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Core bacterial communities dominated *Purus frumentum* biomass under different green manure returning amounts in saline-alkali soil

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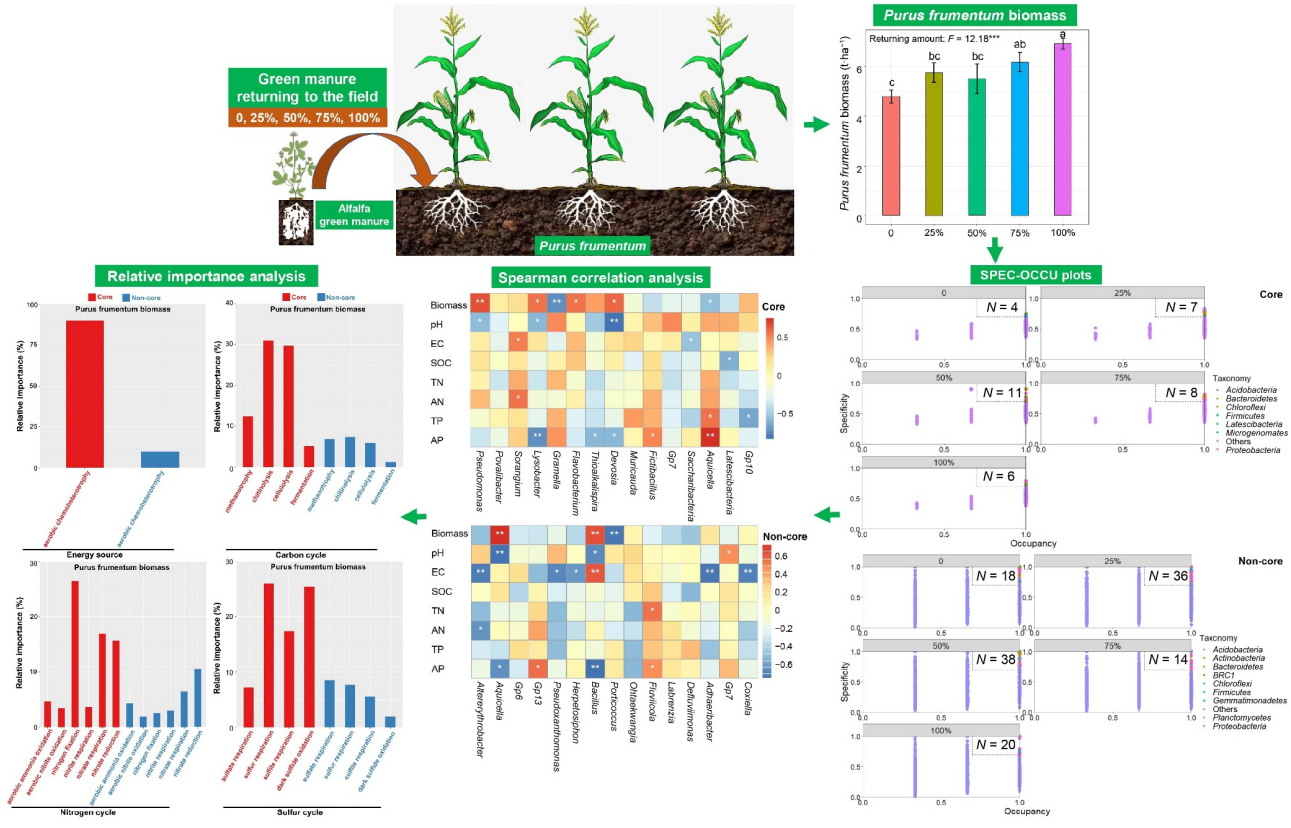
ABSTRACT



- *P. frumentum* biomass could be improved by appropriating returning measures.
- *P. frumentum* biomass was excellent in 75% alfalfa returning amount.
- Key species of bacteria differed among the alfalfa returning amounts
- The relationship of core bacteria and their potential ecological functions are more close to biomass.

The use of green manure returning to field is a common practice in conservation tillage. However, there is limited research on how different amounts of alfalfa can affect saline-alkali soil properties, bacterial community characteristics, and subsequent productivity. In this study, five different amounts of alfalfa return were investigated to understand the biological relationships between rhizospheres soil properties, bacterial communities, potential functions, and the *Purus frumentum* biomass. The results showed that the biomass was highest when 75% of the alfalfa was returned to the field. This particular amount was associated with relatively low soil pH and electrical conductivity. Additionally, it increased the relative abundance of beneficial bacterial taxa in both core and non-core bacteria. Statistical analysis revealed significant differences in both core (RANOSIM = 0.871, *P* = 0.001) and non-core (RANOSIM = 0.947, *P* = 0.001) bacterial communities among the different amounts of alfalfa

return based on non-metric multidimensional scaling analysis. Core bacterial taxa and their potential ecological functions were more closely related to plant biomass compared to non-core bacteria based on correlation analysis and multiple regression analysis. Therefore, our results indicate that optimizing the amount of alfalfa return can improve subsequent plant biomass. Regulating soil physicochemical properties and influencing core microbial community structure are of great significance for soil functional stability and crop productivity sustainability.

Keywords alfalfa, green manure, core bacteria, FAPROTAX, saline-alkali soil

1 Introduction

Salt alkali stress is a significant factor that affects sustainable agricultural development, and its improvement and utilization are crucial (Lu et al., 2021). Salt-tolerant green manure cultivation is considered an effective measure for the sustainable utilization and stable development of saline alkali land, as it provides both production and ecological value without the complexity, high cost of inorganic amendments, and risk of secondary pollution (Redondo-Gómez et al., 2021). Alfalfa (*Medicago sativa* L.) is a perennial legume plant widely cultivated as a high-quality livestock feed (Zhang et al., 2020a) and is also used as a green manure in agricultural planting systems (Han et al., 2022). The utilization of alfalfa green manure has improved the efficiency of phosphorus utilization in rice fields and reduced phosphorus loss (Gao et al., 2022). In addition, numerous studies have focused on the impact of returning alfalfa green manure on subsequent crop yields, greenhouse gas emissions, and soil nutrient availability (Gao et al., 2016; Yang et al., 2023). However, the comprehensive effect of different amounts of alfalfa return to farmland on soil microflora and subsequent crop yield in saline-alkali soil remains unclear. This knowledge gap hinders our understanding of the role of green manure return in enhancing soil fertility in saline alkali soil and our understanding of the relationship between microbial communities and ecosystem functions. Soil microorganisms encompass a wide range of species and play a crucial role in mediating approximately 80%－90% of soil processes. They contribute to various soil functions, including the key biogeochemistry cycling of organic matter and nutrients, as well as the maintenance of soil structure and soil ecosystem stability (Sengupta and Dick, 2015; Zhou et al., 2022a). In the field of ecology, core microorganisms are generally defined as a group of two or more shared members screened based on Venn diagrams in a specific habitat, and are widely distributed in microbial communities at different locations (Shade and Handelsman, 2012). The concept of core microorganisms focuses on persistent members of microbial communities that appear in nearly all combinations related to specific hosts, and it is gaining popularity. Identifying the core bacterial community may be an important step in identifying key members of the bacterial community that maintain plant health and development (Liu et al., 2021).

Researchers have identified a series of dominant bacteria in various soils worldwide by screening for high relative abundance and widespread distribution, indicating the presence of core groups in different soil ecosystems (Lemanceau et al., 2017; Jiao et al., 2019). Others have verified the existence of core microorganisms based on network analysis and found potential migration of core microbial communities driven by warming conditions (Wang et al., 2021b). In addition, soil core microorganisms help prevent the spread and evolution of pathogens in agricultural ecosystems (Toju et al., 2018). However, it is currently unclear whether core microorganisms are present in saline alkali soil under different alfalfa returning amounts and whether they play a dominant ecological role. Considering that the composition of soil microbial communities in saline-alkali soil may vary depending on the amount of returning alfalfa green manure, it is essential to address whether there are differences in core microbial taxa or abundance between groups. These are key questions that need to be investigated. Gaining a deeper understanding of the potential ecosystem functions of core microorganisms would be valuable in addressing challenges in agricultural production, ecological environmental remediation, and human health from a microbial perspective. The FAPROTAX (Functional Annotation of Prokaryotic Taxa) is a useful tool for predicting the ecological potential functions of bacterial and archaeal taxa identified through amplicon sequencing. FAPROTAX is a database constructed based on published literature, which maps microbes (bacteria and archaea) to metabolic or ecological related functions, such as nitrification, sulfur respiration, and methanotrophy (Louca et al., 2016). This indicates that if all cultivated objects of a taxon, both cultivated and uncultured members, are able to perform a function, then the function will be assigned to all members of the taxon (Sansupa et al., 2021). However, conducting phylogenetic surveys of communities by reconstructing unobserved states (PICRUSt) (Langille et al., 2013) and predicting functional profiles from macro-genomic 16S rRNA fragment gene data (Tax4Fun) (Asshauer et al., 2015) were based on the detected gene content of the taxonomic group to predict function. In this regard, FAPROTAX has demonstrated advantages in predicting the biogeochemistry cycling functions of environmental samples (Louca et al., 2016; Varela et al., 2018; Deng et al., 2019). For instance, FAPROTAX

was used to evaluate the potential function of microbial inoculation and fertilization on soil bacteria in carbon and nitrogen cycling (Wang et al., 2018a; Gao et al., 2019a; Li et al., 2019). Studies have shown that *Spartina alterniflora* invasion can stimulate soil denitrification and nitrification (Li et al., 2019). Other research has found that straw incorporation and nitrogen fertilizer have significant effects on nitrogen fixation and hydrocarbon degradation. Similarly, soil tillage and conversion of forest farmers have been found to increase soil nitrification and denitrification functions (Merloti et al., 2019). While FAPROTAX may not capture the functional phenotype of all microbial taxa, it has been demonstrated to allocate functions to prokaryotic taxa from aquatic and terrestrial sources (especially soil). FAPROTAX can also highlight potential functions related to biogeochemistry dynamics, such as comparing the functional characteristics of the carbon and nitrogen cycles under different treatments in non-aquatic samples (Merloti et al., 2019; Sansupa et al., 2021).

In this study, a field experiment was conducted to investigate the biological relationships among rhizospheres soil properties, core and non-core bacterial sub-communities, their potential functions, and *Purus frumentum* biomass. Four different amounts of alfalfa green manure returning, with non-returning as the control, were set up. The study aimed to address the following questions: (1) Which alfalfa green manure returning amount optimizes the yield of *P. frumentum* biomass? (2) Are there significant differences in core and non-core bacterial sub-community structures and potential functions among the different treatments? Do core microorganisms play a more important ecological role?

2 Materials and methods

2.1 *Medicago sativa* and *Purus frumentum* seeds

For our experiment, we employed *Medicago sativa* (Aurora) and *Purus frumentum*, which were provided by Zhengzhou Kaiyuan Grass Industry Technology Co., Ltd. The germination rates for *Medicago sativa* (Aurora) and *Purus frumentum* were > 95% and 90%, respectively.

2.2 Field site and experimental design

The field experiment was conducted in Tiaozini, Dongtai, Jiangsu Province, China (32°51′14" N, 120°56′16" E), from October 2021 to October 2022. The characteristics of this region were described in our previous research (Zhang et al., 2023). The soil chemical properties were as follows: pH 8.89, electrical conductivity 290.33 μS cm^{-1}, soil organic matter 7.83 g kg^{-1}, total phorsphorus (P) 0.62 g kg^{-1}, total nitrogen (N) 0.63 g kg^{-1}, available P 18.98 mg kg^{-1}, soil available N 66.92 mg kg^{-1}. For the experiment, the cultivation of *M. sativa* was performed in late October 2021. A total of 15 plots, each measuring 2 m × 2 m, were set up. The sowing density was 2.5 g m^{-2}, with a sowing depth of 3 cm and a row spacing of 25 cm. *M. sativa* was harvested in May 2022, and the aboveground biomass was recorded (266.11 g m^{-2}). Then, *M. sativa* with different amounts was returned to the field following a completely randomized design: 1) non-returning (0%); 2) 25% returning; 3) 50% returning; 4) 75% returning; 5) 100% returning. After 30 days of decomposition, *P. frumentum* was planted. No additional fertilization was applied during the growth of *M. sativa* and *P. frumentum*. Each treatment received the same daily agricultural management practices, such as irrigation and weeding.

2.3 Collection of aboveground and rhizosphere soil samples

For *P. frumentum*, the aboveground and rhizosphere soils were simultaneously sampled. For the aboveground parts, we randomly harvested one subplot measuring 0.5 m × 0.5 m, and oven-dried the samples at 65°C for 72 h before weighing. Moreover, the rhizosphere soil samples were obtained according to Fan et al. (2020a). The 15 samples collected were thoroughly homogenized and passed through a 2.0 mm sieve. Each sample was then divided into two subsamples. One subsample was air-dried at room temperature for soil properties analysis, while the other was stored at −80°C for DNA extraction.

2.4 Soil properties determination

Soil pH was determined using a PB-10 pH meter (Sartorius, Germany) with a soil-water slurry (1:5, w/v). Soil electrical conductivity (EC) was determined using a conductivity meter (B-173; HORIBA, Kyoto, Japan). Soil organic carbon (SOC) was determined using an Elementar Analyzer (Vario EL III, Germany). Soil total nitrogen was assayed by a Kjeltec Analyzer (FOSS Tecator, Hoganas, Sweden). Soil available nitrogen was measured following the method described by Shi (1996). The determination of soil total phosphorus and available phosphorus (Olsen-P) was performed according to the procedures outlined by Olsen and Sommers (Olsen SR, 1982) and (Watanabe and Olsen, 1965), respectively.

2.5 Soil genomic DNA extraction

The soil total genomic DNA was extracted using 0.25 g of fresh soil and a MoBio Powersoil™ DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). The concentration of soil DNA (A260/A280 ratio) was determined by a Nanodrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The qualified soil total DNA was then stored at −80 °C for subsequent analysis.

2.6 High-throughput sequencing and processing

The V4-V5 regions of the 16S rRNA gene were profiled using primers 515F (5′-GTG CCA GCM GCC GCG GTA A-3′) and 907R (5′-CCG TCA ATT CMT TTR AGT TT-3′) (Jiang et al., 2018). PCR amplification was carried out in an ABI GeneAmp 9700 PCR thermocycler (Vernon, CA, USA). PCR reactions were performed in a 20-μL system with 5 μL of 5 × FastPfu Buffer, 0.5 μL of FastPfu polymerase, 2 μL of 2.5 mM dNTPs, 0.2 μM of forward (1 μL) and reverse (1 μL) primers, about 10 ng of template DNA, and ddH_2O added to a final volume under the following thermal cycling conditions: 3 min at 95°C, followed by 32 cycles of 30 s at 95°C (denaturation), 30 s at 62°C (renaturation), 30 s at 72°C (extension), and 5 min at 72°C, and finally cooled to 4°C at the end. The PCR products were purified, quantified, and subjected to gel recovery. The purified PCR amplicons were sequenced using the Illumina HiSeq (2 × 250 bp) platform (Illumina, USA) at Genesky Biotechnologies Co., Ltd. (Shanghai, China). The acquired amplicon sequence variants (ASVs) were processed and detected using the DADA2 plugin (Forster et al., 2019). The cutadapt plugin was utilized to trim adapters and primer sequences from the ASV sequences. The remaining high-quality ASV sequences were assigned to taxonomic lineages according to a 0.8 confidence threshold using the RDP (version 11.5) within the SILVA (release 128) database (Wang et al., 2007). All raw sequence data have been stored in the National Center for Biotechnology Information (NCBI) with the BioProject accession number PRJNA960924.

2.7 Statistical analysis

To determine the differences between different treatments, one-way analysis of variance (ANOVA) was performed, followed by multiple comparisons using the Tukey HSD tests ($P < 0.05$). Prior to the formal analysis, appropriate transformations were applied to the data to ensure data normality and homogeneity of variance. IBM SPSS Statistics (23.0) software was used for all statistical analyses. 1752180 sequence subsets (19009 ASVs) were randomly selected from each sample for subsequent downstream analysis to standardize inter-sample sequencing. Core bacteria were screened based on two criteria: high abundance (systematic type ranking in the top 10% of total abundance) and relative universality (frequency of occurrence > 80%). These criteria were used to identify ASVs that were both abundant and widespread across different soil samples (Delgado-Baquerizo et al., 2018; Wang et al., 2021b; Jiao et al., 2022a). A total of 681 ASVs were denoted as the core taxa. The Specific Occupancy (SPEC-OCCU) plot method was used to identify potential key species (specialist species) in a bacterial community (Gweon et al., 2021). Specificity is defined as the average abundance of species (S) in different returning amounts (T); occupation is defined as the relative frequency of species (S) appearing in different returning amounts:

$$\text{Specificity} = \frac{\text{Nindividuals}_{S,T}}{\text{Nindividuals}_{S}}$$

$$\text{Occupancy} = \frac{\text{Nsites}_{S,T}}{\text{Nsites}_{T}}$$

$\text{Nindividuals}_{S,T}$ is the mean number of individual ASV S across all samples of returning amounts (T); Nindividuals_{S} is the sum of the mean number of individual S over all returning amounts (T); $\text{Nsites}_{S,T}$ is the number of samples in T where S is present; Nsites_{T} is the total number of samples in T. These two metrics are then used as axes in the SPEC-OCCU plot. The threshold for specialist species is specificity and occupancy greater than or equal to 0.7.

The “metaMDS” function of the “vegan” package was used to analyze the relationship between soil bacterial communities among different treatments based on Bray−Curtis distance. Non-metric multidimensional scaling (NMDS) was used for visualization. Analysis of Similarities (ANOSIM) was used to analyze the significant differences between core and non-core bacterial communities under different treatments. Spearman analysis was used to evaluate the correlation between various indicators. The Prokaryotic Taxa Functional Annotation (FAPROTAX) database was used to predict the potential ecological function of bacterial taxa identified through 16S rRNA amplicon sequencing. The relative importance of potential ecological functions on corn yield was evaluated based on a multiple regression model analysis using the “lm” function and “calc. relimp” function based on the “stats” and “relapmo” R packages.

3 Results

3.1 Variation of *P. frumentum* biomass and soil physico-chemical properties in different alfalfa returning amounts

Results of the one-way ANOVA showed significant ($F = 12.18$, $P < 0.001$) differences among different alfalfa returning amount treatments for *P. frumentum* biomass at harvest (Fig. 1). The largest *P. frumentum* biomass was observed in the 100% returning amount of alfalfa to the field (T4, 6.91 t ha^{-1}), which was significantly ($P < 0.05$) higher than that of the non-returning amount (CK, 4.79 t ha^{-1}), 25% returning amount (T1, 5.75 t ha^{-1}), and 50% returning amount (T2, 5.51 t ha^{-1}) treatments, but excluding 75% returning amount of alfalfa to the field (T3, 6.18 t ha^{-1}). Treatment T3 increased *P. frumentum* biomass by 1.29, 1.07, and 1.12 times compared to CK, T1, and T2, respectively. Additionally, the *P. frumentum* biomass under T3 significantly ($P < 0.05$) increased by 29.02% compared to

CK. However, no significant differences were observed among CK, T1, and T2. Different alfalfa returning amounts resulted in significantly (P < 0.05) different physicochemical properties (Table 1). It was observed that soil pH decreased when alfalfa was returned to the field compared to the non-returning treatment. The EC value under T3 was significantly (P < 0.05) lower than in the other treatments. However, no significant differences were found in the content of SOC, AN, and TP among the groups, while TN and AP were lower under T3 and T4 compared to the other treatments.

3.2 Core and non-core bacterial community composition in different alfalfa returning amounts

Non-metric multidimensional scaling (NMDS) analysis revealed that soil core (R = 0.871, P = 0.001) and non-core (R = 0.947, P = 0.001) bacterial communities for different alfalfa returning amounts formed obvious clusters in the ordination space (Figs. 2A, 2B), with significant differences

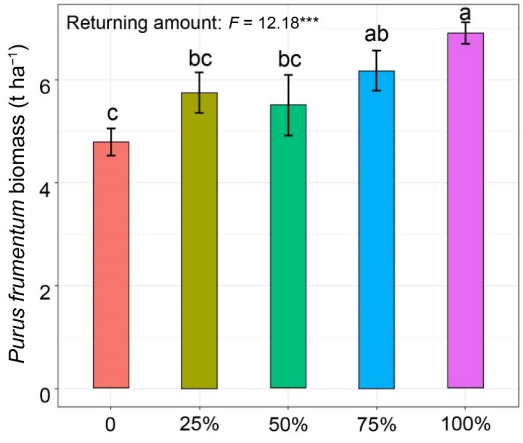


Fig. 1 Comparison of *P. frumentum* biomass in different alfalfa returning amount. 0, 25%, 50%, 75%, 100% represent different alfalfa returning amount, respectively. The overall differences among treatments were estimated based on parametric one-way analysis of variance (ANOVA). Additionally, different lowercase letters (a, b, c) mean significant differences among treatments (P < 0.05, multiple comparison with Tukey HSD test). Error bars represent the standard errors. As the same below.

at taxonomic levels based on analysis of similarities (ANOSIM). In addition, significant differences were observed in the beta diversity of core bacteria under different returning amounts, with the lowest diversity observed in the 75% returning amount treatment. Non-core taxa exhibited a similar trend, which is significantly higher than that of core microbiota (Fig. S1).

The dominant bacterial genera across all treatments were *Gemmatimonas*, *GP6*, and *Pseudomonas* in both core and non-core bacteria (Fig. S2). Additionally, different alfalfa returning amounts resulted in significant (P < 0.05) differences in the relative abundance of bacterial genera (Tables 2, 3). For core bacteria, the largest relative abundance of *Pseudomonas* was observed in the T4 treatment (3.06%), which was significantly higher than that in T1 (2.22%) and T2 (2.17%), excluding T3 (2.82%). In addition, the relative abundance of *Gemmatimonas* in T3 was higher than that of CK, T1, T2, and T4 by 1.05, 1.25, 1.14, and 1.01 times, respectively (Table 2). For non-core bacteria, the highest relative abundance of *Pseudomonas* was observed in the T3 treatment, followed by the T4 treatment (Table 3). The relative abundance of dominant genera in the core microbiota was significantly higher than that in the non-core microbiota under different alfalfa returning amounts, such as *Gemmatimonas* and *Pseudomonas* (Table S1).

3.3 The specificity and occupancy of differential species in different alfalfa returning amounts

To assess the distribution and specificity of ASVs under different alfalfa returning amounts, a two-dimensional SPEC-OCCU plot was utilized (Fig. 3). In addition, potential key species within the group were identified, with a threshold of specificity and occupancy greater than or equal to 0.7 (dotted boxes in Fig. 3). In the analysis of core taxa, the number of specialist ASVs differed among the alfalfa returning amounts with an increasing trend in terms of richness from non-returning (4 ASVs represent), 25% returning (7 ASVs), 50% returning (11 ASVs), 75% returning (8 ASVs) to 100% returning (6 ASVs), representing 0.3%, 0.8%, 0.9%,

Table 1 Soil physicochemical properties under different alfalfa returning amount in saline alkali soil field experiment.

Treatments	pH (H_2O)	EC (μS cm^{-1})	SOC (g kg^{-1})	TN (g kg^{-1})	AN (mg kg^{-1})	TP (g kg^{-1})	AP (mg kg^{-1})
0	9.02(0.02)ab	278(4.51)b	5.22(0.34)a	0.67(0.02)ab	52.09(3.71)a	0.78(0.02)a	19.74(0.53)ab
25%	8.99(0.01)bc	293(7.23)ab	6.16(0.1)a	0.72(0.01)a	56.32(0.62)a	0.80(0.02)a	21.47(0.54)a
50%	9.08(0.01)a	284(11.53)ab	5.7(0.31)a	0.64(0.01)b	48.49(0.2)a	0.73(0.01)a	18.58(0.75)b
75%	8.98(0.02)bc	256.67(6.49)b	5.9(0.48)a	0.66(0.02)ab	51.52(2.02)a	0.73(0.01)a	18.05(0.33)b
100%	8.95(0.01)c	321(12.01)a	5.83(0.41)a	0.66(0.02)ab	54.5(0.84)a	0.75(0.02)a	14.24(0.22)c

Note: 0, 25%, 50%, 75%, 100% represent different alfalfa returning amount, respectively; EC, conductivity; SOC, soil organic carbon; TN, soil total nitrogen; AN, alkali-hydrolyzable nitrogen; TP, soil total phosphorus; AP, available phosphorus. Additionally, different lowercase letters (a, b, c) mean significant differences among treatments (P < 0.05, multiple comparison with Tukey HSD test). Error bars represent the standard errors.

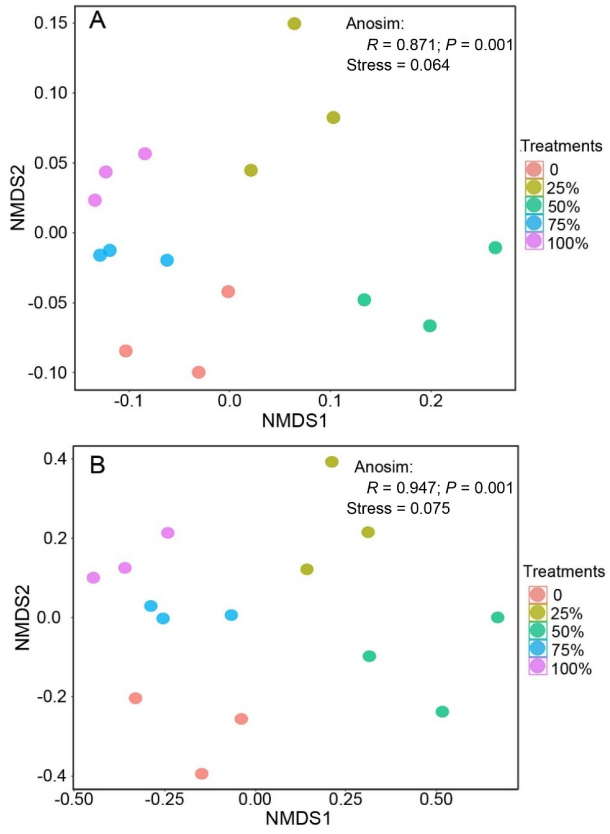


Fig. 2 Non-metric multidimensional scaling (NMDS) showed the structure of soil core (A) and non-core (B) bacterial communities in different alfalfa returning amount. ANOSIM analysis was used to test the difference between each treatment. ANOSIM: An analysis of similarity.

3.0%, and 0.4% of the total sequences, respectively (Fig. 3A).

For non-core taxa, the richness of specialist ASVs from non-returning (18 ASVs represent), 25% returning (36 ASVs), 50% returning (38 ASVs), 75% returning (14 ASVs) to 100% returning (20 ASVs) represented 0.5%, 1.1%, 1.8%, 0.2%, and 0.5% of the total sequences, respectively (Fig. 3B). In addition, *Proteobacteria* was found in all specialist groups across returning amounts in both core and non-core taxa, and it accounted for a large proportion (Fig. S3). Moreover, the alpha diversity (Shannon, Chao1 index) of key species within the core and non-core microbial communities was calculated. The relative importance of these key species to *P. frumentum* biomass was evaluated based on multiple regression model analysis. The results showed that the alpha diversity within the core microbial communities exhibited a stronger relationship with *P. frumentum* biomass (Fig. S4).

3.4 Relationships among soil physicochemical properties, bacterial key species, and *P. frumentum* biomass

Spearman correlation analysis was conducted to evaluate the biological associations among soil physicochemical properties, bacterial key species, and *P. frumentum* biomass. For physicochemical properties, only soil pH showed a significant ($r = -0.54$, $P < 0.05$) correlation with *P. frumentum* biomass (Table S2). For core bacteria, *Pseudomonas* ($r = 0.65$, $P < 0.01$), *Lysobacter* ($r = 0.56$, $P < 0.05$), *Flavobacterium* ($r = 0.55$, $P < 0.05$), and *Devosia* ($r = 0.60$, $P < 0.05$)

Table 2 Core bacterial relative abundance at genus level under different alfalfa returning amount in saline alkali soil field experiment.

Genus	0	25%	50%	75%	100%
Gemmatimonas	4.15(0.22)a	3.5(0.04)b	3.82(0.07)ab	4.37(0.08)a	4.32(0.16)a
Thiobacillus	3.35(0.5)a	1.63(0.21)b	1.6(0.15)b	2.91(0.17)a	2.25(0.14)ab
Gp6	2.55(0.19)a	2.34(0.08)a	2.68(0.12)a	2.51(0.11)a	2.51(0.15)a
Pseudomonas	2.5(0.16)ab	2.22(0.26)b	2.17(0.13)b	2.82(0.05)ab	3.06(0.17)a
Muriicola	2.75(0.05)	2.67(0.12)a	1.76(0.13)b	2.37(0.11)a	2.54(0.1)a
Aridibacter	1.62(0.17)a	1.47(0.08)a	1.82(0.13)a	1.83(0.03)a	1.87(0.17)a
Lysobacter	1.88(0.12)ab	1.11(0.08)c	1.52(0.14)bc	2.07(0.11)a	1.78(0.08)ab
Ohtaekwangia	1.69(0.09)ab	1.6(0.04)ab	1.81(0.08)a	1.46(0.07)b	1.8(0.08)ab
Pontibacter	1.58(0.28)bc	2.15(0.13)ab	2.52(0.09)a	1.23(0.04)c	1.6(0.09)bc
Chryseolinea	1.47(0.08)abc	1.63(0.21)ab	1.79(0.11)a	1.21(0.03)bc	0.96(0.03)c
Gimesia	1.32(0.1)a	1.46(0.1)a	1.11(0.1)a	1.48(0.03)a	1.34(0.04)a
Povalibacter	1(0.15)a	0.9(0.08)a	0.8(0.12)b	1.38(0.05)a	1.07(0.12)a
Gp10	0.84(0.06)a	0.86(0.01)a	1.07(0.16)a	0.97(0.04)a	0.89(0.07)a
Latescibacteria	0.72(0.12)b	0.68(0.05)b	0.57(0.05)b	1.17(0)a	1.25(0.15)a
Steroidobacter	0.99(0.08)a	0.79(0.11)ab	0.64(0.03)b	0.84(0.07)ab	1.03(0.05)a

Note: 0, 25%, 50%, 75%, 100% represent different alfalfa returning amount, respectively; Different lowercase letters (a, b, c) mean significant differences among treatments ($P < 0.05$, multiple comparison with Tukey HSD test). Error bars represent the standard errors.

Table 3 Non-core bacterial relative abundance at genus level under different alfalfa returning amount in saline alkali soil field experiment.

Genus	0	25%	50%	75%	100%
Gp6	2.7(0.43)a	1.57(0.19)a	1.98(0.39)a	2.81(0.11)a	2.57(0.33)a
Gemmatimonas	2.06(0.21)ab	1.87(0.13)b	2.46(0.22)ab	2.34(0.1)ab	2.77(0.07)a
Pseudomonas	0.91(0.2)ab	0.46(0.12)b	0.56(0.17)b	1.64(0.18)a	0.99(0.1)ab
Opitutus	1.02(0.09)a	0.98(0.14)a	1.2(0.15)a	1.13(0.15)a	0.83(0.03)a
Ohtaekwangia	0.71(0.2)a	1.01(0.24)a	1.3(0.11)a	0.82(0.09)a	0.96(0.25)a
Gp10	0.69(0.07)a	0.98(0.17)a	0.97(0.23)a	1.06(0.13)a	0.65(0.13)a
Povalibacter	0.44(0.04)a	0.55(0.13)a	0.92(0.15)a	0.63(0.23)a	0.65(0.09)a
Blastopirellula	0.86(0.04)a	0.73(0.04)ab	0.59(0.02)bc	0.51(0.06)c	0.51(0.02)c
Gimesia	0.57(0.13)ab	0.7(0.08)ab	0.93(0.13)a	0.35(0.11)b	0.45(0.05)ab
Altererythrobacter	0.48(0.05)a	0.49(0.07)a	1.75(0.99)a	0.45(0.1)a	0.3(0.12)a
Haliangium	0.59(0.06)a	0.71(0.11)a	0.42(0.05)a	0.68(0.02)a	0.58(0.08)a
Gp16	0.77(0.08)a	0.39(0.09)ab	0.18(0.06)b	0.68(0.01)a	0.56(0.16)ab
Gp7	0.52(0.16)ab	0.73(0.05)ab	0.49(0.03)ab	0.47(0.07)b	0.86(0.02)a
Litorilinea	0.5(0.07)a	0.71(0.05)a	0.61(0.05)a	0.37(0.06)a	0.54(0.17)a
Nitrospira	0.66(0.09)a	0.42(0.08)a	0.4(0.13)a	0.49(0.04)a	0.6(0.16)a

Note: 0, 25%, 50%, 75%, 100% represent different alfalfa returning amount, respectively; Different lowercase letters (a, b, c) mean significant differences among treatments ($P < 0.05$, multiple comparison with Tukey HSD test). Error bars represent the standard errors.

exhibited significant positive correlations with *P. frumentum* biomass. Conversely, *Gramella* ($r = -0.71$, $P < 0.01$) and *Aquicella* ($r = -0.54$, $P < 0.05$) showed significantly negative correlations with *P. frumentum* biomass. Additionally, different bacterial genera displayed diverse responses to the same physicochemical properties. For instance, the relative abundance of *Aquicella* responded positively to TP, while *Gp10* responded negatively (Fig. 4A). For non-core bacteria, *P. frumentum* biomass exhibited significant positive correlations with *Gp6* ($r = 0.79$, $P < 0.01$) and *Fluviicola* ($r = 0.70$ $P < 0.01$), while it showed a significant negative correlation with *Porticoccus* ($r = -0.69$, $P < 0.01$). Moreover, the same bacterial genera displayed diverse reactions to different physicochemical properties. For example, the soil pH and AP showed significantly negative correlations with the relative abundance of *Bacillus*, whereas *Bacillus* showed a significant positive relationship with the EC content (Fig. 4B).

3.5 Predictive functional profiling of core and non-core bacteria in different alfalfa returning amounts and its relationship with *P. frumentum* biomass

The potential ecological functions of key species within the core and non-core bacteria were determined based on the FAPROTAX database. It was found that different alfalfa returning amounts resulted in significant ($P < 0.05$) differences in the potential functions of bacterial genera (Table S3, S4). Multiple linear regression was employed to assess the relative importance of the same potential functions of core and non-core bacteria to *P. frumentum* biomass, which included energy source, carbon cycles, nitrogen cycles, and sulfur cycles (Fig. 5). In terms of energy source, aerobic chemoheterotrophy exhibited a higher relative importance in the core bacterial group (90%) compared to the non-core bacterial group (10%) (Fig. 5A). For the carbon cycle, the potential function of core bacteria dominated *P. frumentum* biomass, such as methanotrophy, chitinolysis, cellulolysis, and fermentation (Fig. 5B). For the nitrogen cycle, core bacteria involved in nitrogen fixation and nitrate reduction exhibited a greater explained degree of variance in *P. frumentum* biomass compared to non-core bacteria (Fig. 5C). For the sulfur cycle, the potential functions of core bacteria, such as sulfur respiration, sulfite respiration, and dark sulfide oxidation had a higher explained degree of variance in *P. frumentum* biomass compared to non-core bacteria, while sulfate respiration showed the opposite trend (Fig. 5D).

4 Discussion

The impact of straw returning is complex and can vary depending on the duration of the experiment, the amount of straw returned, and differences in soil physicochemical properties (Zhang et al., 2008; Qin et al., 2015). Previous studies have shown that straw returning can improve corn yield (Qin et al., 2015; Gao et al., 2019b). Nevertheless, it is important to control the amount of straw returned to the field to avoid potential adverse effects on early seedling growth and final crop yield (Zhang et al., 2014). Our findings

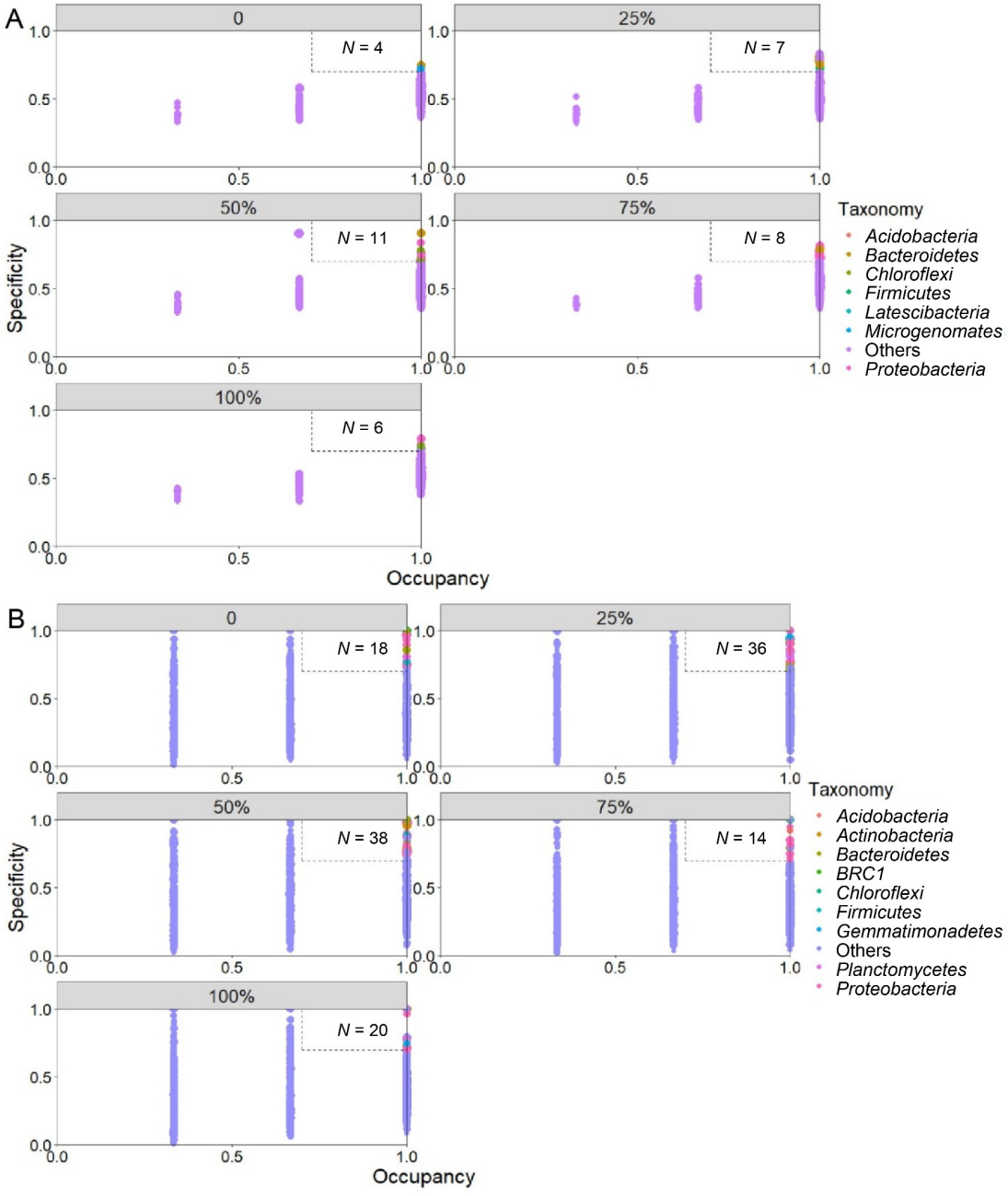


Fig. 3 The specificity and occupancy rate of ASV in core and non-core bacterial taxa under different returning amounts. Specificity is defined as the average abundance of species in different returning amounts; Occupation is defined as the relative frequency of species appearing in different returning amounts. The threshold for key species is specificity and occupancy greater than or equal to 0.7.

suggest that a 75% or 100% alfalfa returning amount is more beneficial for improving *P. frumentum* biomass. Similar results have been reported in previous studies, where returning 50% or 75% of straw significantly improved soil fertility and increased crop yield in rice-wheat rotation systems (Xu et al., 2015). However, other studies have shown that using 50% straw mulch with subsoil tillage significantly increased spring corn yield in the Huang-huai-hai region (Tao et al., 2015). There are several possible reasons for these discrepancies. First, differences in the species and varieties of green manure used can significantly affect aboveground and root biomass, as reported in previous research (Monirifar et al., 2020; Meza et al., 2022). Secondly, variations in abiotic factors such as soil texture and temperature can lead to changes in soil microorganisms and enzyme activities involved in straw decomposition (Yang et al., 2020a; Qin et al., 2021). In addition, the variations in *P. frumentum* biomass under different alfalfa returning amounts may be the result of a combination of abiotic factors (such as soil pH) and biological factors (such as

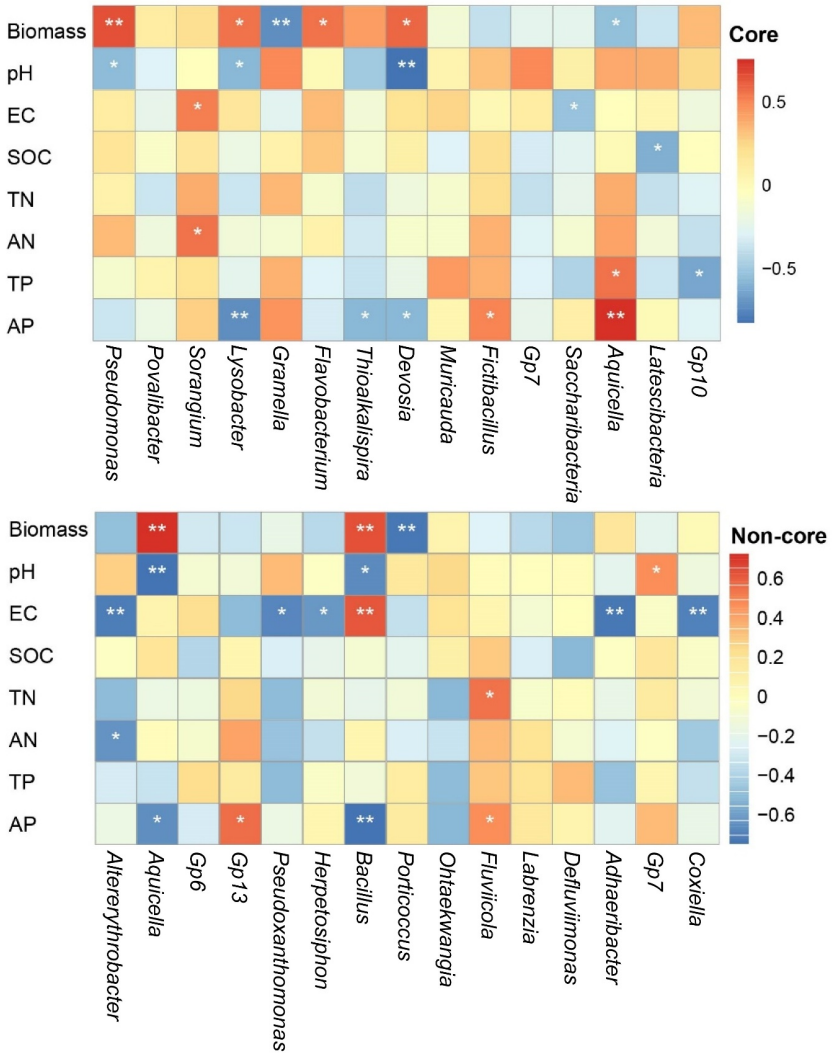


Fig. 4 Relationship between *P. frumentum* biomass, soil physicochemical properties and relative abundance of core (A) and non-core (B) bacteria at the genus level based on spearman correlation analysis. EC, conductivity; SOC, soil organic carbon; TN, soil total nitrogen; AN, alkali-hydrolyzable nitrogen; TP, soil total phosphorus; AP, available phosphorus.

specific microorganisms).

The practice of straw returning to the field has been found to have a significant impact on changes in soil physicochemical properties (Akhtar et al., 2018; Dong et al., 2023), which is consistent with our results (Table 1). Soil pH and electrical conductivity (EC) are important indicators for evaluating saline alkali soil and the degree of soil salinization (Zhang et al., 2020b). Previous studies have demonstrated that planting green fertilizers such as alfalfa on saline alkali soil can decrease soil pH (Li et al., 2018), as well as reduce soil salinity and soluble salt ion concentration to some extent (Gelaye et al., 2019). In addition, an appropriate returning amount to the field can help inhibit soil surface salinity, which may be closely related to the quality of the returned dry matter and its degradation and conversion rate in the soil (Li et al., 2018; Fan et al., 2020b). The decrease in soil nutrient content with increased alfalfa returning amounts (75% and 100% returning amount) may be attributed to both abiotic and biological factors. First, the increased straw returning can lead to higher greenhouse gas emissions (such as N_2O) (Huang et al., 2017), and straw decomposition can consume soil nitrogen nutrients (Wang et al., 2018b). Secondly, there are significant differences in the response of soil bacterial community composition to the amount of straw returning (Jin et al., 2023), and these differences tend to increase with higher quantities (Yang et al., 2020b), which is consistent with our results. In addition, previous studies have shown that the composition of microbial communities is closely related to crop productivity (Delgado-Baquerizo et al., 2018). Lastly, an increase in the returning amount may alter specific microbial taxa in the soil, such as *Pseudomonas* and *Gemmatimonas*, thereby promoting the absorption of soil nutrients by aboveground plants (Li et al., 2017; Shah et al., 2022).

The interaction between plants and soil benefitting microorganisms helps to improve plant performance in vari-

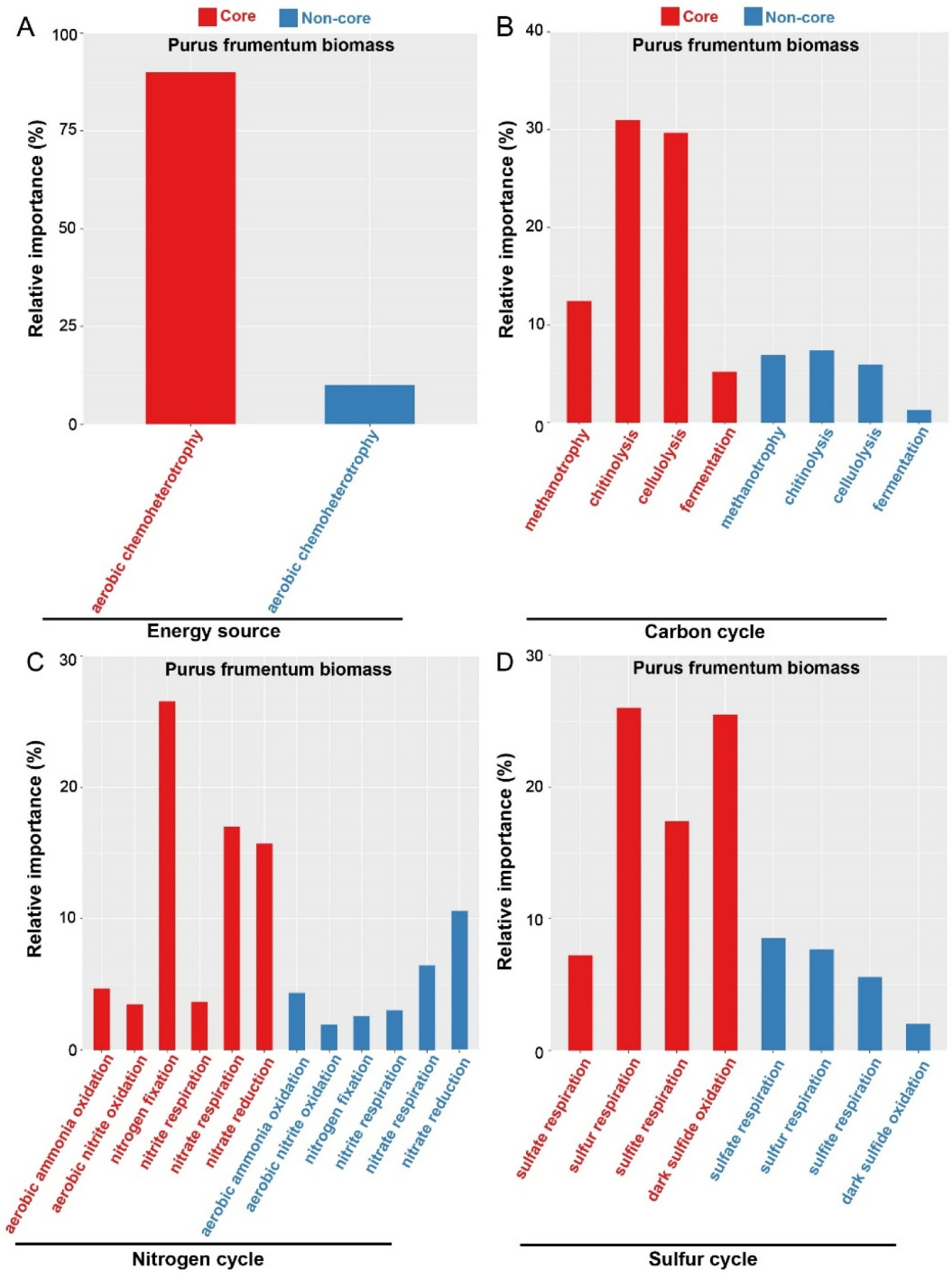


Fig. 5 Quantifying the relative importance of the potential ecological functions (energy source, carbon cycle, nitrogen cycle, sulfur cycle) of core and non-core bacteria to *P. frumentum* biomass under different alfalfa returning amount based on multiple linear regression.

ous environmental conditions (Li et al., 2022; Zhou et al., 2022b). *Gemmatimonas*, for example, is associated with the decomposition of complex organic matter and the cycling and transformation of soil nutrients, such as carbon, nitrogen, and phosphorus (Xu et al., 2023). *Pseudomonas*, a common growth promoting bacterium in the rhizosphere, is closely related to lignin degradation and plant health (Khan et al., 2023). *Povaliactor* has demonstrated tolerance to metal elements in soil ecological restoration (Deng et al., 2020). Based on the above literature, these results suggest that these bacteria could be potential beneficial contributors. Overall, our study revealed that the higher relative abundance of beneficial bacteria taxa (such as *Pseudomonas*) was higher in the 75% alfalfa returning amount compared to

other treatments in both core and non-core bacteria. In addition, the interaction between microorganisms also plays an important role in regulating plant nutrient absorption and in influencing plant growth and development. For example, the use of artificial recombinant microbiota (SynComs) has been shown to downregulate the expression of nitrate transporter genes and nitrate reductase genes, inhibiting direct nitrogen absorption by plant roots and related metabolic pathways, while improving biological nitrogen fixation (Wang et al., 2021a). In addition, rhizosphere microbes can produce plant hormones to regulate plant flowering signaling pathways (Rodriguez et al., 2019) or induce gene upregulation/downregulation of carbon/nitrogen metabolism and other related pathways to influence plant growth and root development (Sun et al., 2020). In our study, both *Lysobacter* in the core microbiota and *Bacillus* in the non-core microbiota were found to be closely related to corn yield. *Lysobacter* has been shown to produce various extracellular enzymes that contribute to plant disease control, as well as secondary metabolites with antibiotic and biosurfactant functions in fungal antagonistic effects. Therefore, they have shown great potential in improving agricultural productivity, plant health, and in inhibiting pathogenic microorganisms. Additionally, previous studies have demonstrated the importance of core taxonomic taxa in enhancing wheat yield and quality (Zheng et al., 2023), which is consistent with our research.

FAPROTAX has emerged as a promising tool for predicting the ecological functions of bacterial and archaea taxa derived from 16S rRNA amplicon sequencing (Sansupa et al., 2021). In our study, we observed significant differences in the potential functions of soil bacteria under different alfalfa returning amounts to the field, which was consistent with previous research. It has been found that there is considerable spatial heterogeneity in soil potential functions when all straw is returned to the field (Liu et al., 2022). The surface placement of a large amount of straw reduces the contact between straw and soil particles, thereby reducing the sensitivity of microorganisms to microbial decomposition (Helgason et al., 2009). Additionally, core microorganisms are essential components of holobionts and play important roles in various evolutionary processes, such as enrichment, selection, and inheritance (Lemanceau et al., 2017). Previous studies have also demonstrated that core and non-core microorganisms exhibit different responses to environmental changes while contributing to ecosystem functions (Jiao et al., 2022b). This is consistent with our results. We observed that, among the four potential ecological functions predicted, the contribution of core bacteria to corn yield was generally greater than that of non-core bacteria, indicating the important role of core microorganisms in maintaining soil function and crop productivity (Fan et al., 2020a).

5 Conclusions

In conclusion, our study found a significant difference in *P. frumentum* biomass under different alfalfa returning amounts. The highest *P. frumentum* biomass was observed with the 75% alfalfa returning amount, which alleviated the degree of soil salinization to some extent and increased the relative abundance of soil beneficial bacterial taxa, thereby promoting the absorption of soil nutrients by *P. frumentum*. The core and non-core bacterial community composition and key species types varied among different alfalfa returning amounts. Additionally, core bacterial taxa and their potential ecological functions are more closely related to *P. frumentum* biomass compared to non-core bacteria. These results highlight the importance of core microbial taxa in maintaining crop production and suggest the possibility of improving biomass by optimizing cultivation management and regulating core taxa through green manure returning amount to the field. Therefore, we recommend including core microbial communities as a key factor in policies and management approaches to maintain ecosystem stability and sustainability. This research provides a reference basis for formulating strategies to improve plant productivity and ecosystem function.

Competing interests

The authors declare no competing interests.

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Electronic supplementary material

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