

# SOIL ORGANISM DIVERSITY AND FUNCTIONS IN PLASTIC SHED AND OPEN FIELD SOILS UNDER DIFFERENT CULTIVATION METHODS

TAO, J.<sup>1,3\*</sup> – CHEN, J.<sup>2</sup> – DU, S. Y.<sup>1,3</sup> – CHEN, X.<sup>1,3</sup> – LI, S. Y.<sup>1,3</sup> – ZHOU, J.<sup>1,3</sup>

<sup>1</sup>*Key Laboratory of Environmental Changes and Natural Disaster, Ministry of Education, Beijing Normal University, Beijing 100875, China*

<sup>2</sup>*Key Laboratory for Ecology and Pollution Control of Coastal Wetlands (Environmental Protection Department of Jiangsu Province), School of Environmental Science and Engineering, Yancheng Institute of Technology, Yancheng 224051, China*

<sup>3</sup>*Faculty of Geographical Science, Beijing Normal University, Beijing 100875, China*

*\*Corresponding author*

*e-mail: juntao@bnu.edu.cn; phone: +86-10-588-00336*

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**Abstract.** The aim of this study was to investigate the influence of four different cropping management practices in plastic shed soils and open-field soils under organic cultivation or conventional cultivation on soil biodiversity and functions. Soils under plastic sheds and open-field were sampled in Beijing, China, in areas where organic cultivation had been conducted for 5 years and conventional cultivation had also been employed. The results showed that plastic shed production resulted in lower soil bacterial richness and diversity than open-field but higher soil mesofaunal Shannon diversity and Pielou's species evenness indexes. Plastic shed production decreased soil urease and phosphate activities compared to open-field under conventional cultivation. Organic cultivation resulted in higher soil urease, phosphatase and catalase activities than conventional cultivation except for soil urease activity in open soil. Plastic shed soils exhibited significantly higher soil electric conductivity (EC) and NO<sub>3</sub>-N contents than open-field soils only under conventional cultivation. By contrast, organic cultivation decreased soil EC and nitrate contents compared to conventional cultivation in plastic shed soils. Organic cultivation resulted in higher soil bacterial OTU, Shannon diversity, Chao1 and ACE indexes than conventional cultivation, but significant differences were observed in the open-field soils.

**Keywords:** *soil bacterial, soil mesofaunal, enzyme activity, cultivation practices, plastic shed production*

## Introduction

Rapid economic development and increasing living standards in China have encouraged the rapid development of plastic shed production. Consequently, the land area used for plastic shed production in China has rapidly increased in recent years. The total area of vegetable crops planted under plastic shed production in China is estimated to reach 3.70 million ha (Luo et al., 2020), and China has become the country that produces the most vegetable crops via plastic shed production worldwide (Duffy, 2017). Moreover, most of the newly developed plastic shed production land has been converted from croplands (Min et al., 2011). However, increasing concerns have been raised regarding soil degradation after plastic shed production. Plastic shed production systems are usually characterized by controlled environmental conditions and can strongly alter soil environmental conditions such as soil temperature and moisture. The higher evapotranspiration observed in plastic shed soils compared with open-field soils can also influence the distribution of the soil solution in the soil profile (Ge et al., 2010). For

example, soil salinity is significantly higher in plastic shed its than in open-field its (Ju et al., 2007), which potentially affects soil biodiversity and functions in plastic shed its (Paoletti, 1999; Jiang et al., 2015). Soil microorganisms and fauna are an integral part of the soil ecosystem due to their high biodiversity (Van Straalen, 1998; Nannipieri et al., 2003; Andre et al., 2002). They play vital roles in maintaining soil functions such as nutrient cycling, organic matter decomposition, bioremediation and plant growth (Berg, 2009) that are critical for soil sustainability and health (Bhat, 2013; Goldford et al., 2018). Although those affecting these organisms are a relevant concern in plastic shed production, they are still poorly studied, especially regarding the effects on soil biodiversity and function.

In addition, to obtain a higher vegetable crop yield, large amounts of water, chemical fertilization and insecticides are applied in plastic shed production. These conventional agricultural practices may not only increase salt accumulation (Liu et al., 2005), soil acidification, nutrient imbalances and environmental pollution but also alter soil biodiversity and functions (Coolon et al., 2013; Bender et al., 2016). For instance, insecticide application may directly promote changes in population biodiversity and dynamics by killing components of the soil microbial and faunal community (Giller et al., 1997). As a consequence of the decline in biodiversity, increasing concern has arisen about the sustainability of farming practices (Hole et al., 2005). Organic cultivation is considered an important tool for combatting the negative effects of conventional methods in which the inputs are substituted to meet regulations (Geiger et al., 2010; Goldberger, 2011). Many researchers have reported that organic cultivation practices have positive effects on soil microbial populations, diversity and activities (Clark et al., 1998; Girvan et al., 2004; Ponce et al., 2011). A study by Mäder et al. (2002) showed that organically cultivated soils exhibit greater faunal diversity than conventionally cultivated soils. Two well-known meta-analyses conducted by Hole et al. (2005) and Bengtsson et al. (2005) demonstrated the benefits of organic cultivation on soil fauna communities. Soil enzyme activities in soils under organic cultivation were shown to be higher than those under conventional cultivation. Although the majority of research has shown increased soil organism diversity in soils from organic cultivation systems compared to those from conventional cultivation, some studies have obtained different results. A study of Shannon et al. (2002) showed that the differences in the microbial communities of soils under organic and conventional cultivation were subtle rather than dramatic. Some studies have found that organic field soils exhibit lower arthropod diversity than conventional field soils (Shah et al., 2003; Ponce et al., 2011). The inconsistent results between studies suggest that the benefits of organic cultivation for soil biodiversity may vary according to factors such as management systems, climate and crop type (Hole et al., 2005). For example, organic cultivation in plastic shed soils exhibited fewer examples of specific studies than in open-field soils. Soil biodiversity, including that of both microorganisms and fauna, has been less well studied under organic cultivation in plastic shed fields (Madzaric et al., 2018), and there are a lack of studies concerning soil enzyme activity.

In this study, we characterized soil bacterial and mesofaunal diversity and measured the activities of soil extracellular enzymes and soil properties in plastic shed and open-field soils under organic and conventional cultivation. We hypothesized that (1) plastic shed production would decreased the diversity of soil organisms and enzyme activities compared with open-field under conventional cultivation, but the negative effects may be alleviated by organic cultivation, and (2) organic cultivation increases soil

biodiversity and enzyme activities compared to conventional cultivation, but plastic shed production may reduce these positive effects. Furthermore, this investigation could help farmers to improve their practices and enable stakeholders to develop future strategies for soil organism diversity and functioning and sustainable agriculture by saving inputs and preventing environmental damage.

## Methods and material

### Soil sampling

The study was conducted at Horticultural Farms located at two sites in Shunyi, Beijing, China, in March 2016. The climate in the region is a warm temperate subhumid climate, with 80% of annual precipitation (610 mm) falling from June to August. The mean annual air temperature is 11.5 °C, and the total annual sunshine (hours) is 2750 h.

The soils were sampled when the final harvest of the crops was performed to avoid the effects of direct fertilization during the next growing season. The fields managed under an organic farming approach were located in Beiwu County (40° 04' N, 116° 49' E). Crops have been grown under greenhouse conditions at these sites, typically in plastic sheds and open fields. All of these fields were located near each other within a continuous field area of approximately 11 ha. They were conventionally cultivated for several years before being converted to an organic system in 2010. On the organic horticultural farm, no chemical fertilizers or pesticides were used. The fields managed under the conventional farming approach were located in Lisui County (40° 05' N, 116° 45' E). These fields have been conventionally cultivated for more than 6 years. Crops in these areas have been grown under greenhouse conditions, typically in plastic sheds and in open-field soils. Further details of the major crops that were grown and fertilizer applied to the soils in the various fields are provided in *Table 1*.

**Table 1.** Characteristics of the land management practices investigated in this study

Treatments	Major crop	Fertilizer	Other farming practices
Plastic shed field + organic cultivation	Rotation: <i>Solanum lycopersicum</i> , <i>Capsicum annuum</i> , <i>Solanum integrifolium</i>	Organic fertilizer included: Total N 1.94 g/kg, Total P 0.58 g/kg, Total K 0.84 g/kg. Organic matter 182.45 g/kg; Application amount: 10000-12000 kg/ha	Soil disinfestation: irrigated and covered with plastic film lasted for a period of four weeks in July every year (Huang et al., 2019); Pollination method: bee-pollinated (drone density: 1200 individual/ha); Pest control: stick insect net in yellow; Weed control: manual weeding
Open field + organic cultivation	Corn	Organic fertilizer was same as above; Application amount: 5000-6000 kg/ha	Soil disinfestation: no; Pollination method: Natural pollination; Pest control: no; Weed control: manual weeding
Plastic shed field + conventional cultivation	<b>Rotation:</b> <i>Solanum lycopersicum</i> , <i>Capsicum annuum</i> , <i>Solanum integrifolium</i>	Chemical fertilizer was composed of diammonium phosphate and compound fertilizer (6:1), and included: total nutrients ≥ 680 g/kg, N 180 g/kg, P <sub>2</sub> O <sub>5</sub> 460 g/kg, K <sub>2</sub> O 70 g/kg; Application amount of basic fertilizer: 450 kg/ha; Application amount of supplement fertilizer: 360 kg/ha	Soil disinfestation: no Pollination method: hand pollination; Pest control: Chemical insecticide (neonicotinoids, pyrethroid, benzoylurea and carbamate); Weed control: herbicide (organophosphorus and ether-derivative)
Open field + conventional cultivation	Corn	Chemical fertilizer was same as above; Application amount of basic fertilizer: 200 kg/ha; Application amount of supplement fertilizer: 180 kg/ha	Soil disinfestation: no Pollination method: hand pollination; Pest control: chemical insecticide (neonicotinoids, pyrethroid, benzoylurea and carbamate); Weed control: herbicide (organophosphorus and ether-derivative)

Three 10-cm-deep soil cores (5 cm diameter) were taken from the three subplots and mixed to form a composite soil sample. After removing visible plant roots and stones, the composite samples were passed through a 2-mm sieve and divided into two halves. The first half was air-dried and subsequently stored at 4 °C for the analyses of soil physicochemical properties. The second half of the soil samples was packed in polyethylene bags and immediately stored at -20 °C until DNA extraction. Next-generation high-throughput sequencing was applied to determine the composition and diversity of soil bacterial communities.

### ***Bacterial community***

Soil genomic DNA was extracted from 0.5 g of dried soil per sample with the Fast DNA Spin Kit (MP, Biomedicals, USA) following the manufacturer's protocol. The extracted DNA was diluted to 10 ng/μL, checked using 1% agarose gel electrophoresis, and stored at -20 °C until PCR analysis. Soil bacterial communities were evaluated by amplifying the V4 region of the 16S rRNA gene using the primer set 515F (5'-GTGCCAGCMGCCGCGG TAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT -3').

All PCR amplifications were performed in a 30-μL reaction volume containing 15 μL Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 0.2 μM forward/reverse primers, and 10 ng template DNA (Xiong et al., 2012). The PCR conditions were as follows: 98 °C for 1 min; 30 cycles of 10 s at 98 °C, 30 s at 50 °C and 30 s at 72 °C, and finally 5 min at 72 °C (Xue et al., 2017). Amplicons (200–400 bp) were confirmed on 2% EtBr agarose gels and purified using a GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Carlsbad, CA, USA). Following quantitation, equal concentrations of the purified amplicons were combined in a single tube. Sequencing libraries were generated with an NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer's protocol, and index codes were added. Library quality was assessed on a Qubit 2.0 Fluorimeter (Thermo Fisher Scientific, Carlsbad, CA, USA) and an Agilent Bioanalyzer 2100. The pooled amplicons were subjected to paired-end sequencing on the Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA) (Caporaso et al., 2012).

The paired-end reads were merged with FLASH, a fast and accurate tool designed specifically for overlapping reads. Sequence reads were assigned according to sample-specific barcodes. The sequences were analyzed in QIIME (Quantitative Insights into Microbial Ecology) with in-house Perl scripts for calculating alpha- (within-sample) and beta- (between-sample) diversity. The reads were passed through QIIME quality filters before `pick_de_novo_otus.py` was used to select operational taxonomic units (OTUs) with an OTU table. Sequences showing 97% nucleotide similarity were assigned to the same OTUs. A representative sequence for each OTU was screened and used to assign the taxonomic composition in the Greengenes (bacterial 16S rRNA) databases.

### ***Mesofaunal community***

Modified Tullgren funnel extractors were used for collecting the soil mesofauna. The extracted samples were collected from one soil core (10 × 10 cm), and the depth of the soil core was 15 cm. These samples were taken back to the laboratory and extracted for 48 h at 28 °C. A total of 24 soil mesofauna samples were collected (4 treatments × 3 duplications × 3 plots). All of the soil fauna samples were preserved in 75% alcohol.

The soil fauna were counted under an OLYMPUS SZX16 stereoscopic microscope (Olympus Co., Tokyo, Japan) and were identified to the family level or to the suborder if identification to the family level was not possible (Yin, 1998; Zheng and Gui, 1999). Soil fauna community diversity was quantified using the Shannon-Wiener index ( $H'$ ), Pielou evenness index ( $J$ ) and Menhinick richness index ( $d$ ) (Huang et al., 2006).

### ***Soil properties and enzyme activity***

The soil analysis followed the 'Analysis of soil characteristics' guidelines (Lu, 2000). The organic matter content was analyzed in ground soil by using the Walkley and Black dichromate oxidation method. Soil pH was measured in a 1:5 soil:water (distilled water) slurry using a glass electrode. Available P was extracted with 0.5 mol/L NaHCO<sub>3</sub> by the Olsen method. Available K was extracted with 1 mol/L NH<sub>4</sub>OAc and was determined in all pot soil samples. Soil electrical conductivity determined with a conductivity meter following extraction using a 1:5 soil:water suspension. Soil mineral N was extracted from 40 g equivalent dry soil with 100 ml of a 1 M KCl solution. NO<sub>3</sub><sup>-</sup> was determined with an AA3 continuous flow analyzer. Microbial biomass carbon and microbial biomass nitrogen were determined using the chloroform fumigation extraction methods of Vance et al. (1987) and Potthoff et al. (2003).

Soil enzyme activities were analyzed and assayed as described by Guan (1986). Dedyrogenase (DED) activity (mg TPF g<sup>-1</sup>) was determined by the reduction of triphenyl tetrazolium chloride (1%) to triphenyl formazan. Protease (PRO) activity (mg tyrosine g<sup>-1</sup>) was measured by the determination of the amino acid release after the incubation of samples with sodium caseinate (2%). Phosphatase (PHO) activity (mg phenol g<sup>-1</sup>) was estimated by the determination of phenol release after the incubation of samples with phenyl disodium phosphate (0.5%). Urease (URE) activity (mg NH<sub>4</sub><sup>+</sup> g<sup>-1</sup>) was measured by the determination of NH<sub>4</sub><sup>+</sup> released in the hydrolysis reaction after the incubation of samples with urea (1%). Catalase (CAT) activity was measured by back-titrating residual H<sub>2</sub>O<sub>2</sub> with KMnO<sub>4</sub>.

### ***Statistical analysis***

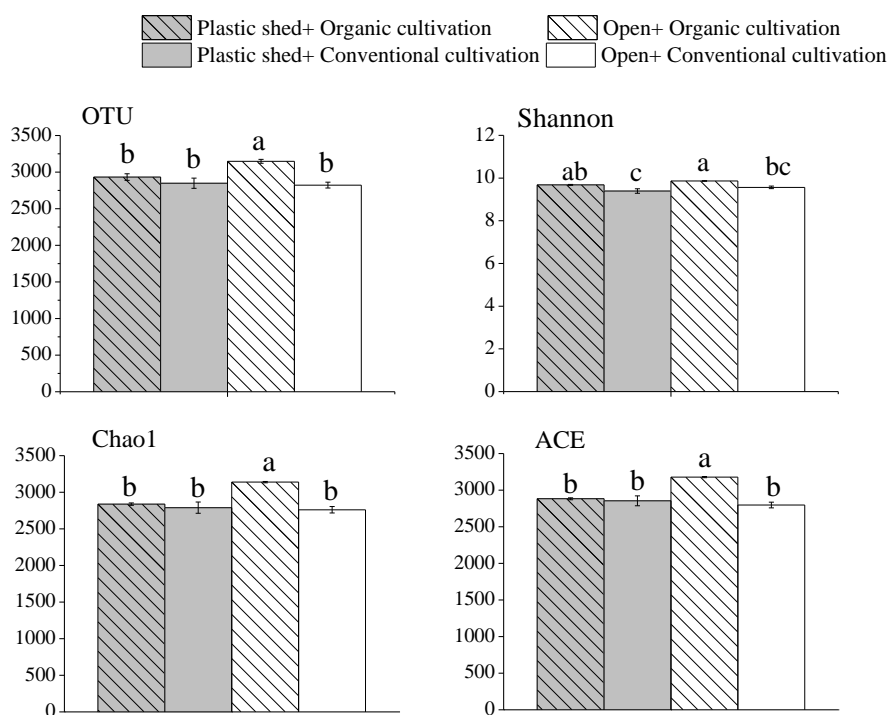
Two-way analysis of variance (ANOVA) was used to determine the effects of plastic shed and organic cultivation on soil electrical conductivity, NO<sub>3</sub><sup>-</sup>-N, soil organic matter, available P, available K, microbial biomass carbon and microbial biomass nitrogen, urease, protease, phosphatase, dehydrogenase and catalase activities, and the soil bacterial OTU, Shannon and Chao1, soil mesofauna Shannon, Pielou and Menhinick indexes. Duncan's test was used to examine the significant differences in the mean values between different treatments at a probability level of 0.05 for detecting significant differences. Redundancy analysis (RDA) and the linear canonical community ordination method were used to visualize the relationships between the response variable values (soil bacterial and mesofaunal diversity), the environmental parameters and the samples with CANOCO 5.0 software (Microcomputer Power Inc., Ithaca, NY). For the RDA in this study, soil bacterial and mesofaunal diversities were used as the explained variables. Seven environmental factors (SOM, NO<sub>3</sub><sup>-</sup>-N, EC, AP, AK, MBC and MBN) were used as the explanatory variables. Additionally, redundancy analysis (RDA) and the linear canonical community ordination method were used to visualize the relationships between the response variable values (soil enzyme activities), the environmental parameters and the samples with CANOCO 5.0 software

(Microcomputer Power Inc., Ithaca, NY). For the RDA in this study, soil enzyme activities were used as the explained variables. Seven environmental factors (SOM, NO<sub>3</sub>-N, EC, AP, AK, MBC and MBN) and seven soil bacterial and mesofaunal diversity indexes (Bac-OUT, Bac-Shannon, Bac-Chao1, Bac-ACE, Fau-Shannon, Fau-Pielou and Fau-Menhinick) were used as explanatory variables.

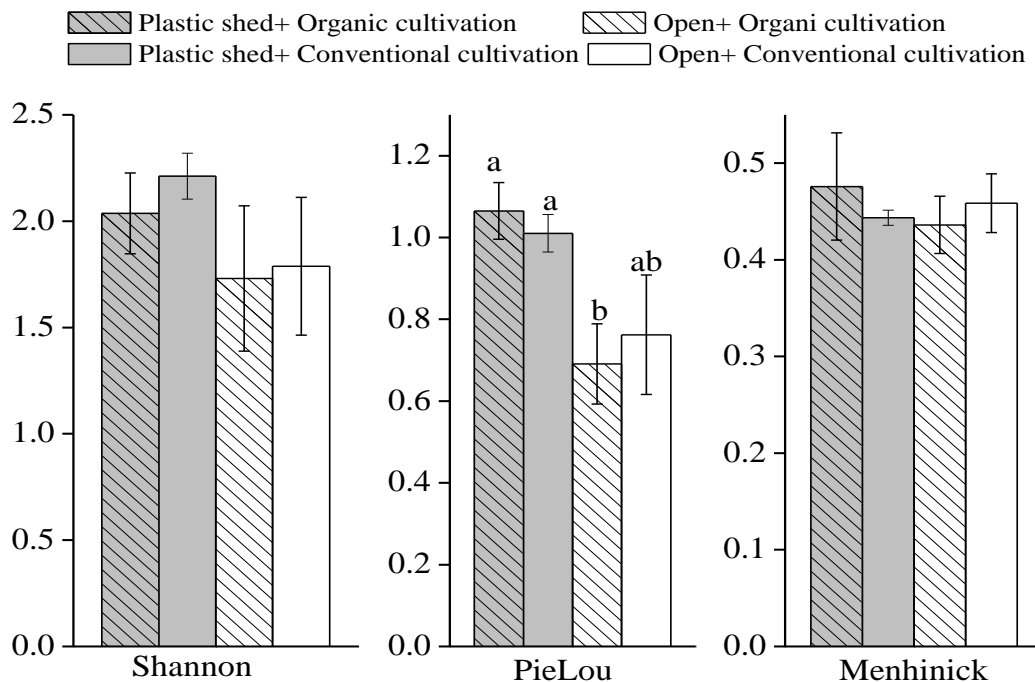
## Results

### *Diversity of the soil bacterial and mesofaunal communities*

The soil bacterial Shannon diversity associated with plastic shed production was lower than that associated with open-field under both organic and conventional cultivation. Plastic shed production decreased the bacterial OTU, Chao1 and ACE evenness indexes compared to open-field only under organic cultivation (*Fig. 1*). Organic cultivation increased bacterial Shannon diversity compared to conventional cultivation in both plastic shed soil and open-field soils. Organic cultivation increased the bacterial OTU, Chao1 and ACE evenness indexes compared to conventional cultivation only in the open-field soils (*Table 2; Fig. 1*). Plastic shed production resulted in higher soil mesofaunal Shannon diversity than open-field under both organic and conventional cultivation. Plastic shed production resulted in a higher soil mesofaunal Pielou index than open-field under both organic and conventional cultivation, but a significant difference was observed only for organic cultivation. Organic cultivation did not affect soil mesofaunal diversity in either plastic shed or open-field soils (*Table 2; Fig. 2*).



**Figure 1.** The OTU, Shannon, Chao1 and ACE index of soil bacteria in the following treatments: plastic shed and open-field soils under organic and conventional cultivation. Vertical lines indicate standard deviation of the mean. Values with different letters differ significantly at  $p < 0.05$  across different treatments



**Figure 2.** The Shannon, PieLou and Menhinick index of soil mesofauna in the following treatments: plastic shed and open-field soils under organic and conventional cultivation. Vertical lines indicate standard deviation of the mean. Values with different letters differ significantly at  $p < 0.05$  across different treatments

**Table 2.** *F* values and error from two-way ANOVA on the effects of plastic shed and organic cultivation and their interactions on the diversity of soil bacterial and mesofaunal community in the all treatments

Treatment	df	The diversity of soil bacterial community				The diversity of soil mesofaunal community		
		OTU	Shannon	Chao1	ACE	Shannon	Pielou	Menhinick
Plastic shed (PS)	1	3.85	8.23*	8.93*	8.95*	1.98	10.11*	0.15
Organic cultivation (ORG)	1	18.30**	22.32**	21.72**	26.23**	0.20	0.01	0.01
PS×ORG	1	6.35*	0.01	13.03**	19.43**	0.05	0.41	0.60
Error	8							

\* Significant at the 0.05 probability level  
 \*\* Significant at the 0.01 probability level  
 \*\*\* Significant at the 0.001 probability level

### Soil enzyme activities

Plastic shed production decreased soil urease, protease and phosphatase activities compared to open-field only under conventional cultivation (Table 3). Under organic cultivation, plastic shed production resulted in higher urease, protease and phosphatase activities than open-field, but the only significant difference was found for protease activity (Table 3). Compared with open-field, plastic shed production increased soil dehydrogenase activity under conventional cultivation and decreased soil dehydrogenase activity under organic cultivation. Compared to conventional cultivation, organic cultivation decreased soil urease and protease activities in open-field soils but increased their activities in plastic shed soils. Organic cultivation

increased soil phosphatase, dehydrogenase and catalase activities compared to conventional cultivation in both plastic shed and open-field soils (Table 3).

### Soil properties

Plastic shed production significantly increased soil EC and NO<sub>3</sub><sup>-</sup>-N concentrations compared to open-field only under conventional cultivation. Organic cultivation significantly decreased soil EC compared to conventional cultivation in both plastic shed and open-field soils (Table 4; Fig. 3). Organic cultivation significantly decreased soil NO<sub>3</sub><sup>-</sup>-N compared to conventional cultivation only in association with plastic shed production. Plastic shed production significantly increased SOM, AP and AK concentrations compared to open-field under both organic and conventional cultivation. Organic cultivation significantly increased soil SOM and AK concentrations compared to conventional cultivation only in plastic shed soils (Table 4; Fig. 3). Plastic shed production increased soil MBC and MBN compared to open-air vegetation under both organic and conventional cultivation (Table 5). Organic cultivation produced significant soil MBN compared to conventional cultivation only in open-air vegetation, while there was no difference in soil MBN between organic and conventional cultivation under plastic shed production (Table 5).

**Table 3.** The mean value ( $\pm$ SE) of soil urease, protease, phosphatase, dehydrogenase and catalase activity in the following treatments: plastic shed and open-field soils under organic and conventional cultivation

	Urease activity (mg/g)	Protease activity (mg/g)	Phosphatase activity (mg/g)	Dehydrogenase activity (mg/g)	Catalase activity (ml/g)					
Plastic shed + organic cultivation	87.57 $\pm$ 11.7 ab	116.2 $\pm$ 7.03 a	113.8 $\pm$ 8.03 a	79.03 $\pm$ 6.41 b	189.5 $\pm$ 20.4 ab					
Plastic shed + conventional cultivation	79.76 $\pm$ 29.6 b	12.24 $\pm$ 1.18 d	20.45 $\pm$ 3.53 c	54.21 $\pm$ 4.95 c	111.6 $\pm$ 20.9 b					
Open-field + organic cultivation	55.86 $\pm$ 15.1b	61.42 $\pm$ 1.97 c	108.2 $\pm$ 4.64 a	97.46 $\pm$ 6.96 a	230.0 $\pm$ 33.6 a					
Open-field + conventional cultivation	119.1 $\pm$ 4.14 a	80.33 $\pm$ 5.94 b	58.77 $\pm$ 9.20 b	16.87 $\pm$ 3.07 d	113.3 $\pm$ 39.1 b					
Analysis of variance										
	df	F	df	F	df	F	df	F	df	F
Plastic shed (PS)	1	0.05	1	1.94	1	5.83*	1	2.89	1	0.50
Organic cultivation (ORG)	1	2.44	1	80.4***	1	111.3***	1	89.88***	1	10.77*
PS $\times$ ORG	1	4.01	1	167.6***	1	10.53*	1	25.15**	1	0.43

Values with different letters within a column show means with treatment-specific significant differences ( $p < 0.05$ ; Duncan test). The lower part of the table shows F-values from the analysis of variance; degrees of freedom \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

**Table 4.** F values and error from two-way ANOVA on the effects of plastic shed and organic cultivation and their interactions on soil electrical conductivity (EC), pH, NO<sub>3</sub>-N, SOM, available P and available K in plastic shed and open-field soils under organic and conventional cultivation

Treatment	df	EC	pH	NO <sub>3</sub> -N	SOM	AP	AK
Plastic shed (PS)	1	15.14**	0.87	93.85***	584.1***	91.76***	203.8***
Organic cultivation (ORG)	1	301.03***	0.67	72.30***	158.8***	1.81	11.60**
PS $\times$ ORG	1	35.95***	0.09	62.50***	177.4***	0.09	37.47***
Error	8						

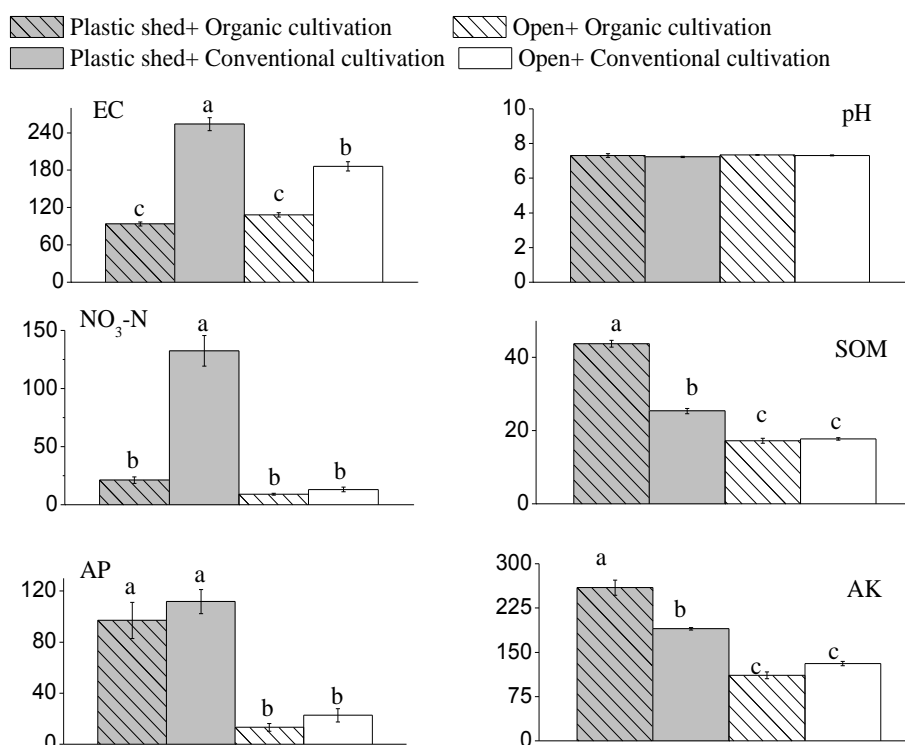
\*Significant at the 0.05 probability level  
\*\*Significant at the 0.01 probability level  
\*\*\*Significant at the 0.001 probability level



**Table 5.** The mean value ( $\pm$ SE) of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in the following treatments: plastic shed and open-field soils under organic and conventional cultivation

	MBC		MBN	
Plastic shed + organic cultivation	165.57 $\pm$ 21.68 a		36.82 $\pm$ 2.85 a	
Plastic shed + conventional cultivation	137.98 $\pm$ 18.06 a		30.68 $\pm$ 2.38 a	
Open-field + organic cultivation	66.44 $\pm$ 2.54 b		22.24 $\pm$ 1.69 b	
Open-field + conventional cultivation	63.18 $\pm$ 8.14 b		13.37 $\pm$ 1.49 c	
Analysis of variance				
	df	F	df	F
PS	1	34.79***	1	53.73***
ORG	1	1.09	1	11.90**
PS $\times$ ORG	1	0.68	1	0.39

Values with different letters within a column show means with treatment-specific significant differences ( $p < 0.05$ ; Duncan test). The lower part of the table shows F-values from the analysis of variance; degrees of freedom \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

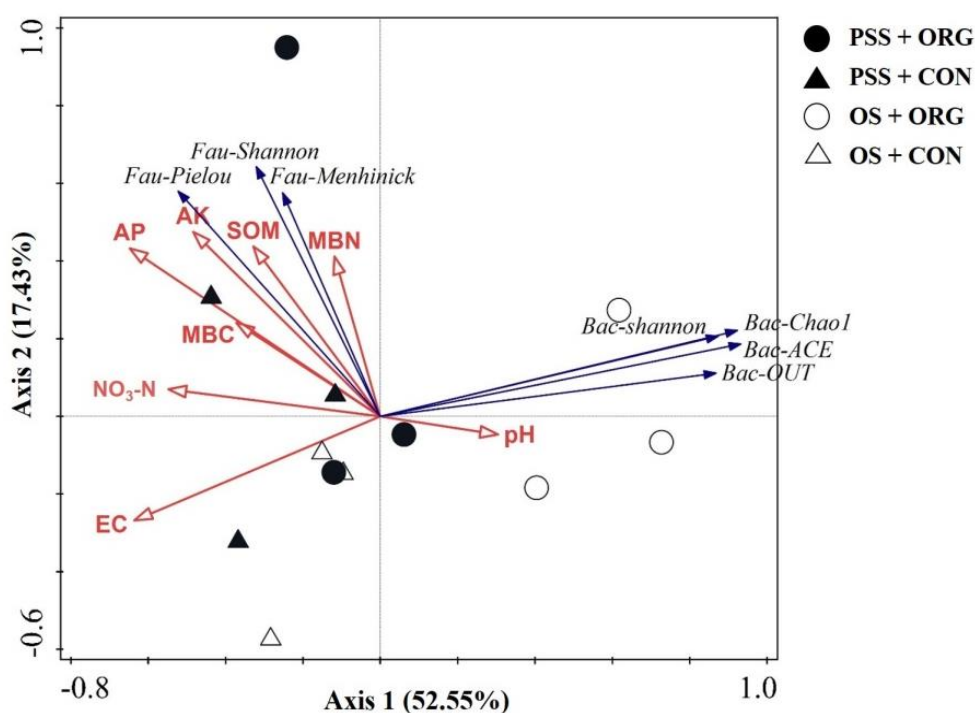


**Figure 3.** Soil electric conductivity (EC), pH, NO<sub>3</sub>-N, organic matter (SOM), available P (AP) and available K (AK) in the following treatments: plastic shed and open-field soils under organic and conventional cultivation. Vertical lines indicate standard deviation of the mean. Values with different letters differ significantly at  $p < 0.05$  across different treatments

### Correlations among soil organism diversity, soil enzyme activities and soil properties

The first ordination RDA axis explained 52.55% of the variation in the soil bacterial and mesofaunal data, and the second axis explained 17.43% (Fig. 4). The RDA

suggested that the soil available P (which explained 26.8% of the variance,  $P = 0.022$ ) was the most important parameter contributing to the diversity of the soil bacteria and mesofauna, followed by soil EC (which explained 24.1% of the variance,  $P = 0.028$ ), and  $\text{NO}_3\text{-N}$  (which explained 19.0% of the variance,  $P = 0.07$ ) (Fig. 4). The first ordination RDA axis explained 57.00%, and the second axis explained 31.20% of the variation in the soil enzyme data (Fig. 5). The RDA suggested that the soil EC (which explained 55.0% of the variance,  $P = 0.002$ ) was the most important parameter contributing to soil enzyme activity, and thereafter, the most important parameters were the soil  $\text{NO}_3\text{-N}$  (which explained 47.7% of the variance,  $P = 0.01$ ), Bac-Shannon (which explained 40.1% of the variance,  $P = 0.004$ ), Bac-Chao1 (which explained 25.4% of the variance,  $P = 0.042$ ), Bac-ACE (which explained 24.9% of the variance,  $P = 0.046$ ), and Bac-OTU (which explained 21.8% of the variance,  $P = 0.072$ ) (Fig. 5).

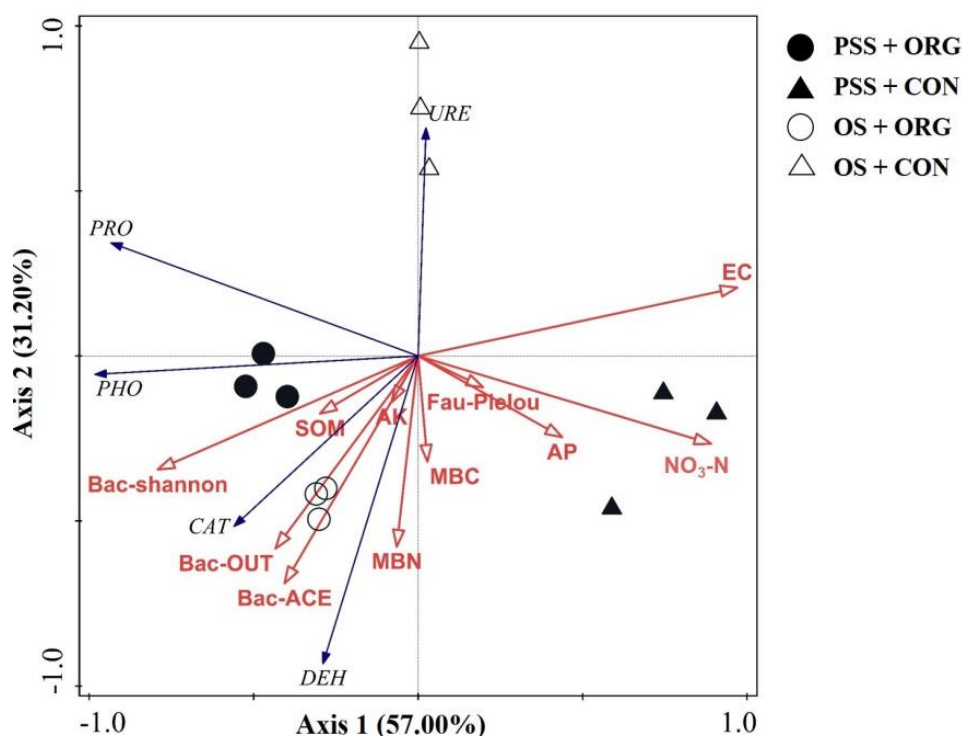


**Figure 4.** Redundancy analysis between soil bacterial and mesofaunal diversity and soil environmental parameters. Bac-Shannon bacterial Shannon diversity index; Bac-Chao1, bacterial Chao1 index; Bac-ACE, bacterial ACE index; Fau-Shannon, mesofaunal Shannon diversity index; Fau-Pielou, mesofaunal Pielou index; Fau-Menhinick, mesofaunal Menhinick index; SOM, soil organic matter;  $\text{NO}_3\text{-N}$ , nitrate; EC, electric conductivity; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; Bac-OUT, bacterial OUT; PSS + ORG, plastic shed soils + organic cultivation; PSS + CON, plastic shed soils + conventional cultivation; OS + ORG, open-field soils + organic cultivation; OS + CON, open-field soils + conventional cultivation

## Discussion

The soil bacterial diversity and richness index in plastic shed soils were lower than those in open-field soils. This may be due to changes in soil environmental factors that play important roles in shaping the bacterial community composition (Horner-Devine et al., 2004). Plastic shed production not only directly changed soil temperature and

moisture but also indirectly altered soil nutrients, which contributed to the changes in the soil bacterial community. In the present study, we observed that soil bacterial diversity was significantly negatively correlated with soil available P, EC and NO<sub>3</sub>-N content. Our results partly agree with the findings of Ma et al. (2018) and Chen et al. (2019), who found that NO<sub>3</sub>-N and electrical conductivity were the most important soil properties controlling the variation in bacterial community structure. The results of previous studies have shown that high soil nutrient levels in plastic shed soils could decrease soil bacterial diversity (Ramirez et al., 2010; Sun et al., 2015; Chen et al., 2019). Although the present study did not measure environmental conditions, several studies have suggested that the soil temperature and moisture content in plastic shed soils are higher than those in open-field soils (Chen et al., 2008). Soil bacteria that are adapted to the high temperatures and moisture levels in plastic shed soils will be the dominant population, which may result in a decrease in bacterial diversity. Organic cultivation increased bacterial Shannon diversity compared to conventional cultivation in both plastic shed and open-field soils. This result is consistent with the findings of many previous studies showing that organic cultivation usually results in much higher soil biodiversity compared to conventional cultivation. The increase in bacterial diversity also increases the resilience of soils, leading to improved soil health (van Bruggen and Semenov, 2000).



**Figure 5.** Redundancy analysis between soil enzyme activities and soil environmental parameters. DEH, dehydrogenase; URE, urease; PRO, protease; PHO, phosphatase; CAT, catalase; SOM, soil organic matter; NO<sub>3</sub>-N, nitrate; EC, electric conductivity; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; Bac-OUT, bacterial OUT; Bac-Shannon bacterial Shannon diversity index; Bac-Chao1, bacterial Chao1 index; Bac-ACE, bacterial ACE index; Fau-Shannon, mesofaunal Shannon diversity index; Fau-Pielou, mesofaunal Pielou index; Fau-Menhinick, mesofaunal Menhinick index. PSS + ORG, plastic shed soils + organic cultivation; PSS + CON, plastic shed soils + conventional cultivation; OS + ORG, open-field soils + organic cultivation; OS + CON, open-field soils + conventional cultivation

Plastic shed production resulted in higher soil mesofaunal diversity and evenness indexes compared to open-field under both organic and conventional cultivation. This result is consistent with the findings of previous studies (Shah et al., 2003; Li and Gu, 2009; Ponce et al., 2011) in which the authors speculated that this change may be due to the improvement of soil nutrients. In the present study, we observed that the Shannon, Pielou and Menhinick indexes of the soil mesofauna were positively correlated with soil available P, soil microbial biomass carbon, microbial biomass nitrogen and nitrate contents. However, other researchers have shown that plastic shed production leads to lower Shannon diversity and Pielou evenness of the soil fauna community compared to open-field (Dong et al., 2008; Wang, 2009). This may occur because the soil macrofauna is more severely affected by plastic shed production than the meso- and microfauna (Postma-Blaauw et al., 2010; Ponge et al., 2013). The nonsignificant differences in mesofaunal diversity and evenness indexes between organic and conventional cultivation were not consistent with the findings of many studies (Cotes et al., 2010; Jiang et al., 2015) that have shown increased diversity of the soil fauna (arthropods) under organic cultivation. Such nondiscriminating results could be attributed to the heterogeneity of organic practices applied within soil systems as well as climate parameters and the different responses of species to management disturbance (Hole et al., 2005; Bengtsson et al., 2005; Gkisakis et al., 2016)

In soils, enzymes play an essential role in mediating biochemical transformations and nutrient cycling and can thus be used as a sensitive index to monitor changes in soil microbial activity and functioning. Under conventional cultivation, plastic shed production decreased soil urease, protease and phosphatase activities compared to open-field. These results suggest a decrease in soil function related to N and P transformation (Sinsabaugh et al., 2008). However, under organic cultivation, we observed that plastic shed production resulted in higher soil urease, protease and phosphatase activities compared to open-field. These results indicated that organic cultivation could alleviate the adverse effect of plastic shed production on soil enzyme activities related to soil N and P. In the present study, we observed higher soil urease, protease, phosphatase, dehydrogenase and catalase activities under organic cultivation than under conventional cultivation in plastic shed soils. These results indicated that the soils of organic cultivation systems exhibit higher overall microbial activity and a higher capacity to cleave proteins and organic phosphorus. In the present study, soil enzyme activities showed no significant correlation with most of the examined soil properties. These results differ from those of a previous study showing that soil properties such as available P and N exhibit close relationships with soil enzyme activities (Ling et al., 2014). However, the present study indicates that soil enzyme activities are significantly positively correlated with bacterial the Shannon diversity, Chao1 and ACE indexes. The results were in accordance with the findings of Chen et al. (2019) showing that soil enzyme activities were correlated with the microbial diversity index. The findings of Carrara et al. (2018) also showed that extracellular enzyme activities were significantly correlated with the bacterial community composition. This may be explained by the results of a study suggesting that a change in microbial diversity impacts soil functions such as enzyme activities (Colombo et al., 2016). This also supports the conclusion that the microbial community composition is more important than soil nutrient properties in influencing soil functioning (Nannipieri et al., 2012; Stark et al., 2014). In addition, the present study revealed that soil enzyme activities were significantly negatively correlated with soil electrical conductivity and NO<sub>3</sub>-N. These results indicated that the

accumulation of salt in the soil impaired soil enzyme activities (Tejada et al., 2006; Tripathi et al., 2007).

Under conventional cultivation, plastic shed production significantly increased soil EC compared to open-field. These results indicated that under conventional cultivation, plastic shed production causes soil salinization. This agrees with the findings of a previous study (Ju et al., 2007). These results may be due to the following two reasons. First, under conventional cultivation, heavy application rates of chemical fertilizers in vegetable production cause excessively high nutrient accumulation in soil. Soil nitrate is one major factor leading to soil salinization, which occurs easily in plastic shed soils due to the heavy use of fertilizers (Shi et al., 2009). In the present study, plastic shed production significantly increased the soil NO<sub>3</sub>-N concentration compared to open-field only under conventional cultivation. The accumulated NO<sub>3</sub>-N content reflects the impact of chemical fertilizers on soil EC. Second, plastic shed production usually causes higher evapotranspiration compared with open-air fields (Han et al., 2009), which induces the upward movement of soil water and the soil solution from subsoil, resulting in the accumulation of soil salt ions in topsoil (Ge et al., 2010). However, under organic cultivation, the soil EC values were not different between the plastic shed and open-field soils. These results indicated that organic cultivation can significantly alleviate soil salinization in plastic shed soil. Organic cultivation can reduce soil nitrate accumulation, which may improve denitrification processes (Huang et al., 2019). In the present study, significantly lower soil EC and nitrate contents were observed under organic cultivation than under conventional cultivation. Together, the available evidence suggests that the observed effects of organic cultivation management are attributable to its roles in alleviating soil salinization (or nitrate accumulation), which likely benefit from the alteration of soil N-cycling processes. The soil organic matter, available K and available P contents observed in association with plastic shed production were significantly higher than those associated with open-field under both organic and conventional cultivation. These results indicate that the nutrient contents of the plastic shed soils were maintained at high levels to achieve sustained soil chemical fertility (Chen et al., 2019; Xie and Tan, 2001; Yang et al., 2011). The soil organic matter and available K contents under organic cultivation were significantly higher than those under conventional cultivation associated with plastic shed production. These results occurred because N fertilizer in organic shed production systems is generally replaced with organic fertilizer, which includes carbon and K in different forms.

Plastic shed production increased soil microbial biomass carbon and microbial biomass nitrogen compared to open-field under both organic and conventional cultivation. These findings are consistent with the results of several previous studies (Yu, 2007) and may be due to the higher soil nutrient contents associated with plastic shed production compared with open-field (Mele and Crowley, 2008; Zhong et al., 2010). In the present study, we observed that soil microbial biomass carbon and microbial biomass nitrogen were correlated with soil nutrients such as soil organic matter, NO<sub>3</sub><sup>-</sup>-N, available P and available K. Organic cultivation significantly increased soil microbial biomass carbon and microbial biomass nitrogen compared to conventional cultivation in both plastic shed and open-field soils, with the exception of soil microbial biomass nitrogen associated with plastic shed production. These results are in agreement with other studies (Liu et al., 2007; van Diepeningen et al., 2006). Under organic cultivation, more organic carbon there is applied to fields to maintain the organic matter content in soils, which may simultaneously increase the microbial biomass.

## Conclusion

This research provides evidence that plastic shed soils exhibit lower soil bacterial richness and diversity than open-field soils but present higher soil mesofaunal Shannon diversity and Pielou's species evenness indexes. Plastic shed production significantly decreased soil urease and phosphate activities compared with those in open soil only under conventional cultivation. Organic cultivation mostly resulted in higher bacterial abundance and diversity, soil microbial biomass and enzyme activities compared with conventional cultivation, but significant differences in some parameters were observed only in the open-field soils. Changes in soil enzyme activities occurred and were tightly linked to bacterial diversity and soil electrical conductivity, rather than to soil nutrient properties. Plastic shed production increased soil nutrient properties but caused soil salinization (mainly because of soil nitrate accumulation) under conventional cultivation, which may cause groundwater pollution. Organic cultivation decreased the soil EC and nitrate contents and increased soil organic matter and available K contents compared with conventional cultivation in plastic shed soils. Together, these results indicated that organic cultivation could be used to minimize the negative impacts of plastic shed production, particularly to decrease soil EC and nitrate contents and enhance soil functions, and the showed that the positive impacts of organic cultivation on soil microbial diversity and functions were reduced by plastic shed production. Further studies are still needed to determine the long-term effects of plastic shed production systems on soil nutrients, biodiversity and functions under different management practices.

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