**Chemical structure predicts the effect of plant-derived low molecular weight compounds on soil microbiome structure and pathogen suppression**

Yian Gu1,2, Xiaofang Wang1, Tianjie Yang1, Ville-Petri Friman1,3, Stefan Geisen4,5, Zhong Wei1,\*, Yangchun Xu1,\*, Alexandre Jousset1,6, Qirong Shen1

1 Nanjing Agricultural University, Jiangsu Key Laboratory for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing, 210095, PR China

2 Huaiyin Normal University, Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, Jiangsu Collaborative Innovation Center of Regional Modern Agriculture & Environmental Protection, Huaian, 223300, PR China

3 University of York, Department of Biology, Wentworth Way, York, YO10 5DD, UK

4 Netherlands Institute of Ecology (NIOO-KNAW), Department of Terrestrial Ecology, PO Box 50, 6700 AB Wageningen, The Netherlands

5 Department of Plant Science, Laboratory of Nematology, Wageningen University, 6700 ES Wageningen, the Netherlands

6 Utrecht University, Institute for Environmental Biology, Ecology & Biodiversity, Padualaan 8, 3584CH Utrecht, The Netherlands

\* Corresponding authors

E-mail address: weizhong@njau.edu.cn (Zhong Wei) or ycxu@njau.edu.cn (Yangchun Xu), Tel.: +86 025 84396291; fax: +86 025 84396260.

**Abstract**

1. Plant-derived low molecular weight compounds play a crucial role in shaping soil microbiome functionality. While various compounds have been demonstrated to affect soil microbes, most data are case-specific and do not provide generalizable predictions on their effects. Here we show that the chemical structural affiliation of low molecular weight compounds typically secreted by plant roots – sugars, amino acids, organic acids and phenolic acids – can predictably affect microbiome diversity, composition and functioning in terms of plant disease suppression.

2. We amended soil with single or mixtures of representative compounds, mimicking carbon deposition by plants. We then assessed how different classes of compounds, or their combinations, affected microbiome composition and the protection of tomato plants from the soil-borne *Ralstonia solanacearum* bacterial pathogen.

3. We found that chemical class predicted well the changes in microbiome composition and diversity. Organic and amino acids generally decreased the microbiome diversity compared to sugars and phenolic acids. These changes were also linked to disease incidence, with amino acids and nitrogen-containing compound mixtures inducing more severe disease symptoms connected with a reduction in bacterial community diversity.

4. Together, our results demonstrate that low molecular weight compounds can predictably steer rhizosphere microbiome functioning providing guidelines to engineer microbiomes based on root exudation patterns by specific plant cultivars or crop regimes.

**KEYWORDS**

Chemical structure, Plant-derived low molecular weight compounds, Soil microbiome, Soil suppressiveness

**1 INTRODUCTION**

The soil and root-associated microbiome comprise an essential component of plant health ([Berendsen, Pieterse & Bakker, 2012](#_ENREF_4); [Paredes & Lebeis, 2016](#_ENREF_38)) and there is a growing interest in harnessing the benefits of soil microbiome for sustainable food production ([Chaparro, Sheflin, Manter & Vivanco, 2012](#_ENREF_9); [de Vries, Griffiths, Knight, Nicolitch & Williams, 2020](#_ENREF_12)). In this interaction, plants shape the surrounding microbial communities by secreting low molecular weight organic compounds to the rhizosphere ([Carvalhais *et al.*, 2015](#_ENREF_8)), which can enrich distinct microbial groups and promote their activity ([Eilers, Lauber, Knight & Fierer, 2010](#_ENREF_16)). In turn, enriched microorganisms can have a positive effect on the plant performance ([Berendsen *et al.*, 2012](#_ENREF_4)). Over time, this process can lead to selection for specific rhizosphere microbial communities that are beneficial for the plant and can remain in the soil even between the crop seasons leading to a phenomena called plant-soil feedbacks or soil legacy effects ([Kardol, Bezemer & Van Der Putten, 2006](#_ENREF_28)).

Recent studies suggest that secretion of specific antimicrobial compounds by competing bacteria can change microbial community activity and composition via interference competition ([Mehrabi *et al.*, 2016](#_ENREF_37); [Wei *et al.*, 2019](#_ENREF_55)). Similar effects could also be triggered by plant-derived compounds that can shape the microbial community structure via both facilitative and inhibitory effects ([Jousset, Schmid, Scheu & Eisenhauer, 2011](#_ENREF_27); [Zwetsloot, Kessler & Bauerle, 2018](#_ENREF_62)). For example, plant-derived root exudates form an important source of nutrients for the rhizosphere bacteria. These low molecular weight organic compounds typically consist of amino acids, sugars, phenolic acids, and organic acids ([Bais, Weir, Perry, Gilroy & Vivanco, 2006](#_ENREF_2); [Badri, Chaparro, Zhang, Shen & Vivanco, 2013](#_ENREF_1)). While sugars and organic acids represent a source of carbon, amino acids are important for providing microbes nitrogen, phenolics typically inhibit microbial activity and this effect is often taxa-specific ([Badri *et al.*, 2013](#_ENREF_1)). These compounds can then increase the relative abundance of certain microbes in the available species pool that can in turn provide benefits for the plant in terms of growth and immune activation ([Hu *et al.*, 2018](#_ENREF_25); [Rolfe, Griffiths & Ton, 2019](#_ENREF_42)). Such cascades can also be triggered by pathogenic bacteria. For example, the foliar pathogen *Pseudomonas syringae* pv *tomato* (Pst DC3000) can indirectly change the microbial community composition by shifting *Arabidopsis thaliana* root exudation, which will improve disease resistance during subsequent plant generation ([Yuan *et al.*, 2018](#_ENREF_60)). Similarly, it has been shown that *Ralstonia solanacearum* plant pathogen invasion can trigger changes in the tomato root exudation patterns leading to reduced microbial diversity via increased exudation of phenolic compounds that are also harmful for the pathogen ([Gu *et al.*, 2016](#_ENREF_23)). As a result, breeding new plant cultivars with improved ability to actively recruit beneficial microbes using secreted low molecular weight organic compounds could be an important avenue for sustainable crop production ([Perez-Jaramillo, Mendes & Raaijmakers, 2016](#_ENREF_39); [Preece & Penuelas, 2020](#_ENREF_40)). While considerable body of research exists on the role of single molecules in isolation ([Eilers *et al.*, 2010](#_ENREF_16); [Hu *et al.*, 2018](#_ENREF_25)), or root exudates as a whole ([Badri *et al.*, 2013](#_ENREF_1)), there is less information on the role of specific classes of compounds on the microbiome composition and disease suppression. As a result, we lack a holistic framework that could predict how certain compounds or compound combinations might change microbiome functioning. Here we present an experimental framework allowing the prediction of how plant-derived low molecular weight organic compounds shape the microbiome diversity and functioning on the basis of their chemical structure.

To achieve this, we first screened the effect of a range of molecules belonging to different chemical classes, that are typically secreted by plants, on microbiome composition by amending natural soil with a total of 48 low molecular weight compounds falling into four chemical functional classes: amino acids, sugars, phenolic acids and organic acids (applied twice a week for a total of six weeks; soil conditioning experiment). We then assessed the changes in microbiome biomass, composition and diversity and tested if the compound-mediated effects could predict the functioning of microbial communities in terms of suppression of soil-borne bacterial pathogen *R. solanacearum* in the tomato rhizosphere - an economically important pathogen causing bacterial wilt ([Genin, 2010](#_ENREF_21)). We hypothesized that 1) different functional chemical classes would have distinct effects on the soil microbiome structure by selectively recruiting and repelling different bacterial taxa, and that, 2) these changes would correlate with microbiome ability to suppress plant pathogen, providing a predictive framework for steering rhizosphere microbiome functioning based on low molecular weight compounds.

**2 MATERALS AND METHODS**

**2.1 Preparation and assembly of low molecular weight organic compounds**

We selected 48 low molecular weight organic compounds (12 sugars, 12 amino acids, 12 organic acids and 12 phenolic acids; Supporting information, Table S1) typically found in tomato tissues or root exudates. In addition to all 48 mono-compounds, we assembled 16-compound mixtures using 54 independent low molecular weight compound combinations (Table S2). Each combination was randomly composed of four sugars, four amino acids, four organic acids and four phenolic acids, and each of the 48 carbon resources was included in a total of 18 different combinations. Each compound, or compound mixture, was prepared as a standardized solution of 1.3125 g C L-1 in 20% (v:v) methanol, which is an established procedure used in several studies bringing the compromise between solubilization of all substances and a minimal impact on soil microbiome ([Qu & Wang, 2008](#_ENREF_41); [Lanoue *et al.*, 2009](#_ENREF_32); [Zhou & Wu, 2012](#_ENREF_61); [Badri *et al.*, 2013](#_ENREF_1)). Each solution was adjusted to a pH of 7.0 to prevent differences in acidity from biasing the results in microbiome assembly ([Fierer & Jackson, 2006](#_ENREF_20)). None of the compounds had a charge, and were mostly complex and inert carbon compounds. As a result, no signs of chemical reactions (e.g., gas or precipitation production or color change) were observed when preparing solution of compound mixtures. As methanol is also a carbon source and could potentially affect the community composition, we included a methanol-only treatment to account for its effect. Water-only treatment was also included to compare the effects of water-only and solvent-only treatments on soil bacterial community composition.

**2.2 Screening the effects of different compounds in soil conditioning experiment**

We conditioned soil with different low molecular weight compounds to assess their effect on microbiome composition and abundance using a topsoil (0-20 cm) collected from a tomato field in Qilin (Nanjing, China; 118°57’E, 32°03’N). This field has been infested with the bacterial plant pathogen *R. solanacearum* for more than 15 years. The soil is a yellow-brown earth (Udic Argosol) with pH of 5.4, total C of 19.7 g kg-1, total N of 6.3 g kg-1, available K of 178 mg kg-1, available P of 172.9 mg kg-1, and organic matter content of 24.6 g kg-1.The soil was sieved (< 4 mm) and homogenized thoroughly. The soil was then divided to replicate pots (35 cm × 25 cm × 10 cm) each containing 600 g (dry weight) of soil for the experiment. The effect of each compound or compound mixture was tested independently using the following experimental design: four replicates were used for control (methanol-only) and three replicates for each of the single compound (48 treatments) and 16-compound treatments (54 treatments) (Table S2). Each replicate consisted of two independent technical replicate pots to ensure we had enough conditioned soil for the second experiment (soil suppressiveness). As the daily input of carbon secreted by tomato has not previously been reported, we used estimates derived from maize and oat in sand soils: 0.05-0.1 mg C d-1 g-1 ([Trofymow, Coleman & Cambardella, 1987](#_ENREF_50); [Iijima, Griffiths & Bengough, 2000](#_ENREF_26); [Baudoin, Benizri & Guckert, 2003](#_ENREF_3)). This is likely to be conservative estimate as the amount of carbon in root exudates of tomato has been reported to be greater compared to maize, wheat and barley ([Whipps, 1987](#_ENREF_58)). To mimic natural exudation, we applied low molecular weight organic compound mixtures twice a week at every 84 hours for a total of six weeks. At each time of application, every pot received 0.075 mg C g-1 soil d-1 of low molecular weight organic compounds (120 ml per pot). The soils were then thoroughly mixed with sterile 5 mL tips to ensure even distribution of compounds. Pots were maintained in the greenhouse with natural temperature variation ranging between 20-32 °C and soil samples were collected after 6 weeks of conditioning for DNA extraction for the bacterial community composition analysis as described below.

**a) Soil DNA extraction**

Four soil cores (1 cm diameter × 10 cm deep) were collected from each pot and soil samples from two pots were mixed together resulting in three and four biological replicates for carbon and control treatments, respectively. We used 0.5 g subsamples of homogenized soil cores for DNA extraction with MoBio PowerSoil DNA extraction kit (Carlsbad, CA, USA) following the manufacturer’s instructions. The quantity and quality of DNA was measured using a NanoDrop (ND2000, ThermoScientific, DE, USA) spectrophotometer and aliquots of DNA were used for quantitative PCR analyses (qPCR) and 16S rRNA amplicon sequencing.

**b) Quantification of total bacterial abundances and pathogen density in the soil samples**

Quantitative PCR assays were performed to determine 1) *R. solanacearum* pathogen densities and 2) total bacterial biomass using the SYBRPremix Ex TaqTM Kit (Takara, Dalian, China) and a 7500 Fast Real-Time PCR System (Applied Biosystems, CA, USA). The specific primer sets Rsol\_*fli*C ([Schonfeld, Heuer, van Elsas & Smalla, 2003](#_ENREF_46)) and universal primer sets Eub338/Eub518 ([Fierer, Jackson, Vilgalys & Jackson, 2005](#_ENREF_19)) were used to target the *R. solanacearum fli*C gene and V4 regions of the bacterial 16S rRNA genes, respectively. Plasmid standard (pMD 19-T vector; Takara, Dalian, China) was generated from cloned target genes (*fli*C gene or 16S rRNA gene) using the *R. solanacearum* strain QL-Rs1115, which is the dominant genotype in the field from where the soil was originally collected ([Wei *et al.*, 2011](#_ENREF_57)). The standard curves were generated according to a previously published protocol ([Cao *et al.*, 2011](#_ENREF_6)) and each individual sample was measured in triplicate.

**c) Determining bacterial community diversity and composition in the soil samples**

We used 16S rRNA amplicon sequencing to determine bacterial community composition and diversity in the soil samples. Three DNA products from each mono-compound and 16-compound carbon treatment replicates were pooled into one. As a result, the differences in bacterial community diversity and composition were compared at carbon class and combination level (N = 12 and N = 54 for each mono and 16-compound carbon treatments). The V4 region of the bacterial 16S rRNA genes was amplified using a primer set 563F and 802R ([Cardenas *et al.*, 2010](#_ENREF_7)) with attached Illumina flow cell adapters under optimized PCR conditions described previously ([Gu *et al.*, 2016](#_ENREF_23)). The PCR products were purified using an AxyPrep PCR Clean-up Kit (Axygen Biosciences, Union City, CA, USA), DNA quantity determined with QuantiFluorTM-ST (Promega, WI, USA) and final samples sequenced using Illumina MiSeq sequencing Shanghai (2x250PE, Biozeron Bio-technology Co., Ltd). Sequence reads were processed using `the UPARSE pipeline ([Edgar, 2013](#_ENREF_14)). Briefly, read pairs from each sample were assembled and low-quality nucleotides (maximal expected error of 0.25), short reads (< 200 bp) and singletons removed. Sequence reads were then clustered into operational taxonomic units (OTUs) using 3% dissimilarity cutoff point and chimeric sequences removed using UCHIME ([Edgar, Haas, Clemente, Quince & Knight, 2011](#_ENREF_15)). We obtained between 25,013 to 38,779 (mean = 31,032) quality-filtered sequences for all the soil samples. The representative sequences and OTU tables were then analyzed using Mothur ([Schloss *et al.*, 2009](#_ENREF_45)), the sampling depth was equalized using the lowest number of sequences detected in any of the samples (25,013) and taxonomic assignment performed using the RDP 16S rRNA classifier ([Wang, Garrity, Tiedje & Cole, 2007](#_ENREF_54)).

**2.3 The suppression of bacterial wilt disease incidence**

In the second experiment, we assessed how soil conditioning affected microbiome functioning in terms of bacterial wilt disease suppression. We first quantified *R. solanacearum* densities in each sample at the end of the soil conditioning experiment using serial dilution plating on selective SMSA media ([Elphinstone, Hennessy, Wilson & Stead, 1996](#_ENREF_17)). We then adjusted the *R. solanacearum* densities to 5 × 106 CFU g-1 soil in every treatment using drenching method (see below) two days after the end of soil conditioning experiment using overnight cultures of *R. solanacearum* QL-Rs1115 strain previously isolated from the same field ([Wei *et al.*, 2011](#_ENREF_57)). Prior the experiment, one single colony was grown overnight in nutrient broth (NB) media, harvested by centrifugation (10,000×g for 8 min) and washed twice with a sterile saline solution (0.9% NaCl). Pathogen stock culture density was then estimated based on optical density (OD600) and further verified by serial dilution count assay on NB media. 50 mL of the suspension (diluted based on formerly dilution count results on selective SMSA media) was used to drench soils. Soils were incubated for three days to allow the stabilization of the inoculated pathogen and serial dilution and plating used to verify that pathogen densities remained within one order of magnitude of inoculated levels. Tomato plants were prepared for the inoculation as follows. Tomato seeds (*Solanum lycopersicum* cv. ‘Jiangshu’) were surface-sterilized with 3% NaClO for 5 min, rinsed four times in sterile distilled water and germinated in the dark at 30 °C for two days. Germinated seeds were then sowed in nursery pots (6 cm × 6 cm × 6 cm) in nursery substrate (Huainong, Huaian soil and fertilizer institute, Huaian, China). After ten days of growth (corresponding to five days after the end of soil conditioning experiment), each tomato seedling was gently washed and four seedlings transplanted to larger pot (35cm × 25 cm × 10 cm) containing 600 g dry weight of homogenized soil originating from soil conditioning experiment. Both technical replicates from the soil conditioning experiment were used and eight seedlings from two technical replicates were considered as one biological replicate. Four replicates were used for control (methanol-only, N = 4) and three replicates for mono-compound (N = 144) and 16-compound mixtures (N = 162) resulting in 2480 plants (620 pots with 4 seedlings each). It should be noted that no low molecular weight compounds were added to the soil in the second experiment. Similar to the soil conditioning experiment, disease suppression experiment was conducted in a greenhouse with a natural temperature variation ranging between 25-35 °C. The severity of bacterial wilt disease was recorded 40 days after transplantation as a disease index on a scale of 0-4 (0 = no wilting, 1 = 1-25% of leaves wilted, 2 = 26-50% of leaves wilted, 3 = 51-75% of leaves wilted and 4 = 76-100% of leaves wilted). Disease incidence was then determined as = [ ∑ (number of diseased plants in given disease index × given disease index) × (total number of plants × highest disease index)-1] × 100% ([Chen *et al.*, 2013](#_ENREF_11)).

**2.4 Statistical analyses**

Analysis of variance (ANOVA, Tukey’s honestly significant difference test) and Student’s *t* test were used to compare mean differences between the treatments using SPSS (v. 19). The soil microbiome composition was ordinated by principal coordinates analysis (PCoA) based on unweighted UniFrac distances (phylogeny-based distance metric) ([Lozupone & Knight, 2005](#_ENREF_36)) and microbiome compared using analysis of molecular variance (AMOVA). Principal component analysis (PCA) was also used to summarize variation in the composition of soil bacterial communities using CANOCO ([Etten, 2005](#_ENREF_18)). In this analysis, the top 10% of most significant OTUs (based on linear discriminant analysis where LDA scores > 3 using Mothur ([Schloss *et al.*, 2009](#_ENREF_45))) were included in the PCA and the input data was log transformed before the analysis. The OTUs that were significantly associated with the solvent-only and water-only control treatments were screened using DESeq2 ([Love, Huber & Anders, 2014](#_ENREF_35)). Structural equation modeling (SEM) was conducted with R package lavaan ([Rosseel, 2012](#_ENREF_43)) to compare the direct and indirect effects (via soil microbiome) of plant-derived compounds on disease incidence and to visualize effects as simple networks. The first step of SEM requires establishing an initial model based on theoretical causal relationships between variables ([Delgado-Baquerizo *et al.*, 2016](#_ENREF_13); [Trivedi *et al.*, 2016](#_ENREF_49)). The fit of the initial model is then determined and adjusted in case of a poor fit ([Lamb, 2008](#_ENREF_31); [Latz, Eisenhauer, Rall, Scheu & Jousset, 2016](#_ENREF_33)). In this study, the initial SEMmodels were generated based on relationships between low molecular weight compound classes and ratio of nitrogen addition, soil bacterial community diversity and the severity of bacterial wilt disease (Fig. S2). The fit of the initial models were tested using χ2-test (the model has a good fit with low χ2 value (~ ≤ 2) and high *p*-value (traditionally > 0.05)) and the root mean square error of approximation (RMSEA; the model has a good fit with low RMSEA value (~ ≤ 0.05) and high *p*-value (traditionally > 0.05)) ([Delgado-Baquerizo *et al.*, 2016](#_ENREF_13)). The total observed bacterial community richness (Sobs index) was used as input data for bacterial community diversity in SEMs.

**3 RESULTS**

**3.1 The chemical class of plant-derived compounds predicts the abundance, richness and composition of soil bacterial communities**

Most plant-derived compounds had a strong impact on total bacterial abundances and community richness after 6 weeks of soil conditioning (Fig. 1 and Fig. S3). The amendment of 16-compound mixture, organic acids and amino acids generally increased bacterial abundances but decreased the richness of soil bacterial communities relative to the control treatment (Fig. 1a-b). In contrast, sugars and phenolic acids had no clear effects on the abundance and richness of bacterial communities (*p* > 0.05 in all pairwise comparison). Thirty-six of the 48 molecules, including all the organic acids and amino acids, increased the total bacterial abundances, while five sugars (Melibiose, D-Galactose, Sucrose, L-Rhamnose and D-Mannitol) and five phenolic acids (Coumaric acid, Salicylic acid, Gallic acid, Syringic acid, and Cinnamic acid) decreased the total bacterial abundances (Fig. S3a). Six of the 48 carbons (one sugar: L-Rhamnose and five phenolic acids: Benzoic acid, p-Hydroxybenzoic, Cinnamic acid, Coumaric acid, and Salicylic acid) increased bacterial community richness, while other compounds decreased bacterial community richness (Fig. S3b).

The methanol-only treatment had slight effects on soil bacterial community composition compared to the water control (*p* = 0.03, analysis of molecular variance (AMOVA); Fig. S1a and External Databases S1): it enriched a few OTUs belonging to Gemmatimonadetes and Candidatus Saccharibacteria and decreased some OTUs belonging to Planctomycetes (Fig. S1b). The methanol had however smaller effects compared to those of plant-derived compounds (Fig. S1c-d) and we thus used this treatment as a baseline in further analyses. Principal coordinates analysis revealed that the chemical class significantly explained the differences in bacterial community composition (d.f. = 5, MS = 0.51, *p* < 0.001, Fs = 3.1, AMOVA; Fig. 1c). Compared to the control treatment, amino acids (d.f. = 1, MS = 0.38, *p* < 0.001, Fs = 2.3, AMOVA with post hoc test) and 16-compound mixtures (d.f. = 1, MS = 0.48 , *p* < 0.001 , Fs = 2.7, AMOVA with post hoc test) had similar (d.f. = 1, MS = 0.19, *p* = 0.266, Fs = 1.1, AMOVA with post hoc test) and relatively strongest impacts on the soil bacterial community composition followed by organic acids (d.f. = 1, MS = 0.23, *p* = 0.009, Fs = 1.6, AMOVA with post hoc test; Fig. S4). In contrast, phenolic acids (d.f. = 1, MS = 0.19, *p* = 0.006, Fs = 1.4, AMOVA with post hoc test) and sugars (d.f. = 1, MS = 0.14, *p* = 0.106, Fs = 1.1, AMOVA with post hoc test) had relatively smaller effects on soil bacterial community composition.

Overall, addition of compounds mainly affected the relative abundance of Proteobacteria, Bacteroidetes, and Actinobacteria (LDA, Fig. 2 and External Databases S2), with each chemical class being associated with a specific subset of species. For example, 16-compound mixtures were associated with relatively high proportion of OTUs belonging to Proteobacteria and Firmicutes (83.3%), phenolic acids with Actinobacteria (48%) and amino acids and organic acids with Bacteroidetes (55.6% and 35.7%, respectively). While amino acids and 16-compound mixtures were associated with a specific subset of species (Fig. 2), they mainly enriched OTUs belonging to Proteobacteria and Actinobacteria compared to other compounds (Fig. S5). Based on PCA results, we identified twenty genera that significantly explained most of the variation in the soil bacterial community composition (Fig. S6). For example, sugar treatments showed higher relative abundance of *Bradyrhizobium* (F5,100 = 21.3, *p* < 0.001, ANOVA), *Flavitalea* (F5,100 = 12.8 , *p* < 0.001, ANOVA), and *Spartobacteria* (F5,100 = 21.9, *p* < 0.001, ANOVA), while amino acids and the 16-compound mixtures enriched the relative abundance of *Mycobacterium* (F5,100 = 15.5, *p* < 0.001, ANOVA). Compared to phenolic acids treatments, organic acids enriched the relative abundance of *Achromobacteria* (Student’s *t* test, *p* < 0.001) and decreased the relative abundance of *Terrabacter* (Student’s *t* test, *p* = 0.001) and *Ornithinibacillus* (Student’s *t* test, *p* < 0.001).

**3.2 Effect of soil conditioning on pathogen densities**

Soil conditioning had contrasting effects on pathogen densities varying from a 4-fold reduction to 70-fold increase compared to the control treatment, and these effects were driven by specific compounds instead of chemical class (Fig 3a and S7a). Specifically, four sugars (Melibiose, Sucrose, L-Rhamnose and D-Mannitol), two amino acids (Citrulline, β-Alanine) and one phenolic acid (Coumaric acid) clearly decreased the pathogen densities. In contrast, 37 compounds including all the tested organic acids increased the pathogen densities (Fig. S7a). Pathogen densities were strongly correlated with total bacterial densities (Table 1), indicating that the introduced compounds acted as a general growth stimulants or inhibitors instead of specifically affecting the pathogen.

**3.3 Effect of soil conditioning on disease suppression by the soil microbiome**

Soil conditioning further led to a strong variation in microbiome functioning in terms of bacterial wilt disease suppression. The soils conditioned with amino acids, and in some cases with 16-compound mixtures, showed a sharp increase in disease incidence compared to the other treatments (ANOVA with Tukey post hoc test; Fig. 3b, Table S3). More specifically, 12 carbons including five sugars (Sucrose, Maltose, L-Rhamnose, D-Xylose and L-Arabinose), four organic acids (Succinic acid, Pyruvic acid, Lactic acid and Acetic acid) and three phenolic acids (Coumaric acid, Ferulic acid, and Cinnamic acid) significantly decreased the disease incidence (Fig. S7b). In contrast, 23 carbons including all the amino acids increased the disease incidence (Fig. S7b). The variation in disease incidence was explained by soil conditioning-mediated changes in microbiome species richness and total bacterial abundances (Fig. 3c, Table 1). For example, amino acids treatment was associated with a decrease in microbiome diversity and a subsequent increase in disease incidence.

**3.4 The rate of nitrogen addition played a key role in determining soil bacterial community diversity and functioning**

Because amino acids were the only compound class containing nitrogen, we assessed if its effects on soil bacterial community diversity and functioning were due to nitrogen fertilization. We first calculated the amount of nitrogen present in different amino acids and 16-compound mixtures, and analyzed its relationship with bacterial community abundance, diversity and functioning (effect on disease incidence). We found that the nitrogen addition rate correlated positively with the total bacterial abundances (Fig. 4a), and negatively with bacterial community richness (Fig. 4b). No significant correlation was observed between nitrogen addition rate and *R. solanacearum* abundances at the end of soil conditioning experiment (Fig. 4c). However, nitrogen addition rate was positively associated with the disease incidence (Fig. 4d).

**3.5 Changes in microbiome composition can protect plants from bacterial wilt**

We used structural equation modeling (SEMs) to describe the direct and indirect effects of soil conditioning on soil microbiome composition and functioning (effect on disease incidence). Two SEMs were constructed based on chemical class and nitrogen content of the organic compounds. The final SEMs provided a good fit with our data based on RMSEA and χ2-test (Fig. 5). In the first SEM model, we found that organic and amino acid treatments were directly linked with high soil bacterial abundances and low bacterial community richness during the soil conditioning experiment (Fig. 5a). Moreover, amino acids were directly linked with a high disease incidence at the end of the greenhouse experiment (Fig. 5a). Crucially, bacterial community richness measured at the end of the soil conditioning experiment was negatively linked with pathogen densities at the end of the subsequent greenhouse experiment (Fig. 5a). In the second SEM, the nitrogen addition rate was negatively linked with bacterial community richness and positively linked with soil bacterial abundances at the end of soil conditioning experiment (Fig. 5b). Moreover, mono-compounds and 16-compound mixtures with high nitrogen contents were positively correlated with a high disease incidence at the end of the greenhouse experiment.

**4 DISCUSSION**

In this study, we explored the effects of different chemical classes of typical plant-derived compounds on soil microbiome composition, abundance and disease suppression. We observed that while *R. solanacearum* pathogen density was generally promoted by amendments, this increase was not always associated with high disease incidence. Instead, nitrogen rich amino-acids and 16-compound mixtures had positive effects on the disease incidence and that this effect was driven by changes in the total bacterial biomasses and microbiome diversity leading to a loss of protective function of the soil microbiome.

Each chemical compound class had a specific effect on the microbial community. Amino acids and organic acids had greater effects on the soil bacterial community composition than sugars and phenolic acids. This is in agreement with previous observations showing that organic acids can cause larger shifts in the dominant soil bacterial taxa compared to sugars ([Shi *et al.*, 2011](#_ENREF_47)). One explanation for this could be that sugars mainly promote bacterial growth having only small effects on secondary metabolism that might be important for triggering competition-mediated shifts in microbial communities ([Yang *et al.*, 2019](#_ENREF_59)). Phenolic compounds are relatively hard to decompose by most microorganisms ([Krastanov, Alexieva & Yemendzhiev, 2013](#_ENREF_29)), which may explain their observed low impact on microbial communities. In contrast to the general class-level patterns, five specific phenolic-compounds increased bacterial community diversity, which is in line with a previous study by [Badri *et al.* (2013](#_ENREF_1)), who found that phenolic-related compounds present in the natural root exudates positively correlate with a higher number of bacterial OTUs compared to sugars, sugar alcohols or amino acids. However, we also found that some other phenolic compounds reduced bacterial diversity, potentially due to negative effects on competitively dominant bacterial groups: many phenolic compounds have shown to be antimicrobial ([Lanoue *et al.*, 2009](#_ENREF_32)) and could thus directly suppress some microorganisms via antibiosis. Interestingly, while bacterial communities were the most similar between amino acid and 16-compound mixture treatments, clear difference were found suggesting that addition of diverse mix of compounds also shapes the microbial community complexity.

The induced shifts in soil microbiome composition during the soil conditioning experiment predicted well the subsequent changes in microbiome-mediated disease suppression. Specifically, nitrogen-rich amino acids and mixtures correlated positively with increased disease incidence and reduced bacterial community diversity. Nitrogen fertilization was recently shown to abolish microbiome-mediated plant protection in the tomato leaf phyllosphere ([Berg & Koskella, 2018](#_ENREF_5)), while the negative correlation between disease incidence and bacterial community diversity also supports our findings ([Van der Heijden *et al.*, 1998](#_ENREF_51); [van Elsas *et al.*, 2012](#_ENREF_52)). Mechanistically, surplus of nitrogen could have stimulated fast-growing r-strategists that were able to outcompete K-strategists during the soil conditioning experiment when the nitrogen was likely a limiting resource in the absence of deposition from plants ([Sinclair & Rufty, 2012](#_ENREF_48)). This could have led to reduced diversity and potentially reduced invasion resistance by leaving niche space vacant for the pathogen ([van Elsas *et al.*, 2012](#_ENREF_52); [Wei *et al.*, 2015](#_ENREF_56)). Alternatively, Species-poor communities could have exerted weaker interference competition by producing a less diverse set of antibacterial pathogen-inhibiting compounds ([Van Elsas *et al.*, 2007](#_ENREF_53)). Together, our results indicate that the composition of plant-derived low molecular weight organic compounds can predictably drive changes in microbiome composition and functioning in relation to disease suppression.

While low molecular weight compounds are typically degraded easily by soil microbes ([Kuzyakov & Domanski, 2000](#_ENREF_30)), we cannot rule out that some amendments remained in the soil having cascading effects during the plant protection experiment. Additionally, the root exudates secreted by tomato plants likely had further impact on the soil microbiome and disease incidence during the second experiment. However, as we used the same tomato plant cultivars in all treatments, this unlikely created any bias to our results which more likely stemmed from differences in microbial community composition and abundances between compound treatments. Further complexity could have risen by pathogen-mediated shifts in root exudation patterns. For example, it has previously shown that the presence of *R. solanacearum* can promote the exudation of phenolic compounds having predictable effects on rhizosphere microbiome composition ([Gu *et al.*, 2016](#_ENREF_23)) and further studies are thus needed to understand how plant diseases emerge as a result of interactions between the pathogen, microbiome and plant root exudation patterns. In the future, it is also important to take into account the concentration, stoichiometry and dynamics of plant root exudation, which are highly variable and dependent on plant species, abiotic factors and interactions with the microbiome ([Haichar *et al.*, 2008](#_ENREF_24); [Sasse, Martinoia & Northen, 2018](#_ENREF_44)). The effects of plant-derived low molecular weight molecules on soil microbiome composition and plant functioning has previously been shown to depend on their concentration ([Zhou & Wu, 2012](#_ENREF_61); [Chen, Yu, Zhou & Wu, 2018](#_ENREF_10)) with the same compound having concentration-dependent positive or negative effects on the pathogen growth ([Ling *et al.*, 2013](#_ENREF_34)). Potential emergent properties of compound mixtures could further increase the difficulty in predicting the root exudate effects on microbiome functioning.

In conclusion, our study demonstrates that plant-derived compounds can have a profound effect on microbiome structure and function. Furthermore, we show that the effect of different compounds can be predicted on the basis of their chemical structure, and specifically, that nitrogen-rich compounds can reduce microbiome ability to protect plants against pathogen. These results suggest new possibilities for the application of fertilizers. While, nitrogen is essential for plant growth, it can also indirectly promote disease by reducing the natural protection offered by the soil microbiome ([Berg & Koskella, 2018](#_ENREF_5)). This pattern could also potentially explain the positive relationship between bacterial wilt disease occurrence and excessive use of chemical fertilizers ([Ghorbani, Wilcockson, Koocheki & Leifert, 2009](#_ENREF_22)). In this case, application of less nitrogen-rich fertilizer could be considered to offset the crop losses caused by plant pathogens. Moreover, new plant varieties, or cropping regimes, that increase temporal and spatial variation in root exudation patterns could be used to stimulate species-rich microbial communities that can naturally suppress pathogens. Together, our study provides the basis for a framework at sustainably enhancing plant health by engineering natural root-associated microbiomes based on plant root exudation patterns.

**SUPPORTING INFORMATION**

Additional results tables, figures and databases needed to evaluate the conclusions in the paper are present in the Supporting information.

**ACKNOWLEDGEMENTS**

This research was financially supported by the National Key Basic Research Program of China (2015CB150503), the Natural Science Foundation of Jiangsu Province (BK20181068, BK20170085), the National Natural Science Foundation of China (41471213, 31801952) and the Natural Science Research Program of Huaian (HAB201829). A.J. is supported by the Netherlands Organisation for Scientific Research (NWO) project ALW.870.15.050 and the Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) project 530-5CDP18. Ville-Petri Friman is funded by the Royal Society (grant nos. RSG\R1\180213 and CHL\R1\180031) and jointly by a grant from UKRI, Defra, and the Scottish Government, under the Strategic Priorities Fund Plant Bacterial Diseases programme (BB/T010606/1) at the University of York. S.G. is supported by an NWO-VENI grant from the Netherlands Organisation for Scientific Research (016.Veni.181.078).

**CONFLICT OF INTEREST**

The authors declare no competing interest.

**AUTHORS’ CONTRIBUTIONS**

YAG, YCX, and ZW conceived the ideas and designed methodology; YAG, XFW collected the data; YAG, VPF, and AJ analyzed the data. YAG, VPF, and AJ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

**DATA AVAILABILITY STATEMENT**

All of the sequencing data have been deposited in the DDBJ SRA under the accession number PRJNA394815.

**R****EFERENCES**

Badri, D.V., Chaparro, J.M., Zhang, R., Shen, Q., & 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** (7), 4502-4512. doi: 10.1074/jbc.M112.433300

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., & Vivanco, J.M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology,* **57** (1), 233-266. doi: 10.1146/annurev.arplant.57.032905.105159

Baudoin, E., Benizri, E., & Guckert, A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biology and Biochemistry,* **35** (9), 1183-1192. doi: 10.1016/S0038-0717(03)00179-2

Berendsen, R.L., Pieterse, C.M., & Bakker, P.A. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science,* **17** (8), 478-486. doi: 10.1016/j.tplants.2012.04.001

Berg, M., & Koskella, B. (2018). Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. *Current Biology,* **28** (15), 2487-2492.e2483. doi: 10.1016/j.cub.2018.05.085

Cao, Y., Zhang, Z., Ling, N., Yuan, Y., Zheng, X., Shen, B., & Shen, Q. (2011). *Bacillus subtilis* SQR 9 can control *Fusarium* wilt in cucumber by colonizing plant roots. *Biology and Fertility of Soils,* **47** (5), 495-506. doi: 10.1007/s00374-011-0556-2

Cardenas, E., Wu, W.M., Leigh, M.B., Carley, J., Carroll, S., Gentry, T., ... Tiedje, J.M. (2010). Significant association between sulfate-reducing bacteria and uranium-reducing microbial communities as revealed by a combined massively parallel sequencing-indicator species approach. *Applied and Environmental Microbiology,* **76** (20), 6778-6786. doi: 10.1128/AEM.01097-10

Carvalhais, L.C., Dennis, P.G., Badri, D.V., Kidd, B.N., Vivanco, J.M., & Schenk, P.M. (2015). Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. *Molecular Plant-Microbe Interactions,* **28** (9), 1049-1058. doi: 10.1094/MPMI-01-15-0016-R

Chaparro, J.M., Sheflin, A.M., Manter, D.K., & Vivanco, J.M. (2012). Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils,* **48** (5), 489-499. doi: 10.1007/s00374-012-0691-4

Chen, S., Yu, H., Zhou, X., & Wu, F. (2018). Cucumber (*Cucumis sativus* L.) seedling rhizosphere *Trichoderma* and *Fusarium* spp. communities altered by vanillic acid. *Frontiers in Microbiology,* **9**, 2195. doi: 10.3389/fmicb.2018.02195

Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., & Guo, J.h. (2013). Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environmental Microbiology,* **15** (3), 848-864. doi: 10.1111/j.1462-2920.2012.02860.x

de Vries, F.T., Griffiths, R.I., Knight, C.G., Nicolitch, O., & Williams, A. (2020). Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science,* **368** (6488), 270-274. doi: 10.1126/science.aaz5192

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., ... Singh, B.K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications,* **7** (1), 10541. doi: 10.1038/ncomms10541

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods,* **10** (10), 996-998. doi: 10.1038/nmeth.2604

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics,* **27** (16), 2194-2200. doi: 10.1093/bioinformatics/btr381

Eilers, K.G., Lauber, C.L., Knight, R., & Fierer, N. (2010). Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biology and Biochemistry,* **42** (6), 896-903. doi: 10.1016/j.soilbio.2010.02.003

Elphinstone, J., Hennessy, J., Wilson, J., & Stead, D. (1996). Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *EPPO Bulletin,* **26** (3-4), 663-678. doi: 10.1111/j.1365-2338.1996.tb01511.x

Etten, E.V. (2005). Multivariate analysis of ecological data using CANOCO. *Austral Ecology,* **30** (4), 486-487. doi: 10.1111/j.1442-9993.2005.01433.x

Fierer, N., Jackson, J.A., Vilgalys, R., & Jackson, R.B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology,* **71** (7), 4117-4120. doi: 10.1128/AEM.71.7.4117-4120.2005

Fierer, N., & 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** (3), 626-631. doi: 10.1073/pnas.0507535103

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist,* **187** (4), 920-928. doi: 10.1111/j.1469-8137.2010.03397.x

Ghorbani, R., Wilcockson, S., Koocheki, A., & Leifert, C. (2009) Soil management for sustainable crop disease control: a review. *Organic farming, pest control and remediation of soil pollutants*, pp. 177-201.Springer.

Gu, Y., Wei, Z., Wang, X., Friman, V.-P., Huang, J., Wang, X., ... Jousset, A. (2016). Pathogen invasion indirectly changes the composition of soil microbiome via shifts in root exudation profile. *Biology and Fertility of Soils,* **52** (7), 997-1005. doi: 10.1007/s00374-016-1136-2

Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., ... Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *The ISME journal,* **2** (12), 1221-1230. doi: 10.1038/ismej.2008.80

Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., ... Erb, M. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications,* **9** (1), 2738. doi: 10.1038/s41467-018-05122-7

Iijima, M., Griffiths, B., & Bengough, A.G. (2000). Sloughing of cap cells and carbon exudation from maize seedling roots in compacted sand. *New Phytologist,* **145** (3), 477-482. doi: 10.1046/j.1469-8137.2000.00595.x

Jousset, A., Schmid, B., Scheu, S., & Eisenhauer, N. (2011). Genotypic richness and dissimilarity opposingly affect ecosystem functioning. *Ecology Letters,* **14** (6), 537-545. doi: 10.1111/j.1461-0248.2011.01613.x

Kardol, P., Bezemer, T.M., & Van Der Putten, W.H. (2006). Temporal variation in plant–soil feedback controls succession. *Ecology Letters,* **9** (9), 1080-1088. doi: 10.1111/j.1461-0248.2006.00953.x

Krastanov, A., Alexieva, Z., & Yemendzhiev, H. (2013). Microbial degradation of phenol and phenolic derivatives. *Engineering in Life Sciences,* **13** (1), 76-87. doi: 10.1002/elsc.201100227

Kuzyakov, Y., & Domanski, G. (2000). Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science,* **163** (4), 421-431. doi: 10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R

Lamb, E.G. (2008). Direct and indirect control of grassland community structure by litter, resources, and biomass. *Ecology,* **89** (1), 216-225. doi: 10.1890/07-0393.1

Lanoue, A., Burlat, V., Henkes, G.J., Koch, I., Schurr, U., & Röse, U.S. (2009). *De novo* biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytologist,* **185** (2), 577-588. doi: 10.1111/j.1469-8137.2009.03066.x

Latz, E., Eisenhauer, N., Rall, B.C., Scheu, S., & Jousset, A. (2016). Unravelling linkages between plant community composition and the pathogen-suppressive potential of soils. *Scientific Reports,* **6** (1), 23584. doi: 10.1038/srep23584

Ling, N., Zhang, W., Wang, D., Mao, J., Huang, Q., Guo, S., & Shen, Q. (2013). Root exudates from grafted-root watermelon showed a certain contribution in inhibiting *Fusarium oxysporum* f. sp. *niveum*. *PLoS One,* **8** (5), e63383. doi: 10.1371/journal.pone.0063383

Love, M.I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology,* **15** (12), 550. doi: 10.1186/s13059-014-0550-8

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology,* **71** (12), 8228-8235. doi: 10.1128/AEM.71.12.8228-8235.2005

Mehrabi, Z., McMillan, V.E., Clark, I.M., Canning, G., Hammond-Kosack, K.E., Preston, G., ... Mauchline, T.H. (2016). *Pseudomonas* spp. diversity is negatively associated with suppression of the wheat take-all pathogen. *Scientific Reports,* **6**, 29905. doi: 10.1038/srep29905

Paredes, S.H., & Lebeis, S.L. (2016). Giving back to the community: microbial mechanisms of plant–soil interactions. *Functional Ecology,* **30** (7), 1043-1052. doi: 10.1111/1365-2435.12684

Perez-Jaramillo, J.E., Mendes, R., & Raaijmakers, J.M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology,* **90** (6), 635-644. doi: 10.1007/s11103-015-0337-7

Preece, C., & Penuelas, J. (2020). A return to the wild: root exudates and food security. *Trends in Plant Science,* **25** (1), 14-21. doi: 10.1016/j.tplants.2019.09.010

Qu, X.H., & Wang, J.G. (2008). Effect of amendments with different phenolic acids on soil microbial biomass, activity, and community diversity. *Applied Soil Ecology,* **39** (2), 172-179. doi: 10.1016/j.apsoil.2007.12.007

Rolfe, S.A., Griffiths, J., & Ton, J. (2019). Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. *Current Opinion in Microbiology,* **49**, 73-82. doi: 10.1016/j.mib.2019.10.003

Rosseel, Y. (2012). lavaan: An R package for structural equation modeling. *Journal of Statistical Software,* **48** (2), 1-36. doi: 10.18637/jss.v048.i02

Sasse, J., Martinoia, E., & Northen, T. (2018). Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science,* **23** (1), 25-41. doi: 10.1016/j.tplants.2017.09.003

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., ... Robinson, C.J. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology,* **75** (23), 7537-7541. doi: 10.1128/AEM.01541-09

Schonfeld, J., Heuer, H., van Elsas, J.D., & Smalla, K. (2003). Specific and sensitive detection of *Ralstonia solanacearum* in soil on the basis of PCR amplification of *fliC* fragments. *Applied and Environmental Microbiology,* **69** (12), 7248-7256. doi: 10.1128/aem.69.12.7248-7256.2003

Shi, S., Richardson, A.E., O'Callaghan, M., DeAngelis, K.M., Jones, E.E., Stewart, A., ... Condron, L.M. (2011). Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiology Ecology,* **77** (3), 600-610. doi: 10.1111/j.1574-6941.2011.01150.x

Sinclair, T.R., & Rufty, T.W. (2012). Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics. *Global Food Security,* **1** (2), 94-98. doi: 10.1016/j.gfs.2012.07.001

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., ... Singh, B.K. (2016). Microbial regulation of the soil carbon cycle: evidence from gene-enzyme relationships. *ISME Journal,* **10** (11), 2593-2604. doi: 10.1038/ismej.2016.65

Trofymow, J., Coleman, D., & Cambardella, C. (1987). Rates of rhizodeposition and ammonium depletion in the rhizosphere of axenic oat roots. *Plant and Soil,* **97** (3), 333-344. doi: 10.1007/BF02383223

Van der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., ... Sanders, I.R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature,* **396** (6706), 69-72. doi: 10.1038/23932

van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V., & Salles, J.F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National academy of Sciences of the United States of America,* **109** (4), 1159-1164. doi: 10.1073/pnas.1109326109

Van Elsas, J.D., Hill, P., Chroňáková, A., Grekova, M., Topalova, Y., Elhottová, D., & Krištůfek, V. (2007). Survival of genetically marked Escherichia coli O157: H7 in soil as affected by soil microbial community shifts. *The ISME journal,* **1** (3), 204-214. doi: 10.1038/ismej.2007.21

Wang, Q., Garrity, G.M., Tiedje, J.M., & Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology,* **73** (16), 5261-5267. doi: 10.1128/AEM.00062-07

Wei, Z., Gu, Y., Friman, V.-P., Kowalchuk, G.A., Xu, Y., Shen, Q., & Jousset, A. (2019). Initial soil microbiome composition and functioning predetermine future plant health. *Science Advances,* **5** (9), eaaw0759. doi: 10.1126/sciadv.aaw0759

Wei, Z., Yang, T., Friman, V.-P., Xu, Y., Shen, Q., & Jousset, A. (2015). Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nature Communications,* **6** (1), 8413. doi: 10.1038/ncomms9413

Wei, Z., Yang, X., Yin, S., Shen, Q., Ran, W., & Xu, Y. (2011). Efficacy of *Bacillus*-fortified organic fertiliser in controlling bacterial wilt of tomato in the field. *Applied Soil Ecology,* **48** (2), 152-159. doi: 10.1016/j.apsoil.2011.03.013

Whipps, J. (1987). Carbon loss from the roots of tomato and pea seedlings grown in soil. *Plant and Soil,* **103** (1), 95-100. doi: 10.1007/BF02370673

Yang, C., Dong, Y., Friman, V.-P., Jousset, A., Wei, Z., Xu, Y., ... Hart, M. (2019). Carbon resource richness shapes bacterial competitive interactions by alleviating growth-antibiosis trade-off. *Functional Ecology,* **33** (5), 868-875. doi: 10.1111/1365-2435.13292

Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., ... Shen, Q. (2018). Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome,* **6** (1), 156. doi: 10.1186/s40168-018-0537-x

Zhou, X., & 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** (10), e48288. doi: 10.1371/journal.pone.0048288

Zwetsloot, M.J., Kessler, A., & Bauerle, T.L. (2018). Phenolic root exudate and tissue compounds vary widely among temperate forest tree species and have contrasting effects on soil microbial respiration. *New Phytologist,* **218** (2), 530-541. doi: 10.1111/nph.15041

**F:\paper\RE\idea1\idea2\sob-pco1\2017年10月南京会议后修改\汇总\Next\图表-LM\re-2018.12.11\图表\2019.2.24投稿\Main figures and tables\审稿回复\Fig. 1-n.tif**

**FIGURE 1** Effects of the chemical class of mono-compounds (sugars, organic acids, amino acids and phenolic acids) and 16-compound mixtures on soil bacterial abundances (a), bacterial community richness (b) and bacterial community composition (c). In (a) and (b), plots show medians with interquartile range, and small letters denote for statistically significant differences between mono-compound and 16-compound mixture treatments (Tukey’s test). The red dashed line indicates the mean of the solvent-only control treatment (n = 4). Panel (c) shows the percentage of explained variation based on PCo 1 and 2 based on unweighted Unifrac distance metric and means with 95% confidence intervals. Different symbols and colors denote for different compound treatments

F:\paper\RE\idea1\idea2\sob-pco1\2017年10月南京会议后修改\汇总\Next\图表-LM\re-2018.12.11\图表\2019.2.24投稿\Main figures and tables\审稿回复\Fig. 2.tif

**FIGURE 2** Bacterial taxa specifically associated with different mono-compound chemical classes (sugars, organic acids, amino acids and phenolic acids) and 16-compound mixture treatments. The top 10% significant OTUs with LDA scores > 3 were used in the analysis. Histograms show LDA scores of enriched bacteria at the genus level, while the pie plots show the percentage of enriched bacteria at the phylum level

F:\paper\RE\idea1\idea2\sob-pco1\2017年10月南京会议后修改\汇总\Next\图表-LM\re-2018.12.11\图表\2019.2.24投稿\Main figures and tables\审稿回复\Fig. 3-n.tif

**FIGURE 3** Effects of mono-compound (sugars, organic acids, amino acids and phenolic acids) and 16-compound mixtures on pathogen abundances (a), bacterial wilt disease incidence (b) and correlation between the disease incidence and bacterial community richness at the end of the soil conditioning experiment (c). In (a) and (b), plots show medians with interquartile range and small letters denote for statistically significant differences between mono-compound and 16-compound mixture treatments (Tukey’s test). The red dashed line indicates the mean of the solvent-only control treatment (n = 4) and the line in panel (c) shows the linear regression fitting

F:\paper\RE\idea1\idea2\sob-pco1\2017年10月南京会议后修改\汇总\Next\图表-LM\re-2018.12.11\图表\2019.2.24投稿\EST投稿\Fig. 4.tif

**FIGURE 4** Correlations between total bacterial abundances (16S rRNA gene abundances; a), bacterial community richness (Sobs index; b), pathogen abundance (c) and disease incidence (d) with the nitrogen addition rate. Bacterial abundances and microbiome diversity were quantified based on 16S rRNA gene abundances and as bacterial community richness (Sobs index), respectively. Lines in all panels show linear regression fitting

F:\paper\RE\idea1\idea2\sob-pco1\2017年10月南京会议后修改\汇总\Next\图表-LM\re-2018.12.11\图表\2019.2.24投稿\Main figures and tables\审稿回复\Fig. 5.tif

**FIGURE 5** Structural equation models (SEMs) linking the compound classes (a) and nitrogen addition rate (b) with changes in bacterial community composition in the soil conditioning experiment and disease suppression. The top levels denote for compound class and nitrogen addition rate, the middle levels effects observed at the end of the soil conditioning experiment, and the bottom level effects observed at the end of the greenhouse experiment. Bacterial abundances and microbiome diversity were quantified based on 16S rRNA gene abundances and bacterial community richness (Sobs index), respectively. Green and red arrows represent positive and negative effects between variables, respectively. Numbers beside the arrows denote for standardized path coefficients and arrow widths correspond to the strength of path coefficients. Non-significant relationships are not shown

**TABLE 1** Results of linear models explaining variation in pathogen abundances at the end of soil conditioning experiment and disease incidence at the end of greenhouse experiment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Response variable | Predictor variable | d.f. | *F* | *p* |
| Pathogen abundance | Bacterial community richness | 1 | 0.54 | 0.46 |
|  | Bacterial community composition | 1 | 3.21 | 0.08 |
|  | Bacterial abundance | 1 | 37.56 | **< 0.001** |
|  | Residuals | 98 |  |  |
| Disease incidence | Bacterial community richness | 1 | 20.58 | **< 0.001** |
|  | Bacterial community composition | 1 | 0.35 | 0.55 |
|  | Bacterial abundance | 1 | 4.16 | **0.04** |
|  | Pathogen abundance | 1 | 1.75 | 0.19 |
|  | Residuals | 97 |  |  |

*Note*: Significant effects are highlighted in bold.