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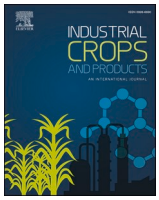

Long-term effects of nitrogen and phosphorus fertilizers on rhizosphere physicochemical characteristics and microbial composition in alfalfa

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ABSTRACTS

Repeated fertilizer applications to different monoculture cropping systems can alter soil nutrients and microbial community structure. Here we investigate the impact of long-term (4 year) distinct nitrogen (N) and phosphorus (P) fertilizer treatments on rhizosphere physicochemical characteristic and soil microbial community composition in an alfalfa (*Medicago sativa* L.) cropping systems. N and P fertilizer significantly influenced the physicochemical properties and stoichiometry of alfalfa rhizosphere soil. Nevertheless, N and P fertilizers application on the rhizosphere bacterial and fungal community structures were inconsistent. Fertilizer application minimally metamorphose the rhizosphere bacteria and fungi richness (Sobs index) and diversity (Shannon index). Non-metric multidimensional scaling analysis (NMDS) revealed that fertilizer treatments have no significant influence the fungal community, however, they significantly altered the bacterial community. Bacterial dominant phyla, *Actinobacteriota*, *Acidobacteriota*, *Chloroflexi,* and *Gemmatimonadota* changed significantly, indicating that the composition of the bacterial community was more responsive to fertilizer application when compared to fungal community composition. Spearman correlation analysis demonstrated no significant correlation amidst soil factors and bacterial diversity, conversely, bacterial richness, fungal diversity and richness were significantly modified by soil factors (AP, AN, and C/N). Network analysis indicated that N application reduced the positive associations between bacteria and fungi, whereas P application enhanced the positive associations. In conclusion, fertilization changes soil fertility of alfalfa fields and the bacterial community composition. Additionally, tests on phosphate solubilizing bacteria (PSB) isolated from the rhizosphere soil of alfalfa demonstrated that these bacteria could significantly enhance the biomass of alfalfa.

1. Introduction

Soil microorganisms contribute to soil structure, health, fertility, nutrient cycling, carbon sequestration, plant growth, and are essential for ensuring sustainability, and resilience of agroecosystems (Gao et al., 2024). Soil microorganisms serve as key indicators of soil quality, and their abundance, activity, and composition are essential factors affecting agricultural land productivity (Rachwał et al., 2021; Schloter et al., 2018). Soil microorganisms can regulate soil properties and thereby increase crop yields (Jiang et al., 2025). For example, soil microbes facilitate the turnover of soil organic matter, which is essential for crop growth and yield, soil fertility, water retention, soil structure, microbial activity, and carbon sequestration, while, microbial death/turnover, forms significant soil carbon and nutrient reserves (Bar-On et al., 2018; Mao et al., 2024; Sokol et al., 2022). However, soil texture and farm management practices may alter soil microbial communities and structure (Abdul Rahman et al., 2021). Since, soil microbial respond and 'adjust' to plant cover, environmental conditions, and cultural/management practices (Schloter et al., 2003).

Fertilization is an important crop management practice that improves crop growth and yields, and soil health and quality. Fertilizers containing nitrogen (N) and phosphorus (P) are often applied in excess as they are vital for crop growth, and are frequently limited/depleted (or are perceived to be) in crop production systems (Lu and Tian, 2017; Waqas et al., 2020). Nevertheless, the prolonged overuse of N and P fertilizers may adversely impact soil structure, microbial biomass, diversity, and community composition (Chen et al., 2023; Geisseler et al., 2017). In that, N application has been announced to reduce soil

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microbial diversity, relying on the type and amount of N utilized, and the intensity of the application method (soil disturbance) (Yang et al., 2020). However, it has also been shown that N application increases soil microbial diversity (Tang et al., 2024). Long-term N application may also affect structure of microbial communities. Such as, N fertilizers significantly enhanced the proportion of bacterial, including, *Aspergillus*, *Actinobacteria*, and ureolytic flora, however, it resulted in a reduction of *Acidobacteria* abundance (Dai et al., 2018; Sun et al., 2019b,a; Wang et al., 2018). In reaction to the enrichment of soil N, the proportion of ascomycetes in the fungal community showed a notable increase (Song et al., 2023). Whereas, P alters the soil microenvironment, and has been reported to affect soil microorganisms differently depending on available P, the amount of P applied, soil pH (Li et al., 2021; Wang et al., 2018). Research has reported P application can influence microbial communities (Ikoyi et al., 2018), where high-abundance bacterial and fungal colonies were more susceptible to P fertilizer inputs when compared to low-abundance colonies (Liu et al., 2023b). The effects of various phosphorus fertilizer sources on the composition and abundance of soil microbial communities exhibited significant differences (Devisme et al., 2024). P fertilization has been demonstrated to significantly increase the proportion of Gram-positive bacteria to that of Gram-negative bacteria (Liu et al., 2022). In conclusion, N and P fertilizers can affect the composition and structure of microbial communities by modifying the preferences of soil microorganisms (Hai et al., 2024).

Studies have investigated the impacts of lasting individual applications of N or P on microbial communities and soil health (Liu et al., 2023a; Song et al., 2023; Sun et al., 2019a), however, microbial feedback to prolonged combined N and P or balanced fertilization is less well understood. Balanced fertilizer application is a best management practice that improves nutrient use efficiency, crop growth, yield, and quality, and soil health and microbial function (Zhang et al., 2021). Chronic unbalanced fertilizer application are a major driver of shift in soil microbial community (Eo and Park, 2016). For instance, N deficiency exerted the most pronounced influence on bacterial communities, while P deficiency significantly impacted network structure and bacterial activity (Ma et al., 2019). This is mainly because single or unbalanced nutrient inputs alter soil physicochemical properties and soil stoichiometric ratios, resulting in nutrient limited microbial growth, which in turn alters their community composition (Morris et al., 2022; Tang et al., 2022). Moreover, the sensitivity of soil microbial activity to nitrogen and phosphorus fertilizers varies (Tang et al., 2024). It revealed the effect of N fertilizer on microbial genes involved in soil functions diminishes with the addition of P (Su et al., 2015), suggesting that N-P rationing is favourable to soil microbial and their functions.

Alfalfa (*Medicago sativa* L.) is a high-yielding, palatable, perennial legume forage crop, with elevated nutritional value (Wang and Zhang, 2023), which serves as a valuable source of high-quality forage for the livestock industry and also contributes significantly to soil fertilization and ecological resilience. Numerous research has probed the impacts of fertilization on alfalfa growth, performance, persistence, yield and environmental improvement (Zhao et al., 2023; Gu et al., 2023; Hu et al., 2024). Similarly, inter-root microbial communities have been recognized as potential candidates for sustainable agriculture and studies have shown that *Pseudomonas* sp., *Paraclostridium* sp., *Stenotrophomonas* sp., *Serratia* sp., *Enterobacter* sp., *Pantoea* sp. are crucial for improving photosynthetic capacity, crop yield and stress tolerance (Guan et al., 2023; Ji et al., 2024; Lu et al., 2024; Priya et al., 2022). Plant growth promoting rhizobacteria (PGPR) can reduce the reliance on chemical fertilizers, maximize crop yields, and enhance soil nutrient cycling (Zhang et al., 2025; Zhao et al., 2024). Consequently, the study examined the influence of lasting N and P rationing on soil microbial communities and the physicochemical properties within an alfalfa production system. In addition, PGPR were isolated the alfalfa field and their biotrophic effects were evaluated in a pot experiment. This research deepens our insight into the mechanisms through which fertilization impacts perennial crop soils in dryland agriculture and the development of potential microbial agents for sustainable agriculture.

2. Materials and methods

2.1. Experimental Site

The experimental location was at the Water-Saving Irrigation Experimental Station of Shihezi University in Shihezi, Xinjiang (44°18′29″ N, 86°03′45″ E, 412 m a.s.l.), China. Climatic conditions were characterised as temperate, continental, and arid. Average annual temperature, sunshine duration, and precipitation were, 7.2 °C, 2865 h, and 215 mm, respectively. The soil physicochemical properties have been detailed in the previous study reported by Sun et al. (2022).

2.2. Field experiment design

The plot (4 m × 6 m = 24 m^2) experiment was conducted using a two-factor randomized block design, with three N levels: 0 (N_0), 60 kg·ha^{-1} (N_1) and 120 kg·ha^{-1} (N_2), and four P_2O_5 levels: 0 (P_0), 50 (P_1), 100 (P_2) and 150 kg·ha^{-1} (P_3) (Table 1), with 3 blocks. Fertilizer was administered in a dropwise manner with water during the branching stage following the regrowth of alfalfa, 3–5 days after cutting of the 1st, 2nd and 3rd crops.

Alfalfa (WL366HQ) was manually strip-seeded at 18.0 kg·ha^{-1}. To isolate each plot and stop the surface and subsurface flow of nutrients and water between treatments, a 1 m wide walkway was established between each plot. Drip irrigation was buried 10 cm underground, and spaced 60 cm apart.

2.3. Sampling and measurements for field

2.3.1. Soil sample collection

Briefly, uniform alfalfa plant was selected, plant debris was removed from, and a small excavation (20 cm deep) was dug around each alfalfa root system May 3, 2023. Alfalfa plants were then carefully removed from each hole, gently agitated to eliminate loose soil, then remaining soil retained on the root system was then carefully collected for analysis.

2.3.2. Determination of soil indicators

In summary, soil pH and electrical conductivity (EC) were assessed using a soil-water ratio of 1:5. Soil water content (SWC) was assessed using the drying technique. Soil organic carbon (SOC) was determined by the high-temperature externally heated potassium dichromate oxidation-volume method, soil TN by Kjeldahl N determination, TP by the molybdenum-antimony antimony colorimetric method, alkali-hydrolyzable nitrogen (AN) was measured using the alkali N-proliferation method, while available phosphorus (AP) was determined through the sodium bicarbonate extraction followed by molybdenum-antimony colorimetric analysis (Lu, 2000). The ratios of SOC to TN (C/N), SOC to TP (C/P), and TN to TP (N/P) were calculated by measuring the

Table 1
Specific application amounts of N fertilizer and P fertilizer.

Treatments		N (kg·ha^{-2})	P_2O_5 (kg·ha^{-2})	Urea (kg·ha^{-2})	Monoammonium phosphate (kg·ha^{-2})
N_0	P_0	0	0	0	0
	P_1	0	50	51.0	96.2
	P_2	0	100	25.6	192.3
	P_3	0	150	0	288.5
N_1	P_0	60	0	130.4	0
	P_1	60	50	181.4	96.2
	P_2	60	100	156.0	192.3
	P_3	60	150	130.4	288.5
N_2	P_0	120	0	260.9	0
	P_1	120	50	311.9	96.2
	P_2	120	100	286.4	192.3
	P_3	120	150	260.9	288.5

individual contents of SOC, TN, and TP.

2.3.3. High-throughput sequencing

Total genomic DNA from the microbial community was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) in collaboration with Shanghai Meiji Bio-medical Technology Co (Shanghai, China). PCR amplification of the V3-V4 variable region of the 16S rRNA gene was carried out following the method described by Liu et al. (2016).

2.4. Isolation and characterization of alfalfa growth promoting rhizobacteria

2.4.1. Isolation of phosphate solubilising bacteria

Phosphate solubilizing bacteria were obtained from soil by dilution plating method (Ba et al., 2020), briefly, 1 g of the collected alfalfa rhizosphere soil, subjected to various fertilization treatments, was precisely measured and introduced into a test tube containing 9 ml of sterile saline solution. The mixture was then placed on a shaking table for 30 minutes, allowed to settle, and the supernatant was carefully aspirated. Subsequently, 1 ml of the supernatant was transferred into another test tube containing 9 ml of sterile saline solution, and mixed well to make a 10^{-2} dilution. Dilutions were made sequentially as describe to achieve: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}. Using a pipette 100 μl dilution (10^{-6}, 10^{-7} and 10^{-8}) was added into the organic and inorganic phosphorus bacterial medium, respectively, and incubated at 36 ℃ for 3 days and observed the appearance of lysophosphorus circle. Colonies observed with larger phospholysis circle were further screened and purified for 4–5 generations by plate delineation method, and the 5 strains with the largest phospholysis circle were selected for subsequent analysis. All strains were stored and preserved in 50 % glycerol solution (1:1) and frozen (-80 ℃).

2.4.2. Physiological and biochemical characterization of Phosphate Solubilizing Bacteria (PSB)

The selected strains were subjected to catalase test, V-P test, citrate test, urease test, starch hydrolysis test, imdole test, nitrate reduction test, methyl red test and oxidase test (Bergey, 1994). Purified single colony spots were inoculated into CAS assay medium for 3 days. Colony diameter was measured against the diameter of the orange-red halo. The IAA was measured by the approach of Yim et al. (2009). The above OD_{600}= 0.5 bacterial sap was inoculated into LB liquid medium (100 ml) containing L-tryptophan 500 μg/ml, and incubated continuously at 36 ℃, 180 r·min^{-1} for 3 days, and repeated for 3 times. The LB liquid broth was centrifuged (5000 r·min^{-1}) for 15 min and the supernatant was mixed with Sackowski's colorant 1:1 and then left to stand for 30 min to determine the OD_{530}.

2.4.3. Quantitative determination of the phosphorus solubilizing capacity of Phosphate Solubilizing Bacteria (PSB)

Five bacterial strains were inoculated into LB liquid medium and activated for 24 h. The OD_{600} value of the bacterial suspension was determined and adjusted to the OD_{600}= 0.5. One percent was respectively added into 100 ml of the liquid medium for the de-organized and de-inorganized P bacteria and cultured continuously at 36 ℃ and 180 r·min^{-1} for 7 days. Then LB liquid was centrifuged (10000 r·min^{-1}) for 10 min, and the supernatant was diluted to determine the AP (Lu, 2000), and the pH was also determined.

2.4.4. Seed germination test

Five strains of the preserved bacteria were inoculated into LB liquid medium for 24 h after activation, and the concentration of the bacteria solution was adjusted to about 10^8 CFU·ml^{-1} with saline solution (adjusted after calculating the concentration of the bacteria solution using plate counting method) used as the 'seed' solution, and LB without bacteria was used as the control. Sterilized alfalfa seeds were soaked in different seed solutions for 2 h and then ~40 seeds were place on a double-layer filter paper in petri dishes, with three replications per treatment. Petri dishes were incubated in a light incubator for 7 days. chlorophyll content, Seed germination, and total shoot length (sum of shoot and root length) were determined (Li, 2000).

2.4.5. Pot experiment

The pot experiment consisted of a total of 6 treatments: i) control (no bacteria) (CK); ii) Y316 bacterial solution (Y316); iii) Y015 bacterial solution (Y015); iv) W003 bacterial solution (W003); v) Y014 bacterial solution (Y014); and vi) W006 bacterial solution (W006). Sterilized alfalfa seeds were evenly placed (April 27, 2024) on a substrate containing a mixture of sterilized soil and vermiculite (3:1), and then lightly covered with a shallow layer of sterilized soil. Soil for this portion of the study was collected from at the Water-Saving Irrigation Experimental Station of Shihezi University. The soil was divided, and sterilized for later use. The physicochemical properties of the soil were: SOC= 13.90 g·kg^{-1}, TN= 1.16 g·kg^{-1}, AN= 92.56 mg·kg^{-1}, TP= 0.89 g·kg^{-1}, AP= 29.53 mg·kg^{-1}, pH= 7.95, EC= 536 uS·cm^{-1}.

Germinated alfalfa seedlings were allowed to develop to the three-leaf stage and were then thinned down to seven plants in each pot. Bacterial solution (10 ml of 10^8 CFU ml^{-1}) was then slowly applied next to each alfalfa plantlet using a sterile syringe. Alfalfa plants were reinoculated at 7 days as described previously, while CK treatment was inoculated with an equal amount of sterilized LB medium (2 inoculations per crop), and the procedure was repeated four times.

2.4.6. Indicators and methods for pot experiment

Alfalfa chlorophyll *a*, chlorophyll *b*, and carotenoid content were determined (Li, 2000) at harvest for the 1st and 2nd crop on June 16 and July 14, 2024, respectively Alfalfa plant height, stem thickness, and aboveground biomass were also determined. Samples were then dried and ground to determine crude protein (CP), crude ash (Ash), plant P, acid detergent fiber (ADF), and neutral detergent fiber (NDF) (Lu, 2000).

2.5. Data analysis

Soil microbial α-diversity (Sobs and Shannon indices), β-diversity, and correlation of soil physicochemical properties with soil microbial composition (Spearman) analyses were determined on the Majorbio Cloud Platform. Soil physicochemical properties, microbial diversity, and composition graphing were done in Origin (OriginPro® 2022b, OriginLab Corporation, Northampton, MA, USA). For soil microbial co-occurrence network analysis, firstly, spearman correlation analysis was achieved in the online analysis platform (http://www.cloud.biomicroclass.com), then species with $|r| > 0.6$, $P < 0.05$ were selected, and finally the network was visualized by Gephi 0.9.2. Two-factor ANOVA was performed using SPSS 26.0 for N and P application levels, and Duncan's method was employed to assess the soil physicochemical properties, as well as in diversity and community composition.

3. Results

3.1. Changes in soil physicochemical properties under different Nitrogen and Phosphorus fertilization

N and P significantly ($P < 0.05$) affected soil pH, EC, SWC, SOC, TN, TP, AP, C/N, C/P, and N/P (Table 2). N fertilizers did not significantly affect SWC, while P fertilizers did not significantly affect pH (Table 2). Further analysis showed that under N_0 and N_1, EC exhibited decreasing trend with increasing P application rates (excluding N_1P_3), but under N_2, P_2 and P_3 were higher than P_0. At the identical N level, P application increased SWC. Similarly, the P application increased SOC content and the P_1, P_2 and P_3 were significantly ($P < 0.05$) greater than the P_0. In the N_0 and N_1, P application increased the TN content. Under the N_0, N_1 and N_2, P application increased soil TP content, and the P_1, P_2 and P_3 were

Table 2
ANOVA of the effect of fertiliser application on soil physicochemical properties.

Treatments	pH	EC	SWC	SOC	TN	TP	AP	AN	C/N	C/P	N/P
		($uS{\cdot}cm^{-1}$)	(%)	($g{\cdot}kg^{-1}$)	($g{\cdot}kg^{-1}$)	($g{\cdot}kg^{-1}$)	($mg{\cdot}kg^{-1}$)	($mg{\cdot}kg^{-1}$)			
N fertilizer rates (N)											
N_0	8.58 ±	164.83 ±	6.72 ±	11.93 ±	1.38 ±	0.33 ±	12.14 ±	79.00 ±	8.65 ±	35.79 ±	4.16 ±
	0.11 A	12.53 C	0.78 A	1.29 C	0.09 C	0.02B	3.00 A	11.00 A	0.74 A	1.38B	0.35 C
N_1	8.56 ±	177.67 ±	6.76 ±	13.29 ±	1.84 ±	0.33 ±	12.05 ±	85.60 ±	7.23 ±	40.06 ±	5.56 ±
	0.05AB	18.70B	0.21 A	1.18B	0.13B	0.03B	1.63 A	12.89 A	0.48B	1.59 A	0.39B
N_2	8.51 ±	189.83 ±	6.94 ±	14.48 ±	2.11 ±	0.36 ±	12.20 ±	84.68 ±	6.89 ±	40.74 ±	5.99 ±
	0.05B	15.83 A	0.34 A	1.77 A	0.18 A	0.04 A	2.90 A	6.66 A	0.99B	2.48 A	0.63 A
P fertilizer rates (P)											
P_0	8.51 ±	180.22 ±	6.41 ±	11.18 ±	1.68 ±	0.30 ±	10.21 ±	82.61 ±	6.81 ±	37.34 ±	5.60 ±
	0.04a	12.68a	0.24c	0.73c	0.31b	0.01d	1.54b	6.57a	0.98c	1.84b	0.98a
P_1	8.57 ±	160.56 ±	6.75 ±	13.05 ±	1.73 ±	0.32 ±	12.67 ±	86.13 ±	7.65 ±	40.25 ±	5.35 ±
	0.03a	8.29b	0.28b	1.50b	0.30b	0.01c	2.59a	12.25a	0.85b	3.99a	0.97ab
P_2	8.54 ±	179.78 ±	6.83 ±	14.41 ±	1.79 ±	0.36 ±	13.12 ±	86.20 ±	8.19 ±	39.67 ±	4.93 ±
	0.06a	17.17a	0.50b	1.39a	0.32ab	0.02b	2.06a	9.29a	1.00ab	2.41a	0.82c
P_3	8.57 ±	189.22 ±	7.23 ±	14.30 ±	1.90 ±	0.37 ±	12.51 ±	77.60 ±	7.72 ±	38.2 0±	5.06 ±
	0.13a	22.22a	0.63a	1.04a	0.35a	0.02a	2.91a	12.67a	1.11a	2.26b	0.89bc
Source of variance											
N	0.027	< 0.001	0.132	< 0.001	< 0.001	< 0.001	0.985	0.307	< 0.001	< 0.001	< 0.001
P	0.126	< 0.001	< 0.001	< 0.001	0.003	< 0.001	0.024	0.327	< 0.001	< 0.001	0.002
N × P	0.006	< 0.001	< 0.001	< 0.001	0.131	0.017	0.022	0.882	0.057	0.016	0.031

significantly greater ($P < 0.05$) than the P_0. At N_1 and N_2, the AP under P_1, P_2 and P_3 was higher than that of P_0, and the difference between the AP under P_2 and P_3 and that of P_0 was significant at the N_2 ($P < 0.05$). The AN under N and P treatments were not significant. At the N_0, N_1, N_2, P raised C/N, while at the same P level (P_0, P_1, P_2 and P_3), N application decreased C/N. Under the N_0, the difference between C/P under each P application was not significant. Within the same P application level, C/P in N_1 and N_2 was significantly ($P < 0.05$) higher than that under N_0. Under the same N application level (excluding N_1P_1), the N/P under P_1, P_2 and P_3 was lower than that under the P_0 (Fig. 1).

Distinct capital letters represent significant differences ($P < 0.05$) among various nitrogen (N) application, and different lowercase letters indicate significant differences ($P < 0.05$) among various phosphorus (P) applications. Data are means ± sem (n = 3). N_0, N_1, and N_2 represent different N applications, each containing four different P applications. P_0, P_1, P_2, and P_3 represent different P applications, each containing three applications. EC, electrical conductivity; SWC, soil water content; SOC, soil organic carbon; TN, total N; TP, total P; AN, alkali-hydrolyzable N; AP, available P.

N_0, N_1, and N_2 represent different nitrogen (N) applications, each containing four different phosphorus (P) applications. P_0, P_1, P_2, and P_3 represent different phosphorus applications, each containing three applications. Data are means ± sem (n = 3).

3.2. Soil microbial community diversity

N and P fertilizers do not significantly altered the soil bacteria and fungi of Shannon index and Sobs index. Moreover, as a whole, the Sobs index varied similarly to the Shannon index (Figs. 2a-1) Unlike bacterial α-diversity, the interaction of N and P influenced the fungal Sobs index ($P < 0.05$) but did not affect the Shannon index, as revealed by two-way ANOVA. However, the one-way ANOVA showed that P_2 significantly ($P < 0.05$) decreased the Shannon index when contrasted with P_0 in the N_2 and N_1 increased the Shannon index when compared with N_0 in the P_0 and P_2 levels. There were 508 genera of bacteria in each fertilization treatment, and 18 genera were unique to N_2P_0 under different fertilization treatments. N_0P_3 is the least unique genus, with 3 species. Similarly, fungi share 95 genera, with the largest number of genera unique to N_2P_1 at 9. N_0P_1 is the least endemic, with 2 species (Fig. 3a-b). The NMDS analyses indicated that varying nitrogen and phosphorus fertilizers significantly influenced the β-diversity of soil bacteria, whereas fungal β-diversity remained largely unaffected (Fig. 3c-d).

3.3. Composition of soil microbial communities

The number of soil bacterial sequences and OTUs was 75441–84292 and 1803–1923, respectively (Table 3). The dominant soil bacterial phyla (> 0.01 %) were *Actinobacteriota* (21.17–32.67 %), *Proteobacteria* (16.25–25.06 %), *Acidobacteriota* (10.60–22.35 %), *Chloroflexi* (10.28–13.12 %), *Gemmatimonadetes* (4.37–7.00 %), *Bacteroidota* (2.73–5.92 %), *Firmicutes* (1.58–9.21 %), *Myxococcota* (1.81–2.98 %), *Methylomirabilota* (1.28–2.32 %) and unclassified Bacteria (0.83–1.36 %) in all soil samples. These 10 phyla accounted for 93.47–95.68 % of the sequences (Fig. 4). At the genus level, there were 508 common species under each fertilization, N_2P_0 had the most unique species and N_0P_3 had the least (Fig. 3a).

The one-way ANOVA showed that most of the bacterial phyla abundance was not shifted by N_0 and N_2. Under the N_1, *Actinobacteriota* in P_2 was significantly ($P < 0.05$) larger than P_0, P_1 and P_3. The maximum relative abundance of *Acidobacteriota* and *Chloroflexi* was observed at the P_3 and was significantly ($P < 0.05$) greater than the P_0. Similarly, most of the bacterial phyla abundance did not differ among different N level at the same P level (Fig. 4).

The quantity of soil fungal sequences and OTUs ranged from 90671–110502 and 236–303, respectively (Table 3). The dominant soil fungal phyla (> 0.01 %) were *Ascomycota* (72.12–92.15 %), *Mortierellomycota* (5.09–15.97 %), *Basidiomycota* (1.44–10.92 %), and Unclassified_k_Fungi (0.95–2.56 %), and no significant (Fig. 4). At the genus level, there were 95 common species under each fertilization, N_2P_1 had the most unique species, N_0P_1 and N_2P_0 had the least (Fig. 3b).

3.4. Co-occurrence network of soil microbial communities

With increased N application rate, the average degree, network density, number of links and proportion of positive decreased and then increased, however, the N_1 and N_2 were lower than N_0. However, N_1 improved modularity and N_2 improved clustering coefficient (Fig. 5) (Table 4). At the same time, both N_1 and N_2 increased the average path length. With increased P application rate, clustering coefficient, network density and number of links decreased, then increased, and then decreased. The average path length and modularity initially increased, subsequently decreased, and then increased again. Different N and P levels also changed the keystone taxa of bacteria-fungi in the co-occurrence network (Table 5).

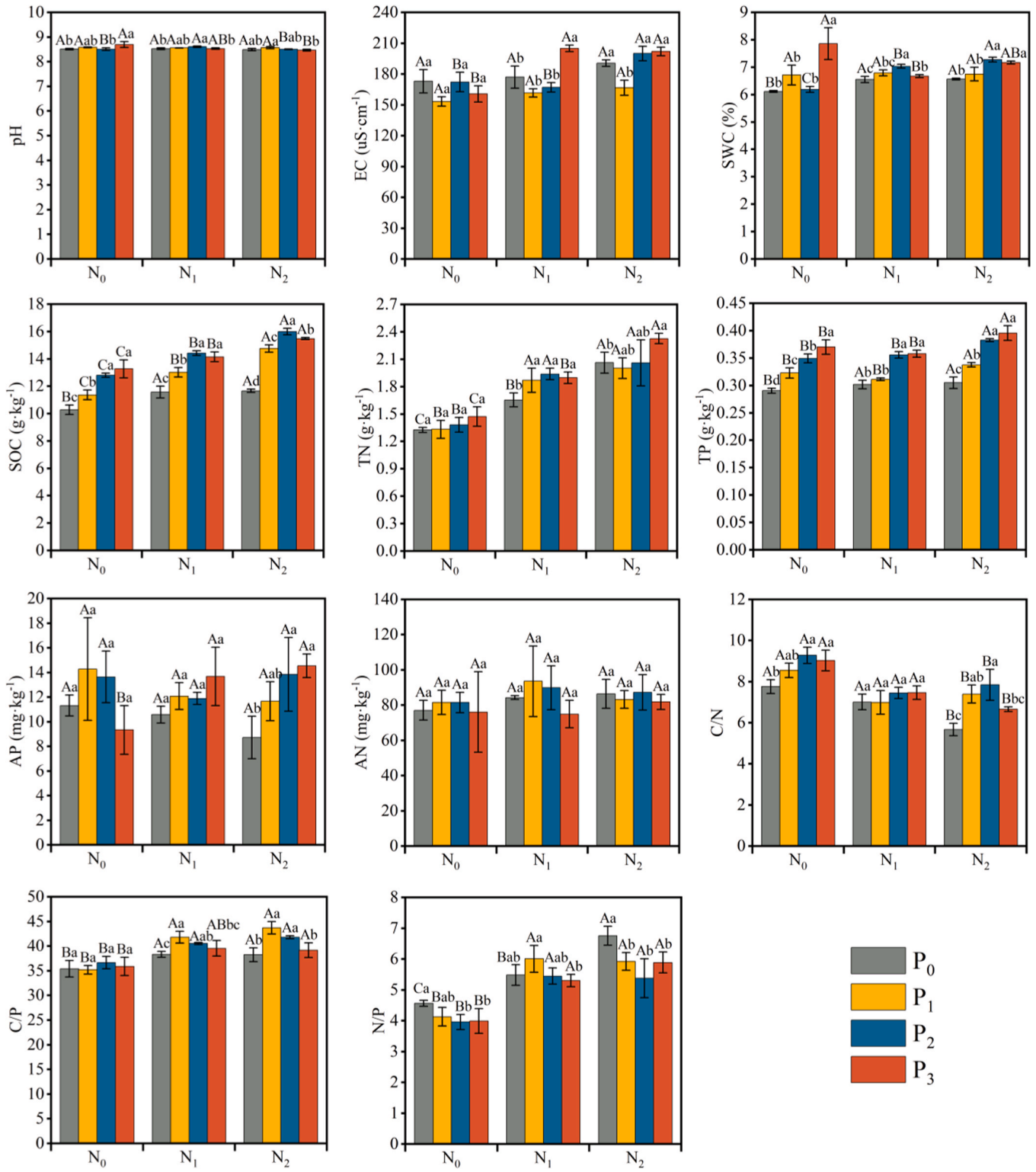

Fig. 1. Soil physical and chemical properties in alfalfa fields with different fertilization treatments. Different lowercase letters indicate significant differences ($P < 0.05$) between different phosphorus (P) applications at the same level of nitrogen (N) applications. Different capital letters indicate significant differences ($P < 0.05$) between different N applications at the same level of P application. The letters appearing in the following charts have the same meaning. Data are means ± sem (n = 3).

3.5. Correlations between soil microbial and physicochemical properties

TP, C/P and N/P were significantly ($P < 0.05$) correlated with *Actinobacteriota*, EC and C/P were significantly ($P < 0.05$) correlated with *Proteobacteria*, and C/N was strongly associated with ($P < 0.05$) *Bacteroidota*. While, TP was significantly ($P < 0.05$) correlated with *Entotheonellaeota*, while AP, SWC and TN were significantly ($P < 0.05$) correlated with *Cyanobacteria* (Fig. 6a). Correlation analysis with the phyla showed that SOC, TP, and C/P were significantly ($P < 0.05$) correlated with *Blastocladiomycota*, and AP, C/N, and N/P were strongly

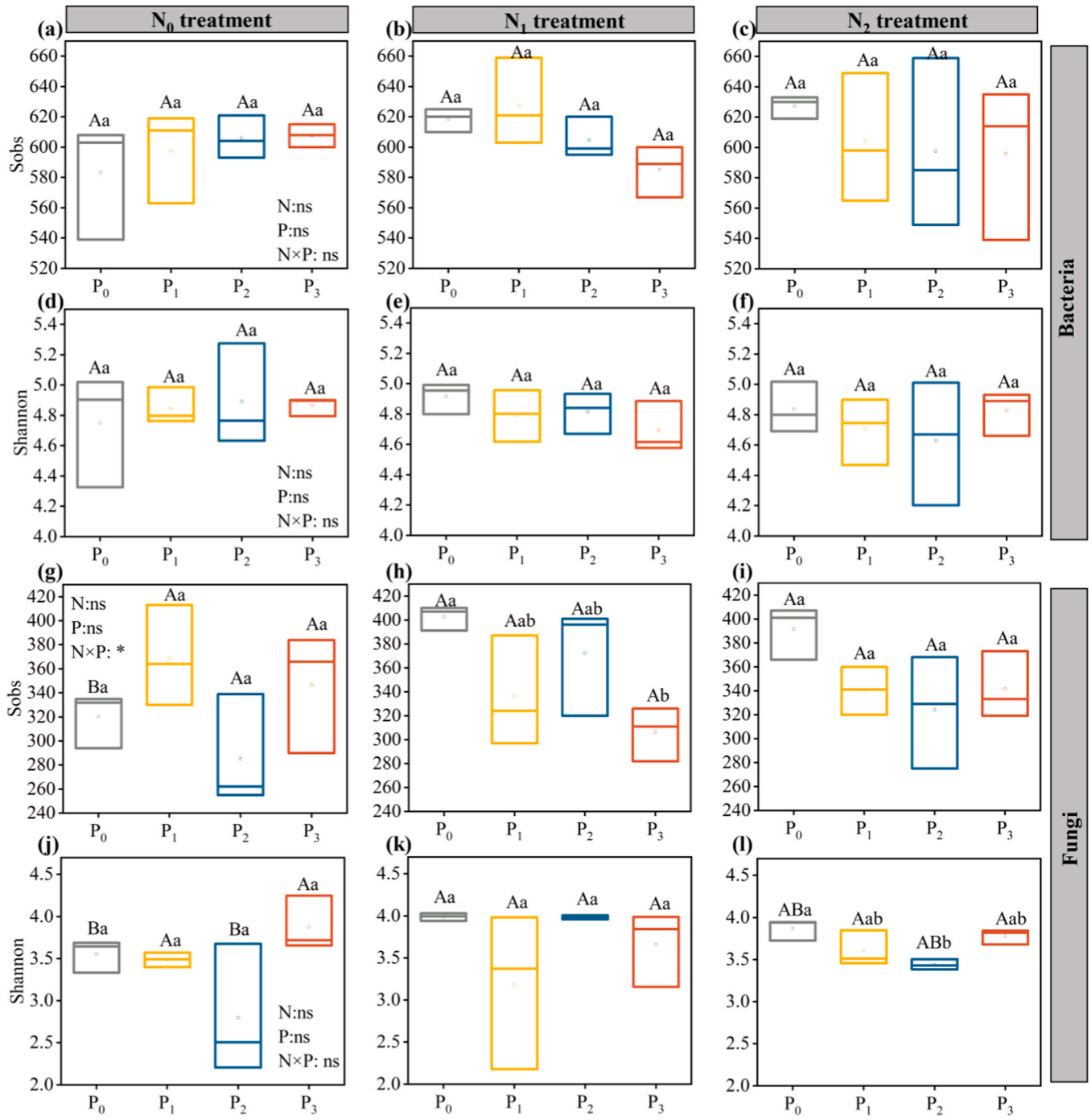

Fig. 2. Soil microbial diversity in alfalfa fields with N and P treatments. a-c represent Sobs index of bacteria in P levels at N_0, N_1, and N_2 fertilizer rates, d-f represent Shannon index of bacteria in P levels at N_0, N_1, and N_2 fertilizer rates, g-i represent Sobs index of fungi in P levels at N_0, N_1, and N_2 fertilizer rates, and j-l represent Shannon index of fungi in P levels at N_0, N_1, and N_2 fertilizer rates. * indicates $P < 0.05$, and ns indicates $P > 0.05$. Data are means ± sem (n = 3).

associated with *Zoopagomycota* (Fig. 6b). Correlation analysis with microbial diversity showed that AN, AP, and C/N was markedly ($P < 0.05$) correlated with the bacterial Sobs index and fungal Sobs index (Fig. 6c).

3.6. Characteristics of phosphate-solubilizing bacteria (PSB)

The P solubilizing capacity of the five strains of PSB screened from alfalfa rhizosphere soil ranged from 19.67 to 375.67 mg·L^{-1}, with Y316 having the strongest P solubilizing capacity. All PSB the ability to produce IAA and siderophore production capacity (Fig. 7). Y316, W003 and W006 were more similar to *Pantoea*, while Y014 and Y015 were more similar to *Enterobacter* (Fig. 8).The physiological and biochemical characteristics of the strains are described in detail in the Table 6.

3.7. Effect of phosphate-solubilizing bacteria (PSB) on alfalfa seed germination

The seed germination test showed that, compared with CK, inoculation with the PSB increased alfalfa shoot total length, seed germination rate, chlorophyll *a*, chlorophyll *b*, carotenoids (except Y316), and chlorophyll a+b content by 9.66 %-20.80 %, 0.56 %-2.88 %, 3.29 %-23.23 %, 3.88 %-29.33 %, 1.04 %-18.70 % and 3.44 %-24.75 %, but

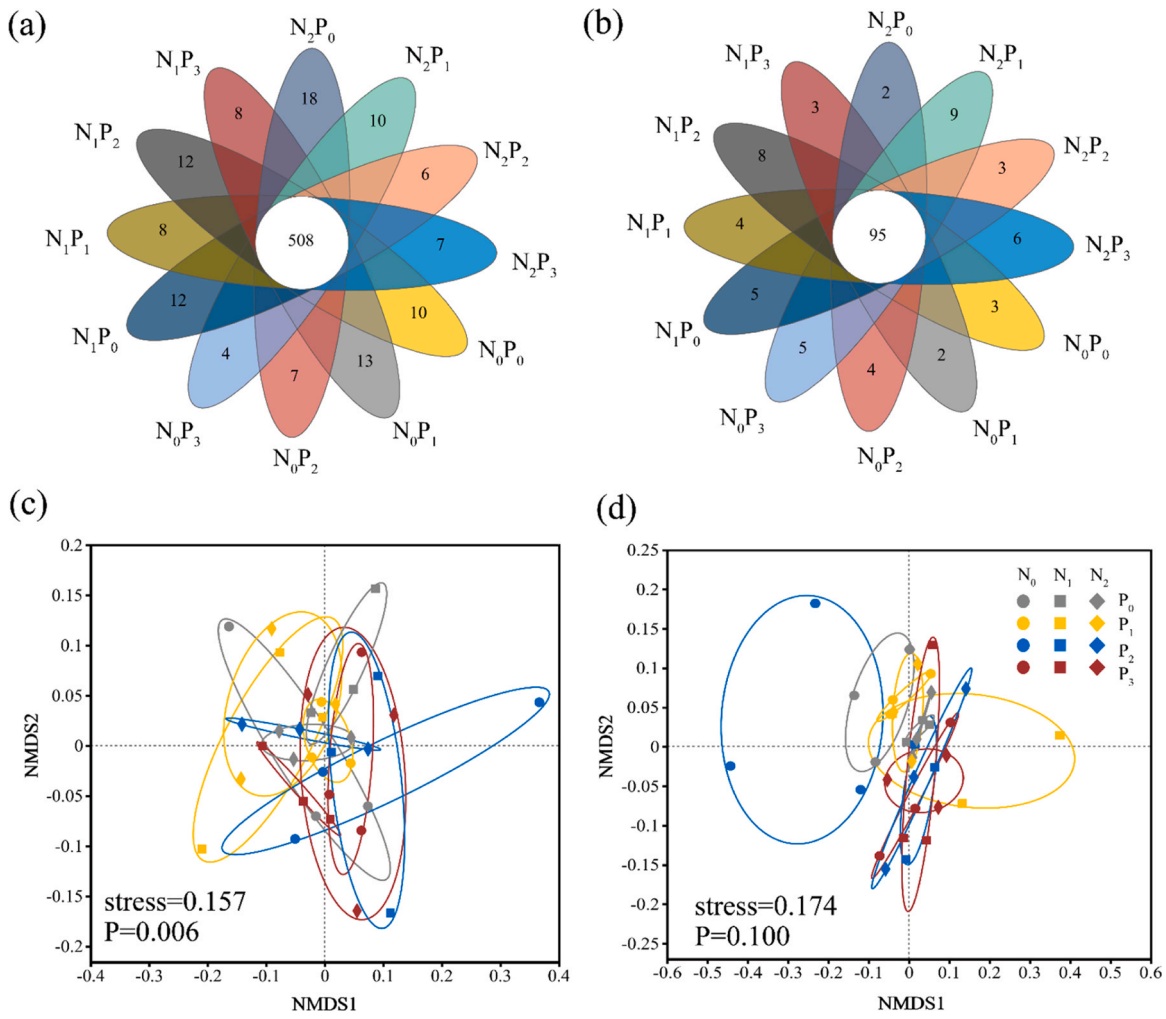

Fig. 3. Analysis of soil microbial composition and β-diversity. (a) and (b) illustrate the quantity of species that are both shared and exclusive to bacteria and fungi at the genus level, and (c) and (d) Present the non-metric multidimensional scaling analysis utilizing the Bray-Curtis distance algorithm for bacteria and fungi at the OUT level.

Table 3
Sequencing results of soil bacterial and fungal communities in alfalfa fields with different fertilization treatments.

Treatments		Bacteria		Fungi	
		Total sequences	Total OTUs	Total sequences	Total OTUs
N_0	P_0	84292 ± 3701	1803 ± 79	90671 ± 13150	253 ± 13
	P_1	80406 ± 3166	1886 ± 48	101978 ± 5006	287 ± 23
	P_2	79111 ± 2512	1882 ± 51	100346 ± 11219	236 ± 19
	P_3	84163 ± 2011	1910 ± 29	95966 ± 9208	265 ± 13
N_1	P_0	79502 ± 3885	1888 ± 35	95825 ± 7178	303 ± 3
	P_1	76565 ± 4426	1868 ± 87	90741 ± 14516	266 ± 32
	P_2	78326 ± 2776	1864 ± 32	102977 ± 12232	286 ± 14
	P_3	80311 ± 2258	1900 ± 48	101347 ± 3849	247 ± 13
N_2	P_0	84031 ± 404	1923 ± 34	102456 ± 7318	300 ± 16
	P_1	79383 ± 4455	1868 ± 82	102393 ± 11012	270 ± 16
	P_2	75441 ± 1794	1873 ± 98	107489 ± 2158	262 ± 27
	P_3	82831 ± 3195	1864 ± 85	110502 ± 4472	269 ± 17
	N	0.122	0.930	0.144	0.189
	P	0.019	0.943	0.490	0.091
	N × P	0.773	0.630	0.854	0.040

treatments were not significant. The combined analysis showed that inoculation with PSB only significantly increased alfalfa shoot total length when compared with CK ($P < 0.05$) (Table 7).

3.8. Effect of PSB on alfalfa growth performance and nutritional quality

Inoculation of PSB significantly increased alfalfa stem thickness, plant height, and aboveground biomass of the 2 cut ($P < 0.05$) (Fig. 9a-c). However, except for crude protein and crude ash of alfalfa from the 1st cut, the inoculation of PSB did not markedly ($P > 0.05$) impact the CP, Ash, plant P content, NDF, ADF, and RFV of alfalfa from the 2 cut (Fig. 9d-i). Overall analysis showed that inoculation with PSB increased alfalfa stem thickness, plant height, aboveground biomass, CP, P content, NDF and ADF by 23.41 %, 22.03 %, 34.24 %, 9.99 %, 9.01 %, 1.46 % and 3.33 %, and decreased Ash and RFV by 1.33 % and 2.74 %.

3.9. Effect of PSB on chlorophyll content of alfalfa

Inoculation with PSB did not significantly alter the chlorophyll contents, and carotenoids of the 1st cut of alfalfa ($P > 0.05$) (Fig. 10a), but significantly increased the chlorophyll *a*, carotenoids, and chlorophyll *a*+b contents of the 2nd cut ($P < 0.05$) (Fig. 10b). Overall analysis showed that inoculation with PSB resulted in 14.26 %, 5.39 %, 12.59 % and 12.03 % chlorophyll *a*, chlorophyll *b*, carotenoids and chlorophyll *a*+b contents, respectively (Fig. 10c).

3.10. Principal component analysis

The growth performance, nutritional quality and chlorophyll content

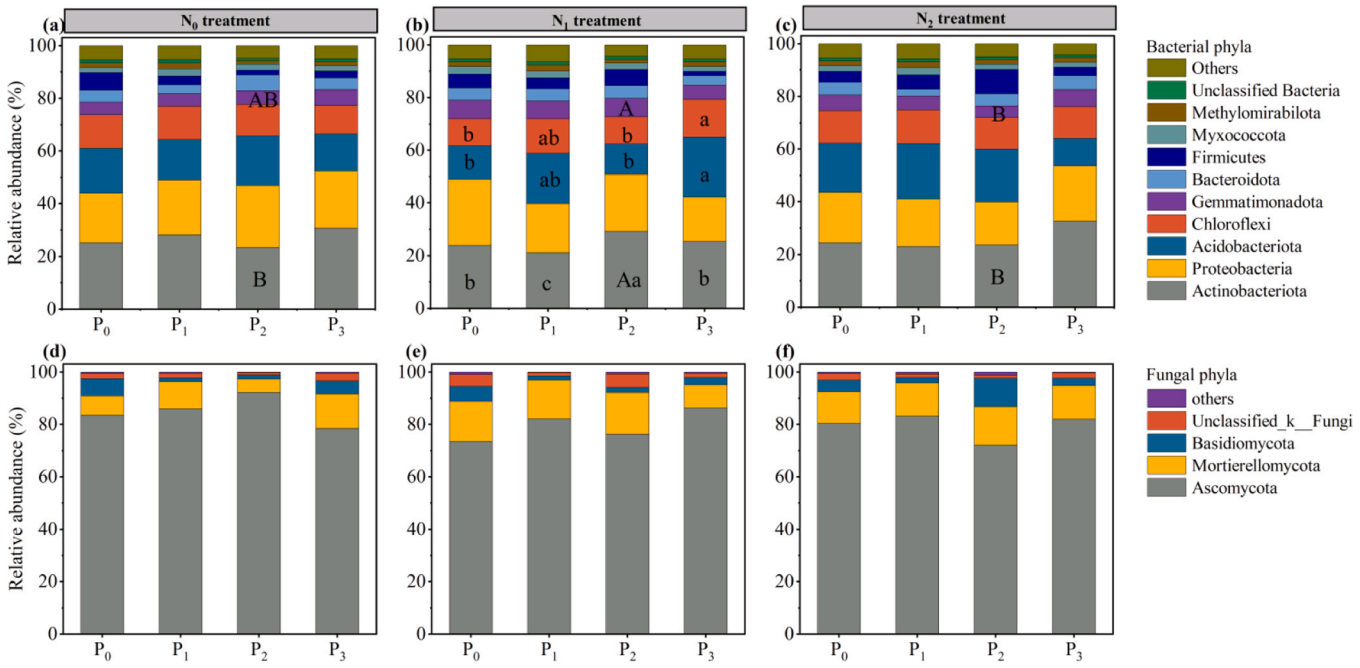

Fig. 4. Variations in the average relative abundances of dominant bacterial phyla (a-c) and fungal phyla (d-f) in response to N and P fertilizers.

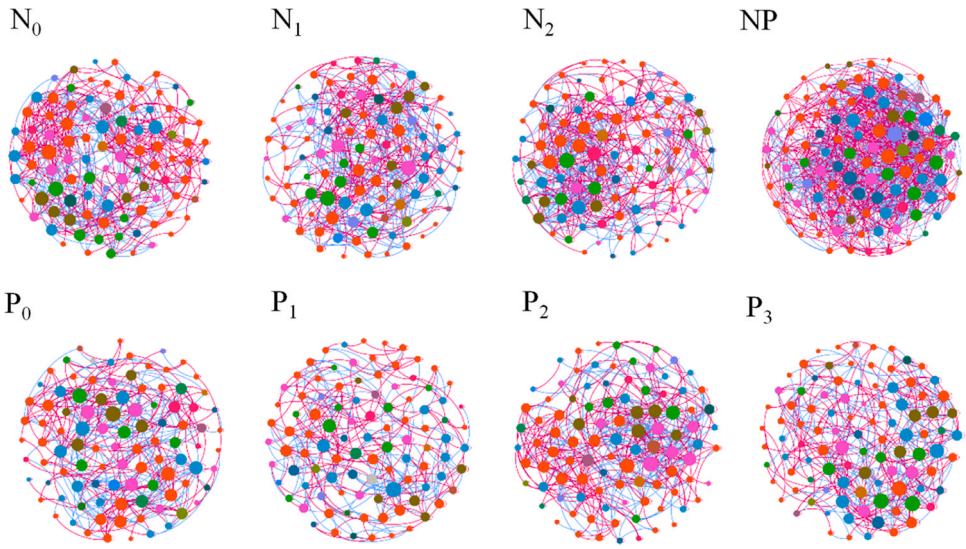

Fig. 5. Bacterial-fungal co-occurrence network analysis (top 50 bacterial abundance and top 50 fungal abundance) at the different N application rates and P application rates. The size and color of the points indicate the species weight at the genus level, with larger and darker points representing higher weight values of the species within the co-occurrence network. The red lines represent positive associations between species, and the blue lines represent negative associations between species. N_0, N_1, and N_2 represent different nitrogen (N) applications, each containing four different phosphorus (P) applications. P_0, P_1, P_2, and P_3 represent different P applications, each containing three applications. NP encompasses all fertilization treatments.

of alfalfa inoculated with different PSB were comprehensively evaluated, and in the 1st crop, W003 treatment performed best, followed by W006 (Fig. 11a and d). Similarly, in the 2nd crop, W003 treatment performed best, followed by W006 (Fig. 11b and e). In the 2-crop integrated analysis revealed that the integrated score of treatments were W006 >W003 >Y316 >Y014 >Y015 >CK (Fig. 11c and f).

4. Discussion

4.1. Effects of nitrogen and phosphorus rationing on soil physicochemical properties

N and P fertilizers are important factors which drive crop growth and soil nutrient content (Lu and Tian, 2017). The present research showed that N fertilization significantly changed soil pH, EC, SOC, TN, TP and stoichiometry, with similar results reported elsewhere (Gu et al., 2023; Song et al., 2023). In that, N and P fertilizer inputs directly increase the TN and TP content of the soil (Gu et al., 2023). Increased available nutrient content in the soil increases the photosynthetic rate of alfalfa,

Table 4
Properties of the co-occurrence networks with Nitrogen (N) application rates and Phosphrous (P) application rates.

Treatment	Clustering coefficient	Average path length	Average degree	Network density	Network diameter	Modularity	Number of nodes	Number of links	Proportion of positive	Proportion of negative
N fertilizer rates (N)										
N_0	0.531	2.681	12.263	0.125	8	0.448	99	607	67.38 %	32.62 %
N_1	0.513	2.769	10.760	0.109	7	0.449	100	538	60.04 %	39.96 %
N_2	0.532	2.689	11.449	0.118	7	0.401	98	561	65.78 %	34.22 %
P fertilizer rates (P)										
P_0	0.570	3.001	9.898	0.102	7	0.474	98	485	61.86 %	38.14 %
P_1	0.528	3.247	8.102	0.084	7	0.542	98	397	63.98 %	36.02 %
P_2	0.610	2.943	10.928	0.114	8	0.462	97	530	64.15 %	35.85 %
P_3	0.559	3.080	9.755	0.101	7	0.507	98	478	64.23 %	35.77 %
N fertilizer rates (N) × P fertilizer rates (P)										
NP	0.485	1.971	21.58	0.218	4	0.271	100	1079	60.43 %	39.57 %

Table 5
Phylum levels of the top 5 keystone taxa at the different N application rates and P application rates.

Rank	N_0	N_1	N_2	NP
1	*Basidiomycota*	*Chloroflexi*	*Ascomycota*	*Myxococcota*
2	*Ascomycota*	*Chloroflexi*	*Gemmatimonadota*	*Acidobacteriota*
3	*Ascomycota*	*Proteobacteria*	*Actinobacteriota*	*Methylomirabilota*
4	*Acidobacteriota*	*Ascomycota*	*Ascomycota*	*Ascomycota*
5	*Actinobacteriota*	*Ascomycota*	*Basidiomycota*	*Ascomycota*
Rank	P_0	P_1	P_2	P_3
1	*Chloroflexi*	*Basidiomycota*	*Methylomirabilota*	*Chloroflexi*
2	*Actinobacteriota*	*Actinobacteriota*	*Chloroflexi*	*Ascomycota*
3	*Chloroflexi*	*Actinobacteriota*	*Proteobacteria*	*Chloroflexi*
4	*Actinobacteriota*	*Proteobacteria*	*Chloroflexi*	*Actinobacteriota*
5	*Actinobacteriota*	*Ascomycota*	*Acidobacteriota*	*Proteobacteria*

Note: keystone taxa is determined according to betweenness centrality values.

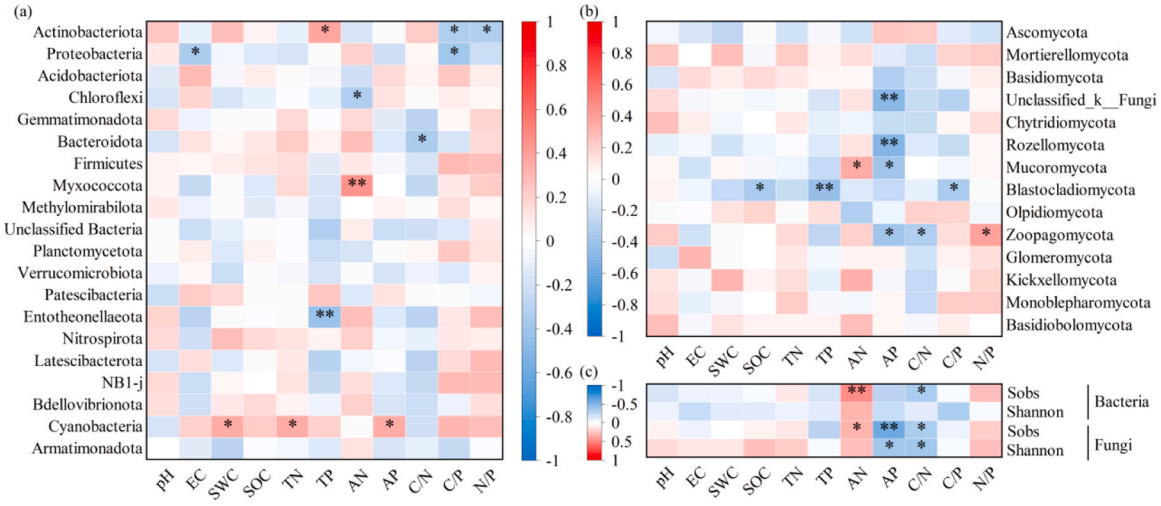

Fig. 6. Spearman's rank correlations between dominant bacterial phyla (a), fungal phyla (b), and diversity index (c) with physicochemical properties across. * indicates $P < 0.05$.

which then increases the amount of alfalfa apoplastic material and decomposition rate, which follows increases SOC content (Sun et al., 2022). Similarly, fertilization may increase the secretion of compounds from root, which in turn promotes SOC sequestration (Panchal et al., 2022; Wang et al., 2023a). However, excessive application of N and P fertilizers can result in luxury consumption of nutrients (more nutrients are taken up than are needed for optimal crop growth/production), which waste resources (fertilizers and application cost increase), potentially increase environmental impacts (nutrient pollution), and create nutrient imbalance and/or toxicity within the production system which may reduce alfalfa nutrient utilization efficiency and yield (Wang et al., 2023c). Similarly, over-fertilization may reduce the SOC sequestration potential, negatively effects soil resilience, and this practice does not support the sustainability of forage cropping systems (Mandal et al., 2020). Long-term input of N and P may also change soil stoichiometric ratios, in the current study, C/N, C/P and N/P (converted to molar ratio) ranged from 6.61 to 10.83, 90.97–112.94 and 10.22–15.51, respectively. With lower C/N and C/P when compared with average values of topsoil (14.4 and 136) at the experiment site, indicating that N and P fertilizer have to some extent improved SOC cycling and soil nutrient effectiveness. In that, the N/P exceeded the average value of the surface soil at the experiment site (9.3) (Tian et al., 2010). Moreover, it is vital to consider the significant social, economic, and environmental impacts of excessive 'on-farm' applying of N and P

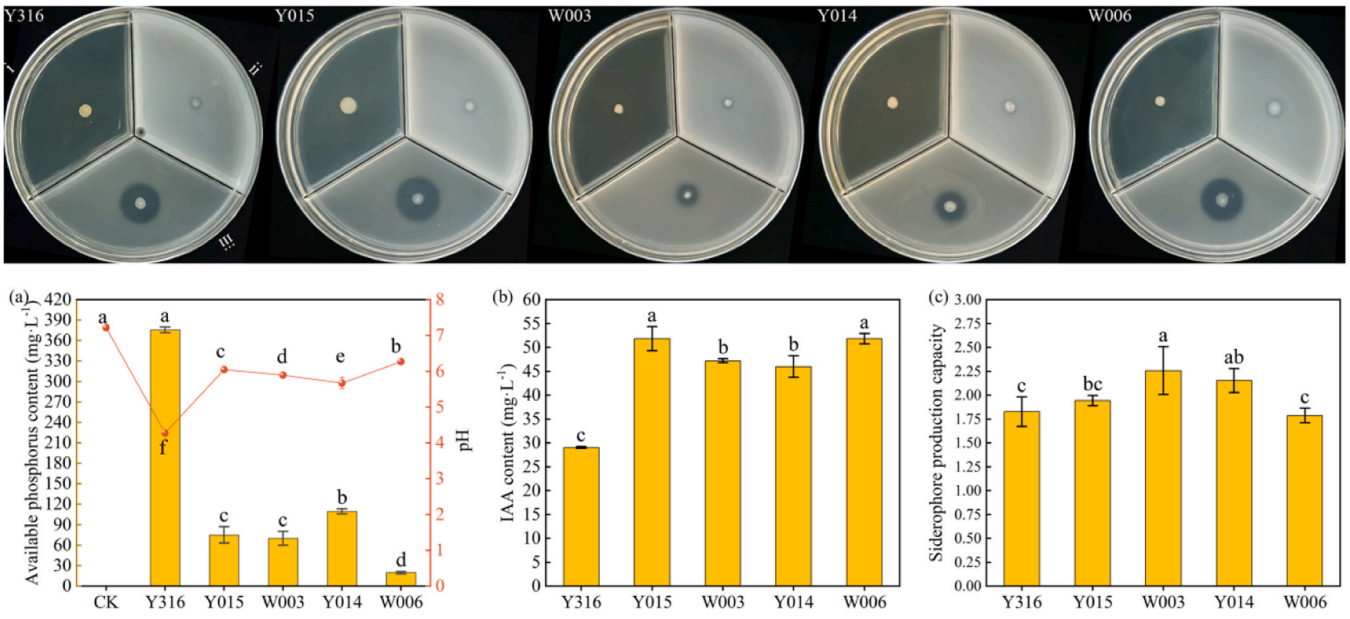

Fig. 7. Physiological and biochemical characteristics of PSB, including i) colonial morphology in LB; ii) qualitative ability to dissolve inorganic phosphorus; iii) qualitative ability to dissolve organophosphorus (24 h); (a) quantitative phosphorus dissolving capacity; (b) IAA production capacity; (c) qualitative siderophore production capacity. Different letters indicated significant difference ($P < 0.05$).

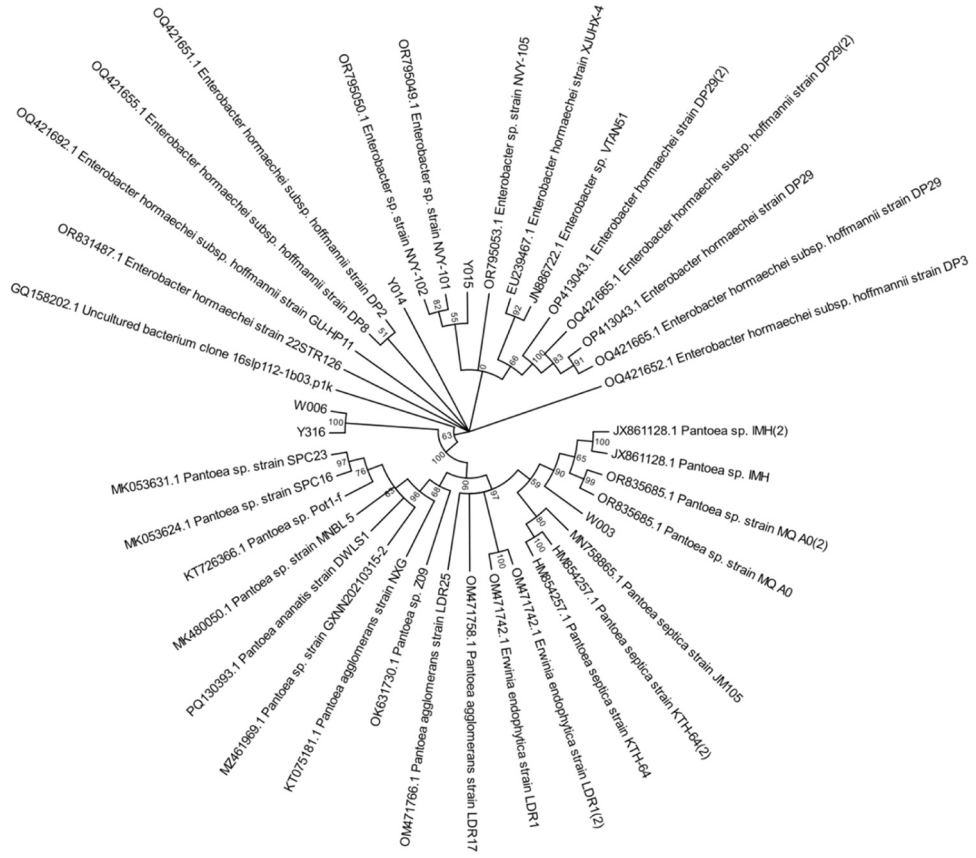

Fig. 8. A phylogenetic tree was constructed for five strains isolated from an alfalfa field, along with reference sequences obtained from NCBI. The tree was generated using the Neighbor-Joining method in MEGA software. The numbers displayed on the branches represent the confidence levels of the relationships between paired sequences, as determined by the bootstrap statistical method.

have from a fertilizer production and distribution/supply chain standpoint, including, increased energy consumption, greenhouse gas emission, water and air pollution, resource depletion, and biodiversity loss, which while beyond the remit of this study, stress the global scale and overall impact of fertilizer use beyond the end-user (farmer) and highlight the need for more sustainable approaches to agricultural fertilizer

Table 6
Physiological and biochemical characteristics of phosphate-solubilizing bacteria (PSB).

Strains	Catalase test	Oxidase test	Citrate test	Starch hydrolysis test	Imdole test	Nitrate reduction test	Urease test	Methyl red test	V-P test
Y316	+	-	-	-	-	+	-	-	+
Y015	-	-	-	-	-	+	+	-	-
W003	+	-	-	-	-	+	+	-	+
Y014	+	-	-	-	-	+	-	-	-
W006	+	-	+	-	-	+	-	-	+

Note: + , positive; -, negative.

Table 7
Effects of phosphate-solubilizing bacteria (PSB) on alfalfa plant height, germination rate and chlorophyll content.

Treatments	Shoot total length (mm)	Germination rate (%)	Chlorophyll *a* (mg/g)	Chlorophyll b (mg/g)	Carotenoid (mg/g)	Chlorophyll *a*+b (mg/g)
CK	51.03 ± 2.50	92.47 ± 3.29	0.75 ± 0.12	0.25 ± 0.05	0.27 ± 0.02	1.00 ± 0.15
Y316	61.23 ± 2.75	95.14 ± 2.19	0.77 ± 0.10	0.26 ± 0.03	0.27 ± 0.03	1.03 ± 0.13
Y015	55.96 ± 1.63	92.99 ± 6.07	0.86 ± 0.12	0.30 ± 0.08	0.30 ± 0.04	1.16 ± 0.20
W003	56.12 ± 4.59	94.00 ± 1.15	0.92 ± 0.14	0.32 ± 0.05	0.32 ± 0.04	1.24 ± 0.19
Y014	61.64 ± 6.99	94.66 ± 3.03	0.78 ± 0.05	0.30 ± 0.02	0.27 ± 0.01	1.08 ± 0.07
W006	55.05 ± 5.02	94.87 ± 2.50	0.85 ± 0.11	0.29 ± 0.05	0.29 ± 0.04	1.14 ± 0.16
P-value	0.081	0.901	0.424	0.490	0.408	0.440
CK	51.03 ± 2.50	92.47 ± 3.29	0.75 ± 0.12	0.25 ± 0.05	0.27 ± 0.02	1.00 ± 0.15
+PSB	58.00 ± 4.86	94.33 ± 3.00	0.84 ± 0.11	0.30 ± 0.05	0.29 ± 0.04	1.13 ± 0.15
P-value	0.030	0.347	0.215	0.142	0.337	0.175

Note: +PSB denotes inoculated phosphate solubilizing bacteria, where the data represented in the 1 row where +PSB is located is the mean of 5 inoculated PSB. Data are means ± sem (n = 3).

use.

4.2. Effect of nitrogen and phosphorus on soil microbial diversity

Soil microbial diversity is fundamental to maintaining subsurface ecosystem balance and soil health and productivity. Fertilizer application alters the soil fertility, which then effects soil microbial diversity (Sabir et al., 2021), with N application exerting the greatest influence on bacterial diversity (Yang et al., 2020). Globally, N fertilizer application may reduce soil microbial diversity, a process which is mainly determined by soil conditions, species of N fertilizer, timing, and rate,and duration of N application as reviewed by Geisseler and Scow (2014). Conversely, in contrast, the results of fertilizer on soil microorganisms were not consistent at the regional scale, with a study showing that the N and P no alter diversity of soil microorganisms (Sun et al., 2023). The N and P fertilizers no change the diversity of soil bacteria and fungi (Shannon index) in alfalfa production, which was consistent with the results of the influence of N and P on the diversity of soil microorganisms in alfalfa (He et al., 2022). However, reports suggest that a balanced P fertilizer has increased microbial diversity (Liu et al., 2023a), but that the only of N has reduced it (Wang et al., 2023b). Unlike the P fertilizer can alleviate the adverse impact of N fertilizer on soil microbial diversity (Yang et al., 2020). Therefore, N and P fertilization may help to maintain soil microbial diversity, depending on soil type, climate, production strategy and management.

In the current study, different fertilizer treatments (N alone, P alone, and P-N combined) effected shifts in soil microbial diversity, mainly through alters in different soil factors (pH, SOC, AP and TN, etc.), however, it diversity may not respond to changes in soil factors in the same way (Yang et al., 2020). For example, the lessening in pH resulting from N fertilizer is a fundamental reason influencing soil bacterial diversity and richness (Wang et al., 2023b), and the rise in effective soil P levels resulting from P application is a significant factor influencing soil microbial diversity (Liu et al., 2023a). Furthermore, it has also been reported that fertilizer application affects changes in soil factors, but does not lead to changes in bacterial and fungal diversity (Bebber and Richards, 2022; Eo and Park, 2016). In the current study, N and P fertilizers significantly altered the physicochemical properties of the soil, however, the Spearman correlation analysis indicated that TN and pH did not exhibit a significant correlation with soil microbial diversity and richness, which may be because fertilization has a minor effect on soil pH, resulting in a reduced effect on soil microorganisms (Chen et al., 2022). Previous work revealed fertilizer application changes soil nutrient content, but might not be the primary influence affecting the diversity of soil microorganisms (Bebber and Richards, 2022; Eo and Park, 2016). In contrast, correlation analysis between soil factors and microbial richness (Sobs index) showed that soil C/N was related with bacterial and fungal richness, therefore, soil stoichiometric properties significantly influence the characteristics of soil microbial (Zhong et al., 2020).

Findings indicate that application of N or P can multiply the complexity of soil microbial networks, however, over fertilizer may negatively affect the microbial networks (Chen et al., 2022). This is primarily that moderate fertilization enhances the population of certain symbiotic microorganisms, which in turn promotes cooperation and competition between microorganisms (Chen et al., 2022; Liu et al., 2021). Whereas in the current investigate, N led to a reduction in soil microbial network complexity and diminished positive associations between bacteria and fungi. Furthermore, P application enhanced the intricacy of the soil microbial network and positive associations between microorganisms. Indicating, improving the steadiness of the soil microbial network is more effectively achieved through N-P rationing (Wu et al., 2023).

4.3. Effect of nitrogen and phosphorus fertilization on soil microbial community composition

The effects of N and P on soil microbial community have been reported to be inconsistent (Wang et al., 2021, 2023b). Additionally, the mechanisms through which fertilization influenced the composition of soil microbial differed, such that, fertilization increased soil nutrient content, leading to changes in bacterial community composition, while, fungal community composition was primarily affected by root N and soluble sugar (Wu et al., 2023). Most current research suggests that excessive or unbalanced fertilizer application may be a key part driving shifts in microbial communities, which in turn regulate crop growth and development (Eo and Park, 2016; Kaminsky et al., 2018; Sun et al., 2019b). Balanced fertilization does not influence microbial

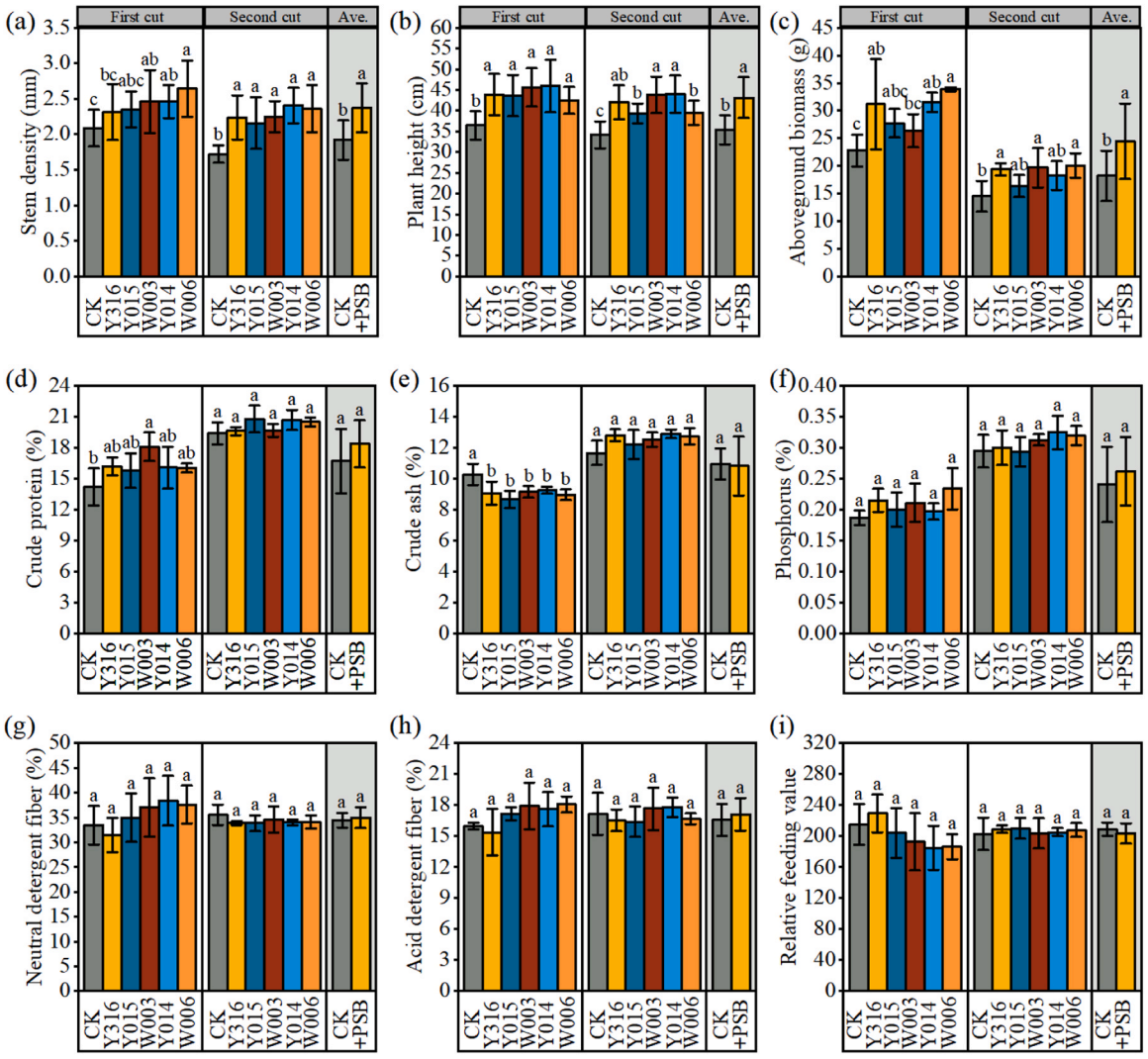

Fig. 9. Changes in growth performance and nutritional quality of alfalfa under conditions of inoculation with phosphate-solubilizing bacteria (PSB)

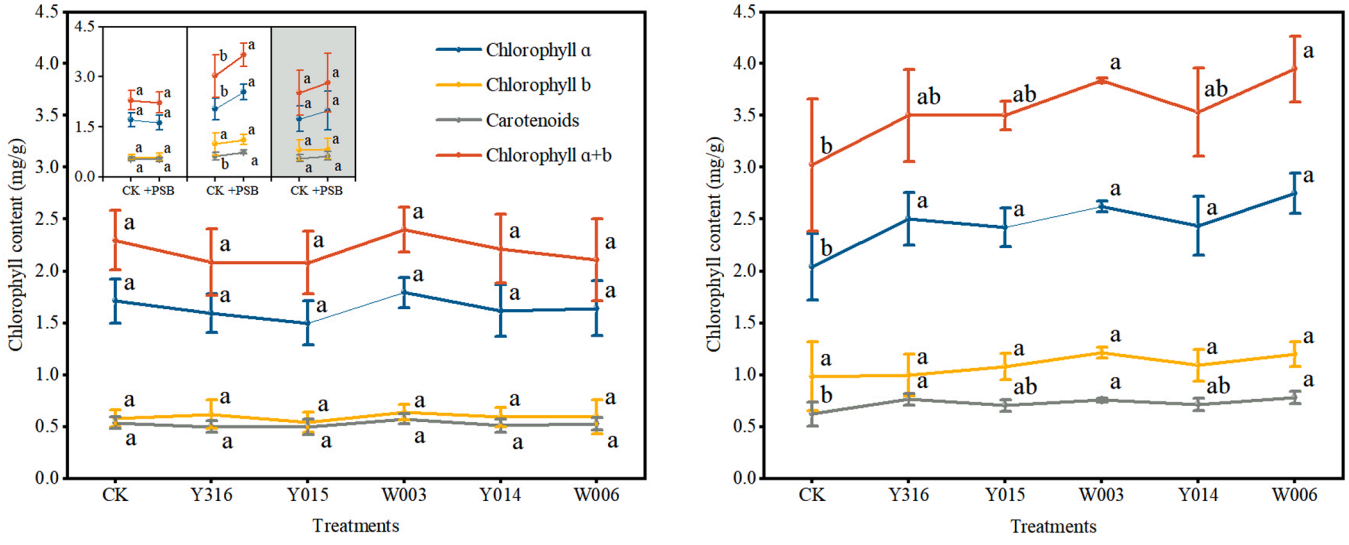

Fig. 10. Changes in chlorophyll content of alfalfa under the condition of inoculation with phosphate-solubilizing bacteria (PSB) Data are means ± sem (n = 4). (a) is the chlorophyll content of the 1st crop, (b) is the chlorophyll of the second crop, and (c) is the average value of the 1st, 2nd and two crops.

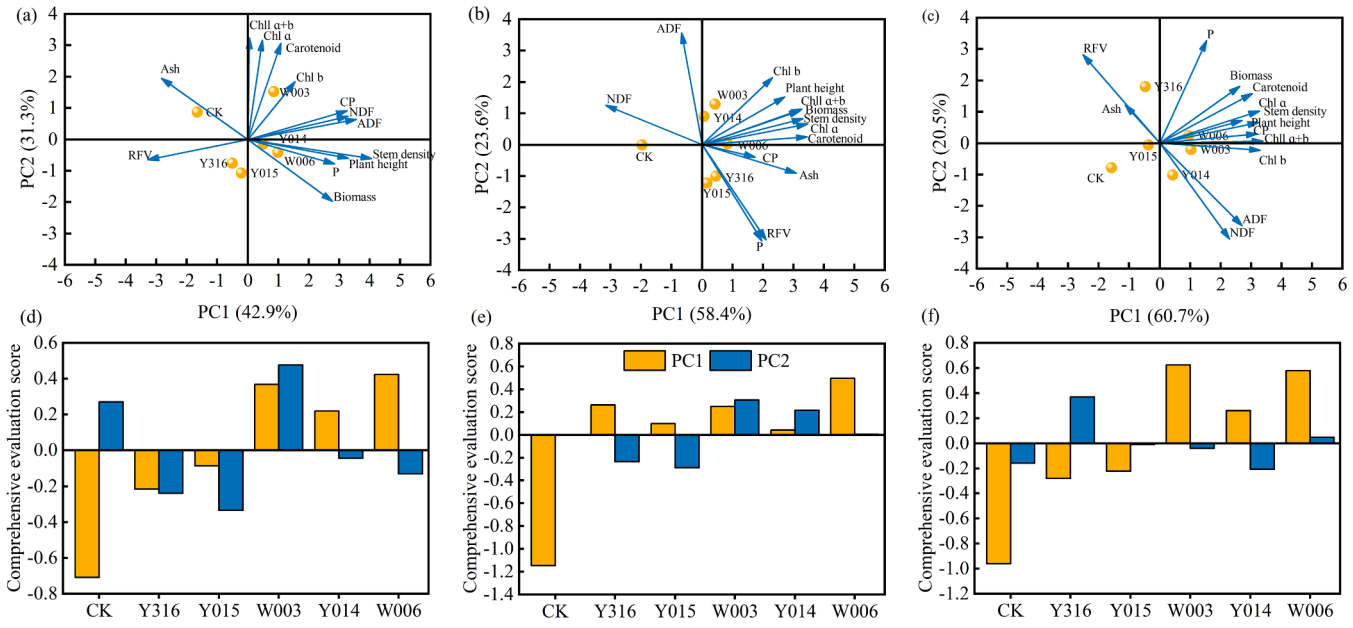

Fig. 11. Principal component analysis of growth performance, nutritional quality and chlorophyll content of alfalfa under inoculation with PSB. Ash, crude ash; CP, crude protein; P, plant phosphorus; NDF, neutral detergent fiber; ADF, acid detergent fiber; RFV, relative feeding value; Biomass, aboveground biomass; Chl a, chlorophyll a; Chl b, chlorophyll *b*, and Chl a+b, chlorophyll a+b.

communities, but alters the expression of functional genes for microbial nutrient cycling to accelerate soil nutrient turnover, which is conducive to improving soil quality and crop yields (Su et al., 2015). The N and P did not result in notable alterations in the fungal community composition. This suggests that N and P were not limiting factors affecting the growth of the fungal community (Chen et al., 2022; Shi et al., 2019). However, N and P fertilizers markedly changed the abundance of the predominant soil bacterial phyla (*Actinobacteriota*, *Acidobacteriota*, *Chloroflexi* and *Gemmatimonadota*). Although N and P altered the abundance of soil microorganisms, and even some of the bacterial phylum levels changed significantly, their abundance there was no clear pattern of it. It may be that in the study the change in soil nutrient content was not related to factors that determined influenced in the soil microbial abundance (Shi et al., 2019). For example, it has been observed that the establishment of alfalfa itself can change it (Qi et al., 2023). Consequently, we propose that the influences of these factors on soil microbial may mitigate the effect of N and P on the same.

Similarly, rhizosphere soil microbial are characterized by the presence of numerous and complex biotrophic bacteria, which are crucial for enhancing plant growth, improving crop nutritional quality and stress tolerance (Behera et al., 2024). More studies have shown that PSB exhibit positive growth-promoting effects in crops (Li et al., 2023a, 2023b; Pan et al., 2024). In this study, we screened five strains of PSB from alfalfa fields with different fertilizer application rates, and subsequent potting tests showed that they had different degrees of growth-promoting effects on alfalfa, among which strain W006 had the best effect and was identified as *Pantoea*. In addition, PSB also can improve the biotic and abiotic stress capacity of plants (Cao et al., 2023; Li et al., 2023b). At present, there are fewer studies on the growth-promoting characteristics and resistance ability of PSB. In the future, greater emphasis may be placed on enhancing the resistance and growth-promoting capabilities of alfalfa through the application of PSB, in order to limit the overuse of fertilizers and achieve sustainable agroecosystems.

5. Conclusion

N and P increased the fertility and altered the microbial community structure of soil. Although, the N and P primarily influenced the composition of the bacterial community, whereas exerting minimal impacts on the fungal community structure, also on bacterial and fungal diversity. Moreover, proper N and P fertilizers facilitated the stability of the microbial network. In addition, our study found that changes in AN and C/N due to fertilizer application were important factors driving changes in bacterial and fungal richness. Finally, our findings indicate that bacterial and fungal community structures do not respond to N and P fertilizer application uniformly, with bacterial communities showing greater sensitivity to fertilizer use. Meanwhile, the potting test of PSB screened from alfalfa rhizosphere soil proved that the inoculation of PSB can significantly increase the biomass of alfalfa, and simultaneously, it could meaningfully augment the nutritional quality of alfalfa. Among them, W006 promotes growth the best. The results of this research assist in comprehending the shifts in soil microbial communities under N and P fertilizer and the application of PSB which can supply theoretical support for the sustainable development of resilient and sustainable agroecosystems.

CRediT authorship contribution statement

Cartmill Andrew D.: Writing – review & editing. **López Ignacio F.:** Writing – review & editing. **Ma Chunhui:** Project administration. **Wei Kongqin:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Zhang Qianbing:** Writing – review & editing, Supervision, Resources, Project administration. **Sun Yanliang:** Formal analysis.

Declaration of Competing 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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Data availability

Data will be made available on request.

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