scientific reports

Check for updates

OPEN

Influence of asparagus straw returns associated with vegetable species on microbial diversity in the rhizosphere

Yuling Yin[1]✉, Yuan Liu[2], Shaochun Luo[1] & Jinsong Zhou[1]

High-throughput sequencing of the 16 S rRNA gene of bacteria and the 18 S rRNA gene of fungi was employed to characterize the compositional diversity of the rhizosphere microbial community in cucumbers (*Cucumis sativus* L.), bitter gourds (*Momordica charantia* L.), and eggplants (*Solanum melongena* L.) with or without asparagus straw return. Asparagus straw return caused significant changes in the bacterial and fungal community composition and abundance of the three vegetables. The dominant bacterial phyla in all the treatments were primarily *Actinobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, and *Proteobacteria*. The dominant fungal phyla were *Ciliophora*, *Ascomycota*, and *Basidiomycota*. The relative abundance of the bacterial phylum *Actinobacteria* was greater in HG1, KG1, and QZ1 and decreased by approximately 20% with asparagus straw return, whereas that of *Firmicutes* was greater in the two treatments to which asparagus straw was added to all three vegetable soils, which were nearly 20–30 times greater than those in HG1, KG1, and QZ1. Asparagus straw return was a crucial factor in the formation of clusters according to the dominant OTUs. Asparagus straw return increased the bacterial diversity in the rhizosphere of eggplants. Special beneficial functional microbes, such as some *Paenibacillus and Cephaliophora_tropica for* cucumbers and *Lysinibacillus* and *Ramlibacter* for eggplants, are affected by certain vegetables. Moreover, the vigour of eggplants treated with asparagus straw return was greater than that of the other vegetables. The fresh weights increased at straw rates of 15 g/kg and 25 g/kg in eggplants (QZ2 and QZ3), with the greatest percentage reaching approximately 200%. Thus, eggplants might be considered a better option for planting after asparagus straw application in the field. Straw return was the main factor affecting rhizosphere microorganisms but it also affected plant species in the soil microbial community, which could allow us to understand the use of straw return.

Keywords Asparagus, Straw return, Microbial community diversity, Vegetables

Abbreviations

HG1	Cucumbers + no asparagus straw added as a control
HG2	Cucumbers + 15 g asparagus straw
HG3	Cucumbers + 25 g asparagus straw
KG1	Bitter gourds + no asparagus straw added as a control
KG2	Bitter gourds + 15 g asparagus straw
KG3	Bitter gourds + 25 g asparagus straw
QZ1	Eggplants + no asparagus straw added as a control
QZ2	Eggplants + 15 g asparagus straw
QZ3	Eggplants + 25 g asparagus straw

Asparagus (*Asparagus officinalis* L.) is an herbaceous perennial vegetable belonging to the Asparagaceae family. It is known as the "king of vegetables" owing to its tender and unique flavour, rich nutritional value, and important bioactive compounds such as rutin and protodioscin, which are beneficial for human health because of their antioxidant activities[1–3]. In 2020, the annual production of asparagus was 7.3 million metric tons (MMTs), with

[1]Institute of Vegetables and Flowers, Jiangxi Academy of Agricultural Sciences, Nanchang, China. [2]Liaoning Saline or Alkaline Land Utilization and Research Institute, Liaoning Academy of Agricultural Sciences, Panjin, China. ✉email: yuling_0_2000@163.com

China being the top producer and accounting for 87% of global production[4]. The asparagus planting area in China has reached 120,000 hectares, accounting for approximately 49% of the world's total, indicating that China is the world's largest asparagus producer and exporter[5]. With the expansion of the asparagus production area, a large amount of asparagus residue is produced every year in China, especially in southern China, given that the asparagus fern is cut twice a year, in summer and in fall. Owing to the shortage in and rising cost of labour, most residues are discarded in the field, which results in a waste of bioresources and environmental pollution. Therefore, straw removal from the field has become a great burden for asparagus producers and is an urgent problem in asparagus production.

Half of agricultural inputs will eventually be converted to crop straw[6]. The utilization of crop straw is a tool to prevent the waste of farmland, water resources, and agricultural costs[7,8]. The current utilization of crop straw in China is not optimal. The effective management of crop residues has become a significant issue faced by the government. Burning is the most popular method for straw disposal by farmers. But straw burning in the field can accelerate soil erosion, environmental pollution, and soil degradation and negatively affect ecosystem functions[9,10] and is now banned by the government. Comprehensive technologies for crop straw utilization are still immature, with high costs and poor effects[10]. Crop straw contains a large amount of organic matter and nutrient elements, including nitrogen, phosphorus, potassium, magnesium, calcium, and sulfur, which are essential nutrient elements for crop growth[11–13]. Directing straw return to the field after crop harvest not only prevents the loss of carbon as CO_2 during incineration but also improves the organic C level of the soil, which directly or indirectly improves soil physics, microbial vitality, and crop root development[14–16]. Straw return fields provide a rich carbon source for soil microorganisms. This practice changes the living environment of microorganisms and the structure of the soil microbial community[17] significantly increasing the amounts of soil bacteria, fungi, and actinomycetes.

Moreover, the plant root-soil interface is a dynamic region in which complex interactions between plant roots, bacteria, and fungi occur. Microbial community compositions change greatly because of their strong selectivity in the environment of plant rhizospheres[18]. Plant-microbe interactions in the rhizosphere also play a significant role in microbial activity[19,20]. The plant genotype can play a significant role in shaping the rhizosphere microbiome, as different plant species and varieties produce different types and amounts of root exudates[21–23]. Rhizosphere microbial communities could respond to both straw return and plant type in the straw return field regime, as crops are usually planted in the field following straw return directly or after the completion of decomposition processing. However, the influence of differences in crops and straw return in the same habitat on microbial communities has rarely been investigated to clarify the suitable crops chosen as candidates planted in fields with straw return. In our study, we analysed the rhizosphere microbial community composition of three species of popular vegetables under various intensities of asparagus straw return. Bacterial 16 S rRNA and fungal 18 S rRNA gene amplicon sequencing were used to explore microbial richness, abundance, and diversity in rhizosphere soil samples. We sought to (1) determine the differential effects of the different asparagus straw return treatments on the rhizosphere microbial community composition of three vegetables and (2) clarify the relationships among the asparagus straw return treatments, vegetable species, and the soil microbial community.

Results

Effect of asparagus straw return on the morphology of three vegetables

The percentages of the increase in plant height and fresh weight compared with those in the absence of asparagus straw return are shown in Fig. 1. The heights and fresh weights of all the vegetables improved with asparagus straw return. The height of the eggplant plants (QZ2) increased by more than 30% at a straw return rate of 15 g/kg. In contrast, bitter gourds (KG2) at a straw return rate of 25 g/kg presented the greatest increase in plant

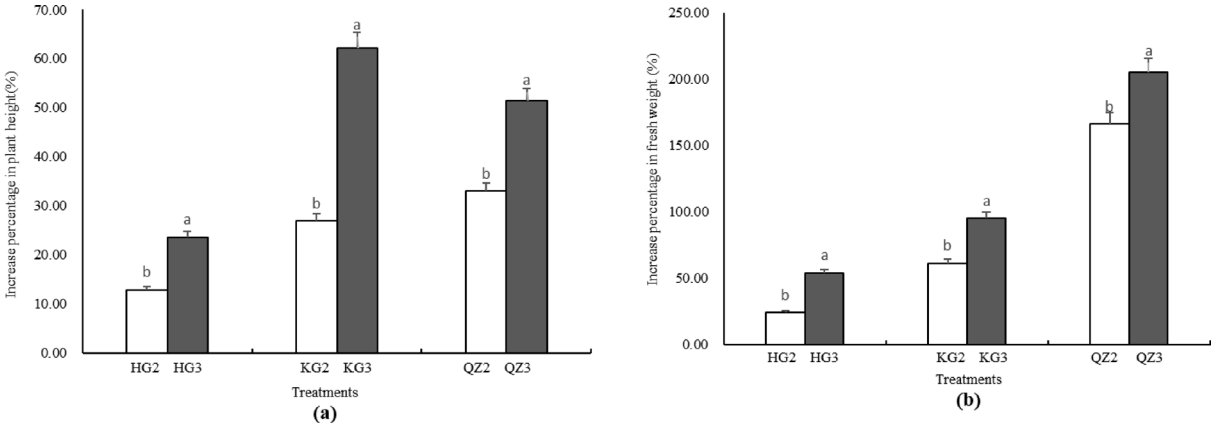

Fig. 1. The percentage increase of three vegetables produced from asparagus straw as a function of plant height (a) and fresh weight (b). HG2 and HG3 indicate cucumbers planted with 15 and 25 g/kg asparagus straw return, respectively. KG2 and KG3 indicate bitter gourds planted with asparagus straw return rates of 15 and 25 g/kg added, respectively. QZ2 and QZ3 indicate eggplants to which 15 and 25 g/kg asparagus straw was added, respectively.

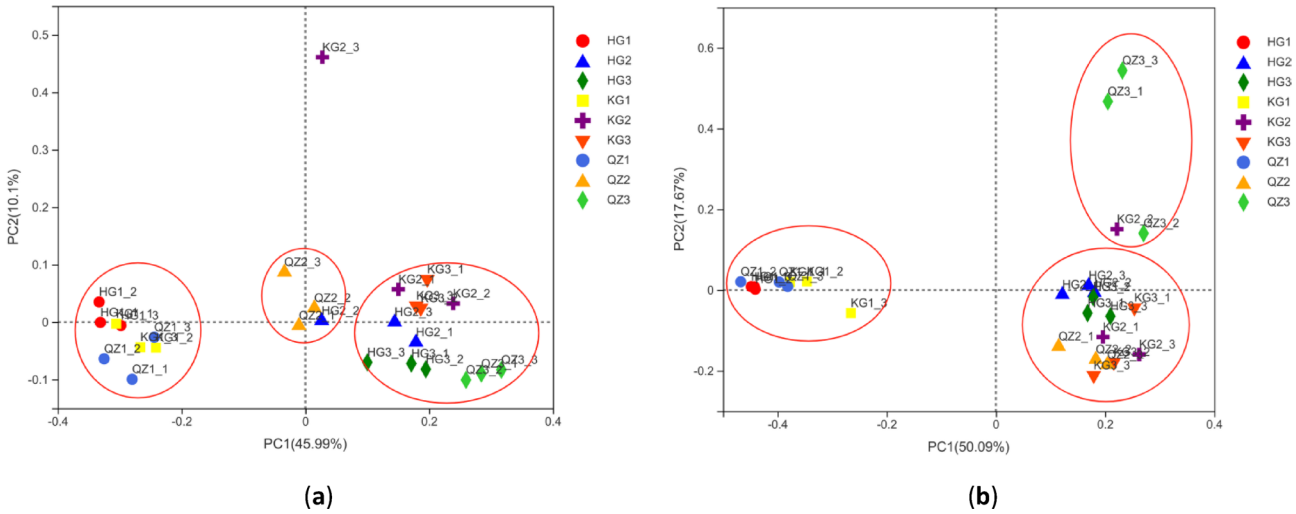

Fig. 2. Principal coordinate analysis (PCoA) ordination highlighting differences in the composition of bacterial (a) and fungal (b) communities (OTUs) in all treatments.

	Statistic *R*	*P*
Bacterial	0.7565	0.001
Fungal	0.8361	0.001

Table 1. Statistical confirmation by analysis of similarity (ANOSIM) for reliability in PCoA.

height (approximately 60%). The fresh weights of eggplants (QZ2 and QZ3) increased at straw return rates of 15 g/kg and 25 g/kg, with the greatest percentage reaching approximately 200%. Overall, eggplant (QZ) height and fresh weight improved the most in response to asparagus straw return.

Distribution of microbial communities

Principal coordinate analysis (PCoA) revealed that the return of asparagus residue resulted in a differential distribution of the microbial community in the rhizospheres of the three vegetables (Fig. 2). According to the distribution of rhizosphere bacterial flora (Fig. 2a), the first two principal components, PC1 and PC2, explained 45.99 and 10.1% of the distribution, respectively. HG1, KG1, and QZ1 were separated from those of the other two treatments. QZ2, QZ3, and HG3 were separated from each other and from the other treatments. KG2, KG3, and HG2 were nearly mixed.

However, according to the distribution of the fungal community in the rhizosphere soil (Fig. 2b), the first two principal components, PC1 and PC2, explained 50.09% and 17.67%, respectively, of the distribution. The HG1, KG1, and QZ1 samples were also separated from those of the other two treatments. With the exception of the QZ3 samples, which were separated from those of the other treatments, the other treatments were largely not separated from each other. Compared with those under the other treatments, the microbial community under the asparagus residue return treatment differed greatly in terms of the different vegetable rhizospheres.

Moreover, different forms of asparagus straw return significantly affected the separation of both communities in our treatments (Table 1), which indicates that the grouping was reasonable and reliable on the basis of the microbial data. The differences in the PCoA results for the bacterial and fungal communities in the rhizosphere soil among the treatments significantly reflected the original conditions of all the treatments. The R values were 0.7565 for the bacterial PCoA and 0.8361 for the fungal PCoA, whereas the p value was 0.001 for both analyses according to an ANOSIM.

Soil microbial community composition

The dominant bacterial phyla included *Actinobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, and *Proteobacteria* (Fig. 3). Notably, the relative abundance of the bacterial phylum *Actinobacteria* was greater in HG1, KG1, and QZ1 and decreased by approximately 20% with asparagus straw return, whereas that of *Firmicutes* was greater in the two treatments to which asparagus straw was added to all three vegetable soils, which was nearly 20–30 times greater than that in HG1, KG1, and QZ1. The relative abundances of the bacterial phyla *Chloroflexi* and *Acidobacteria* decreased by approximately 45% and 33%, respectively, with increasing degree of asparagus straw return. The dominant fungal phyla were *Ciliophora*, *Ascomycota*, and *Basidiomycota* (Fig. 4). The relative abundance of the fungal phylum *Ciliophora* was greater in both HG2 and KG2 treatments, and the relative abundance of *Ascomycota* was highest in the treatment without asparagus residue return and decreased by nearly 14–20% with increasing concentration of returned asparagus straw.

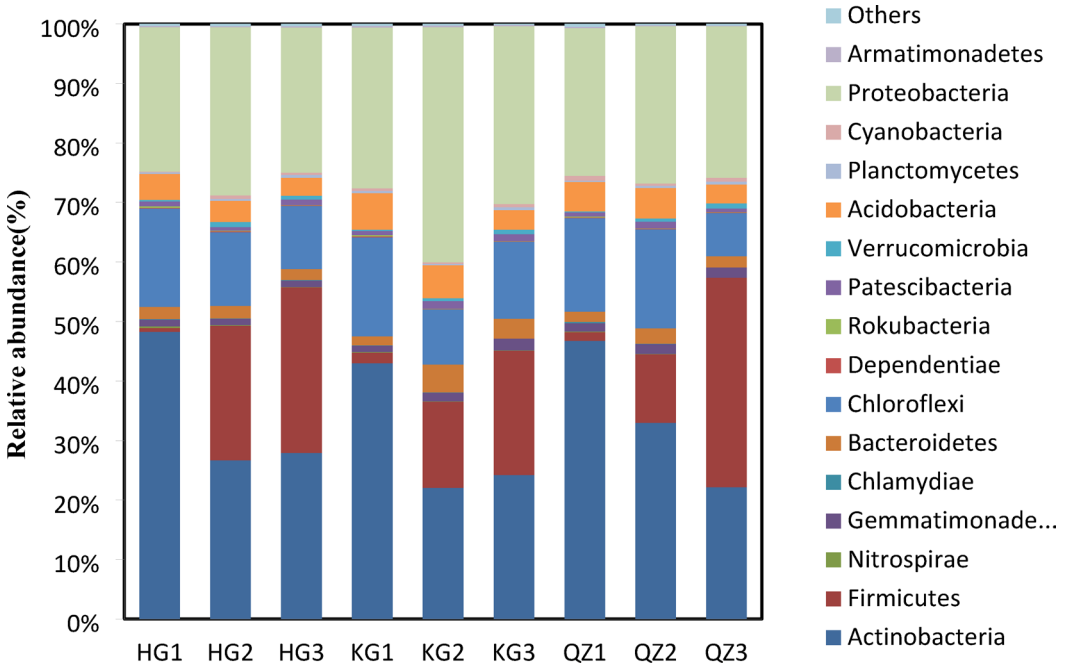

Fig. 3. Relative abundances of bacterial phyla in all the treatment groups. All phyla representing < 0.01% relative abundance were combined as "Others" (Fibrobacteres, *Deinococcus-Thermus*, *Elusimicrobia*, etc.). HG1, HG2 and HG3 indicate cucumbers planted with 0, 15 and 25 g/kg asparagus straw return, respectively. KG1, KG2 and KG3 indicate bitter gourds planted with asparagus straw return of 0, 15 and 25 g/kg, respectively. QZ1, QZ2 and QZ3 indicate eggplants planted with 0, 15 and 25 g/kg asparagus straw return, respectively.

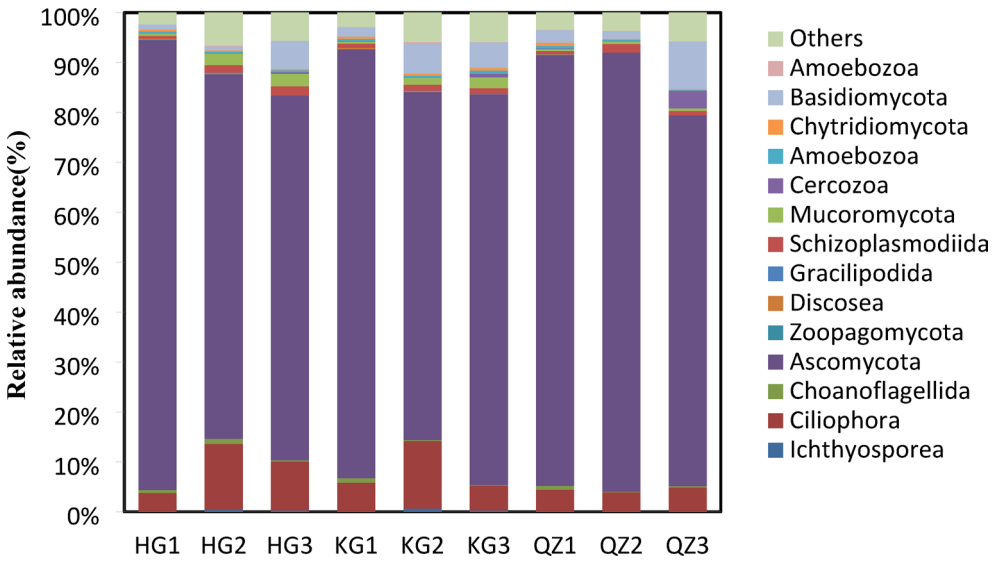

Fig. 4. Relative abundances of fungal phyla in all the treatment groups. All phyla representing < 0.01% relative abundance were combined as "Others" (*Labyrinthulomycetes*, *Blastocladiomycota*, *Protosteliida*, *Ochrophyta*, *Cryptophyceae*, *Acanthocystidae*, *Phragmoplastophyta*, *Cavosteliida*, etc.). HG1, HG2 and HG3 indicate cucumbers planted with 0, 15 and 25 g/kg asparagus straw return, respectively. KG1, KG2 and KG3 indicate bitter gourds planted with asparagus straw return of 0, 15 and 25 g/kg, respectively. QZ1, QZ2 and QZ3 indicate eggplants planted with 0, 15 and 25 g/kg asparagus straw return, respectively.

Bacterial and fungal alpha diversity

Bacterial alpha diversity

Bacterial *alpha* diversity was evaluated using the Sobs, Shannon, Simpson, Ace and Chao indices (Table 2). The bacterial Sobs index values for HG1 were significantly greater than those for HG2 and HG3, whereas those for

Alpha diversity indices	Asparagus straw return (g/kg)	HG	KG	QZ
Sobs	0	1939.33a	1635.00b	1656.00ab
	15	1682.67ab	1493.00bc	1659.00ab
	25	1609.00b	1396.67bc	1319.00c
Shannon	0	5.4537ab	5.4038ab	5.3238b
	15	5.4677ab	5.4263ab	5.6628a
	25	5.4483ab	5.5409ab	5.306b
Simpson	0	0.022b	0.0274ab	0.0389a
	15	0.0162b	0.0139b	0.012b
	25	0.0186b	0.0123b	0.0163b
ACE	0	2552.61a	2192.10ab	2203.04ab
	15	2308.94abc	1933.95abc	2179.88abc
	25	2313.23bcd	1843.68 cd	1756.93d
Chao	0	2522.15a	2170.00abc	2185.82abc
	15	2321.11ab	1963.35bcd	2205.64abc
	25	2206.53abc	1852.05 cd	1764.55d

Table 2. Alpha diversity indices of the bacterial communities in different vegetable rhizospheres under the asparagus straw return treatment. Different capital letters in the same α diversity index represent significant differences at $p < 0.01$, and lowercase letters represent significant differences at $p < 0.05$. HG, KG and QZ indicate cucumbers, bitter gourds and eggplants, respectively, when planted in pots containing returned asparagus straw.

Alpha diversity indices	Asparagus straw return (g/kg)	HG	KG	QZ
Sobs	0	192.33b	213.33ab	200.33ab
	15	229.33a	221.33ab	203.00ab
	25	209.67ab	212.33Aab	197.67ab
Shannon	0	2.5335d	2.8224bcd	2.8069bcd
	15	3.4106a	3.1585ab	2.7118 cd
	25	3.4759a	3.0815abc	2.7971bcd
Simpson	0	0.1952ab	0.1366bc	0.1360bc
	15	0.0859c	0.1294bc	0.2333a
	25	0.0742c	0.1491bc	0.1743ab
ACE	0	223.64a	232.22a	228.20a
	15	253.31a	242.50a	222.79a
	25	230.52a	232.93a	223.30a
Chao	0	233.02a	235.23a	234.41a
	15	256.00a	238.60a	225.23a
	25	227.29a	236.95a	228.15a

Table 3. Alpha diversity indices of the fungal communities in different vegetable rhizospheres under the asparagus straw return treatments. Different capital letters in the same α diversity index represent significant differences at $p < 0.01$, and lowercase letters represent significant differences at $p < 0.05$. HG, KG and QZ indicate cucumbers, bitter gourds and eggplants, respectively, planted in the pots containing asparagus residual treatment.

QZ3 were significantly lower than those for QZ1 and QZ2. When 0 g/kg asparagus straw was added, the bacterial Sobs index values for HG1 were significantly greater than those for KG1, whereas when 25 g/kg asparagus straw was added, the bacterial Sobs index values for HG3 were significantly greater than those for KG3. The bacterial Shannon index for QZ2 was significantly greater than that for the other treatments. The bacterial Simpson index for QZ1 was significantly greater than that for the other treatments. The bacterial ACE indices for HG, KG and QZ in response to the addition of 25 g/kg asparagus straw were significantly lower than those in response to the addition of 0 g/kg asparagus straw, while there were no significant differences among the species of vegetables. The bacterial Chao index for QZ3 was significantly lower than those for QZ1 and QZ2.

Fungal alpha diversity

Fungal *alpha* diversity was evaluated using the Sobs, Shannon, Simpson, ACE and Chao indices (Table 3). The fungal Sobs index values for HG1 were lower than those for the other treatments, while those of the other

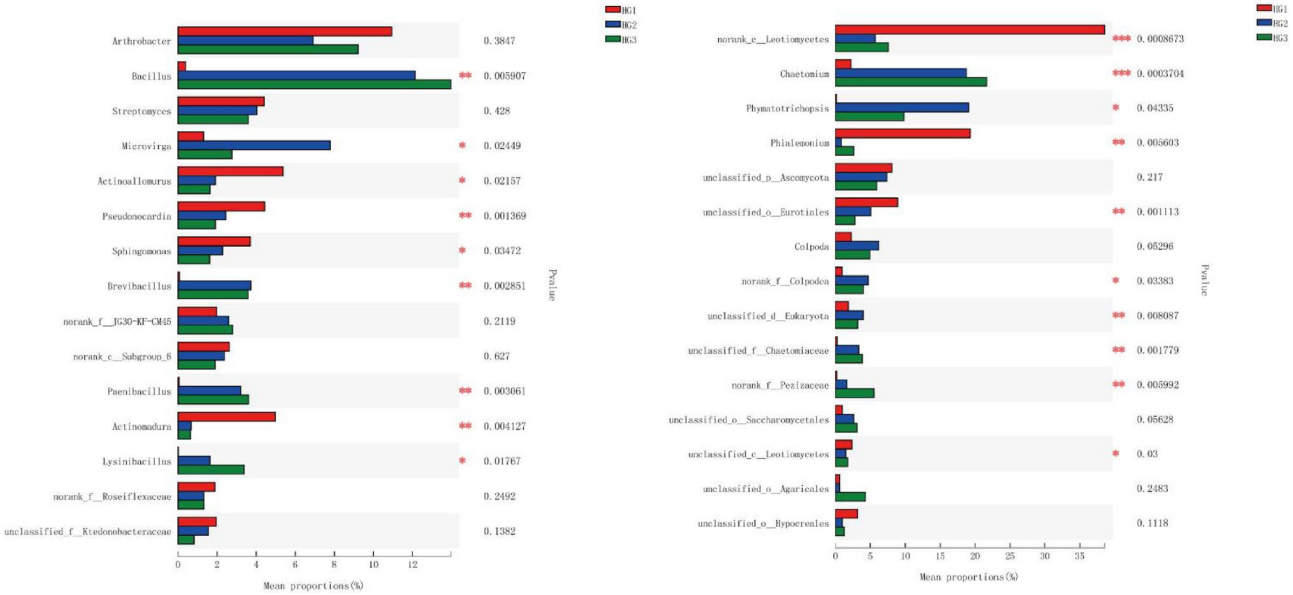

Fig. 5. Relative abundance difference in bacterial genera in the rhizosphere of three vegetables with asparagus straw return. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; and ***$P \leq 0.001$.

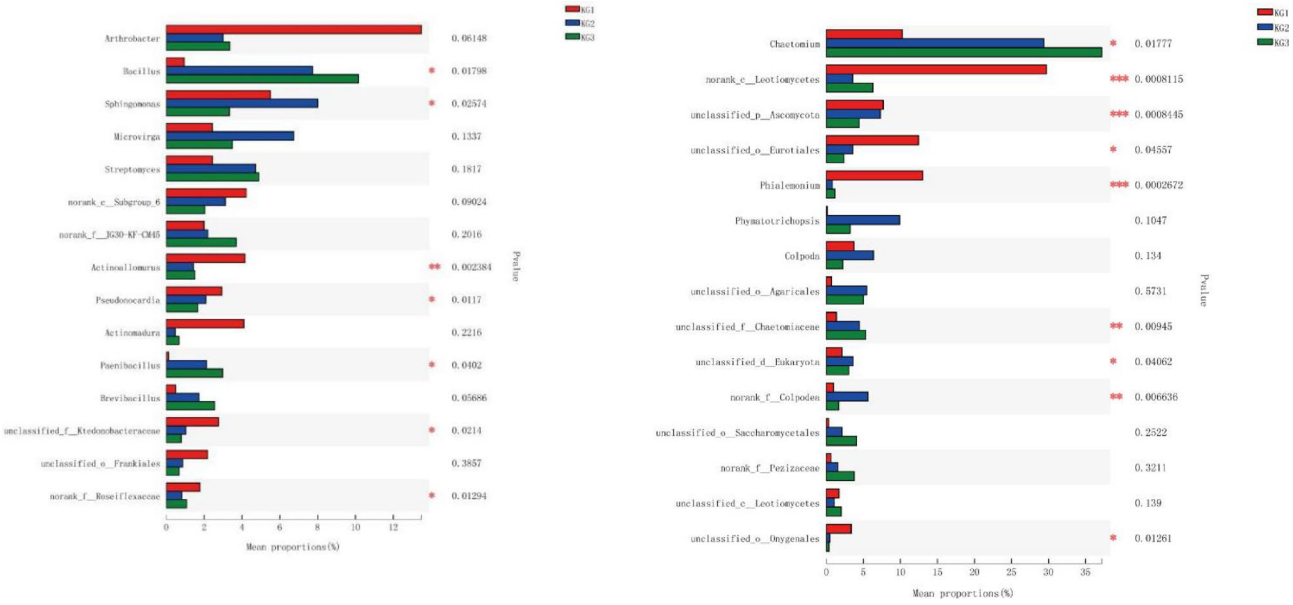

Fig. 6. Relative abundance difference in bacterial genera in the rhizosphere of three vegetables with asparagus straw return. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; and ***$P \leq 0.001$.

treatments were at the same level. The fungal Shannon indices for HG2 and HG3 were significantly greater than that for HG1, while for the same asparagus straw addition treatments, the Shannon indices for HG were significantly greater than those for KG and QZ. The fungal Simpson index for HG1 was significantly greater than that for HG2 and HG3, while that of QZ2 was significantly greater than that of QZ1. Under 15 and 25 g/kg of added asparagus straw, the fungal Simpson index for HG was significantly lower than that under 0 g/kg of returned asparagus straw. The fungal ACE index and Chao1 index did not significantly differ among the treatments.

Varied at the microbial genus level

At the genus level, asparagus straw return significantly affected the bacterial and fungal proportions in the rhizosphere of each vegetable species. Asparagus straw significantly increased bacterial and fungal genera in the rhizospheres of both cucumbers and eggplants, which were greater than those in the bitter gourds (Figs. 5, 6 and

7). The proportions of the bacterial genera *Bacillus*, *Microvirga*, *Brevibacillus*, *Paenibacillus*, and *Lysinibacillus* were significantly greater in the rhizosphere of HG2 and HG3 (cucumbers), whereas *Actinoallomurus*, *Pseudonocardia*, *Sphingomonas*, and *Actinomadura* were significantly lower. At the fungal genus level, the abundances of *Chaetomium*, *Colpodca*, *Eukaryota*, *Chaetomiaceae*, and *Pezizaceae* significantly increased in the rhizosphere of cucumbers with asparagus straw return, whereas the abundances of *Leotiomycetes*, *Phialemonium*, and *Leotiomycetes* significantly decreased (Fig. 5). Moreover, bacterial and fungal genera and their varied trends were similarly influenced by asparagus straw in the rhizosphere of the other two vegetables (Figs. 6 and 7), but the number of bacteria and fungi were lower in the rhizosphere of KG (bitter gourds) (Fig. 6). For a specific microbial genus, the mean proportion of *Bacillus* in the rhizosphere of eggplants with asparagus straw return was approximately 20% (Fig. 7), which was much greater than the 12% reported in the rhizosphere of other vegetables (Figs. 5 and 6). Asparagus straw return significantly decreased the mean proportion of *Leotiomycetes* in the rhizosphere of the three vegetables (Figs. 5, 6 and 7). The mean proportions of *Chaetomium* in the rhizospheres of cucumbers, bitter gourds, and eggplants with asparagus straw return were approximately 25%, 35% and 45%, respectively.

Discussion

Soil microorganisms are highly diverse and are the driving force for the recycling and transformation of soil organic matter and nutrients[24]. Straw return can significantly increase the number of bacteria to improve the community structure and functional diversity of soil microorganisms[25] resulting in the formation of a new microbiota[26]. This treatment not only provides a more appropriate soil physical and chemical environment for microorganisms, which is conducive to their survival, but also provides abundant carbon and nitrogen sources[7,27]. Different straw properties and environmental factors can lead to substantial variation in the diversity of soil microbial communities[28]. In addition to the treatments in which no asparagus straw was added, the distributions of both the soil bacterial and fungal communities differed from those in the treatments to which no asparagus straw was added (Fig. 1), which included all three vegetable straw treatments. The external properties of each vegetable were more vigorous and stronger when asparagus straw was added than when asparagus straw was not added, with results such as greater plant height and fresh weight, especially in the eggplant treatment (Fig. 1). The intensity of straw return has different effects on soil microbial structure and diversity[29]. In general, medium and high amounts of straw return are more attractive to soil microorganisms[15] which may provide many nutrients for feeding these microorganisms. The number of soil bacteria and actinomycetes increased in response to the different amounts of straw returned to the field. The fungal population increased only at a relatively high rate of straw return. A similar phenomenon was found in our study, which revealed that increasing the intensity of asparagus straw treatment significantly increased the bacterial community composition of the three vegetables, whereas the distribution of fungal communities did not significantly differ among the treatments in which asparagus straw was added. Straw usually increases bacterial richness without the emergence of new bacterial species[30,31]. The relative abundance of *Firmicutes* significantly increased after asparagus straw return, whereas its relative abundance in the rhizosphere of the three vegetables without asparagus straw was very low (Fig. 3). *Proteobacteria* were present in all the treatments, and the greatest number of bacteria was detected in the low-intensity asparagus straw return treatment in the HG2, KG2, and QZ2 groups. The most dominant fungal

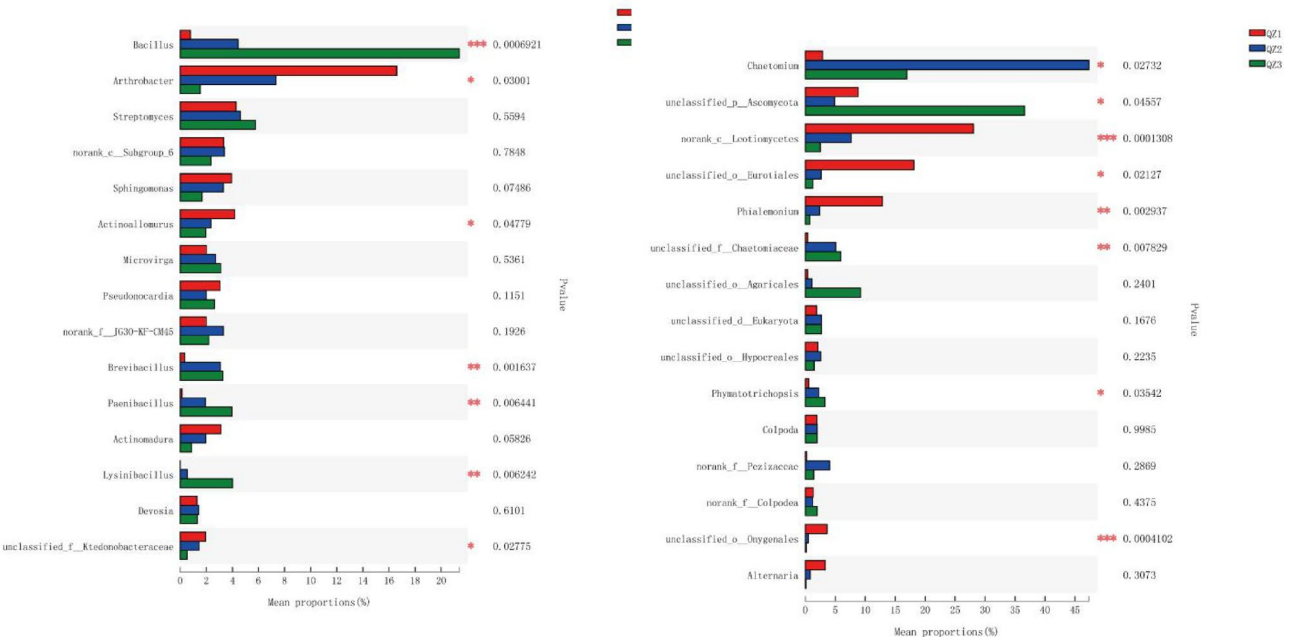

Fig. 7. Relative abundance difference in fungal genera in the rhizosphere of three vegetables with asparagus straw return. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; and ***$P \leq 0.001$.

phylum in all the treatments was *Ascomycota* (Fig. 4). This trend might have occurred because the community is largely restricted by the soil pH[16]. The relative abundance of *Acidobacteria* decreased with increasing intensity of asparagus straw return. A negative correlation was detected between *Acidobacteria* and soil total carbon[32]. The relative abundance of the phylum *Basidiomycota* was high in HG3, KG2, KG3, and QZ3 (Fig. 3) because straw residue degradation affects the abundance of this phylum and can cause rapid metabolism of organic substances in roots[33].

Microbial activity is vigorous in the rhizosphere of plants but varies with the species[22] because root exudate metabolite profiles are distinctive among various crops. Rhizosphere microbial communities also respond significantly to plant types[34]. Natural variation within maize species can be used as an approach for understanding the role of root exudate in microbiome selection, in which a specific degree of root exudation impacts root-associated microbiomes[35]. The fungal composition varied among the species of vegetables (Fig. 2b). Asparagus straw return has a strong influence on microbial species in the rhizospheres of three vegetable species (Supplementary Tables 1 and 2). In terms of bacteria, the abundances of *Paenibacillus_cineris*, *Paenibacillus_faecis*, *Paenibacillus_lactis*, and *Aneurinibacillus* was highly increased in cucumbers (HG) treatment involving asparagus straw return. Some bacteria that have excellent functions in terms of nitrogen fixation, such as *Lysinibacillus*, *Ramlibacter*, *Shimazuella*, and *Bacillusaerophilus*, strongly increased in abundance in *Solanum* eggplant (QZ) treatment. In terms of fungi, *Mucoromycota* (Fig. 3), *Cephaliophora_tropica*, and *Alternaria_alternata* (Supplementary Table 2) were enriched in cucumbers (HGs) and bitter gourds (KGs) with both intensities of asparagus straw treatment, which was lower in the *Solanum* eggplant (QZ) treatment. This finding might be due to the root exudates in cucumbers (HGs) and bitter gourds (KGs) having a positive effect on these plants, whereas *Cephaliophora_tropica* and *Alternaria_alternata* commonly include several fungi that can cause plant diseases. Because the experiment was conducted on vegetables at the seedling stage, disease might not have occurred in bitter gourds. Pathogenic populations with potential harm may increase as favourable conditions for growth are provided by the return of crop straw to soils at relatively high rates and consequently to diseases such as root rot in maize[36] common rot and sharp eyespot in wheat[37].

Plants grown following maize crops were significantly different, with tomatoes showing the greatest differences, which is consistent with the hypothesis that plant species are important in recruiting specific rhizosphere microbial communities[38]. A greater abundance of fungal pathogens was also observed with corn straw return than with wheat straw return in the wheat-corn rotation system[29]. The microbial community was compositionally and functionally distinct and colonized the rhizosphere of tomato plants grown following alfalfa. Asparagus straw return increased the bacterial diversity in the rhizosphere of eggplants. Asparagus straw return decreased the bacterial richness in the rhizospheres of the three vegetables (Table 2). Asparagus straw return increased the fungal diversity in the rhizosphere of cucumbers. Asparagus straw return had no influence on the fungal richness in the rhizospheres of the three vegetables, with no significant changes in the Sobs, ACE and Chao indices (Table 3). The bacterial and fungal diversity are strongly influenced by the vegetable species cucumbers and eggplants. Moreover, at the genus level, the differences in bacteria and fungi were still greater in the rhizospheres of HG and QZ than in those of KG (Figs. 5, 6 and 7). Asparagus straw return increased the relative abundance of dominant microbes, such as *Bacillus* and *Acidobacteria*, which are related to soil pH and play important roles in soil material circulation and ecological environment construction. Moreover, there was a decrease in *Leotiomycetes*, which is a common plant pathogen causing leaf spot disease, cucurbit powdery mildew, and anthracnose[39,40].

Conclusion

Asparagus straw return was the dominant factor affecting the rhizosphere microbial genera, but vegetable species were also the determining factor for significant change. Our results indicate that there is a specific correlation between vegetable species and the influence of crop straw return on the soil microbial community. The straw return rates that could be applied in the field should be based on the crop species planted following straw return. Vegetable species should be considered when asparagus straw is returned. In combination with the growth mass and soil microbial conditions, eggplants should be a better choice to plant in asparagus straw return field. However, the reasons that some special microbes are significantly affected by a given vegetable species require further research to explore how asparagus straw and vegetable species interact with those functional microbes.

Methods

Experimental setup

The experiment was conducted at the experimental station of the Institute of Vegetables and Flowers, Jiangxi Academy of Agricultural Sciences, Jiangxi Province, China (28° 11′ N, 115° 56′ E). Two densities of asparagus straw return to the soil were designed for incorporation. Eggplants (*Solanum melongena* L.), bitter gourds (*Momordica charantia* L.) and cucumbers (*Cucumis sativus* L.) were employed as candidates for microbial diversity assessment in soil treated with asparagus straw return. Eggplant cultivar: Long purple from Jingyan Yinong Seed Co. Ltd.; bitter gourd cultivar: Gan4 from the Jiangxi Academy of Agricultural Sciences; and cucumber cultivar: Jinlv from the Tianjin Academy of Agricultural Science. Seeds of the eggplants, bitter gourds and cucumbers were sown after straw return. Soil samples were collected from the rhizospheres of three vegetable species for microbiological testing.

Asparagus residues were collected from five-year-old asparagus at an asparagus planting site. Spears measuring 35–40 cm in length were harvested. The bottom portion (5–10 cm) of the long asparagus spears was taken as the residue after 30 cm of the top portion was cut for commercial use. The residues were washed, dried at 70℃ in an oven, and ground into a powder using a mini grinder.

Soil treatments (g/kg)	pH	OM (g/kg)	Total *N* (%)	Hydro-*N* (mg/kg)	Available *P* (mg/kg)	Available K (mg/kg)
0	6.26 ± 0.06	7.27 ± 0.13	0.058 ± 0.009	7.76 ± 0.10	11.2 ± 0.70	138 ± 8.76
15	6.55 ± 0.11	10.3 ± 0.17	0.079 ± 0.006	21.3 ± 1.08	11.5 ± 0.73	327 ± 7.85
25	6.45 ± 0.09	15.1 ± 0.15	0.107 ± 0.01	41.4 ± 0.82	15.6 ± 0.90	587 ± 10.13

Table 4. Basic physical and chemical properties of the soil treated with asparagus straw.

Treatment name	HG1	HG2	HG3	KG1	KG2	KG3	QZ1	QZ2	QZ3
Vegetable species planted	Cucumbers			Bitter gourds			Eggplants		
Asparagus straw added, amount per kg soil	0	15 g	25 g	0	15 g	25 g	0	15 g	25 g

Table 5. Treatment details used in the experiments.

The incorporation treatments were as follows: ① no residue added as a control, ② 15 g of residue, and ③ 25 g of residue. One kilogram of soil was mixed with 15–25 g of residue powder, placed into a 7 cm×15 cm plastic pot covered with plastic film (with 20 needle holes on the film), and incubated at 80% field water capacity at a temperature of 30 ℃ for 30 d. Water supplementation was applied after every five days. After 30 d, the covered plastic films were removed for sowing vegetable seeds.

The physical and chemical properties of the asparagus straw return soil used in the study are shown in Table 4.

The vegetables planted after residues were used in the experiment were cucumbers, bitter gourds, and eggplants. The seeds were soaked in water for 24 h and rinsed every 12 h. Then, a 9 cm diameter Petri dish with two layers of moist filter paper was used for seed germination. Uniformly germinated seeds were sown in pots subjected to the three residue treatments. There were three replicates per treatment and ten pots per replicate. The pots were placed in a greenhouse at a temperature of approximately 25:33 °C (dark: light). The plants were all subjected to the same farm practices.

The experiment consisted of three groups and nine treatments (Table 5). The first group included ①cucumbers + no asparagus straw added as a control (HG1), ②cucumbers + 15 g asparagus straw (HG2), and ③cucumbers + 25 g asparagus straw (HG3); the second group included ①bitter gourds + no asparagus straw added as a control (KG1); ②bitter gourds + 15 g asparagus straw (KG2), and ③ bitter gourds + 25 g asparagus straw (KG3); and the third group included ①eggplants + no asparagus straw added as a control (QZ1), ②eggplants + 15 g asparagus straw (QZ2), and ③eggplants + 25 g asparagus straw (QZ3). Each treatment had ten repetitions.

Morphological measurement

Morphological features, including the plant height and fresh weight, were investigated using tape scale measurements and a balance. Plant heights and fresh weights were assessed by measuring all the plants.

Soil sampling

After 30 d, soil samples were collected from each of the nine treatments. The soil samples were collected from each plot in the rhizosphere of the plants. The plants were slightly uprooted from the pots to remove the soil. The soil closely adhering to the roots was brushed off, and the samples were immediately stored at − 80 °C for microorganism analysis.

Soil DNA extraction and high-throughput sequencing

The 0.5 g soil (fresh weight) samples were used for the extraction of the genomic DNA of the microorganisms using an E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) by following the manufacturer's instructions. A NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to determine the quality and concentration of the DNA. The soil bacterial 16 S rRNA gene in the hypervariable V3-V4 regions in the rhizosphere of three vegetables was amplified using the primers 338 F (5′-ACTCCTACGG GAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The soil fungal 18 S rRNA gene in the hypervariable V5-V7 regions in the rhizosphere of three vegetables was amplified using the primers SSU0817F (5′-TTAGCATGGAATAATRRAATAGGA-3′) and 1196R (5′-TCTGGACCTGGTGAGTTTCC-3′). The conditions for amplification by PCR were one cycle at 95℃ for 3 min, 27 cycles at 95℃ for 30 s, at 55℃ for 30 s, and at 72℃ for 45s, with a final step of 72℃ for 10 min, and 10℃ until the reaction was stopped by the user. We conducted three PCRs with a 20 μL final volume mixture including 5×TransStartFastPfu Buffer (4 μL), 2.5 mM dNTPs (2 μL), 5 μM forward primer (0.8 μL), 5 μM reverse primer (0.8 μL), TransStartFastPfu DNA Polymerase (0.4 μL), and 10 ng of template DNA. We extracted the PCR products of the soil DNA from a 2% agarose gel and further purified them using the AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, USA) and then quantified them via QuantiFluor™-ST (Promega, USA) according to the manufacturer's instructions. Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq PE300 and PE250 platform (Illumina, San Diego, USA) for soil bacteria and fungi, respectively, following the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Processing of sequencing data

Sequences from the Illumina MiSeq platform were processed using the QIIME (version 1.70) software package. We employed Trimmomatic for raw fastq files to be demultiplexed and quality-filtered and then FLASH for merging those data using the following standards: (a) truncating the reads at any site receiving an average quality value < 20 over a 50 bp sliding window; (b) keeping the primers exactly matched (nucleotide sequence be allowed having two mismatches) and removing the reads that contained ambiguous bases; (c) merging sequences that overlapped longer than 10 bp on the basis of their overlap sequence; and (d) differentiating samples on the basis of barcodes and primers at the beginning and end of the sequence and adjusting the sequence direction. Zero barcode mismatches were allowed, and 2 primer mismatches were allowed as the maximum. The clustering for operational taxonomic units (OTUs) was conducted with a 97% similarity cut-off by UPARSE (version 7.1 http://drive5.com/uparse/). The significance of the alpha diversity was analysed with DPS 7.5 software with tw o-way ANOVA.

Data availability

Raw sequence data obtained after software processing deposited in NCBI database under accession number of PRJNA983104, PRJNA983108.

Received: 10 September 2024; Accepted: 11 July 2025
Published online: 15 July 2025

References

1. Pellegrini, N. et al. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **133**, 2812–2819. https://doi.org/10.1093/jn/133.9.2812 (2003).
2. Sarabi, B., Hassandokht, M. R., Hassani, M. E. & Ramak, M. T. Evaluation of morphological characteristics of Iranian edible wild asparagus (Asparagus officinalis L). *Iran. J. Hortic. Sci.* **41**, 197–207 (2010). (in Iranian with English abstract).
3. Lee, J. W. et al. Bioactive compounds, antioxidant and binding activities and spear yield of *Asparagus officinalis* L. *Plant. Foods Hum. Nutr.* **69**, 175–181. https://doi.org/10.1007/s11130-014-0418-9 (2014).
4. Statistica, G. Leading Asparagus Producing Countries in 2021 (in Metric Tons). (2023). https://www.statista.com/statistics/279556/global-top-asparagus-producing-countries/. Accessed 06 Jan 2023.
5. Chitrakar, B., Zhang, M. & Adhikari, B. Asparagus (*Asparagus officinalis*): processing effect on nutritional and phytochemical composition of spear and hard-stem by products. *Trends Food Sci. Technol.* **93**, 1–11 (2019).
6. Guan, X. K. et al. Improved straw management practices promote in situ straw decomposition and nutrient release, and increase crop production. *J. Clean. Prod.* **250**, 119514. https://doi.org/10.1016/j.jclepro.2019.119514 (2020).
7. Xu, N., Tan, G. C., Wang, H. Y. & Gai, X. P. Effect of Biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil. Biol.* **74**, 1–8. https://doi.org/10.1016/j.ejsobi.2016.02.004 (2016).
8. Zhang, H. et al. Responses of soil organic carbon and crop yields to 33-year mineral fertilizer and straw additions under different tillage systems. *Soil. Till Res.* **209**, 104943. https://doi.org/10.1016/j.still.2021.104943 (2021).
9. Montgomery, D. R. Soil erosion and agricultural sustainability. *Proc. Natl Acad. Sci.* U. S. A. 104, 13268–13272. https://doi.org/10.1073/pnas.0611508104 (2007).
10. Srinivasan, V., Maheswarappa, H. P. & Lal, R. Long term effects of topsoil depth and amendments on particulate and non-particulate carbon fractions in a Miamian soil of central Ohio. *Soil. Till Res.* **121**, 10–17. https://doi.org/10.1016/j.still.2012.01.014 (2012).
11. Campbell, C. A. et al. Quantifying short-term effects of crop rotations on soil organic carbon in Southwestern Saskatchewan. *Can. J. Soil. Sci.* **80**, 193–202. https://doi.org/10.4141/S99-045 (2000).
12. Beare, M. H., Wilson, P. E., Fraser, P. M. & Butler, R. C. Management effects on barley straw decomposition, nitrogen release, and crop production. *Soil. Sci. Soc. Am. J.* **66**, 84–856. https://doi.org/10.2136/sssaj2002.8480 (2002).
13. Wu, J. S. et al. Effects of rice and wheat straw ditch-buried returns on the soil physical properties of wheat fields. *Acta Ecol. Sin.* **37**, 2066–2075 (2016). (in Chinese with English abstract).
14. Franzluebbers, A. J., Hons, F. M. & Zuberer, D. A. Tillage-induced seasonal changes in soil physical properties affecting CO2 evolution under intensive cropping. *Soil. Till Res.* **34**, 41–60. https://doi.org/10.1016/0167-1987(94)00450-S (1995).
15. Chen, Y., Chang, S. K. C., Chen, J., Zhang, Q. & Yu, H. Characterization of microbial community succession during vermicomposting of medicinal herbal residues. *Bioresour Technol.* **249**, 542–549. https://doi.org/10.1016/j.biortech.2017.10.021 (2018).
16. Cong, P. et al. Changes in soil organic carbon and microbial community under varying straw incorporation strategies. *Soil. Till Res.* **204**, 104735. https://doi.org/10.1016/j.still.2020.104735 (2020).
17. Wang, H. H. et al. Straw incorporation influences soil organic carbon sequestration, greenhouse gas emission, and crop yields in a Chinese rice (Oryza sativa L.)-wheat (*Triticum aestivum* L.) cropping system. *Soil. Till Res.* **195**, 104377. https://doi.org/10.1016/j.still.2019.104377 (2019).
18. Kielak, A., Pijl, A. S., Van Veen, J. A. & Kowalchuk, G. A. Differences in vegetation composition and plant species identity lead to only minor changes in soil-borne microbial communities in a former arable field. *FEMS Microbiol. Ecol.* **63**, 372–382. https://doi.org/10.1111/j.1574-6941.2007.00428.x (2008).
19. Cui, Y. X. et al. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the Northern loess plateau, China. *Soil. Biol. Biochem.* **116**, 11–21. https://doi.org/10.1016/j.soilbio.2017.09.025 (2018).
20. Duan, C. J. et al. Reveal the response of enzyme activities to heavy metals through in situ zymography. *Ecotoxicol. Environ. Saf.* **156**, 106–115. https://doi.org/10.1016/j.ecoenv.2018.03.015 (2018).
21. Vimal, S. R., Singh, J. S., Kumar, A. & Prasad, S. M. Plant genotype-microbiome engineering as nature-based solution (NbS) for regeneration of stressed agriculture: A review. *Sci. Hortic-Amsterdam.* **321**, 112258. https://doi.org/10.1016/j.scienta.2023.112258 (2023).
22. Zhang, B. H., Hong, J. P., Zhang, Q., Jin, D. S. & Gao, C. H. Contrast in soil microbial metabolic functional diversity to fertilization and croprotation under rhizosphere and non-rhizosphere in the coal gangue-land fill reclamation area of loess hills. *PLOS ONE.* **15**, e0229341. https://doi.org/10.1371/journal.pone.0229341 (2020).
23. Zhalnina, K. et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* **3**, 470–480. https://doi.org/10.1038/s41564-018-0129-3 (2018).
24. Zhang, H. & Chu, L. M. Early development of soil microbial communities on rehabilitated quarries. *Restor. Ecol.* **21**, 490–497. https://doi.org/10.1111/j.1526-100X.2012.00917.x (2013).
25. Lou, Y. L. et al. Straw coverage alleviates seasonal variability of the topsoil microbial biomass and activity. *CATENA* **86**, 117–120. https://doi.org/10.1016/j.catena.2011.03.006 (2011).

26. Bai, N. L. et al. Long-term effects of straw return and straw-derived Biochar amendment on bacterial communities in soil aggregates. *Sci. Rep.* **10**, 7891. https://doi.org/10.1038/s41598-020-64857-w (2020).
27. Henriksen, T. M. & Breland, T. A. Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil. *Biol. Fert Soils.* **35**, 41–48. https://doi.org/10.1007/s00374-001-0438-0 (2002).
28. Merilä, P. et al. Soil organic matter quality as a link between microbial community structure and vegetation composition along a successional gradient in a boreal forest. *Appl. Soil. Ecol.* **46** (2), 259–267 (2010).
29. Su, Y. et al. Soil microbial community shifts with long-term of different straw return in wheat-corn rotation system. *Sci. Rep.* **10**, 6360. https://doi.org/10.1038/s41598-020-63409-6 (2020).
30. He, J. Z., Zheng, Y., Chen, C. R., He, Y. Q. & Zhang, L. M. Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches. *J. Soil. Sediments.* **8**, 349–358. https://doi.org/10.1007/s11368-008-0025-1 (2008).
31. Börjesson, G., Menichetti, L., Kirchmann, H. & Kätterer, T. Soil microbial community structure affected by 53 years of nitrogen fertilisation and different organic amendments. *Biol. Fert Soils.* **48**, 245–257. https://doi.org/10.1007/s00374-011-0623-8 (2012).
32. Sun, R. B., Zhang, X. X., Guo, X. S., Wang, D. Z. & Chu, H. Y. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil. Biol. Biochem.* **88**, 9–18. https://doi.org/10.1016/j.soilbio.2015.05.007 (2015).
33. Hanson, C. A., Allison, S. D., Bradford, M. A., Wallenstein, M. D. & Treseder, K. K. Fungal taxa target different carbon sources in forest soil. *Ecosystems* **11**, 1157–1167. https://doi.org/10.1007/s10021-008-9186-4 (2008).
34. Brisson, V. L. et al. Phosphate availability modulates root exudate composition and rhizosphere microbial community in a Teosinte and a modern maize cultivar. *Phytobiomes J.* **6**, 83–94. https://doi.org/10.1094/PBIOMES-06-21-0041-R (2022).
35. Wang, P. et al. Natural variation in root exudation of GABA and DIMBOA impacts the maize root endosphere and rhizosphere microbiomes. *J. Exp. Bot.* **73**, 5052–5066. https://doi.org/10.1093/jxb/erac202 (2022).
36. Govaerts, B. et al. Infiltration, soil moisture, root rot and nematode populations after 12 years of different tillage, residue and crop rotation managements. *Soil. Till Res.* **94**, 209–219. https://doi.org/10.1016/j.still.2006.07.013 (2007).
37. Qi, Y. Z., Zhen, W. C. & Li, H. Y. Allelopathy of decomposed maize straw products on three soil-borne diseases of wheat and the analysis by GC-MS. *J. Integr. Agric.* **14**, 88–97. https://doi.org/10.1016/S2095-3119(14)60795-4 (2015).
38. Samaddar, S., Schmidt, R., Tautges, N. E. & Scow, K. Adding alfalfa to an annual crop rotation shifts the composition and functional responses of tomato rhizosphere microbial communities. *Appl. Soil. Ecol.* **167**, 104102. https://doi.org/10.1016/j.apsoil.2021.104102 (2021).
39. Glawe, D. A. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. *Annu. Rev. Phytopathol.* **46**, 27–51 (2008).
40. Saharan, G. S. & Mehta, N. *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management* (Springer-Verlag GmbH, 2008).

Acknowledgements

This study was funded by the Fundamental Research and Talent Cultivation Foundation of JXAAS (No. JX-SNKYJCRC202406) and the Scientific and Technological Activities of Returned Overseas Fund of MHRSS PRC (201509). Majorbio Bio-Pharm Technology Co. Ltd. kindly provided the protocols and sequencing used in this work.

Author contributions

Y.Y., S.L. Methodology: Y.L., J.Z. Formal analysis: J.Z. Investigation: Y.L. Resources: Y.Y. Data curation: S.L. Writing-original draft: Y.Y. Writing-review & editing: S.L., J.Z. Supervision: S.L. Project administration: S.L. Funding acquisition: Y.Y. All co-authors reviewed the final version and approved the manuscript before submission.

Funding

The Fundamental Research and Talent Cultivation Foundation of JXAAS (JXSNKYJCRC202406), the Scientific and Technological Activities of Returned Overseas Fund of MHRSS PRC (201509).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-11657-9.

Correspondence and requests for materials should be addressed to Y.Y.