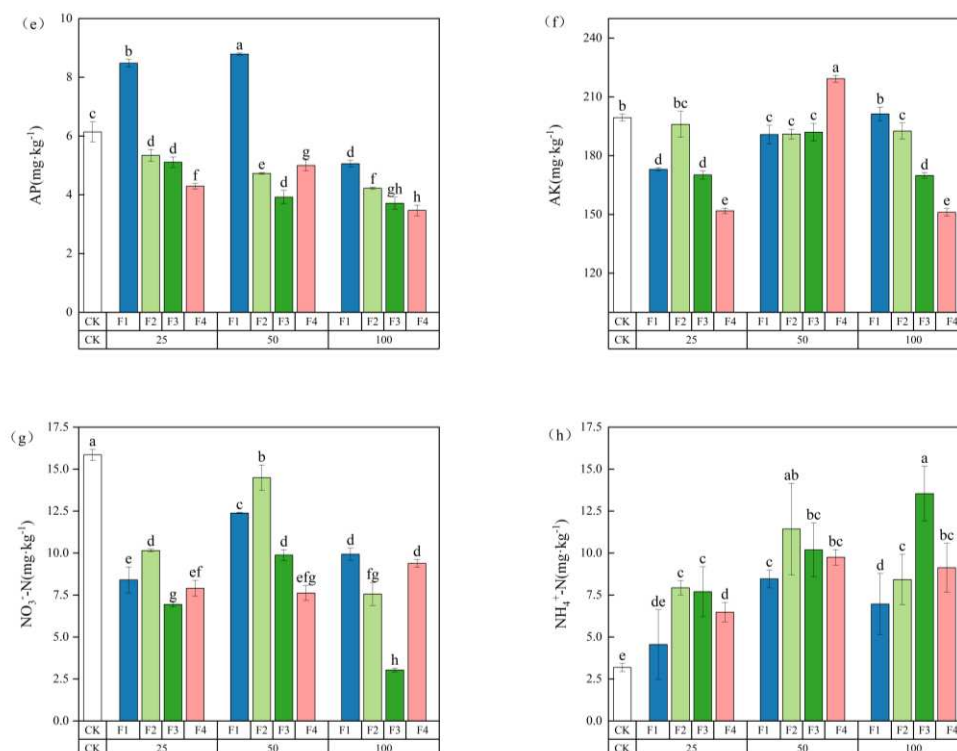


Fig. 2 Effects of carrier formulation and phosphorus concentrations of carrier-based biofertilizers on soil physicochemical properties. (a) Soil organic matter (SOM), (b) total nitrogen (TN), (c) total phosphorus (TP), (d) alkali-hydrolyzable nitrogen (AN), (e) available phosphorus (AP), (f) available potassium (AK), (g) nitrate nitrogen ($\text{NO}_3^- \text{-N}$), and (h) ammonium nitrogen ($\text{NH}_4^+ \text{-N}$). Bars represent means $\pm$ SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$. 25, 50, and 100 represent 25%, 50%, and 100% concentrations of carrier-based biofertilizers, respectively.

Effects of carrier-based biofertilizers and concentrations on soil enzyme activities

The effects of different biofertilizer treatments on soil enzyme activities varied substantially among treatments (Fig. 3). The F3-50 treatment exhibited significantly higher BG activity than all other treatments ($P < 0.05$), reaching $122.88 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ (Fig. 3a). PRO activity in the F4-25 and F4-50 treatments was significantly lower than that of the control ($P < 0.05$) (Fig. 3b). URE activity was significantly higher in the F1-100, F2-100, and F2-50 treatments than that of the control ($P < 0.05$) (Fig. 3c). AKP activity reached its maximum value in the F4-50 treatment ($339.96 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$), which was significantly higher than in all other treatments ($P < 0.05$) (Fig. 3d). LAP activity was significantly higher in the F4-25 treatment than in other treatments (Fig. 3e). NAG activity in the F4-25, F3-50, F4-50, and F4-100 treatments was significantly higher than that in the control ($P < 0.05$) (Fig. 3f).

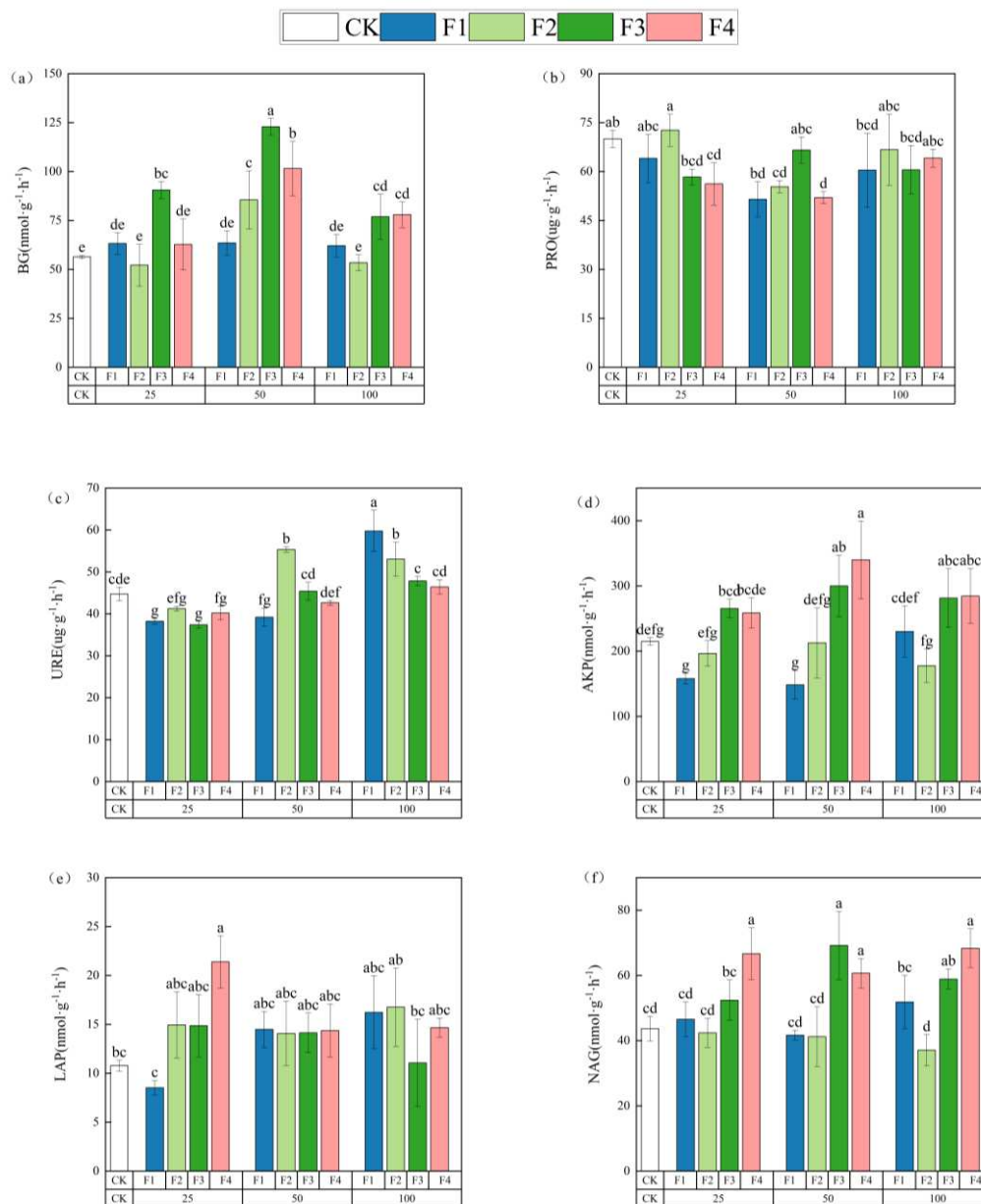


Fig. 3 Effects of carrier formulation and phosphorus concentrations of carrier-based biofertilizers on soil enzyme activities. (a) β -glucosidase (BG), (b) protease, (c) urease (URE), (d) alkaline phosphatase (AKP), (e) leucine aminopeptidase (LAP), and (f) N-acetyl- β -D-glucosaminidase (NAG). Bars represent means $\pm$ SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$. 25, 50, and 100 represent 25%, 50%, and 100% concentrations of the carrier-based biofertilizers, respectively.

Microbial community diversity and composition in different carrier-based biofertilizer treatments

To systematically evaluate the effects of different carrier-based biofertilizers and concentrations on soil microbial communities, microbial responses were analyzed in terms of alpha diversity, beta diversity, and community composition. As revealed by the Chao1 and

Shannon indices, bacterial alpha diversity was not significantly affected by treatments (Fig. 4a). Although some treatments (e.g., the 50% concentration treatments) exhibited variable Chao1 richness compared with the control (CK), the overall changes were relatively small. Similarly, Shannon diversity fluctuated only slightly with treatments, without significant differences ($P = 0.278$).

In contrast, fungal alpha diversity had a more sensitive response to biofertilizer application. The Kruskal-Wallis test indicated significant differences in fungal Chao1 richness among treatments ($P = 0.049$), although no significant pairwise differences were detected by Dunn's test ($P > 0.05$). The Shannon index differed significantly among treatments ($P = 0.003$). At the 25% concentration, several biofertilizer treatments, particularly the F4-25 treatment, showed significantly lower Shannon diversity than the control ($P < 0.05$). However, at the 50% and 100% concentrations, fungal Shannon diversity generally remained comparable to that of the control, and some treatments even showed higher values.

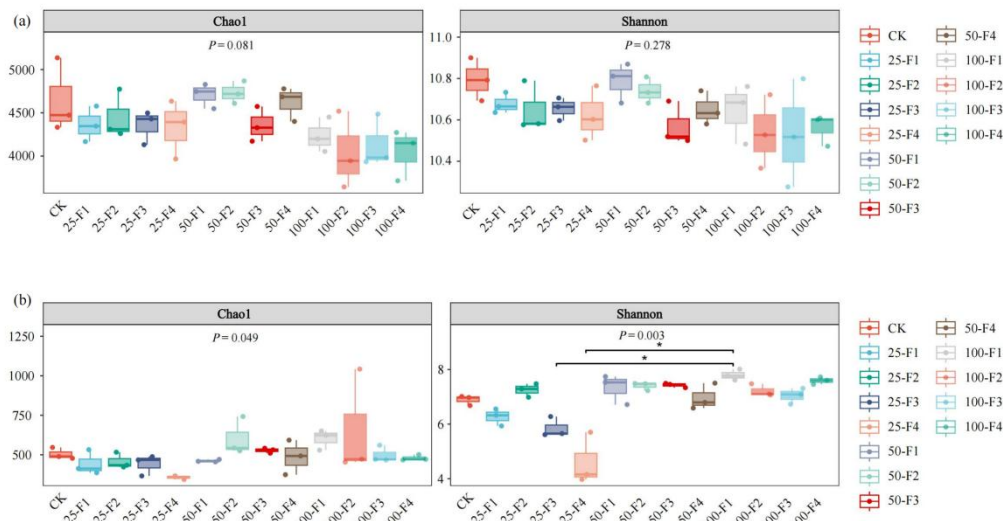


Fig. 4 α -diversity of soil bacterial (a) and fungal (b) communities in different carrier-based biofertilizer treatments. P-values indicate overall differences among treatments based on the Kruskal-Wallis test. Asterisks indicate significant differences between pairwise comparisons determined by Dunn's post hoc test (*, $P < 0.05$; **, $P < 0.01$), whereas comparisons without asterisks indicate no significant differences ($P \geq 0.05$). 25, 50, and 100 represent 25%, 50%, and 100% concentrations of the carrier-based biofertilizers, respectively.

Principal component analysis (PCA) showed that the first and second principal components (PC1 and PC2) explained 33.3% of the bacterial community variation each, with a cumulative explanatory rate of 66.6% (Fig. 5). Different treatments (CK, F1, F2, F3, and F4) showed a certain degree of separation in the two-dimensional space, although with substantial overlap. The F3 treatment showed a relatively distinct shift along the PC1 axis compared with other treatments, but not overall separation.

Compared with bacterial communities, fungal communities exhibited more pronounced separation among treatments. The PC1 and PC2 axes each explained 33.3% of the fungal community variation. The F3 treatment was clearly separable along the PC1 axis, whereas the CK, F1, F2, and F4 treatments overlapped partially with each other but still exhibited discernible groups.

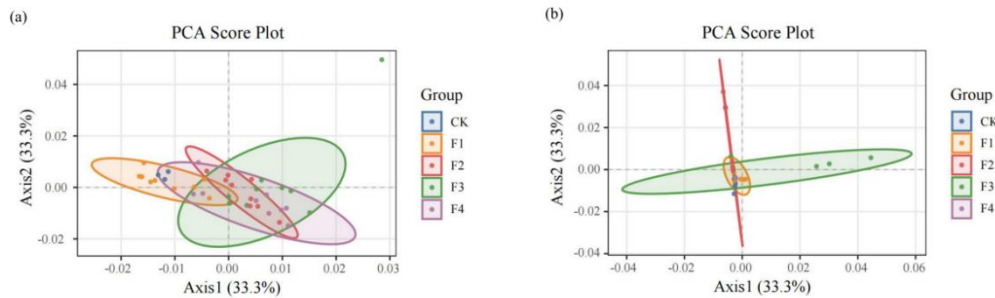


Fig. 5 β -diversity analysis of soil bacterial (a) and fungal (b) communities in different carrier-based biofertilizers treatment.

At the phylum level, bacterial communities were mainly dominated by Proteobacteria, Acidobacteriota, and Actinobacteriota (Fig. 6a), with relatively minor variations in their relative abundances among treatments, indicating an overall stability of bacterial community composition. In contrast, fungal communities were dominated by Ascomycota and Basidiomycota (Fig. 6b), whose relative abundances varied substantially among treatments. Additionally, several low-abundance taxa, such as Mortierellomycota, decreased their relative abundance in some treatments (e.g., F3-25 and F2-100), suggesting shifts in fungal community composition following biofertilizer application.

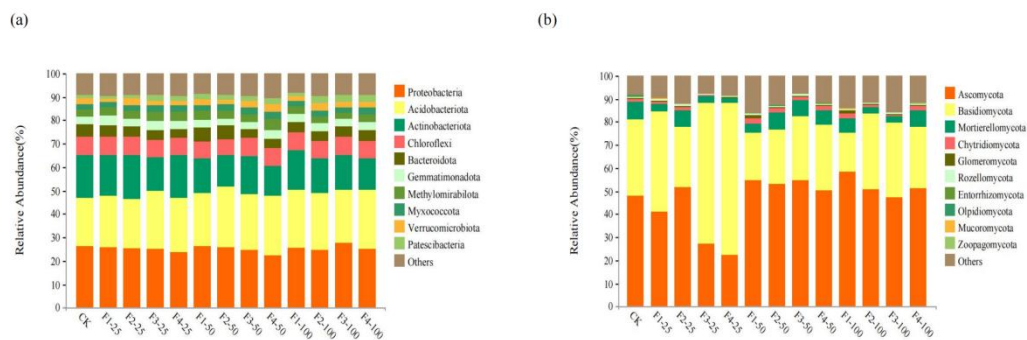


Fig. 6 Relative abundances of dominant bacterial (a) and fungal (b) phyla in different carrier-based biofertilizers treatments.

Microbial taxa identified by LefSe analysis

In the LEfSe analysis taxa with a log10-transformed LDA score > 0.5 were considered significant biomarkers. As shown in Fig. 7, distinct microbial taxa were enriched in different biofertilizer treatments. For bacterial communities (Fig. 7a), the CK was characterized by enrichment of Proteobacteria, Bacteroidota, and Actinobacteriota, with a log10-transformed LDA score of 0.99, 1.03, and 1.10, respectively. The 25% treatments selectively enriched Chloroflexi and Gemmatimonadota, with a log10-transformed LDA score of 0.82 and 1.20, respectively. More treatment-specific biomarkers were identified at the 50% and 100% concentrations. The 50% treatments mainly enriched Verrucomicrobiota, Myxococcota, Acidobacteriota, and Methyloirabilota, with a log10-transformed LDA score of 0.87, 0.94, 1.07, and 1.14, respectively. The 100% treatments significantly enriched Patescibacteria, with a log10-transformed LDA score of 1.15.

For fungal communities (Fig. 7b), the CK enriched Rozellomycota, Mortierellomycota, and Entorrhizomycota, among which Entorrhizomycota exhibited the highest log10-transformed LDA score (1.29). The 25% treatments mainly enriched Mucoromycota and Basidiomycota, with a log10-transformed LDA score of 0.60 and 1.33, respectively. The 50% treatments showed enrichment of Olpidiomyota, Chytridiomycota, Glomeromycota, and Ascomycota, with a log10-transformed LDA score of 0.90, 1.01, 1.05, and 1.25, respectively. The 100% treatments were characterized by enrichment of Zoopagomycota and other fungal taxa (Others), with a log10-transformed LDA score of 0.80 and 1.08, respectively.

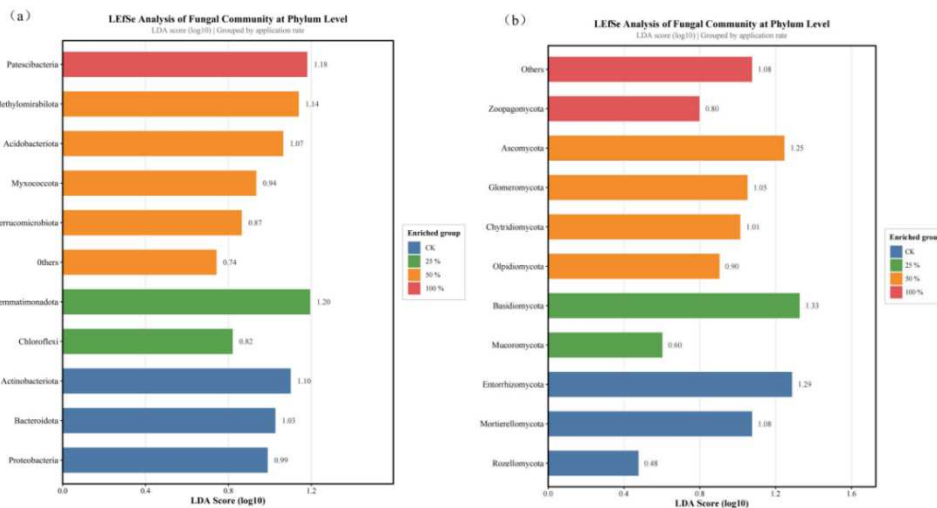


Fig. 7 Bacterial (a) and fungal (b) taxa in different carrier-based biofertilizer treatments identified via LEfSe analysis. Cladograms and LDA score plots illustrate taxa with significant differences among treatments. Taxa were selected based on the Kruskal-Wallis test ($P < 0.05$) and an LDA score > 0.5.

Relationships between microbial communities and environmental factors

Pearson correlation heatmaps (Fig. 8) revealed distinct responses of bacterial and fungal communities to soil environmental variables. For bacterial communities (Fig. 8a), Patescibacteria showed significant positive correlations with $\text{NH}_4^+\text{-N}$, TN, TP, and AN ($P < 0.05$), but significant negative correlations with AP and $\text{NO}_3^-\text{-N}$ ($P < 0.05$). Gemmatimonadota showed significant negative correlations with $\text{NH}_4^+\text{-N}$, AN, TN, and TP, with particularly strong negative correlations with TN and TP ($P < 0.01$), whereas it showed a highly significant positive correlation with AP ($P < 0.01$). Bacteroidota was positively correlated with AP ($P < 0.01$) and $\text{NO}_3^-\text{-N}$ ($P < 0.05$), but negatively correlated with SOM ($P < 0.05$).

For fungal communities (Fig. 8b), Basidiomycota exhibited a highly significant negative correlation with AK ($P < 0.01$), and also showed significant correlations with $\text{NO}_3^-\text{-N}$ and TP. Mucoromycota showed significant negative correlations with SOM and TN, but was positively correlated with AP ($P < 0.05$). Entorrhizomycota was significantly positively correlated with $\text{NO}_3^-\text{-N}$ and negatively correlated with $\text{NH}_4^+\text{-N}$ ($P < 0.05$). Ascomycota exhibited significant positive correlations with TP and highly significant positive correlations with AK ($P < 0.01$). Mortierellomycota showed significant positive correlations with AK and highly significant positive correlations with $\text{NO}_3^-\text{-N}$ ($P < 0.01$). Other fungal taxa showed a significant positive correlation with $\text{NH}_4^+\text{-N}$, while Chytridiomycota and Olpidiomyota were significantly positively correlated with $\text{NO}_3^-\text{-N}$.

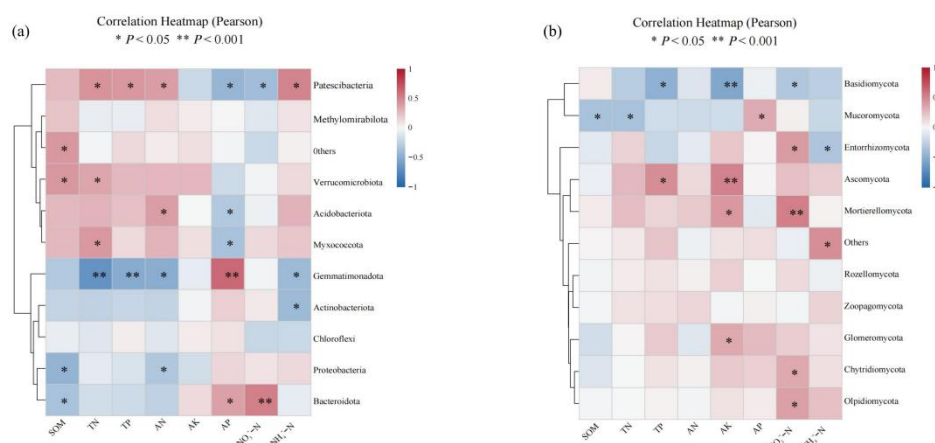


Fig. 8 Pearson correlation heatmap between dominant microbial phyla and soil physicochemical properties. (a) bacterial; (b) fungal. Red and blue indicate positive and negative correlations, respectively. Asterisks indicate significant correlations (*, $P < 0.05$; **, $P < 0.01$).

Correlation analysis between microbial communities, enzyme activities, and AGB (Fig. 9) further indicated close associations between microbial variation and soil ecological processes. For bacterial communities (Fig. 9a), Acidobacteriota showed a significant positive correlation with AKP activity ($P < 0.05$). Methyloirabiolota was significantly positively correlated with AGB, activities of NAG, and AKP ($P < 0.05$) and negative correlations with PRO activity. In contrast,

Gemmatimonadota showed negative correlations with most enzyme activities, including a significant negative correlation with AKP activity ($P < 0.05$) and a highly significant negative correlation with URE activity ($P < 0.01$). Proteobacteria and Bacteroidota were both significantly negatively correlated with NAG and AKP activities ($P < 0.05$).

For fungal communities (Fig. 9b), Basidiomycota showed a significant negative correlation with URE activity, whereas Ascomycota was positively correlated with URE activity ($P < 0.01$). Rozellomycota showed a significant positive correlation with PRO activity ($P < 0.05$).

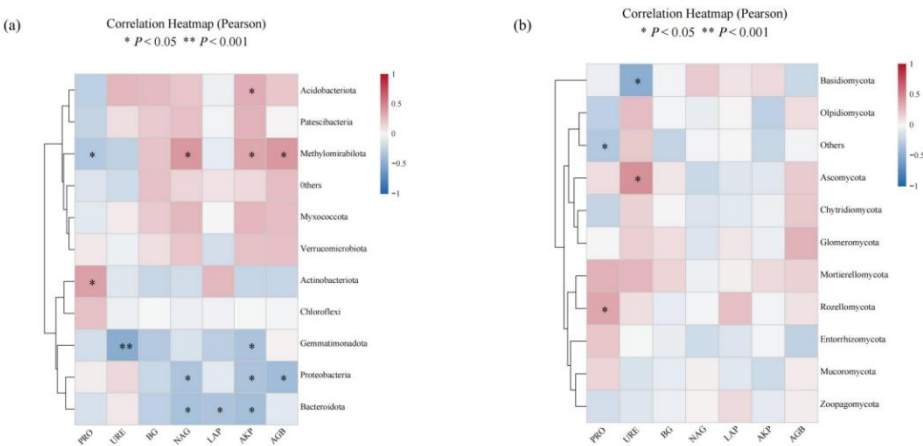


Fig. 9 Pearson correlation between dominant soil microbial phyla and soil enzyme activities and aboveground biomass. (a) bacterial; (b) fungal. Red and blue indicate positive and negative correlations, respectively. Asterisks indicate significant correlations (*, $P < 0.05$; **, $P < 0.01$).

RDA further confirmed the effects of environmental factors on microbial community structures (Fig. 10). For bacterial communities (Fig. 10a), the first two RDA axes explained 37.22% of the total variation (adjusted $R^2 = 0.269$, $P = 0.004$), indicating significant environmental regulation of bacterial community composition. SOM contributed the most to bacterial community variation, represented by the longest vector and mainly distributed along the positive direction of RDA1. Nutrient-related variables, including NAG, AKP, AN, TN, TP, and BG, also showed strong explanatory powers. PRO and LAP were mainly distributed along the negative direction of RDA1 with similar orientations, whereas NO_3^- -N was negatively associated with SOM and NAG along the RDA1 axis.

For fungal communities (Fig. 10b), the first two RDA axes explained 60.76% of the total variation (adjusted $R^2 = 0.455$, $P = 0.001$), which was substantially higher than that observed for bacterial communities. Soil nutrient variables, including AK, TN, TP, NH_4^+ -N, and NO_3^- -N, were important drivers of fungal community composition. PRO was an important enzyme explaining fungal variation, showing a relatively long vector mainly distributed along the positive direction of RDA2. Other enzyme activities, including BG, AKP, NAG, and URE, also exhibited considerable

explanatory effects. Moreover, different biofertilizer treatments were clearly separable in the RDA ordination space, indicating that biofertilizers indirectly influenced fungal community structure through modifications of soil environmental conditions.

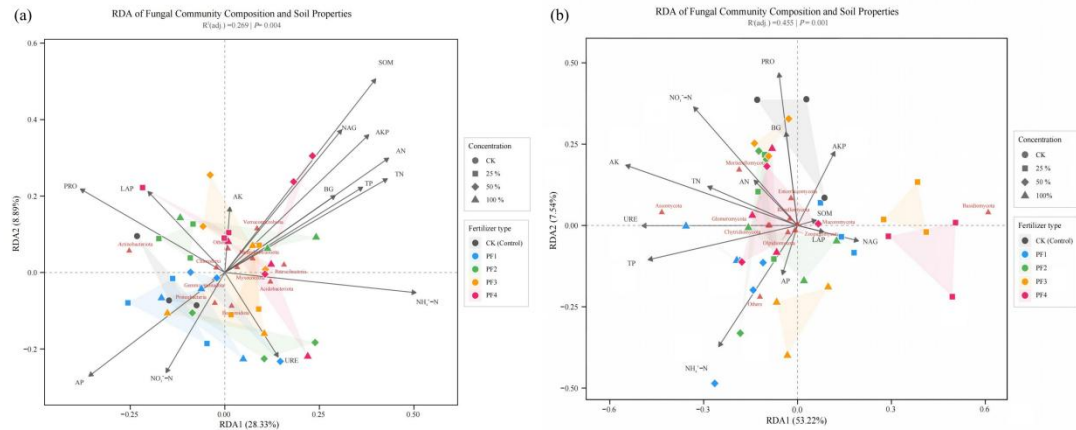


Fig. 10 Relationships between soil microbial communities and environmental variables revealed by redundancy analysis (RDA). **(a)** bacterial community; **(b)** fungal community. Environmental factors are represented by arrows, with their direction and length indicating the strength and correlation with microbial community variation. The percentages of variation explained by RDA axes are indicated on the axes.

Discussion

Biomass-promoting effects of carrier-based biofertilizers

Functional microorganisms can enhance plant growth by improving nutrient availability, modifying rhizosphere environments, and promoting root development (Aloo et al. 2022). However, the field performance of microbial biofertilizers is not solely determined by microbial traits alone but is also affected by carrier properties and concentration (Mitter et al. 2019). Suitable carrier materials can provide protective niches and attachment sites for functional microorganisms, thereby improving microbial survival and maintaining functional activity (Bashan et al. 2014), whereas carrier concentration determines the quantity of microbial inoculants and associated resource inputs into soil (Pagliarini et al. 2023). In degraded alpine meadows characterized by low temperatures, limited nutrient availability, and restricted microbial activity, the effectiveness of functional microorganisms depends largely on whether carrier materials can create suitable conditions for microbial persistence and activity (Li et al. 2024a).

In this study, appropriate carrier-concentration combinations significantly increased aboveground biomass of the artificially seeded grassland ($P < 0.05$), whereas excessive concentration did not promote sustained growth. Two-way ANOVA showed that biofertilizer

concentration had a highly significant effect on aboveground biomass, but not carrier formulation; however, their interaction was highly significant ($P < 0.001$) (Fig. 1). These results directly support our first hypothesis that optimized carrier-concentration combinations enhance biofertilizer performance and improve plant productivity. This finding indicates that the growth-promoting effects of biofertilizers are not independently determined by carrier materials or functional microorganisms, but rather by the interaction and compatibility between carrier properties and inoculation levels (Vassilev et al. 2020).

A clear dose-dependent response was observed among different concentrations. The 50% concentration generally promoted plant growth the strongest, whereas the effectiveness of the 100% concentration declined, indicating that biofertilizers have an optimal concentration range rather than a simple positive relationship between microbial concentration and plant growth (Pantoja-Guerra et al. 2024). The relatively weak response at the 25% concentration may be attributed to insufficient microbial abundance and limited resource inputs, which were unable to effectively alleviate nutrient constraints in degraded soils (O'Callaghan et al. 2022). Conversely, excessive microbial inoculation and organic matter input at the 100% concentration may have altered resource allocation patterns, intensified microbial competition, or temporarily immobilized nutrients, thereby reducing nutrient availability for plants (Bossolani et al. 2023; Kuzyakov and Xu 2013). Therefore, the 50% concentration likely represented the optimal balance among microbial inoculum input, resource release, and plant nutrient demand, maximizing biofertilizer-mediated growth promotion. Previous dose-response studies have also demonstrated that higher inoculation levels do not necessarily have stronger plant growth-promoting effects (Pantoja-Guerra et al. 2024), suggesting that microbial biofertilizer dosage should be optimized rather than simply increased. Furthermore, microbial colonization and functional expression are strongly affected by environmental adaptation and interactions with indigenous microbial communities (Mitter et al. 2021).

Although carrier formulation did not have significant main effects, different carriers showed distinct responses at the same concentration, indicating that carrier composition may affect microbial survival, environmental adaptation, and functional activity, thereby influencing plant growth responses (Sakpirom et al. 2021). Previous studies have shown that carriers not only improve microbial survival but also affect microbial release dynamics, modify rhizosphere conditions, and influence nutrient supply patterns (Bashan et al. 2014). Therefore, the ecological performance of biofertilizers depends ultimately on interactions among carrier composition, concentration, and soil environments (Vassilev et al. 2020). Differences in carrier components can alter organic carbon availability, nutrient supply capacity, microbial attachment environments, and

nutrient release rates (Bolan et al. 2023a), resulting in distinct resource supply patterns and differential plant responses (Khan et al. 2023).

In the present study, the F3 carrier at the 50% concentration resulted in the highest aboveground biomass, suggesting that this formulation may favor microbial activity and nutrient retention the most. This superior performance may be related to the physicochemical properties of F3 carrier components, particularly the role of carbon-based materials in improving microbial habitats (Vassilev et al. 2020) and enhancing nutrient retention capacity (Bolan et al. 2023b). In addition, an appropriate proportion of structural components may reduce short-term nutrient competition caused by rapid nutrient release, allowing nutrients to be more efficiently absorbed into plant-microbe utilization pathways (Bashan et al. 2014; Bolan et al. 2023b). Therefore, the advantage of F3 likely resulted not from a single carrier component, but from the combined effects of different carrier proportions (Tripti et al. 2022).

Although all carrier formulation provided certain protective effects for functional microorganisms, differences in nutrient release patterns and environmental regulation capacities may have resulted in contrasting plant responses (Vassilev et al. 2020). For example, carriers containing abundant readily available organic substrates may rapidly stimulate microbial activation (Vassilev et al. 2020), but excessive resource inputs may intensify competition among soil microorganisms (Bolan et al. 2023b). In contrast, carriers with stronger buffering and protective capacities may maintain microbial activity by improving microbial survival conditions over longer periods (Bolan et al. 2023b). Therefore, the compatibility between carrier formulation and concentration may be more important than the superiority of individual carrier material alone (Vassilev et al. 2020; O'Callaghan et al. 2022). Carriers with excessive proportions of labile organic components may promote rapid microbial activation but also increase short-term resource competition, whereas carriers dominated by stable components may restrict nutrient release and delay microbial activation (Bolan et al. 2023b).

Overall, the superior growth-promoting effect of the F3-50 treatment highlights the importance of optimizing carrier composition and concentration to maximize carrier-based biofertilizer efficiency in degraded alpine meadows. These findings emphasize that the success of microbial restoration strategies depends on achieving an appropriate balance between carrier properties, microbial activity, and resource availability.

Effects of carrier-based biofertilizers on soil nutrient availability and extracellular enzyme activities

In this study, different carrier-based biofertilizer treatments significantly altered soil nutrient availability and nutrient allocation patterns, and exerted differential effects on key nutrient-acquiring enzymes, including AKP, URE, and BG. These responses were jointly affected by carrier formulation and concentration. Several optimal carrier-concentration combinations exhibited enhanced nutrient-cycling-related enzyme activities, suggesting that appropriate biofertilizer inputs may improve soil resource supply capacity by stimulating microbial-mediated nutrient transformation processes and facilitating the conversion of unavailable nutrients into plant-accessible forms (Alori et al. 2017). In contrast, increasing biofertilizer concentrations did not further enhance soil functional properties, possibly because excessive external inputs tipped the balance among nutrient release, microbial demand, and plant nutrient acquisition processes (Pantoja-Guerra et al. 2023; Shahwar et al. 2023).

Phosphorus (P) is one of the most important nutrients limiting plant growth (Pang et al. 2024b). In alpine meadow ecosystems, prolonged exposure to low temperatures, slow mineralization, and organic matter accumulation results in substantial phosphorus accumulation in insoluble inorganic or organically bound forms, leading to generally low phosphorus availability for plants (Pan and Cai, 2023). Phosphate-solubilizing microorganisms can convert unavailable phosphorus into plant-accessible forms through the secretion of organic acids, production of phosphatases, and stimulation of organic phosphorus mineralization processes (Ramos Cabrera et al. 2024), representing an important biological pathway for improving soil phosphorus availability (Pang et al. 2024b). This study found that different carrier-based biofertilizer treatments had differential effects on AKP activity and available AP content. The significant increases in AKP activity and AP in some treatments suggest that functional microbial inoculation may enhance phosphorus mobilization processes in degraded alpine meadow soils (Wu et al. 2025).

However, several treatments (e.g., F3-50, F4-50, F3-100, and F4-100) exhibited relatively high AKP activity without corresponding increases in AP content, and some even showed lower AP levels than the control. Thus, enhanced phosphatase activity does not necessarily guarantee an immediate increase in soil available phosphorus pools. Instead, phosphatase activity may primarily reflect accelerated organic phosphorus mineralization and transformation processes (Li et al. 2021a), representing an adaptive response of plants and microorganisms to phosphorus limitation through enhanced phosphorus acquisition strategies (Manzoor et al. 2022). Under phosphorus-limited conditions, plants and microorganisms commonly increase phosphatase production to promote organic phosphorus mineralization and improve phosphorus acquisition efficiency (Margalef et al. 2021; Sinsabaugh et al. 2022). However, released phosphorus may be rapidly absorbed by plants, assimilated by microorganisms, or re-fixed by soil minerals (Zhou et al.

2022). Therefore, soil AP content represents the residual pool of available phosphorus at a specific moment rather than the overall phosphorus cycling process (Chen et al. 2024). In this study, although the 25% treatments showed relatively high AKP activity, their aboveground biomass remained lower than that of some 50% treatments, further suggesting that plant uptake and microbial immobilization jointly affect soil available phosphorus dynamics (Zhou et al. 2022).

The effects of different carrier formulations on AP accumulation may originate mainly from differences in physicochemical properties among carrier materials. The peat-based substrate used in this study contains abundant organic matter and humified components, which may improve microbial habitats and provide carbon sources for PSB metabolism, thereby indirectly facilitating nutrient transformation (Huang et al. 2021). SMS contains abundant readily decomposable organic compounds, which can stimulate microbial activation and enhance phosphorus cycling-related enzyme activities (Szymański et al. 2022; Sun et al. 2023). Charcoal possesses a large surface area and well-developed pore structure, which facilitate microbial attachment (Bolan et al. 2023a) and improve nutrient retention through adsorption processes (Lehmann and Joseph 2015).

In this study, the relatively high AP content observed in the F1 treatments may be associated with the high proportion of peat-based substrate-derived organic carbon sources and improved microbial habitats (Huang et al. 2021). However, the accumulated available phosphorus was not translated to the highest plant biomass, indicating that increased soil phosphorus availability does not necessarily equate enhanced plant productivity (Liu et al. 2024) because different carrier materials may affect phosphorus transformation through distinct pathways. The F1 carrier may primarily facilitate AP accumulation, whereas the growth-promoting effects of F3 may depend more on improving nutrient transformation processes and enhancing plant nutrient-use efficiency than on simply a higher soil available phosphorus content. These results suggest that carrier-mediated regulation of phosphorus cycling depends not only on phosphorus release capacity but also on microbial metabolic environments (Xiang et al. 2022), nutrient retention characteristics, and plant-microbe nutrient competition processes (Chen et al. 2024).

Apart from phosphorus cycling, carrier-based biofertilizers also influenced soil nitrogen and carbon cycling processes. Soil enzymes represent important biochemical tools used by microorganisms to acquire resources, and their activities reflect microbial strategies for obtaining different limiting nutrients (Sinsabaugh et al. 2022) and the intensity of ecosystem nutrient cycling processes (Burns et al. 2013). This study found that BG, URE, and AKP activities were significantly affected by biofertilizer treatments; however, different enzymes responded distinctly to carrier formulations and concentrations. This indicates that carrier-based biofertilizers do not simply enhance all nutrient cycling processes simultaneously but instead affect soil nutrient

transformation functions through selectively changing nutrient-acquisition enzyme activities (Xiang et al. 2022).

URE activity showed relatively higher values in several 50% and 100% treatments, suggesting that an appropriate biofertilizer input level may enhance potential enzyme activities associated with nitrogen acquisition. URE activity, which catalyzes the hydrolysis of urea to ammonia (NH_3) and carbon dioxide (CO_2), plays an important role in soil inorganic nitrogen formation (Gupta et al. 2023). Previous studies have shown that alleviation of phosphorus limitation can enhance microbial metabolism and further stimulate nitrogen cycling-related enzyme activities (Mehnaz et al. 2019). However, URE activity and NH_4^+ -N concentration were not always synchronized in this study, indicating that enhanced urea hydrolysis capacity does not necessarily and directly rise in soil inorganic nitrogen pools (Burns et al. 2013). Soil inorganic nitrogen availability is jointly affected by mineralization, plant uptake, and microbial assimilation processes (Burns et al. 2013). Moreover, carrier materials may further affect NH_4^+ -N dynamics through adsorption and slow-release effects, leading to asynchronous changes between enzyme activity and inorganic nitrogen concentration (Wang et al. 2021b). For example, charcoal components possess a strong cation exchange capacity, allowing NH_4^+ retention and reducing rapid nitrogen losses, thereby providing a buffering effect (Lv et al. 2021). Thus, different carrier materials may affect soil nitrogen cycling dynamics by altering nitrogen release and retention processes (Xiang et al. 2022).

The initial increase followed by decline in NO_3^- -N concentration suggests that biofertilizers may have promoted nitrogen redistribution processes in soil (Zhang et al. 2022c). This pattern may be attributed to increased organic carbon inputs from carrier materials, which provide available substrates for microorganisms, stimulate microbial growth (Kuzyakov and Xu 2013), and enhance nitrogen assimilation processes, resulting in temporary incorporation of inorganic nitrogen into microbial biomass pools (Chen et al. 2021a). In addition, organic carbon inputs may stimulate denitrification processes and accelerate NO_3^- -N consumption (Dai et al. 2019; Wu et al. 2021). Therefore, reduced NO_3^- -N availability does not necessarily indicate decreased soil nitrogen supply capacity but may reflect enhanced regulation of nitrogen cycling processes following biofertilizer application (Zhang et al. 2022c).

Regarding carbon cycling, BG is a representative carbon-acquiring enzyme, and its activity reflects microbial capacity for utilizing complex organic carbon substrates and their carbon acquisition strategies (Sinsabaugh et al. 2018). In this study, BG activity was the highest in the 50% concentration treatments, suggesting that appropriate biofertilizer inputs enhanced microbial carbon acquisition capacity (Sinsabaugh et al. 2022). Differences in BG activity among carrier

formulations may be traced to the characteristics of organic matter supplied by different materials (Xiang et al. 2022). Carriers such as SMS and peat contain abundant available organic compounds that provide readily decomposable carbon sources and enhance carbon acquisition enzyme activities (Adetunji et al. 2020). Although charcoal has a relatively low decomposition rate, its porous structure provides microbial attachment sites and improves microbial habitats, thereby affecting microbial activity and carbon cycling processes (Yang et al. 2021). Therefore, different carriers may affect microbial carbon utilization strategies by modifying carbon supply patterns (Pathy et al. 2023).

Due to long-term temperature constraints in alpine environments, microbial metabolism and organic matter decomposition processes are relatively slow, prolonging carbon turnover and diminishing soil organic carbon accumulation potential (Xu et al. 2021; Zhou et al. 2021b). Therefore, although carrier-based biofertilizers increased BG activity, changes in SOM were limited in most treatments during the short experimental period, which is consistent with the slow carbon cycling characteristics of alpine meadow ecosystems (Donhauser and Frey 2018; Wang et al. 2022).

Effects of carrier-based biofertilizers on soil microbial community structure and functional groups

Soil microbial communities represent key drivers of nutrient cycling, plant growth, and ecosystem restoration in alpine meadow ecosystems (Delgado-Baquerizo et al. 2020). The structure and function of microbial communities are jointly affected by external microbial inputs, soil resource availability, environmental filtering, and microbial niche differentiation (Trivedi et al. 2020). In degraded alpine meadow ecosystems, slow organic matter turnover at low temperatures further intensifies microbial limitations in carbon, nitrogen, and phosphorus availability (Li et al. 2022), forcing the microbial communities to adapt strongly to the environment through long-term selection. Therefore, external biofertilizer inputs may not only simply increase microbial diversity, but also promote the reassembly of dominant functional taxa by modifying resource supply patterns and microhabitat conditions (Trivedi et al. 2020; Fierer 2022).

In this study, bacterial α -diversity responded weakly to different carrier-based biofertilizers, whereas fungal communities responded to them strongly, suggesting that different microbial groups are distinctly sensitive to external resource inputs due to differences in resource acquisition strategies and ecological functions (Strickland and Rousk 2010; Fierer 2017). Neither bacterial Shannon nor Chao1 indices differed significantly among treatments, while fungal Shannon diversity showed significant variations ($P < 0.05$), indicating that biofertilizer application primarily influenced microbial community composition rather than universally increasing

microbial richness in the short term (Delgado-Baquerizo et al. 2017). Alpine meadow soils are subjected to persistent low temperatures and resource constraints, resulting in relatively stable microbial communities shaped by environmental filtering (Donhauser and Frey 2018; Fierer 2022). Therefore, external resource inputs may have limited effects on microbial richness in the short term but may instead modify community structure through changing resource availability and ecological niches (Delgado-Baquerizo et al. 2017). Moreover, after inoculated functional microorganisms enter soil, their establishment and functional expression may be jointly affected by their interactions with indigenous microbial communities, including competition, facilitation, and niche differentiation, thereby further influencing the direction of community composition changes (Trivedi et al. 2020; O’Callaghan et al. 2022).

PCA analysis further demonstrated that different carrier treatments exhibited certain degrees of separation in the ordination space, with the F3 treatment showing relatively distinct shifts in both bacterial and fungal communities. This pattern suggests that carrier materials may contribute to microbial community differentiation by modifying soil resource availability and microhabitat conditions (Li et al. 2026). Compared with bacterial communities, fungal communities responded more strongly to carrier-based biofertilizer inputs, which was consistent with the variation in fungal α -diversity and the separation pattern observed in PCA. This difference may be associated with contrasting resource utilization strategies among microbial groups (Lehmann et al. 2011). Bacterial communities generally exhibit rapid turnover rates and are more closely associated with changes in readily available nutrients and environmental conditions (Lin et al. 2021), whereas fungi possess extensive hyphal networks and depend more on complex organic substrates for complex organic matter decomposition, making them more sensitive to carbon inputs and microenvironmental changes (Fierer et al. 2023; Baldrian 2021). In this study, different carrier formulations may have generated distinct ecological niches by altering resource supply patterns, soil pore structures, and microenvironmental stability, thereby exerting stronger selective effects on fungal communities (Bolan et al. 2023a). Thus, the stronger fungal response to carrier-based biofertilizers may reflect their greater sensitivity to short-term resource inputs and microenvironmental changes during ecosystem restoration (Zhu et al. 2025).

Community composition analysis at the phylum level further indicated that carrier-based biofertilizers primarily altered soil microbial ecological characteristics through shifts in dominant taxa abundance and community structure (Sun et al. 2022b). Bacterial communities were composed mainly of Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, and Bacteroidota, which are widely involved in organic matter decomposition, nutrient transformation, and plant–microbe interactions (Banerjee and van der Heijden 2023; Barka et al. 2016). Of these

748 taxa, Proteobacteria generally possess a high resource acquisition capacity and respond rapidly to
749 external organic matter inputs and improved nutrient availability, representing typical copiotrophic
750 microbial groups (Fierer 2017). Therefore, variations in Proteobacteria abundance under different
751 resource conditions may reflect changes in microbial resource competition and niche
752 differentiation among treatments (Chen et al. 2021b). In contrast, Acidobacteriota generally
753 exhibit oligotrophic characteristics, and changes in their relative abundance may indicate shifts in
754 microbial niches following improvements in soil resource availability (Kalam et al. 2020).
755 Actinobacteriota possess strong environmental tolerance and the ability to degrade complex
756 organic compounds, making them important functional groups in cold and nutrient-poor
757 ecosystems (Barka et al. 2016). Therefore, differences in resource environments created by
758 carrier-based biofertilizers may alter competitive interactions among dominant bacterial taxa,
759 ultimately shaping distinct microbial community structures (Lin et al. 2021).

760 The phosphate-solubilizing bacteria used in this study belonged to the phylum Proteobacteria
761 (Wu et al. 2025). Proteobacteria maintained relatively high relative abundance across carrier-based
762 biofertilizer treatments and increased in some treatments (F1-50, F3-100). These changes likely
763 reflect broader microbial community responses rather than the direct establishment of inoculated
764 strains, as sequencing represents community composition only at the sampling time point. Instead,
765 the increased abundance of Proteobacteria may reflect the effects of carrier-mediated resource
766 inputs and improved soil conditions, which favor copiotrophic taxa already present in soil (Zhang
767 et al. 2022a) and contribute to resource-driven microbial community shifts (Mendes et al. 2021a).
768 These shifts may further promote organic matter turnover, nutrient transformation, and plant–
769 microbe interactions during alpine meadow restoration (Wang et al. 2021a).

770 Changes in fungal community composition further reflected ecological responses to different
771 resource utilization strategies (Treseder et al. 2021). In this study, fungal communities were
772 mainly dominated by Ascomycota and Basidiomycota. Ascomycota generally possess strong
773 environmental adaptability and an efficient substrate utilization capacity that enable them to
774 respond rapidly to labile organic matter inputs and make them important responders during early
775 ecosystem restoration stages (Tedersoo et al. 2014). In contrast, Basidiomycota are more involved
776 in lignin and complex organic matter degradation, and their dominance often requires longer-term
777 plant residue inputs and the establishment of stable hyphal networks (Treseder and Lennon 2015).
778 Therefore, during the early stage of artificial meadow establishment, external resource inputs may
779 preferentially promote the development of rapidly responding fungal groups such as Ascomycota,
780 whereas fungal taxa associated with complex organic matter decomposition may take longer
781 periods to recover (Clemmensen et al. 2022).

LEfSe analysis further confirmed that different carrier formulations and concentrations exerted selective effects on microbial taxa. The 50% concentration treatments exhibited more differential microbial biomarkers, suggesting that moderate biofertilizer inputs may promote the enrichment of specific microbial groups by modifying resource availability and microenvironmental conditions (Jing et al. 2021). In contrast, the 25% treatments may be insufficient to substantially modify microbial niches, whereas the 100% treatments, despite increasing external resource availability, may have altered microbial competition patterns and reduced the relative advantages of certain taxa under excessive resource supply conditions (Shalev et al. 2026). These results indicate that the ecological effects of biofertilizers are not simply determined by the quantity of introduced microorganisms, but are jointly affected by carrier-mediated habitat modification, resource utilization relationships, and concentration levels (Mendes et al. 2021b; Delgado-Baquerizo et al. 2020).

Associations between soil nutrients, enzyme activities, and microbial community shifts

Pearson correlation analysis and redundancy analysis (RDA) consistently demonstrated that changes in soil resource availability and functional enzyme activities were closely associated with microbial community variations in different carrier-based biofertilizer treatments. Specifically, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, AP, TP, and several enzyme activities were significantly related to dominant bacterial and fungal taxa, suggesting that the effects of carrier-based biofertilizers on microbial communities were not attributable solely to external microbial inputs, but were jointly affected by changes in soil resources and ecological niches (Fierer 2022). These findings support the concept of resource availability-driven microbial community assembly, which proposes that environmental resource availability and niche conditions often exert stronger influences on microbial community structure than microbial input intensity alone (Delgado-Baquerizo et al. 2021; Ning et al. 2020).

For bacterial communities, SOM exhibited a relatively strong explanatory contribution in the RDA analysis (Fig. 10a), indicating that carbon availability may represent an important factor shaping bacterial community variation (Delgado-Baquerizo et al. 2021). In degraded alpine meadows, reduced vegetation cover, decreased plant residue inputs, and low-temperature constraints on organic matter decomposition collectively result in persistent carbon limitations for soil microorganisms (Donhauser and Frey 2018; Liang et al. 2021). Following biofertilizer application, improved soil organic matter availability and resource supply may provide additional metabolic substrates for carbon-limited microorganisms, thereby modifying microbial resource acquisition strategies and competitive interactions (Malik et al. 2020). Previous studies have demonstrated that soil organic carbon not only affects microbial biomass and activity but also

represents a key determinant of microbial community composition and ecosystem functions (Delgado-Baquerizo et al. 2020). Therefore, the observed associations between SOM and dominant microbial taxa in this study suggest that changes in carbon availability may represent an important pathway through which carrier-based biofertilizers influence microbial community restructuring (Zhang et al. 2022d).

Besides carbon availability, soil phosphorus availability also represents an important factor influencing microbial community dynamics (Liu et al. 2021). In this study, AP and TP were significantly correlated with several dominant bacterial and fungal taxa ($P < 0.05$) (Fig. 8), and phosphorus-related variables (AP and TP) exhibited relatively high explanatory contributions in the RDA analysis (Fig. 10), indicating that changes in phosphorus availability may affect microbial resource competition and community assembly processes (Wu et al. 2022). Since phosphorus is essential for microbial growth and metabolism, increased phosphorus availability may not only facilitate plant nutrient acquisition but also modify ecological interactions among microbial taxa (Liu et al. 2021), thereby favoring microorganisms with stronger phosphorus acquisition capacity or resource utilization efficiency (Wu et al. 2022). Therefore, AP, as an indicator of soil available phosphorus supply, may represent an important environmental linkage connecting biofertilizer inputs, microbial community shifts, and vegetation restoration processes (Jing et al. 2021).

In addition to nutrient availability, soil enzyme activities were also closely associated with microbial community restructuring (Yao et al. 2023). The significant relationships between AKP, NAG and URE activities and several dominant microbial taxa (Fig. 9), indicate that microbial resource acquisition strategies may represent an important mechanism underlying community variation (Shu et al. 2023; Yao et al. 2023). Soil enzymes function as key biochemical tools involved in microbial acquisition of carbon, nitrogen, and phosphorus resources. Their activities represent microbial strategies for coping with resource limitations rather than simply manifesting soil fertility status (Moorhead et al. 2016; Sinsabaugh et al. 2022).

Among these enzymes, BG, NAG, URE, PRO, LAP, and AKP are involved in carbon, nitrogen, and phosphorus acquisition processes, respectively, and their activities reflect microbial strategies for acquiring different limiting resources (Yao et al. 2023). BG participates in the degradation of cellulose and other carbon compounds, and increased BG activity indicates enhanced microbial carbon acquisition capacity. However, the absence of significant correlations between BG ($P > 0.05$) and dominant microbial taxa in this study suggests that biofertilizer inputs may have primarily altered carbon acquisition functions rather than directly reshaping dominant microbial composition in the short term (Sinsabaugh et al. 2022). URE is involved in organic

nitrogen mineralization and reflects microbial nitrogen acquisition and transformation processes, whereas AKP is a key phosphorus-cycling enzyme and is strongly associated with several dominant microbial taxa, suggesting that phosphorus cycling may represent an important link connecting microbial functional changes with vegetation recovery (Sinsabaugh et al. 2022). Compared with nutrient concentrations alone, functional enzyme activities may more directly represent microbial ecological functions and therefore provide important insights into the mechanisms underlying microbial differences among treatments (Mori et al. 2021).

Collectively, the obtained results support our third hypothesis that different carrier formulations and concentrations affect microbial community assembly by modifying soil resource availability, thereby influencing the ecological functions of biofertilizers. In this study, the treatment combination with favorable resource supply characteristics (F3-50) exhibited enhanced vegetation recovery accompanied by shifts in microbial community structure and changes in characteristic microbial taxa, indicating its relatively high restoration potential.

Conceptual framework of carrier-based biofertilizers and concentrations promoting restoration of degraded alpine meadows

By integrating the results of soil physicochemical properties, enzyme activities, microbial community structure, and plant growth responses, we proposed a conceptual framework illustrating how different carrier-based biofertilizers promote the restoration of degraded alpine meadows (Fig. 11). Different carrier formulations and concentrations may modify soil resource availability and microhabitat conditions, affect nutrient forms and resource supply patterns, and create more favorable environments for microbial activity (Li et al. 2021b; Li et al. 2024b). Subsequently, carrier-based biofertilizers modify key soil enzyme activities, including BG, URE, and AKP, through alterations in microbial activity, thereby influencing carbon, nitrogen, and phosphorus cycling processes and enhancing nutrient transformation potential (Daunoras et al. 2024). Changes in resource environments further shape bacterial and fungal community assembly and promote the enrichment of specific microbial taxa (Zhang et al. 2022b), ultimately affecting ecosystem functional performance (Jing et al. 2021).

Ultimately, improvements in soil resource availability, microbial community restructuring, and optimization of nutrient cycling processes collectively enhance plant growth and increase aboveground biomass (Wu et al. 2024), thereby promoting the recovery of degraded alpine meadow ecosystem functions. Meanwhile, plant recovery may further improve soil conditions through increased root-derived inputs and organic matter accumulation, facilitating synergistic

interactions among plants, soil, and microbial communities (Bauer et al. 2023; Bossolani et al. 2023).

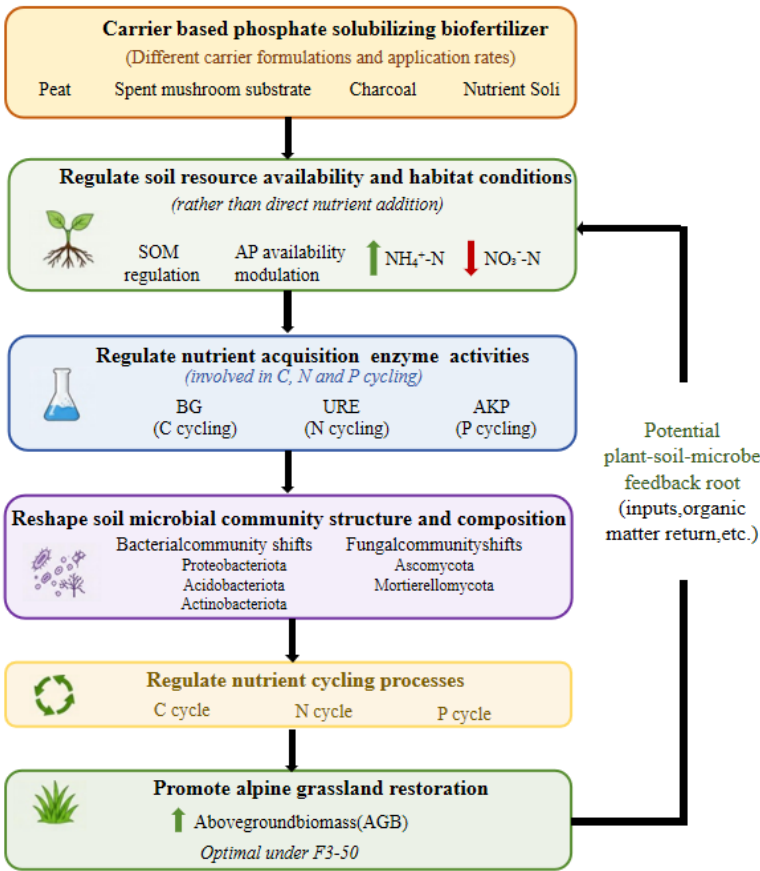


Fig. 11 Framework diagram illustrating the mechanisms by which different carrier-based microbial fertilizers and their concentrations promote the restoration of degraded alpine meadows.

Conclusions

This study demonstrated that different carrier-based biofertilizers and concentrations promoted vegetation recovery in degraded alpine meadows by improving soil resource availability, enhancing soil enzyme activities, and regulating microbial community composition. However, the ecological effects of biofertilizers were jointly determined by carrier characteristics and concentration and did not continuously increase with it. The optimal treatment, particularly the F3-50 formulation, showed greater potential for improving plant growth and soil functions, whereas some high-concentration treatments did not further enhance and even reduced certain ecosystem functional indicators, highlighting the importance of optimizing carrier formulations and concentration for maximizing biofertilizer effectiveness.

Carrier materials not only served as protective matrices for phosphate-solubilizing bacteria

but also affected soil microhabitat conditions and nutrient cycling processes. Changes in soil organic matter, nutrient availability, and C-, N-, and P-acquiring enzyme activities were closely associated with microbial community restructuring, indicating that microbially mediated nutrient cycling represents an important pathway linking biofertilizer with vegetation recovery.

Overall, this study highlights the importance of optimizing carrier composition and concentration to enhance the ecological effectiveness of microbial fertilizers in nutrient-limited alpine ecosystems. These findings provide a theoretical basis for designing functional microbial fertilizers for degraded grassland restoration. Future studies combining long-term field experiments and multi-omics approaches are needed to further elucidate the persistence of introduced functional microorganisms and their contributions to ecosystem restoration processes.

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