Supplementary Materials and Methods

Changes in soil nematode and microbial community in cucumber root-zone soil shaped by intercropping with amaranth

Xu Zhang[1], Mengyuan Song[1], Jiafan Li[1], Xingqun Liu[1], Lihong Gao[1]*, Yongqiang Tian[1]*

[1]Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, College of Horticulture, China Agricultural University, Yuanmingyuan West Road No.2, Haidian District, Beijing 100193, China

*** Correspondence:**

College of Horticulture, China Agricultural University, Yuanmingyuan West Road No.2, Haidian District, Beijing 100193, China

E-mail addresses: tianyq1984@cau.edu.cn (Y. Tian), gaolh@cau.edu.cn (L. Gao)

Supplementary materials and methodology for the Basic properties of bulk soil

The surface soil classified as a loam soil in the experimental greenhouse and the soil of 0-20 cm layer had a pH (in water) of 7.12, an electrical conductivity (EC) value with the extracting ratio of 1:5 (soil/water) of 1284 μs cm^{-1}, and contained 8.5 g kg^{-1} of organic C, 3.196 g kg^{-1} of total nitrogen (N); for the soil available nutrients, the surface soil contained 112.54 mg kg^{-1} of mineral N (nitrate N plus ammonium N), 84.58 mg kg^{-1} of available phosphorus (P) and 392.38 mg kg^{-1} of available potassium (K).

Supplementary materials and methodology for the Soil microbes-related parameters

For analyzing community level physiological profiling (CLPP) of root-zone soil microbial community, a 96-well Eco-Microplate which contained 31 different carbon sources was used. In detail, 5 g soil and 45 mL (1:9, w/v) sterilized water were placed in a 50 mL sterilized tube and vortexed for 1h at 4℃. Subsequently, the homogenized soil solution was diluted using sterilized water up to 10^{-3} fold. Finally, 150 μL soil suspension was added into the Biolog Ecoplate well and incubated at 25℃ in dark. The optical density (OD) values of each well were measured at 590 nm every 24 h using a microplate reader. To eliminate the effects of inoculum density, the optical density value from each well was normalized by the average well color development (AWCD). Plate readings at 96 h (the shortest incubation time that allowed the best resolution among treatments) of incubation were selected to evaluated the soil microbial community carbon source utilization. The normalized values were added up to get the proportion of the AWCD that is attributed to six different guilds (i.e. amides/amines, amino acids,

carbohydrates, carboxylic acids, miscellaneous and polymers). In addition, to measure the diversity index of soil microbial community carbon source utilization, the Shannon-Wiener (SW) index and Simpson index (1/D) was calculated [1].

The 31 kinds of carbon substrates in BIOLOG EcoPlate method.

Chemical guild	Plate number	Substrates
Carbonhydrates	A2	β-methyl-D-glucoside
	A3	D-galactonic acid γ-lactone
	B2	D-xylose
	C2	i-erythritol
	D2	D-mannitol
	E2	N-acetyl-D-glucosamine
	G1	D-cellobiose
	G2	Glucose-1-phosphate
	H1	α-D-lactose
	H2	D,L-α-glycerol phosphate
Miscellaneous	C3	2-hydroxybenzoic acid
	D3	4-hydroxybenzoic acid
Carboxilic acids	B1	Pyruvatic acid methyl ester
	B3	D-galacturonic acid
	E3	γ-hydroxybutyric acid
	F2	D-glucosaminic acid
	F3	Itaconic acid
	G3	α-ketobutyric acid
	H3	D-malic acid
Amino acids	A4	L-arginine
	B4	L-asparagine
	C4	L-phenylalanine
	D4	L-serine
	E4	L-threonine
	F4	Glycyl-L-glutamic acid
Amides/Amines	G4	Phenyl ethylamine
	H4	Putrescine
Polymers	C1	Tween 40
	D1	Tween 80
	E1	α-cyclodextrin
	F1	Glycogen

PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) when no visible amplification was observed from negative control and sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina) and subjected to a single sequencing run on the MiSeq platform (Illumina). For analyzing the soil microbial community, root-zone soil-based Illumina sequences of 16S and ITS were processed and sequentially quality-filtered using Fastp

(version 0.19.6) [2]. Pair-end reads were merged with a minimum overlap using Flash (1.2.11) [3], After removing chimeric sequences, the remaining sequences were binned into OTUs with 97% similarity and the representative sequence for each OTU was taxonomically classified via the Ribosomal Database Project's classifier [4] and the SILVA database (version 138) [5] for bacteria and UNITE (version 8.0) for fungi [6]. All OTUs identified as belonging to chloroplast and mitochondria were removed from the data set. Then, the representative sequences for each OTU were aligned using PyNAST [7] in QIIME [8] and carried out by Uparse software (version 11) [9]. The least number of obtained sequences from all samples was used to eliminate the difference caused by various sequencing depth. Shannon index, Chao1, and Simpson index were applied to directly compare the α-diversity of the root-zone soil microbial community. The non-metric multidimensional scaling was used to assess the β-diversity of microbial community. Linear discriminant analysis (LDA) of effect size (LEfSe) was applied on the OTU table to identify the differentially abundant bacterial taxa (at genus to phylum levels) that significantly change between monocropping and intercropping system. Wilcoxon rank-sum test for pairwise comparison (false discovery rate (FDR) adjusted $p < 0.05$) and the absolute LDA score (>2.5) were used to analyze the statistical significance and strength, respectively. The data were analyzed on the online platform of Majorbio Cloud Platform (www.majorbio.com). The raw sequencing data of the bacteria and fungi were submitted into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA855629).

References for Supplementary Materials

1. Tian, Y.; Zhang, X.; Liu, J.; Gao, L. Effects of summer cover crop and residue management on cucumber growth in intensive Chinese production systems: soil nutrients, microbial properties and nematodes. *Plant Soil* **2011**, *339*, 299–315.
2. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890.
3. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963.
4. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naive bayesian classifier for rapid assignment of RRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267.
5. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **2012**, *41*, D590–D596.
6. Nilsson, R.H.; Larsson, K.-H.; Taylor, A.F.S.; Bengtsson-Palme, J.; Jeppesen, T.S.; Schigel, D.; Kennedy, P.; Picard, K.; Glöckner, F.O.; Tedersoo, L.; et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* **2019**, *47*, D259–D264.
7. Caporaso, J.G.; Bittinger, K.; Bushman, F.D.; DeSantis, T.Z.; Andersen, G.L.; Knight, R. PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics* **2010**, *26*, 266–267.

8. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336.
9. Edgar, R.C. UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads. *Nature methods* **2013**, *10*, 996–998.