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Effects of vanillin on cucumber (*Cucumis sativus* L.) seedling rhizosphere fungal community composition

J.H. Zhang#, H.J. Yu#, X. Ge, D.D. Pan, Y.H. Shen, P.L. Qiao, F.Z. Wu and X.G. Zhou*
Department of Horticulture, Northeast Agricultural University,
Changjiang 600, Xiangfang, Harbin 150030, China
E-mail: zxg2025@163.com

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ABSTRACT

Cucumber seedlings grown in soil were treated with vanillin at 0.1 μmol/g soil and the cucumber rhizosphere fungal composition was analyzed by high-throughput sequencing of total fungal ITS regions. A total of 207,557 quality fungal ITS sequences were obtained and these sequences were classified into more than 390 OTUs at 97% sequence similarity. Vanillin decreased the number of OTUs, ACE, Chao and Shannon indices of cucumber seedling rhizosphere fungal communities. Principal coordinat analysis showed that vanillin changed the cucumber seedlings rhizosphere fungal community composition. Compared with water-treated soils, vanillin-treated soils had higher relative abundances of phylum *Basidiomycota*, classes *Tremellomycetes* and *Ascomycota incertae sedis* but had lower relative abundances of phylum *Zygomycota* and classes *Sordariomycetes* and *Zygomycetes* ($P < 0.05$). Moreover, vanillin promoted the relative abundances of several fungal genera, such as *Fusarium* and *Nectria* spp., but decreased that of *Pseudallescheria* spp.

Key words: Allelopathy, cucumber, *Cucumis sativus* L., fungal community, illumina sequencing, rhizosphere, vanillin

INTRODUCTION

Vegetables production in the greenhouse are often characterized by monocropping, (6,42,48,53), in which the same crop is repeatedly monocropped on the same land. This is not sustainable on long-term, as it reduces the crop yield and quality, and cause 'soil sickness' (6,43,48). Soil sickness has been reported in peanut (*Arachis hypogaea*) (18), corn (*Zea mays*) (14), Jerusalem artichoke (*Helianthus tuberosus*) (46), tea (*Camellia sinensis*) (20) and cucumber (*Cucumis sativus*) (50). Accumulation of phytotoxic compounds (autotoxins) is one of the major factors that contribute to soil sickness (17,34,41,50,55).

Agricultural productivity relies on a wide range of ecosystem services provided by soil microorganisms (1,30,48). Monocropping negatively affects the soil microbial communities i.e. decrease in the activity and diversity of soil microbial communities, change the composition of soil microbial communities and promotes soil-borne pathogens (17,40,48). Phenolic compounds, which are detected in root exudates and decomposing plant debris, are a type of autotoxins that exert detrimental effects on plant growth (19). Recent studies have indicated that phenolic compounds could act as specific substrates or

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*Correspondence author, #Co-first authors signalling molecules for a large group of microbial species in the soil (2,49,55). However, how phenolic compounds affects the soil microbial communities is not well understood.

Phenolic compounds are potential autotoxins of cucumber in both hydroponics and soil conditions (3,45,51). Previously, we found that vanillin changed the soil fungal community structure as estimated by PCR-denaturing gradient gel electrophoresis analysis (52). High-throughput sequencing techniques, such as 454 pyrosequencing and Illumina sequencing, can provide a higher taxonomical resolution and a better understanding of environmental microbial communities than the PCR-based fingerprinting techniques (37,44). This study aimed to further illustrate the taxonomical changes in cucumber rhizosphere fungal communities in response to exogenous vanillin. Cucumber seedlings were treated with vanillin every other day and rhizosphere fungal communities were analyzed 10-days after the treatment by amplicon sequencing of fungal ITS regions.

MATERIALS AND METHODS

Pot experiment

The soil used in this experiment was collected from the undisturbed upper soil layer (0-15 cm) of an open field in our Experimental Station, Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E, mean height above sea level: 127.95 m, annual precipitation: 524.5 mm, maximum and minimum temperature: 36.7°C, -37.7°C). The soil was sandy loam, contained organic matter: 3.67%, available N: 89.02 mg/kg, available P: 63.36 mg/kg, available K: 119.15 mg/kg, EC (1:2.5, w/v): 0.33 mS/cm and pH (1:2.5, w/v): 7.78 (52).

Pot experiment was conducted from July to September 2015. one cucumber seedlings (cv. Jinlv 3) with two cotyledons were transplanted per pot containing 150 g fresh soil. No fertilizer was added. The seedlings were maintained in greenhouse (32°C day/22°C night, relative humidity of 60-80%, 16 h light/8 h dark).

The phenolic compounds decrease rapidly after added into the soil, due to microbial utilization of the compounds under favourable environmental conditions (39). Therefore, vanillin was added to the soil periodically to maintain the desired levels as described before (39). Cucumber seedlings at one-leaf stage were treated with vanillin at 0.1 μmol/g dry soil every two days 5-times**.** Seedlings treated with distilled water served as control. Each treatment had 5-seedlings and replicated thrice. The pH of the vanillin solution was adjusted to 7.0 with 0.1 M NaOH, because the soil pH is a dominant factor that regulates soil microbial communities (13). Soil water content was maintained > 60% of its water holding capacity.

Rhizosphere soil sampling and DNA extraction

One day after the fifth application of vanillin**,** cucumber rhizosphere soil samples were collected from five plants in each replication as described before (46). Total soil DNA was extracted with the Power Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) as per the manufacturer's instructions**.**

Illumina Miseq sequencing and data processing

The ITS1 regions of the fungal rRNA gene were amplified with primer sets of ITS1F/ITS2 as described before (8,48,55). Both the forward and reverse primers also had a 6-bp barcode unique to each sample. Each soil sample was independently amplified, the products of the triplicate PCR reactions were pooled and purified using the Agarose Gel DNA purification kit (TaKaRa). Then, purified amplicons were quantified by a TBS-380 micro fluorometer with Picogreen reagent (Invitrogen, USA), and mixed accordingly to achieve the equal concentration in the final mixture. The mixture was then paired-end sequenced (2×300) on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Raw sequence reads were de-multiplexed, quality-filtered, and processed using FLASH as described before (28,48). Chimeric sequences were identified and removed using USEARCH 6.1 in QIIME (5). Sequences were binned to Operational taxonomic units (OTUs) at 97% sequence similarity with USEARCH using an agglomerative clustering algorithm (11). Then, a representative sequence of each OTU was taxonomically classified through BLAST against the Unite (21) database. The data set was deposited in the NCBI-Sequence Read Archive with the submission Accession Number SRP129904.

Statistical analysis

To avoid potential bias caused by sequencing depth, a random subsampling effort of 29,798 ITS gene sequences per sample was performed. The defined OTUs were used to calculate taxon accumulation curves with the 'vegan' package in 'R' (Version 3.3.1). For alpha diversity analysis, Chao, ACE and Shannon index were calculated using QIIME (5). For beta diversity, principal coordinates analysis (PCoA) was performed to determine differences in microbial community structures based on Bray-Curtis distances with the 'vegan' package in 'R' (Version 3.3.1). Differences in alpha diversity indices and relative abundances of microbial taxa between treatments were analyzed using Welch's t test in 'R' (Version 3.3.1).

RESULTS AND DISCUSSION

Illumina Miseq sequencing data

Across all soil samples, Illumina Miseq sequencing yielded 207,557 quality fungal sequences, with 29,802-44,601 fungal sequences per sample (Mean: 34,593). The average read length of the ITS1 regions was 263 bp. A total of 396 OTUs were identified at 97% sequence similarity. Rarefaction curves of OTUs at 97% sequence similarity of all samples tended to approach the saturation plateau (Fig. 1a). The Good's coverage of each sample, which reflects the captured diversity, was 99.83±0.01% (Fig. 1b). Therefore, our Illumina Miseq sequencing data were enough for community analysis.

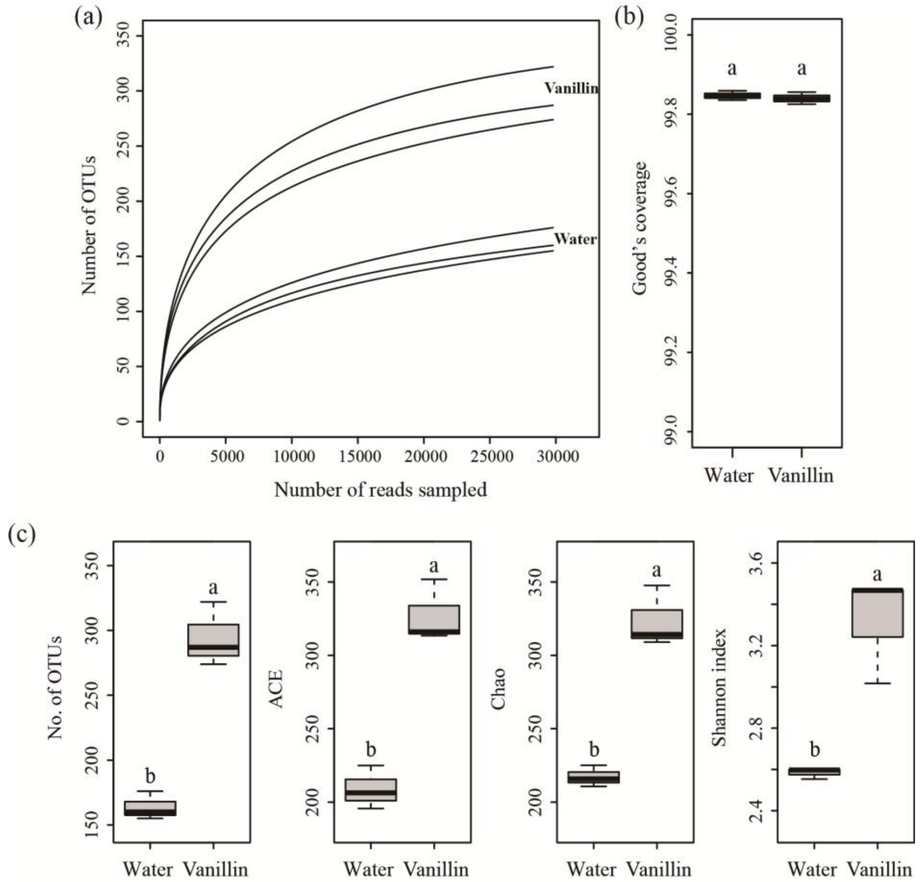

Figure 1. Rarefaction curves of the number of operational taxonomic units (OTUs) (a), The Good's coverage (b) and diversity and richness indices (c) of soil fungal communities in the water and vanillin-treated soils.
OTUs were delineated at 97% sequence similarity. Random subsamples of 29,798 ITS gene sequences per sample were used to generate the rarefaction curves and calculate the Good's coverage, diversity and richness indices. Different letters indicate significant difference based on Welch's *t* test ($P<0.05$).

Alpha and beta diversities of fungal communities

For alpha diversity, the number of OTUs, ACE, Chao and Shannon indices were significantly higher in vanillin-treated soils than in water-treated soils ($P< 0.05$) (Fig. 1c). For beta diversity, PCoA analysis, based on the Bray-Curtis distance dissimilarity, clearly separated vanillin-treated soils and water-treated soils along the first axis (Fig. 2a). The first two axes together accounted for 94.28% of the variation.

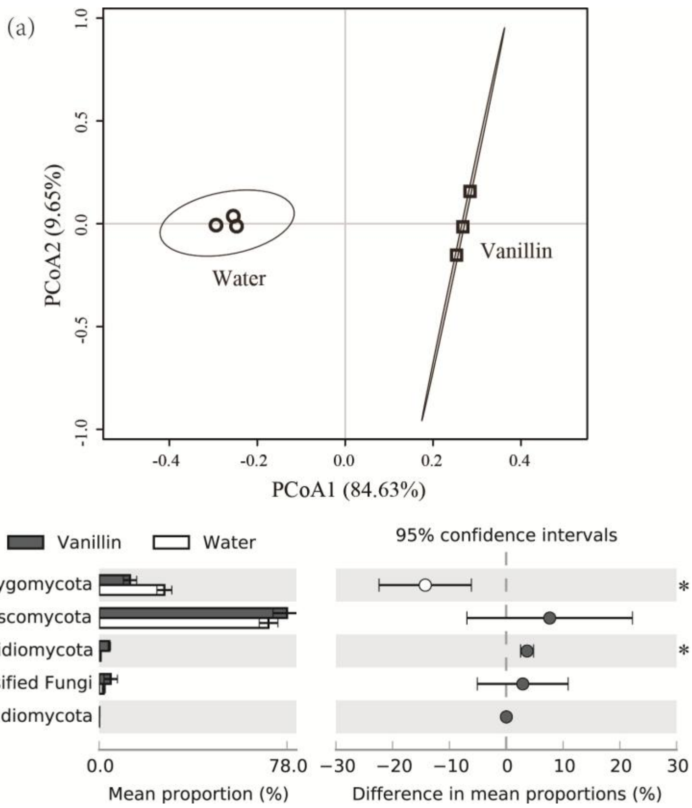

Figure 2. Beta diversities of fungal communities (a) and relative abundances of main fungal phyla (b) in the water and vanillin-treated soils.

Differences in Bray-Curtis distances of fungal communities were visualized by principal component analyses. Ellipses indicate 95% confidence interval for replicates. Values in the bar plot are expressed as mean±standard error. The circles represent the 95% confidence intervals. Asterisks indicate significant difference between treatments based on Welch's *t* test ($P<0.05$).

Taxonomic characteristics of fungal communities

Across all samples, four fungal phyla were detected and 3.4% sequences were unclassified at this level (Fig. 2b). *Ascomycota* and *Zygomycota* were the dominant phyla, which accounted for 74.2% and 20.0% of the total fungal sequences, respectively. Less abundant fungal phyla detected were *Basidiomycota* and *Chytridiomycota*. Compared with water-treated soils, vanillin-treated soils had higher relative abundance of *Basidiomycota* but had lower relative abundance of *Zygomycota* ($P<0.05$).

At the class level, *Sordariomycetes*, *Zygomycetes*, *Leotiomycetes* and *Pezizomycetes* were the dominant classes (average relative abundance >10%) (Fig. 3a). These four classes accounted for more than 91.1% of the bacterial sequences. *Tremellomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Agaricomycetes* and *Ascomycota* *incertae sedis* were also detected at relatively high abundances (average relative abundance > 0.1%) (Fig. 3b). Compared with water-treated soils, vanillin-treated soils had higher relative abundances of *Tremellomycetes*, Unclassified *Ascomycota* and *Ascomycota incertae sedis* but had lower relative abundances of *Sordariomycetes* and *Zygomycetes* (P<0.05).

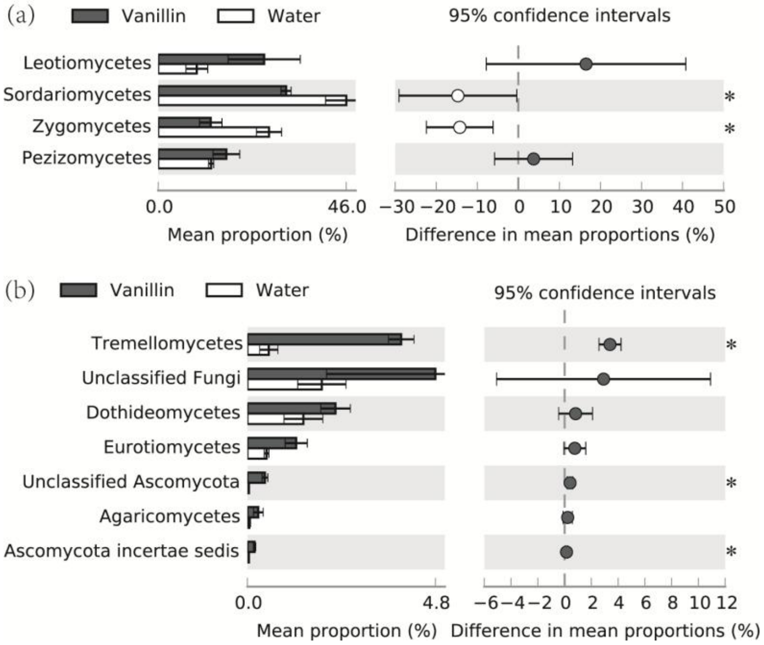

Figure 3. Relative abundances of main fungal classes in the water and vanillin-treated soils.
Fungal classes with average relative abundances >10% (a) and >0.1% (b) were shown in at least one treatment. Values in the bar plot are expressed as mean±standard error. The circles represent the 95% confidence intervals. Asterisks indicate significant difference between treatments based on Welch's *t* test (P<0.05).

More than 140 fungal genera were detected across all samples analyzed (data not shown). Among the classified genera, *Mortierella*, *Pseudallescheria*, *Gibberella*, *Cephaliophora*, *Humicola*, *Cryptococcus*, *Fusarium* and *Pseudaleuria* spp. had mean relative abundances higher than 1.0% (Fig. 4a). Compared with water-treated soils, vanillin-treated soils had higher relative abundances of *Humicola*, *Cryptococcus*, *Fusarium*, *Gibberella*, *Kernia*, *Chaetomium*, *Wardomyces*, *Guehomyces*, *Gymnoascus*, *Myrothecium*, *Stachybotrys*, *Penicillium*, *Acremonium*, *Aspergillus*, *Monographella*, *Pseudeurotium*, *Talaromyces* and *Nectria* spp. but had lower relative abundance of *Pseudallescheria* spp. (P< 0.05) (Fig. 4).

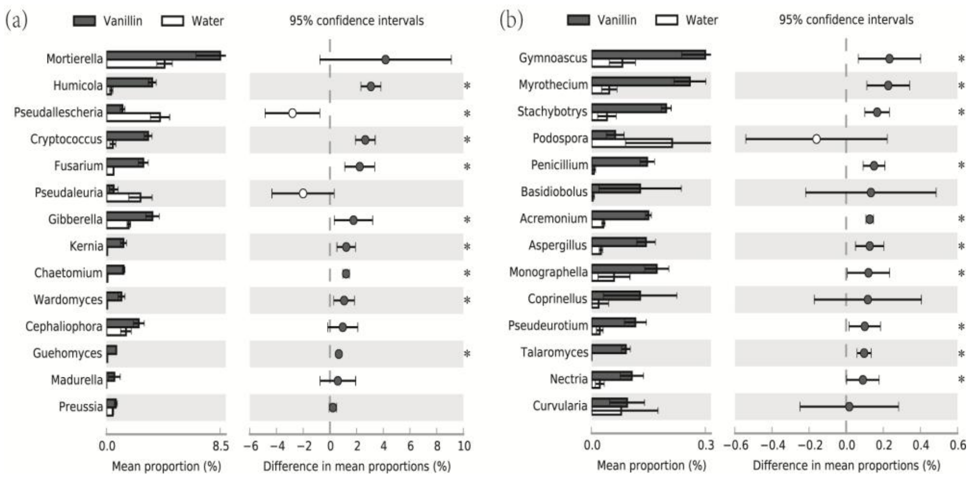

Figure 4. Relative abundances of main classified fungal genera in the water and vanillin-treated soils. Fungal genera with average relative abundances >0.5% (a) and >0.1% (b) were shown in at least one treatment. Values in the bar plot are expressed as mean±standard error. The circles represent the 95% confidence intervals. Asterisks indicate significant difference between treatments based on Welch's *t* test ($P<0.05$).

The concentration of vanillin in cucumber continuous monocropping cropping system was about 0.08 μmol/g soil DW (54). In both natural and agricultural ecosystems, the concentration soil phenolic compounds was shown to ranged from 0.01 to 0.5 μmol/g soil (31,32,54). Therefore, the concentration of vanillin used in this study **(**0.1 μmol/g dry soil) was within the realistic range of concentrations in the soil reported before

Crop disease index and soil-borne pathogens usually increase in continuous monocropping systems (26,48,53). For example, the abundance of soil-borne necrotrophic fungus *Fusarium oxysporum* f.sp. *cucumerinum* Owen (FOC), a yield-limiting factor in cucumber production, was higher in the cucumber monocropping system than in tomato-celery-cucumber-Chinese cabbage rotation system (48). In the present study, vanillin increased the relative abundances of several fungal taxa that contained potential plant pathogens, such as *Fusarium* (teleomorph *Gibberella*) (27), *Myrothecium* (24), *Stachybotrys* (25), *Acremonium* (38), *Aspergillus* (36) *Nectria* spp. (16). This confirmed our previous quantitative PCR analysis, which demonstrated that vanillin at 0.02-0.2 μmol/g soil concentrations increased the *Fusarium* spp. abundance (47).

Some fungi are pathogenic to plants, while some can promote the plant growth or/and inhibit plant pathogens (22,35). In this study, we found that vanillin decreased the relative abundance of *Pseudallescheria* spp. which is inhibitory to the plant pathogens. For example, *P. boydii* produces a fungistatic substance capable of reducing the disease incidence of black leaf spot in spoon cabbage caused by *Alternaria brassicicola* and inhibits the germination of *A. brassicicola* conidia (23). Therefore, the accumulation of vanillin may be linked to the increased plant disease index in monocropping systems by promoting the plant fungal pathogens and inhibiting the plant-beneficial fungi, which should be stressed in the future**.**

Many soil microorganisms use phenolic compounds as carbon resources (29). In this study, vanillin increased the relative abundances of fungal taxa with phenolic compound-degrading capability, such as *Humicola* (9), *Cryptococcus* (7), *Fusarium* (10), *Chaetomium* (15), *Guehomyces* (12), *Myrothecium* (33) and *Penicillium* (teleomorph *Talaromyces*) (10). These results confirmed previous research that showed phenolic compounds can stimulate the rhizosphere phenolic compound-utilizing microorganisms (4).

CONCLUSIONS

The exogenous application of vanillin (an autotoxin of cucumber) changed the cucumber seedling rhizosphere fungal community composition and decreased the fungal community richness and diversity. Importantly, vanillin increased the relative abundances of several fungal taxa that contained potential fungal pathogens (such as *Fusarium* and *Nectria* spp.), but increased the relative abundance of *Pseudallescheria* spp. which has plant pathogen-inhibiting ability. These indicated that, besides its direct toxic effects on cucumber, vanillin also play an important role in the interaction between cucumber and soil fungi. However, the concentration of vanillin used in this experiment may have been slightly higher than that found in the rhizosphere soil but our results suggest the possibility of stimulation of certain fungi by the presence of vanillin in the soil.

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REFERENCES

1. Acosta-Martínez, V. and Cotton, J. (2017). Lasting effects of soil health improvements with management changes in cotton-based cropping systems in a sandy soil. *Biology and Fertility of Soils* **53:** 533-546.
2. Badri, D.V., Chaparro, J.M., Zhang, R., Shen, Q. and Vivanco, J.M. (2013). Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *Journal of Biological Chemistry* **288:** 4502-4512.
3. Blum, U. (1996). Allelopathic interactions involving phenolic acids. *Journal of Nematology* **28:** 259-267.
4. Blum, U., Staman, K.L., Flint, L.J. and Shaffer, S.R. (2000). Induction and/or selection of phenolic acid-utilizing bulk-soil and rhizosphere bacteria and their influence on phenolic acid phytotoxicity. *Journal of Chemical Ecology* **26:** 2059-2078.
5. Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K. and Gordon, J.I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7:** 335-336.
6. Cook, R.J. (2006). Toward cropping systems that enhance productivity and sustainability. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 18389-18394.

7. Cooper, B.H. and Land, G.A. (1979). Assimilation of protocatechuic acid and *p*-hydroxybenzoic acid as an aid to laboratory identification of *Candida parapsilosis* and other medically important yeasts. *Journal of Clinical Microbiology* **10:** 343-345.
8. Crowther, T.W., Maynard, D.S., Leff, J.W., Oldfield, E.E., McCulley, R.L., Fierer, N. and Bradford, M.A. (2014). Predicting the responsiveness of soil biodiversity to deforestation: A cross-biome study. *Global Change Biology* **20:** 2983-2994.
9. Dao, T.H. (1987). Sorption and mineralization of plant phenolic acids in soil. *In: Allelochemicals: Role in Agriculture and Forestry* (Ed., G.R. Waller), p. 358-370. American Chemical Society, Washington DC, USA.
10. DeRito, C.M. and Madsen, E.L. (2009). Stable isotope probing reveals *Trichosporon* yeast to be active *in situ* in soil phenol metabolism. The *ISME Journal* **3:** 477-485.
11. Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460-2641.
12. Fernandez, P.M., Martorell, M.M., Blaser, M.G., Ruberto, L.A.M., de Figueroa, L.I.C. and Mac Cormack, W. (2017). Phenol degradation and heavy metal tolerance of Antarctic yeasts. *Extremophiles* **21:** 445-457.
13. Fierer, N. and Jackson, R.B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 626-631.
14. Gentry, L.F., Ruffo, M.L. and Below, F.E. (2013). Identifying factors controlling the continuous corn yield penalty. *Agronomy Journal* **105:** 295-303.
15. Haider, K. and Trojanowski, J. (1975). Decomposition of specifically ^{14}C-labelled phenols and dehydropolymers of coniferyl alcohol as models for lignin degradation by soft and white rot fungi. *Archives of Microbiology* **105:** 33-41.
16. Han, Y., Liu, X., Benny, U., Kistler, H.C. and VanEtten, H.D. (2001). Genes determining the pathogenicity to pea are clustered on a supernumerary chromosome in the fungal plant pathogen *Nectria haematococca*. *The Plant Journal* **25:** 305-314.
17. Huang, L., Song, L., Xia, X., Mao, W., Shi, K., Zhou, Y. and Yu, J. (2013). Plant-soil feedbacks and soil sickness: From mechanisms to application in agriculture. *Journal of Chemical Ecology* **39:** 232-242.
18. Huang, Y.Q., Han, L.S., Tao, T.T., Yao, Y.C., Yang, J.F., Liang, C.H., Xie, J.H. and Han, X.R. (2017). Bacterial community in peanut soils in various cropping systems. *Allelopathy Journal* **42:** 93-107.
19. Inderjit and Duke, S.O. (2003). Ecophysiological aspects of allelopathy. *Planta* **217:** 529-539.
20. Jia, X.L., Ye, J.H., Zhang, Q., Li, L., Hu, Y.L., Zheng, M.Z., Hong, Y.C., Wang, F.Q. and Wu, C.Z. (2017). Soil toxicity and microbial community structure of Wuyi rock tea plantation. *Allelopathy Journal* **41:** 113-126.
21. Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiß, M. and Larsson, K.H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22:** 5271-5277.
22. Kim, A., Shahzad, R., Kang, S., Khan, A.L., Lee, S., Park, Y., Lee, W. and Lee, I. (2017). *Paenibacillus terrae* AY-38 resistance against *Botrytis cinerea* in *Solanum lycopersicum* L. plants through defence hormones regulation. *Journal of Plant Interactions* **12:** 244-253.
23. Ko, W.H., Tsou, Y.J., Ju, Y.M., Hsieh, H.M. and Ann, P.J. (2010). Production of a fungistatic substance by *Pseudallescheria boydii* isolated from soil amended with vegetable tissues and its significance. *Mycopathologia* **169:** 125-131.
24. Kuti, J.O., Ng, T.J. and Bean, G.A. (1989). Possible involvement of a pathogen-produced trichothecene metabolite in *Myrothecium* leaf spot of muskmelon. *Physiological and Molecular Plant Pathology* **34:** 41-54.
25. Li, S. and Hartman, G.L. (2000). First report of *Stachybotrys chartarum* causing soyabean root rot. *Plant Disease* **84:** 100.
26. Liu, F.F., Zhang, A.H., Lei, F.J., Zhang, J., Xu, Y.H., Yin, M.J. and Zhang, L.X. (2017). Inhibitory effects of *Panax ginseng* stem and leaf ginsenosides against *Fusarium solani*. *Allelopathy Journal* **40:** 163-171.
27. Ma, L.J., Geiser, D.M., Proctor, R.H., Rooney, A.P., O'Donnell, K., Trail, F., Gardiner, D.M., Manners, J.M.

and Kazan, K. (2013). Fusarium pathogenomics. *Annual Review of Microbiology* **67:** 399-416.

28. Magoc, T. and Salzberg, S.L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27:** 2957-2963.
29. Margesin, R., Bergauer, P. and Gander, S. (2004). Degradation of phenol and toxicity of phenolic compounds: A comparison of cold-tolerant *Arthrobacter* sp. and mesophilic *Pseudomonas putida*. *Extremophiles* **8:** 201-207.
30. Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.M., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L. and Bakker, P.A.H.M. (2011). Deciphering the rhizosphere microbiome for disease- suppressive bacteria. *Science* **332:** 1097-1100.
31. Muscolo, A. and Sidari, M. (2006). Seasonal fluctuations in soil phenolics of a coniferous forest: effects on seed germination of different coniferous species. *Plant and Soil* **284:** 305-318.
32. Piotrowski, J.S., Morford, S.L. and Rillig, M.C. (2008). Inhibition of colonization by a native arbuscular mycorrhizal fungal community via *Populus trichocarpa* litter, litter extract, and soluble phenolic compounds. *Soil Biology and Biochemistry* **40:** 709-717.
33. Podieiablonskaia, E.V., Kolomytseva, M.P., Myasoedova, N.M., Baskunov, B.P., Chernykh, A.M., Classen, T., Pietruszka, J. and Golovleva, L.A. (2017). *Myrothecium verrucaria* F-3851, a producer of laccases transforming phenolic compounds at neutral and alkaline conditions. *Microbiology* **86:** 370-376.
34. Politycka, B., Smolen, S., Golcz, A. and Lukaszewicz, S. (2017). Effects of selenium on contents of selected micronutrients and activities of antioxidant enzymes in cucumber roots exposed to *p*-coumaric acid. *Allelopathy Journal* **41:** 101-112.
35. Rahmoune, B., Morsli, A., Khelifi-Slaoui, M., Khelifi, L., Strueh, A., Erban, A., Kopka, J., Prell, J. and van Dongen, J.T. (2017). Isolation and characterization of three new PGPR and their effects on the growth of *Arabidopsis* and *Datura* plants. *Journal of Plant Interactions* **12:** 1-6.
36. Satish, S., Mohana, D.C., Raghavendra, M.P. and Raveesha, K.A. (2007). Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *Journal of Agricultural Technology* **3:** 109-119.
37. Schöler, A., Jacquiod, S., Vestergaard, G., Schulz, S. and Schloter, M. (2017). Analysis of soil microbial communities based on amplicon sequencing of marker genes. *Biology and Fertility of Soils* **53:** 485-489.
38. Schroers, H.J., Geldenhuis, M.M., Wingfield, M.J., Schoeman, M.H., Yen, Y.F., Shen, W.C. and Wingfield, B.D. (2005). Classification of the guava wilt fungus *Myxosporium psidii*, the palm pathogen *Gliocladium vermoesenii* and the persimmon wilt fungus *Acremonium diospyri* in *Nalanthamala*. *Mycologia* **97:** 375-395.
39. Shafer, S.R. and Blum, U. (1991). Influence of phenolic acids on microbial populations in the rhizosphere of cucumber. *Journal of Chemical Ecology* **17:** 369-389.
40. Singh, H.P., Batish, D.R. and Kohli, R.K. (1999). Autotoxicity: Concept, organisms and ecological significance. *Critical Reviews in Plant Sciences* **18:** 757-772.
41. Trezzi, M.M., Vidal, R.A., Balbinot, A.A., Bittencourt, H.V. and Souza, A. (2016). Allelopathy: Driving mechanisms governing its activity in agriculture. *Journal of Plant Interactions* **11:** 53-60.
42. Turrini, A., Sbrana, C., Avio, L., Njeru, E.M., Bocci, G., Barberi, P. and Giovannetti, M. (2016). Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. *Biology and Fertility of Soils* **52:** 643-653.
43. van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J. and Wardle, D.A. (2013). Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology* **101:** 265-276.
44. Vestergaard, G., Schulz, S., Schöler, A. and Schloter, M. (2017). Making big data smart-how to use metagenomics to understand soil quality. *Biology and Fertility of Soils* **53:** 479-484.
45. Yu, J.Q. and Matsui, Y. (1994). Phytotoxic substances in the root exudates of *Cucumis sativus* L. *Journal of Chemical Ecology* **20:** 21-31.
46. Zhou, X., Gao, D., Zhao, M., Zhang, J., Li, L. and Wu, F. (2016). Dynamics of soil bacterial communities in Jerusalem artichoke monocropping system. *Allelopathy Journal* **39:** 167-178.
47. Zhou, X., Jia, H., Ge, X. and Wu, F. (2018). Effects of vanillin on the community structures and abundances of *Fusarium* and *Trichoderma* spp. in cucumber seedling rhizosphere. *Journal of Plant Interactions* **13:** 45-50.

48. Zhou, X., Liu, J. and Wu, F. (2017). Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. *Plant and Soil* **415:** 507-520.
49. Zhou, X., Wang, Z., Pan, D. and Wu, F. (2018). Effects of vanillin on cucumber (*Cucumis sativus* L.) seedling rhizosphere *Bacillus* and *Pseudomonas* spp. community structures. *Allelopathy Journal* **43:** 255-264.
50. Zhou, X. and Wu, F. (2012). Dynamics of the diversity of fungal and *Fusarium* communities during continuous cropping of cucumber in the greenhouse. *FEMS Microbiology Ecology* **80:** 469-478.
51. Zhou, X. and Wu, F. (2012). *p*-Coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of *Fusarium oxysporum* f. sp. *cucumerinum* Owen. *PloS one* **7:** e48288.
52. Zhou, X., Wu, F. and Xiang, W. (2015). Effects of vanillin on rhizosphere microbial communities of cucumber (*Cucumis sativus* L.) seedlings. *Allelopathy Journal* **35:** 49-60.
53. Zhou, X., Yu, G. and Wu, F. (2011). Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. *European Journal of Soil Biology* **47:** 279-287.
54. Zhou, X., Yu, G. and Wu, F. (2012). Soil phenolics in a continuously mono-cropped cucumber (*Cucumis sativus* L.) system and their effects on cucumber seedling growth and soil microbial communities. *European Journal of Soil Science* **63:** 332-340.
55. Zhou, X., Zhang, J., Pan, D., Ge, X., Jin, X., Chen, S. and Wu, F. (2018). *p*-Coumaric can alter the composition of cucumber rhizosphere microbial communities and induce negative plant-microbial interactions. *Biology and Fertility of Soils* **54:** 363-372.