

Planting American ginseng (*Panax quinquefolius* L.) caused soil acidification and bacterial and fungal communities changes

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Abstract

Background American ginseng is an important herb crop and is widely cultivated in China. However, continuous cropping obstacles seriously affect the production of American ginseng, and the reason is still unclear and needs more research. We analyzed the soil microbial alpha diversity and community composition as well as soil physicochemical properties, in bulk soils to assess the changes in soil associated with planting American ginseng.

Results The cultivation of American ginseng resulted in a significant decrease in soil pH. The alpha diversity of soil bacteria and fungi was significantly reduced with the increase of American ginseng planting years. Planting American ginseng also largely altered the community and functional composition of soil bacteria and fungi, in particular, increased the relative abundance of the pathogenic fungus *Fusarium*, and readuced the relative abundance of some beneficial microorganisms, such as *KD4-96*, *RB41* and *Sphingomonas*.

Conclusions Soil acidification, reduction of beneficial taxa and accumulation of fungal pathogens, therefore, may lead to the replantation problem of American ginseng.

Introduction

American ginseng (*Panax quinquefolius* L.), is a perennial herb, which has been widely cultivated in China with a total cultivation area of nearly 3,700 hectares [1]. Due to its anti-inflammatory and antioxidant properties, it is considered to have good therapeutic effects in enhancing central nervous system function, protecting cardiovascular system and improving immunity [2–4]. As an important herbal medicine, the global market of American ginseng reached 85 million US dollars in 2018, and it is estimated that it will reach 7,033 tons in 2019 to 2024 [5]. China is the third largest producer of American ginseng, producing 3,600 tons in 2016 [1], and is the largest national consumer of American ginseng.

However, in China, continuous cropping problems have become a major factor restricting the planting area growth of American ginseng [6, 7]. Moreover, it is estimated that successful replanting needs a crop rotation cycle of at least 30 years [8], which means the land suitable for American ginseng cultivation in China is rapidly decreasing [1].

American ginseng is a perennial herb that grows slowly and usually needs for more than 4 years to achieve optimal medicinal value [9]. However, long-term monoculture can cause obstacles to continuous cropping, mainly manifested as the increase of soil-borne diseases and the significant reduction of crop yield [10]. Continuous cropping obstacle is a common but difficult problem in agriculture, which affect many crops, such as apple, strawberry, alfalfa and grapevine and result in severe losses of crop yield [10–12]. Replanting problems is generally believed to be caused by multiple factors, including the changes of soil properties, autotoxicity, outbreak of soil diseases, and changes in soil microbial communities [6, 13, 14].

Many researches have been conducted to explain the problems that lead to the replanting problem of American ginseng, most of which have focused on allelopathy/autotoxicity, soil nutrient element depletion or soil sickness [15, 16]. For examples, it was found that 9 phenolic compounds identified from fibrous roots of American ginseng significantly reduced the growth of seedlings [17]; in another similar study, XB Bi, JX Yang and W-W Gao [15], has reported 9 phenolic compounds that can inhibited the radicle and shoot growth of American ginseng.

However, soil is a complex and dynamic ecosystem, and can be considered as a microbial seed bank [18, 19]. Soil microbial communities, involved in almost all biogeochemical cycles of terrestrial ecosystems, play an important role in agricultural production, and their biodiversity including species richness, evenness, community composition and functional structure [20, 21], are irreplaceable in maintaining a variety of ecosystem functions and services, such as nutrient cycling, vegetation primary productivity and litter decomposition [22–26].

Therefore, in this study, from the perspective of soil microbial communities, we examined the changes in the alpha diversity of fungi and bacteria, the bacterial and fungal community and functional composition in the soil from American ginseng farmland, where American ginseng has planted for different years (from 0 year to 4 years). This study aimed to find the main factors associated with replanting problems and provided theoretical basis for finding new strategies to solve continuous cropping obstacles.

Materials And Methods

Experimental design and sample collection

In November 2020, the soil samples were collected in Wendeng District, Weihai City, Shandong Province (121°43'E-122°19'E, 36°52'N -37°22' N), one of the main planting areas for American ginseng in China. One crop season of American ginseng usually needs 4 years. Soil samples were random collected from 15 sites in the research area, where American ginseng had been planted for 0–4 years respectively (Y0, Y1, Y2, Y3 and Y4). Y0 were set as the control, where American ginseng had never been planted. There were three replicates for each growth year, resulting in a total of 15 samples. Using five-point sampling method, five soil cores (20 cm deep, 5 cm diameter) were collected from each site by a soil auger. Soil from the five cores were mixed and sieved through a 2 mm mesh.

Soil pH was measured in 1:2.5 (soil: water) water suspensions by a pH meter (310P-02A, Thermo Fisher Scientific, America). The flame photometric method was used to determine soil available potassium (M410, Sherwood Scientific Ltd., Cambridge, UK). Soil inorganic N (NH_4^+ and NO_3^-) was extracted from 10 g (wet mass) fresh soil with 50 ml of 2 mol/L KCl, and then determined using an auto flow injection analyzer (AA3, Bran + Luebbe, Germany).

Soil DNA extraction, PCR amplification and sequencing

For each soil sample, soil microbial genomic DNA was extracted from 0.5 g of mixed soil using the MP FastDNA SPIN Kit for soil according to the manufacturer's instructions (MP Biochemicals LLC, Solon, OH, USA). We quantified the quality and concentration of the extracted through the NanoDrop ND-2000 Spectrophotometer DNA based on 260/230 nm and 260/280 nm absorbance ratios (NanoDrop Technologies Inc., Wilmington, DE, USA). The amplicon method of the 16S and ITS rRNA was used to measured the soil bacterial and fungal diversity and composition. The primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were implemented to amplify the V4 hypervariable regions of the 16S rRNA. The ITS1 region of the fungal rRNA were amplified using primers 2043R (5'-GCTGCGTTCTTCATCGATGC-3') and ITS1F (5'-GTGCCAGCMGCCGCGG-3'). All the PCR reactions were performed in 30 µL reactions containing 0.2 µM of forward and reverse primers, 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs) and about 10 ng of template DNA. The PCR conditions used were as follows: 98 °C for 1 min, 30 cycles of denaturation at 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 30 s, finally extension 72 °C for 5 min. The amplicons were mixed together with the equal volume of loading buffer and detected in a 2% agarose gel by electrophoresis. Equimolar amounts of amplicons were then purified by a Gel Extraction Kit (Thermo Scientific). The Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA) was used for generating the sequencing libraries. The Agilent Bioanalyzer 2100 system and Qubit® 2.0 Fluorometer (Thermo Scientific) were used to assessed the library quality. finally, the library was sequenced through an Illumina HiSeq platform at Shanghai Personalbio Technology Co. LTD, Shanghai, China and 250 bp paired-end reads were obtained.

Sequence Data Processing and Statistical Analysis

The paired-end reads were merged and quality filtered by FLASH (V1.2.7) [27] and QIIME (v1.7.0) [28]. The chimeric sequences were removed using USEARCH software [29]. After removing the chimeric sequences, the OTUs (Operational Taxonomic Units) were clustered at 97% similarity by Uparse (v7.0.1001) [30]. The Silva database [31] for bacteria and Unite database [32] for fungi were used to assign each OTU to taxonomic group.. The sequence numbers of the sample with lowest number of reads (18742 reads for bacteria and 35051 reads for fungi) were used to rarify each sample. The QIIME(V1.7.0) [28] were used to calumniate the alpha-diversity indexes (OTU richness, Shannon diversity and the Chao1 index).

Metabolic potential of functional genes were predicted by the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST2). The 16S rRNA and ITS amplicon sequencing data were against to MetaCyc Metabolic Pathway Database (<https://metacyc.org/>) with known metabolic functions to obtain the prediction of bacterial and fungal metabolic function.

General linear or quadratic model regressions were performed to evaluate the relationship between the the growth years of American ginseng and the soil physicochemical properties (soil pH, soil available potassium, soil inorganic N), soil microbial alpha diversity indices, the relative abundance of dominant (Top 10) bacterial phyla and fungal classes, as well as the relative abundance of bacterial and fungal functional guilds. These data analyses were performed with OriginPro (v 9.0, Massachusetts, USA). The

effects of growth years (Y) on the bacterial and fungal community composition were tested using PERMANOVA (Permutational Multivariate Analysis of Variance) with 999 permutations. To show the relative difference of bacterial and fungal communities and functional composition in different years, we used the NMDS (Non-metric Multidimensional Scaling) plots based on the Bray-Curtis similarity matrix. The RDA (Redundancy Analysis) was further performed to test the effects of soil physicochemical properties on bacterial and fungal communities and functional composition. These statistical analyses were performed in R (V3.5.1; R Core Team. 2013) with vegan package [33]

Results

Soil physicochemical properties

The cultivation of American ginseng resulted in a significant decrease in soil pH. The soil pH was negatively correlated with the growth years of American ginseng ($R^2 = 0.90$; $P < 0.001$; Fig. 1a). In the control fields without American ginseng planting, the soil pH was about 7.46, however, after four years, it dropped to about 4.83. On the other hand, the content of soil available potassium showed positively correlated with the American ginseng growth years and increased with the years of American ginseng cultivated ($R^2 = 0.49$; $P < 0.01$; Fig. 1b). There was no significant relationship between soil inorganic N and American ginseng growth years (Fig. 1c).

Bacterial and fungal alpha diversity

Planting American ginseng had a strong effect on soil microbial alpha diversity (including OTU richness, Shannon index and Chao1 estimator). For bacterial communities, the alpha diversity index showed an obvious linear correlation with the growth years of American ginseng. The OTU richness ($R^2 = 0.79$; $P < 0.001$; Fig. 2a), Shannon index ($R^2 = 0.83$; $P < 0.001$; Fig. 2b) and Chao1 estimator ($R^2 = 0.61$; $P < 0.001$; Fig. 2c) of soil bacteria significantly decreased with the increase of the growth years of American ginseng. Similarly, the OTU richness ($R^2 = 0.51$; $P < 0.01$; Fig. 2d), Shannon index ($R^2 = 0.58$; $P < 0.001$; Fig. 2e) and Chao1 estimator ($R^2 = 0.52$; $P < 0.01$; Fig. 2f) declined with the growth years of American ginseng.

Furthermore, results from a linear regression analysis revealed a remarkable positive correlation between soil pH and the alpha diversity of bacteria and fungi, while soil available potassium was negatively correlated with the alpha diversity of bacteria and fungi (except the Shannon index of fungi). There were no significant correlation between soil inorganic N and the alpha diversity of bacteria and fungi (Table 1).

Table 1

Relationship between soil pH, soil available potassium, soil inorganic N and alpha diversity indices from a linear regression analysis.

		Soil pH	Soil available potassium	Soil inorganic N
Bacteria	OTU Richness	0.78***	-0.72**	-0.16
	Shannon	0.85***	-0.83***	-0.24
	Chao1	0.65**	-0.65*	-0.08
Fungi	OTU Richness	0.64**	-0.56*	0.05
	Shannon	0.67**	-0.49	-0.01
	Chao1	0.65**	-0.57*	0.05
The correlation coefficients (Pearson's r) are presented with the level of significance. *P < 0.05; **P < 0.01; ***P < 0.001.				

Bacterial and fungal communities and functional composition

The NMDS plot revealed that the distribution of bacterial and fungal communities and functional composition was clear separated by years of American ginseng cultivation (Y). The multivariate permutational analysis of variance (PERMANOVA) results also showed that the bacterial and fungal communities and functional composition varied significantly among different planting years and the years of American ginseng cultivation had a high explanation. (Fig. 3a-d). These results implied that planting American ginseng significantly changed the community and functional composition of soil bacteria and fungi.

Further analysis at different taxa classification levels found that, at the bacterial phyla level, the top 10 dominated phyla were Proteobacteria (23.4%-25.3% of total relative abundance), Actinobacteria (21.6%-23.7%), Acidobacteria (13.4%-20.1%), Chloroflexi (9.0%-16.8%), Firmicutes (3.3%-6.6%), Gemmatimonadetes (3.0%-4.8%), Bacteroidetes (2.3%-4.2%), Patescibacteria (1.0%-1.4%), Rokubacteria (0.1%-2.1%) and Verrucomicrobia (0.6%-1.1%) (Fig. 4a). For fungi, the most abundant sequences were dominated by the phylum Ascomycota (48.3%-73.8%), while the phyla Mortierellomycota and Basidiomycota represented only 1.9%-30% and 7.1%-14% reads, respectively (Fig. 4b).

At the genus level, a linear regression analysis was used to calculate the relationship between the relative abundance of dominant bacterial and fungal genus (mean relative abundance > 1%) and American ginseng growth years. The results revealed that relative abundance of bacterial *Aquabacterium* and *Acidotherrmus* increased with the American ginseng growth years, while the relative abundance of *KD4-96*, *RB41*, *Rokubacteriales* and *Sphingomonas* decreased (Fig. 5). At fungal genus level, the relative abundance of *Mortierella* and *Fusarium* showed positive relationship with the years of American ginseng

planted, and the relative abundance of *Pseudaleuria*, *Lophotrichus* and *Saitozyma* showed negative relationship with the years of American ginseng planted (Fig. 6).

The RDA results revealed that soil pH and available potassium observably affected soil bacterial community and functional composition. For both bacteria and fungi, soil pH, with highest explanations, was the main factor to influence their community composition and function (Table 2).

Table 2
Results from the RDA (Redundancy analysis) of soil bacterial and fungal community composition and functional composition with soil properties.

	Soil properties	Explains (%)	P
Bacterial community composition	pH	21.9	< 0.01
	Available potassium	13.8	< 0.01
	Inorganic N	6.4	0.684
Bacterial functional composition	pH	28.7	< 0.01
	Available potassium	17.9	< 0.01
	Inorganic N	9.2	0.216
Fungal community composition	pH	12.4	< 0.01
	Available potassium	9.8	< 0.05
	Inorganic N	7.8	0.028
Fungal functional composition	pH	41.2	< 0.01
	Available potassium	22.0	< 0.05
	Inorganic N	2.1	0.950

Discussion

Our results indicate that planting American ginseng had strong negative influences on bacterial and fungal alpha diversity, and the longer the planting years, the more diversity decreased. The main reason may be that the cultivation of American ginseng led to soil acidification, and the microbial communities were sensitive to soil pH changes. It is well-known that soil pH is the main factor affecting the diversity of soil microbial communities in different ecosystems [34–37]. The effects of soil pH on soil microorganisms can be divided into direct effects and indirect effects. Soil pH can directly affect soil microbial communities cause most soil microbial groups have an optimal pH range for growth, and if soil pH exceeds this range, microbial growth will be limited and therefore their diversity will be reduced. [36, 38]. The indirect effects mean that soil acidification can adversely affect the growth of soil bacterial communities by increasing the solubility of some toxic metals (like Al, Fe, and Cu) in soil, [35, 39]. Since

the bacteria were more sensitive to pH changes, the diversity of bacterial community decreased significantly when soil pH decreased after the planting of American ginseng. Consequently, planting American ginseng by decreasing soil pH reduced the soil bacterial and fungal alpha diversity.

In this research, planting American ginseng distinctly altered the soil bacterial and fungal community composition and functional composition, which may be an important reason for continuous cropping obstacle. For bacterial communities, our results revealed that the relative abundance of *Acidothermus* largely increased with the American ginseng growth years. This genus is acidophilic, as studies have found, for example, that a single species named *A. cellulolyticus*, is thermophilic, acidophilic, and capable of producing many thermostable cellulolytic enzymes [40, 41]. In this study, *Acidothermus* could gradually become the dominant genus because the cultivation of American ginseng led to soil acidification, which made these acidophilus bacteria in an advantageous position in competition with other bacteria.

On the other side, the relative abundance of *KD4-96*, *RB41*, *Rokubacteriales* and *Sphingomonas* decreased with the American ginseng growth years, however, most of them are beneficial microbe for soil health. For example, *RB41* plays an important role in maintaining soil biogeochemical cycling under long-term high pollution stress [42, 43]. Study on the rhizosphere soil of *Fritillaria ussuriensis* with different health levels, N Jiao, XS Song, RQ Song, DC Yin and X Deng [44] found that the *RB41* genus in the healthy soil had the highest relative abundance. *Sphingomonas* from Proteobacteria can degrade some toxic substances in soil, such as diphenylether, atrazine and phenylurea herbicide isoproturon, and promote crop resistance to a variety of pathogens [1, 45]. Furthermore, it was found that *KD4-96* was a kind of heavy metal-resistant bacterium, which played a key role in the biotransformation process of heavy metals [46, 47].

Planting American ginseng also largely changed the community structure and functional composition of soil fungi, especially increased the relative abundance of pathogenic fungi, like *Fusarium*. In this studies, cultivation of American ginseng accumulated the *Fusarium*, a plant damaging pathogen. *Fusarium* can infect many plant species and causing root rot and wilt diseases [48]. In an earlier study from the diseased roots of American ginseng, ZK Punja [49] isolated *Fusarium solani*, *F. oxysporum*, *F. avenaceum*, and *F. equiseti*. Later, W Bi, J Chen, X Jiao and W Gao [50] further confirmed that *F. solani* and *F. oxysporum* were the main pathogens of root rot.

In addition, *Mortierella* was the most abundant fungal genus in this research. Previous studies have confirmed that *Mortierella* can be isolated from the roots of red-skin Asian ginseng (*P. ginseng*) [51]. Our results suggest that the relative abundance of *Mortierella* increases with the years of American ginseng cultivation, and that even reached more than 30% in the fourth year of planting ginseng. However, the causal relationship between *Mortierella* and ginseng red-skin is still uncertain. Therefore, future studies should focus on whether the increase in the abundance of *Mortierella* is one of the reasons for the continuous cropping obstacle of American ginseng.

Conclusion

In this research, the alpha diversity of bacteria and fungi decreased with the increase of American ginseng planting years. American ginseng culture also significantly altered the community and functional composition of soil bacteria and fungi. Particularly, the relative abundance of some beneficial soil microbes, such as *KD4-96*, *RB41* and *Sphingomonas*, gradually decreased with the increase of American ginseng planting years. On the other hand, after planting American ginseng, the relative abundance of some pathogenic fungi, such as *Fusarium* significantly increased. In addition, the cultivation of American ginseng resulted in soil acidification, which was significantly associated with the decrease of soil microbial diversity and changes in community and functional composition after American ginseng cultivation, suggesting that soil pH was a key environmental factor affecting soil microbial communities in American ginseng cultivation farmland. Therefore, we speculated that the reason for continuous cropping obstacle should be that the cultivation of American ginseng caused soil acidification, which reduced the diversity of soil microbial communities and the selection of pathogens was more inclined to choose instead of antagonistic strains, which ultimately led to replanting problems; and in terms of evolutionary selection, pathogens were more likely to be selected than antagonistic strains. In summary, our results indicated that soil acidification and the changes of bacterial and fungal communities maybe the main reason caused replanting problems of American ginseng. This study contributes to an improved understanding of the replanting problems of American ginseng and provide a theoretical basis for soil improvement after the harvest of American ginseng.

Declarations

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Availability of data and materials

The data presented in this study are available upon request from the corresponding author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

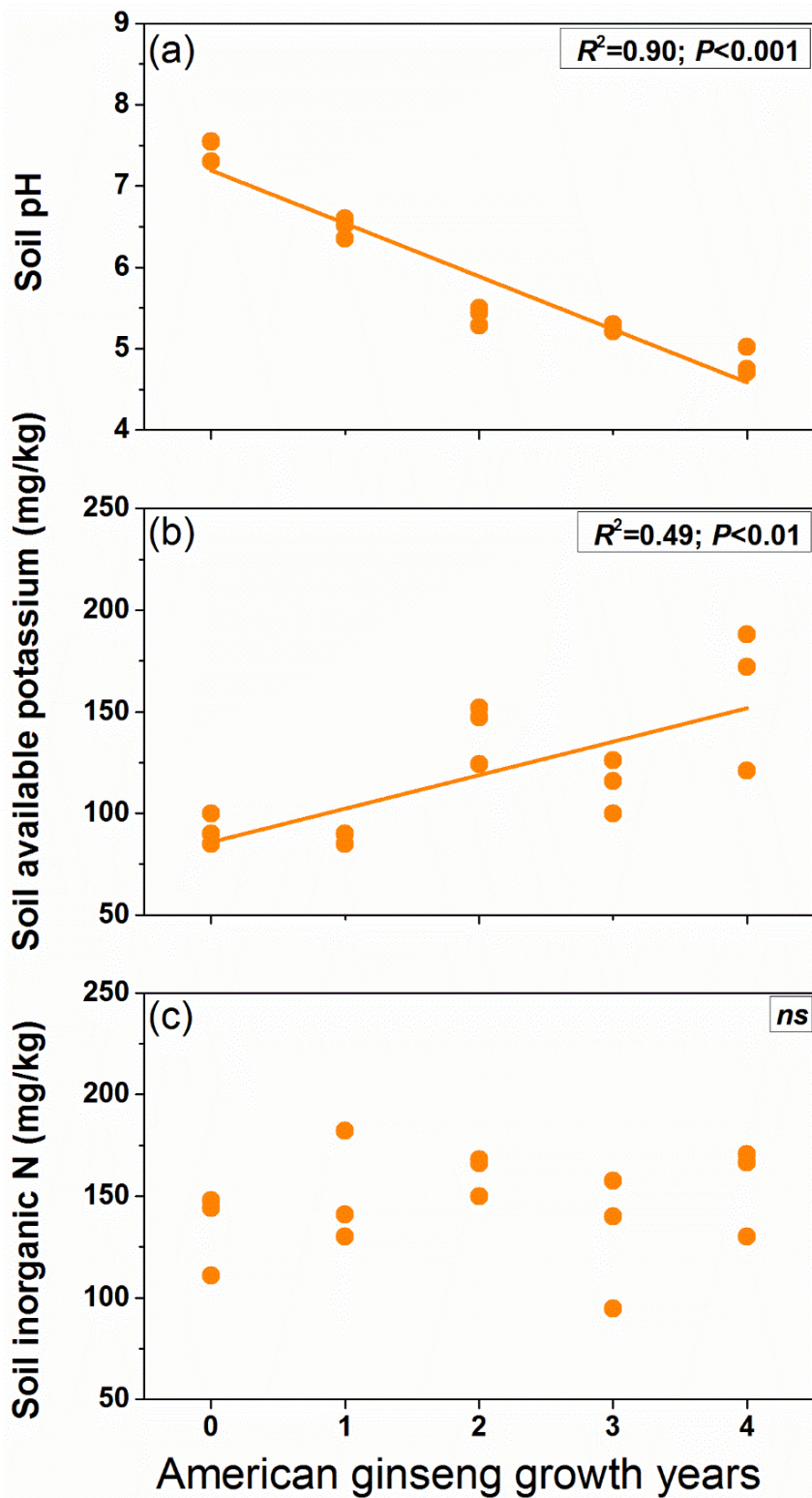


Figure 1

The relationship between Soil properties and American ginseng growth years. For each response variable, regression was estimated using a linear model with years of American ginseng cultivation as a continuous predictor. R^2 is coefficient of determination; P is significance of the regression, *ns* means not significant.

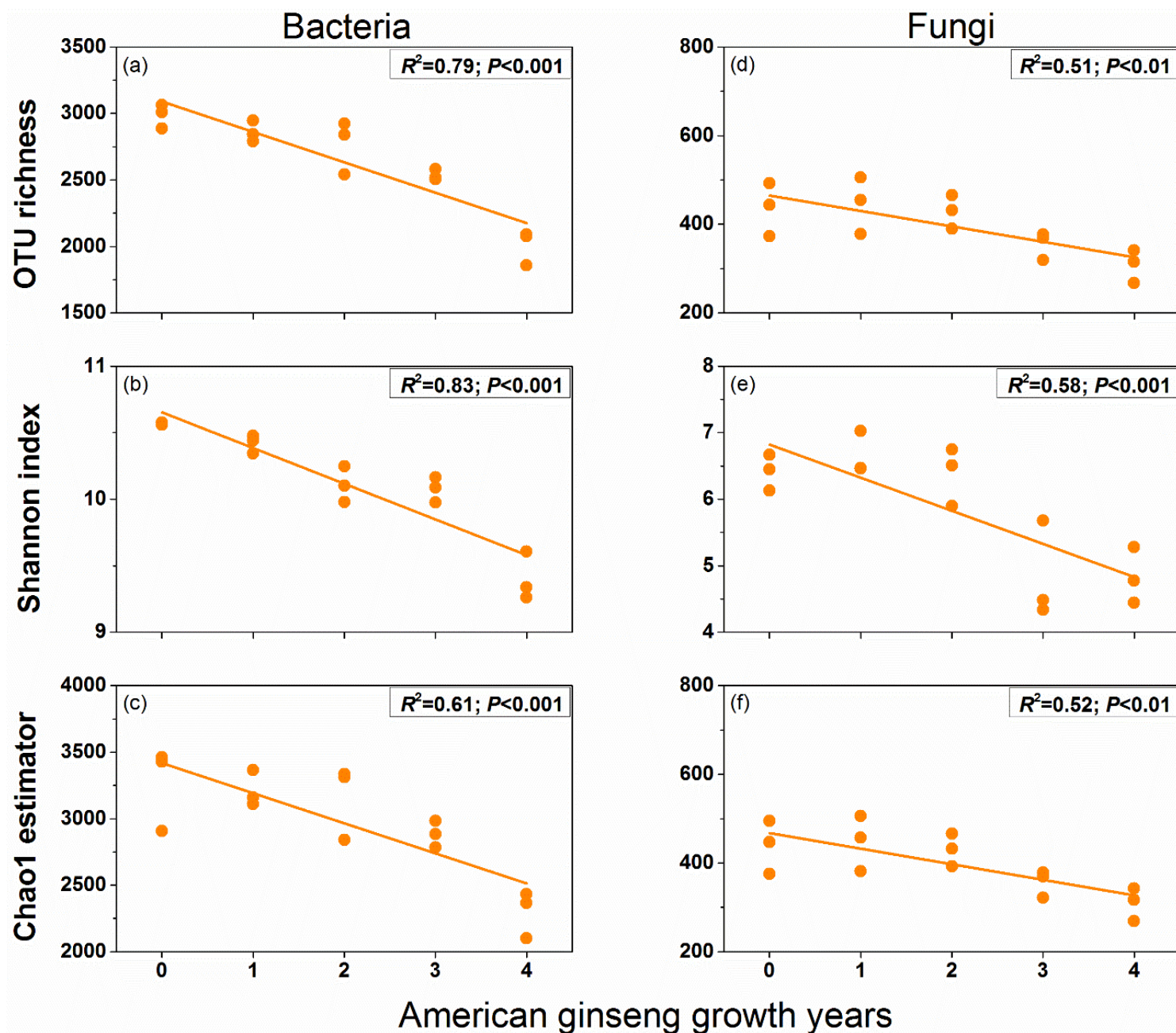


Figure 2

The relationship between soil bacterial and fungal alpha diversity indices and American ginseng growth years. For each response variable, regression was estimated using a linear model with years of American ginseng cultivation as a continuous predictor. R^2 is coefficient of determination; P is significance of the regression.

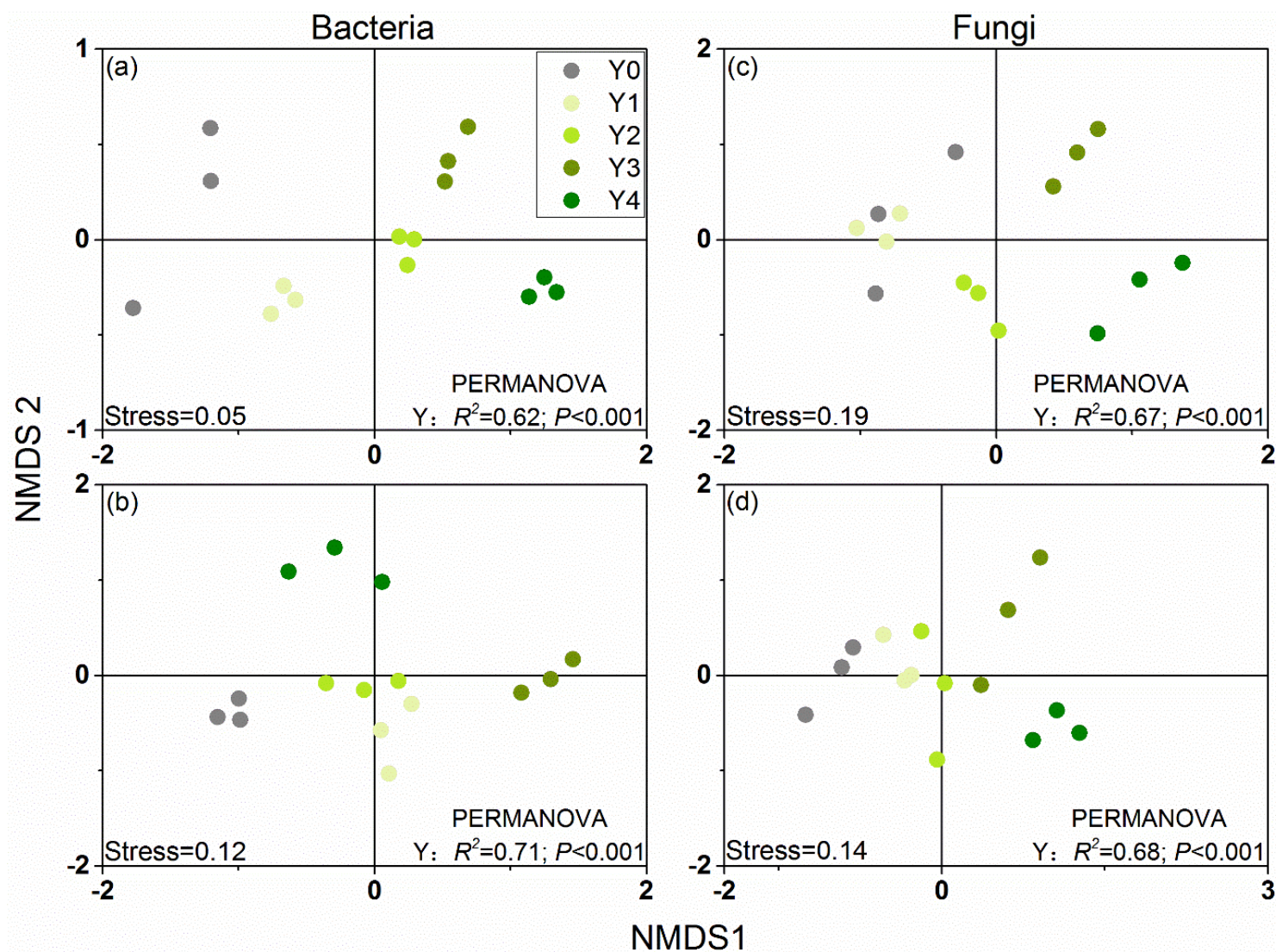


Figure 3

Nonmetric multidimensional scaling (NMDS) ordination of soil bacterial community composition (a), functional composition (b), fungal community composition (c) and functional composition (d). Results (R^2 and P -value) of multivariate permutational analysis of variance (PERMANOVA) on the effects of years of American ginseng cultivation (Y) also shown.

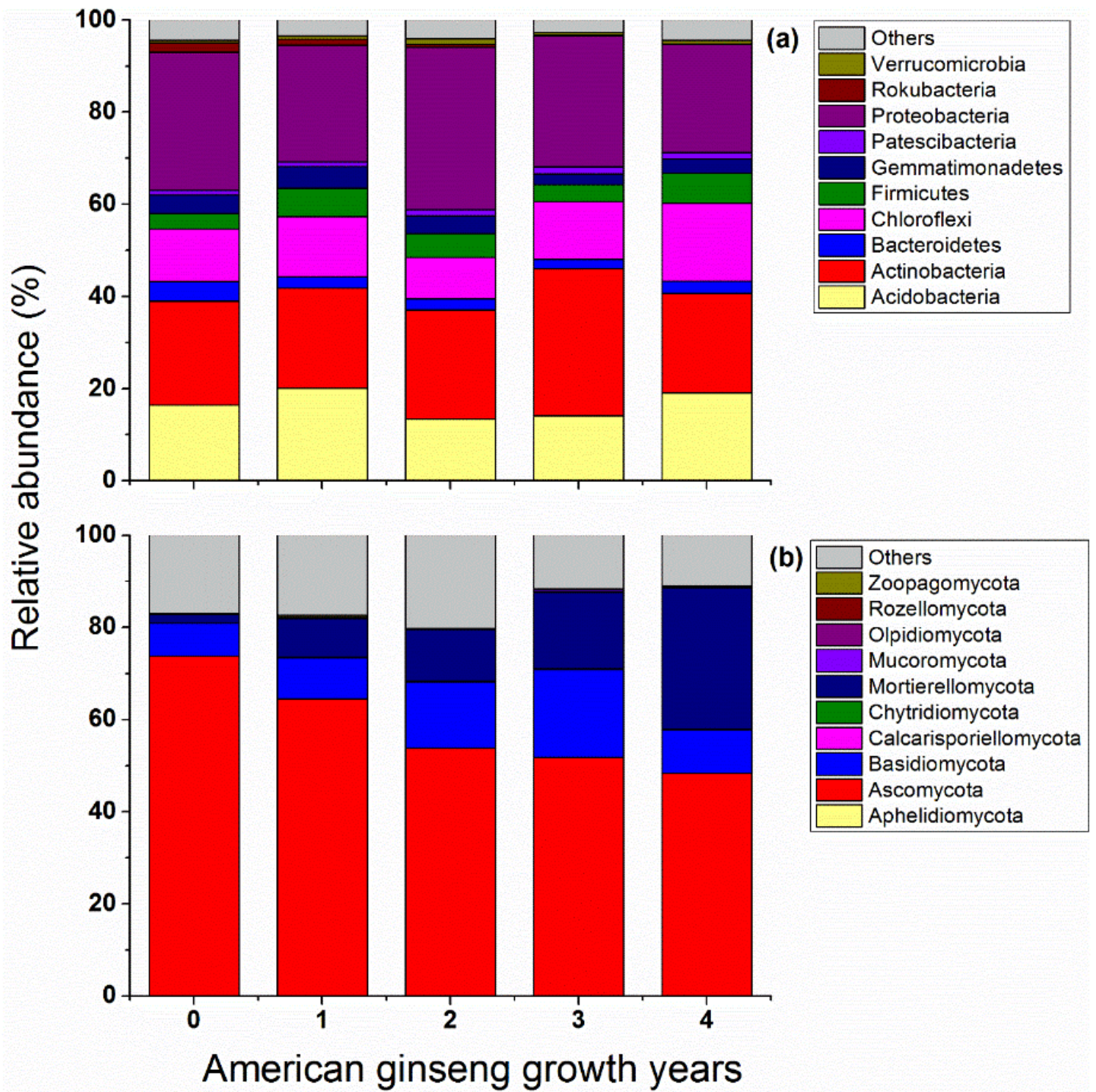


Figure 4

The relative abundance of dominant (top 10) bacterial phyla (a) and fungal phyla (b) in different American ginseng growth years.

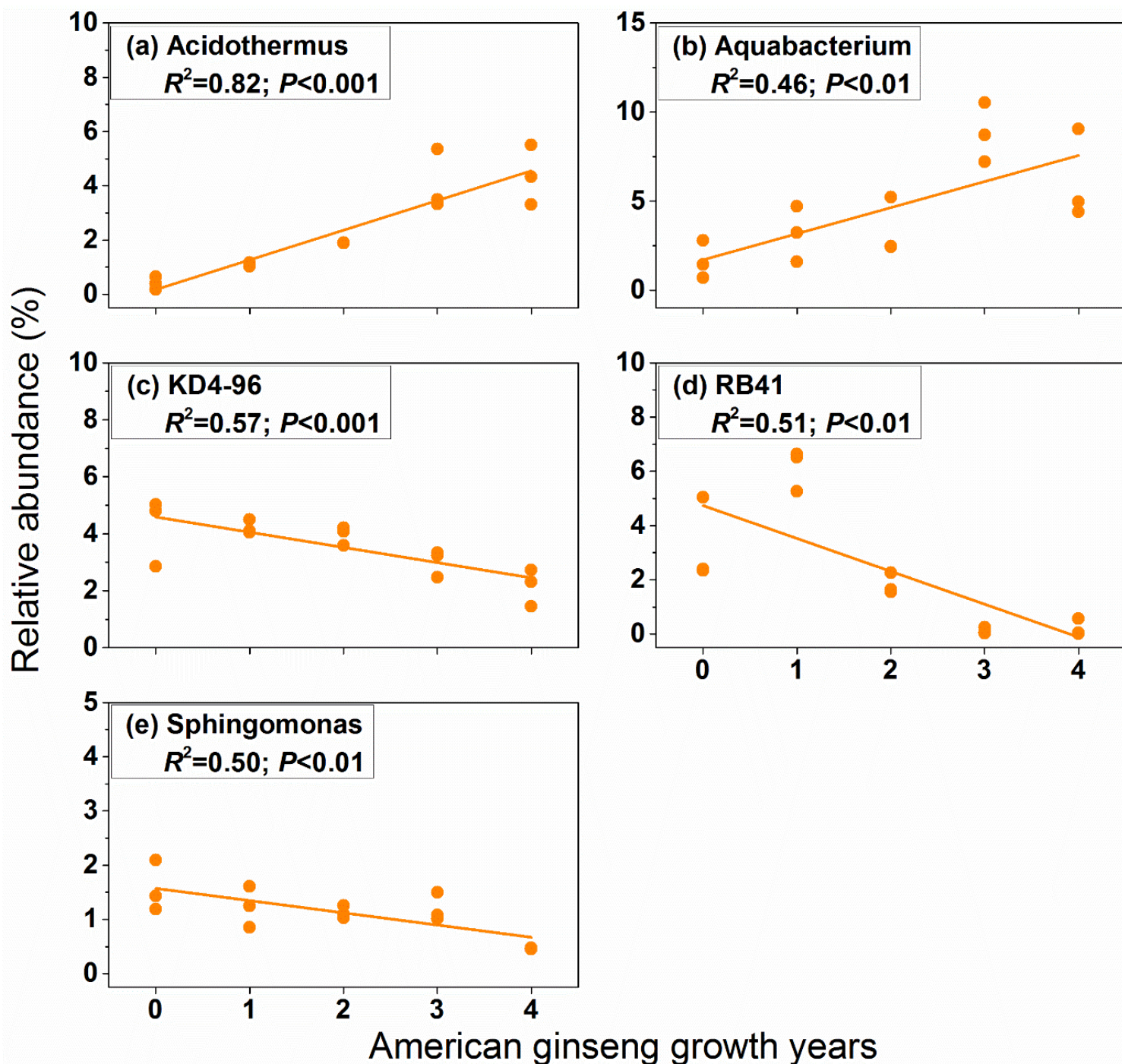


Figure 5

The relationship between the relative abundance of dominant bacterial genus (mean relative abundance >1%) and American ginseng growth years. For each response variable, regression was estimated using a linear model with years of American ginseng cultivation as a continuous predictor. R^2 is coefficient of determination; P is significance of the regression. Only these phyla significantly differed in different sampling times are shown.

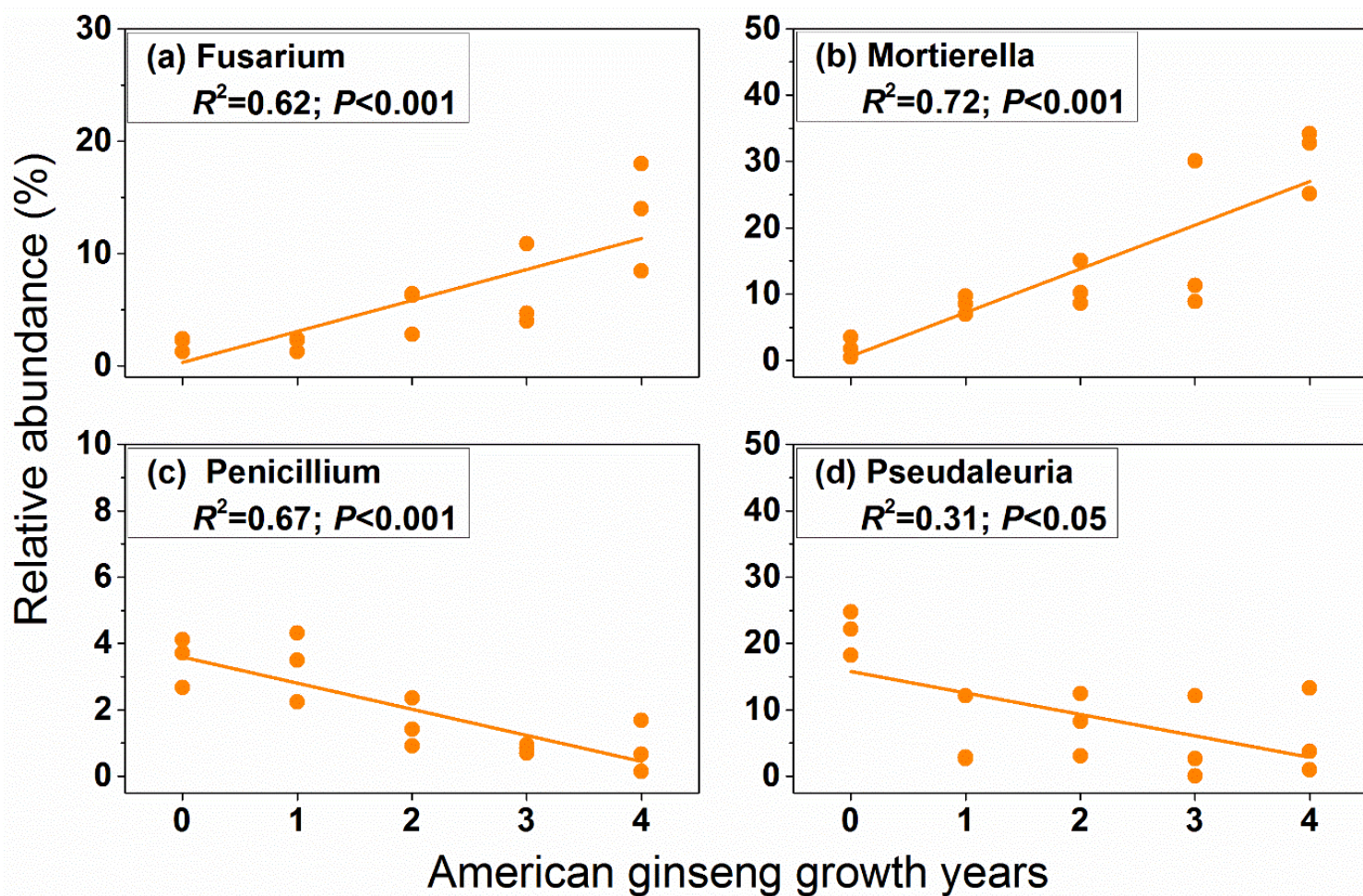


Figure 6

The relationship between the relative abundance of dominant fungal genus (mean relative abundance >1%) and American ginseng growth years. For each response variable, regression was estimated using a linear model with years of American ginseng cultivation as a continuous predictor. R^2 is coefficient of determination; P is significance of the regression. Only these phyla significantly differed in different sampling times are shown.