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Short communication

Soil microbial carbon metabolism reveals a disease suppression pattern in continuous ginger mono-cropping fields

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ABSTRACT

Microbial competition for carbon sources is associated with disease suppression, and continuous mono-cropping stimulates certain patterns of carbon deposits. In this study, we investigated five fields from three ginger mono-cropping durations (over 35 years, approximately 15 years and 3 years) and two disease conditions (wilt-suppressive and wilt-conducive) at two collection time points (fallow and harvest). We measured the bacterial community and overall microbial carbon metabolism ability using high-throughput sequencing and Biolog EcoPlates. The results showed that the metabolic capabilities of soil microbes at harvest time were higher than at fallow time. The distribution of the bacterial community and the soil microbial metabolic diversity were significantly affected by both factors of cropping years and disease conditions at harvest time. Moreover, potentially beneficial microbes more enriched in wilt-suppressive soils than in wilt-conducive soils and L-arginine and 4-hydroxybenzoic acid were significantly metabolized in suppressive samples. We further confirmed that L-arginine and 4-hydroxybenzoic acid were only slightly metabolized by the pathogen. Taken together, these results suggest that the addition of L-arginine could enrich the beneficial microbes, while metabolism of 4-hydroxybenzoic acid could indicate the disease conditions of soil.

1. Introduction

Soil environments are typically oligotrophic, where microbial competition for limited nutrients (e.g. carbon sources) happens intensively (Lemanceau et al., 2014). Long-term inputs of fertilizers and deposits of monocultures may accelerate the specific formation of the assembly, diversity, functioning and evolution of microbial communities (Geisseler and Scow, 2014). However, these mono-cropping systems may also have the chance to increase the soil immunity to create sustainability for disease suppression (Raaijmakers and Mazzola, 2016). Ginger (*Zingiber officinale*) is susceptible to various diseases, especially after continuous mono-cropping (Liu et al., 2017). *Ralstonia solanacarum*, the causal agent of bacterial wilt, can cause a heavy loss in ginger production. In this study, we investigated two growing time points (fallow and harvest) of long-term (over 35 years), middle-term (approximately 15 years) and short-term (3 years) continuous ginger-cropping fields. For the long-term and middle-term fields, we found wilt-suppressive and wilt-conducive sites. We hypothesized that (a) different monoculture years generated different soil microbial communities and their own carbon metabolism characteristics, and (b) several carbon sources would be selected as the indicators for deciphering the disease suppression pattern, considering microbial community-level physiological profiles (CLPPs).

2. Materials and methods

2.1. Site description and sampling

Soil samples were collected from an agricultural field site owned by the Changling farm cooperative in Rongchang County, Chongqing city, China (29°30′6.50″ N, 105°22′41.13″ E). The field sites and managements are described in detail in the Supplementary Data. The long-term monoculture fields started ginger cropping in the 1970s, the middle-term monoculture fields started in 2000 and short-term monoculture fields started in 2012. According to the survey performed in 2014, we chose the long-term and middle-term monoculture fields with over 40% disease incidence of ginger wilt as disease-conducive fields (long-term wilt-conducive, LC; and middle-term wilt-conducive, MC), whereas sites without apparent disease were treated as disease-suppressive fields (long-term wilt-suppressive, LS; middle-term wilt-suppressive, MS; and short-term wilt-suppressive, SS).

We collected soil from each field in early March (fallow time) and middle August (harvest time) of 2015. Each field had three replicates. Because there was no ginger planting in March, we gathered soil

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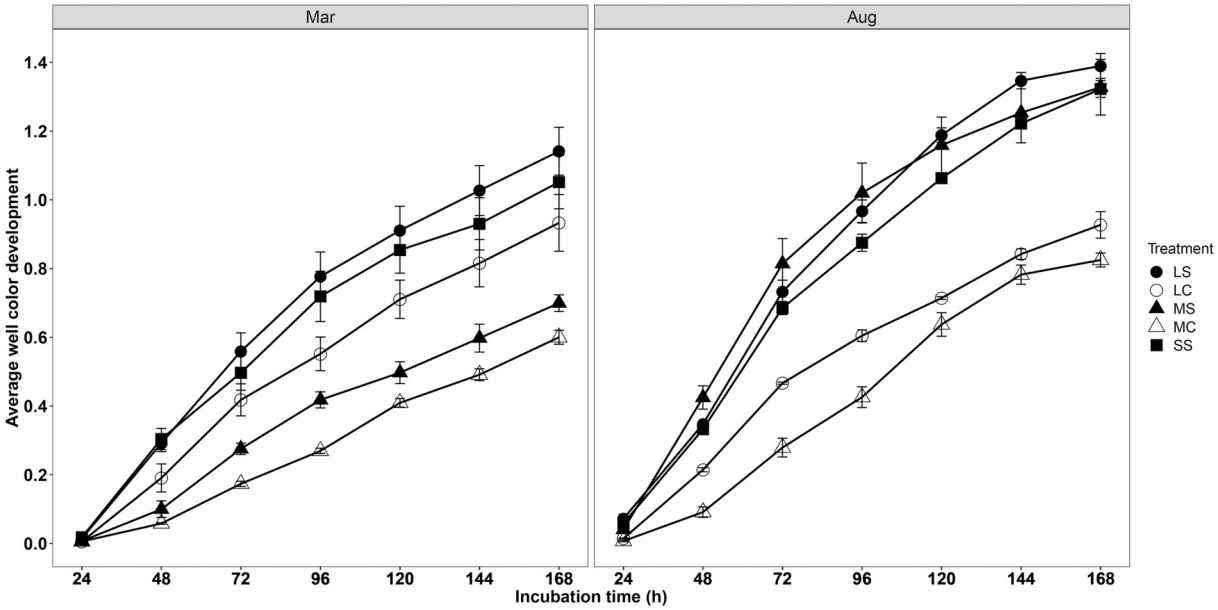

Fig. 1. Average well colour development (AWCD) values of microbial communities in soil extracts collected in March and August using Biolog EcoPlates. LS, long-term continuous cropping and wilt-suppressive soils; LC, long-term continuous cropping and wilt-conducive soils; MS, middle-term continuous cropping and wilt-suppressive soils; MC, middle-term continuous cropping and wilt-conducive soils; SS, short-term continuous cropping and wilt-suppressive soils.

samples at a depth of 5–25 cm. For the August soil samples, we collected the soil close to the rhizome of ginger at a depth of 5–25 cm. The soil samples were sieved through a 2.0-mm sieve and stored at 4 °C for further analysis. Physical and chemical analyses on each soil sample from March were performed using standard methods (Bengtson et al., 2012) in the soil analysis laboratory of Southwest University, China (Table S1). Plants with classical wilt symptoms were collected from the LC and MC fields in August. Details on pathogen isolation and identification are described in the Supplementary Data.

2.2. Microbial community-level physiological profiling

The Biolog substrate utilization assay (Insam, 1997) was performed on the day of sampling to avoid changes in bacterial communities during storage of the soil. Biolog EcoPlates (EcoPlate™, Biolog Inc., Hayward, CA, USA) were used to determine the microbial community level physiological profiles based on the carbon source utilization pattern for each soil sample according to Zhang et al. (2017).

2.3. Soil total community DNA extraction, 16S rRNA gene amplification and sequencing

The soil samples collected in August were subjected to total community DNA extraction using an Omega Biotek Soil DNA kit (Omega Biotek, USA). Amplification and purification were conducted for fifteen samples following the description in Zhang et al. (2017). The purified 16S rRNA gene fragments were sequenced using an Illumina Miseq platform in Majorbio Biopharm Technology Co., Ltd., Shanghai, China. Raw sequencing data were deposited in the NCBI Short Read Archive, BioProjectID PRJNA533499, accession number SRP193048.

2.4. Selected carbon source metabolism assay

Effect of the carbon source on the pathogen metabolism was assayed according to the methods described by Robert et al. (1989) with amendments. Briefly, bacterial isolates, isolated from ginger rhizomes in LC and MC fields, with an initial OD_{600nm} at 1.0 were used to inoculate the MP medium (Plener et al., 2009) that was supplemented with various carbon sources (D-glucose, 4-hydroxybenzoic acid, L-arginine, or L-threonine) at a final concentration of 20 mM and pH 7.0. Treatment with the same volume of sterile water was used as a negative control. Cell density was detected by measuring the value of OD_{600nm} every 8 h during the 144-h cultivation. Each treatment had three replicates.

2.5. Statistical analyses

Sequencing data analyses were performed in R using the normalized reduced data set (28,145 usable reads per sample) with 1412 OTUs and 15 samples, and individual analyses are described in detail in the Supplementary Data.

3. Results and discussion

3.1. Soil properties and pathogen identification

The ginger cropping fields of the Changling area were all acidic soils, in which the pH value varied between 3.80 and 4.03 (Table S1). Moreover, the short-term monoculture field had the highest organic matter, lowest soil CN ratio and highest available K, among other properties (Table S1). These data suggest that the nutrient supply in the short-term monoculture fields is higher than in the other fields. There was no significant difference in soil physicochemical properties between middle-term and long-term continuous mono-cropping fields.

To identify the pathogen causing the wilt symptoms of ginger, bacterial (Fig. S1A) and fungal isolates were retrieved from the rhizomes of diseased ginger in LC and MC fields, respectively. All bacterial colonies were identified as *Ralstonia solanacearum* by using the species-specific primers 759/760 (Fig. S1B). According to the wilt symptoms (Fig. S1C) and the literature (Santhanam et al., 2015), we expected to also find fungal plant pathogens of the genera *Fusarium* and *Alternaria*. However, only *Talaromyces calidicanius* and *Trichoderma* sp. were observed out of ten isolates according to the BLAST database (data not shown). Hence, the causal wilt symptoms may mainly be caused by *R. solanacearum*.

3.2. Duration of monoculture cropping and disease conditions affect the assembly and carbon metabolism of the soil microbial community

Average well colour development (AWCD) values of August samples were relatively higher than those of March samples (Fig. 1). Moreover, in August, the AWCD values of wilt-suppressive soils (LS, MS, and SS) were all significantly higher than that of wilt-conducive soils (LC and MC) after 48 h of incubation ($p < 0.05$; One-way ANOVA, Turkey HSD), whereas no significance was observed with the disease patterns in March. Although the Biolog approach does not necessarily reflect the entire soil microbial community since fast-growing species may dominate the substrate utilization patterns during the incubation period (Ros et al., 2008), microorganisms from fallow soils still can be largely considered at the dormant state during which microorganisms require only basic carbon uptake (Joergensen and Wichern, 2018). Whereas, the soil microorganisms collected at harvest time have already been subjected to a period of adequate root exudates and other nutrient supplements (Khan et al., 2007). Shannon and Simpson indices were both applied for soil microbial metabolic diversity. Both the duration of monoculture cropping and disease conditions significantly affected the soil microbial metabolic diversity; however, only the samples from August were significantly affected by their interactions (Table S2). These data indicated that due to the metabolism of plant-derived compounds, samples collected at harvest time could better explain the microbial metabolic pattern affected by both factors of cropping years and disease conditions than at fallow time.

The composition of the soil bacterial community from different sites in August was tested through 16S rRNA gene sequencing. By comparison, Alpha-proteobacteria was the most dominant class in LS and MS, and Gamma-proteobacteria ranked first in SS followed by Alpha-proteobacteria (Fig. 2A). On the other hand, Acidobacteria were enriched in wilt-conducive samples (LC and MC), which has been reported to closely correlate with soil pH (Bengtson et al., 2012). To determine the main factor driving community composition, principal coordinate and permutational MANOVA (PERMANOVA) analysis using Bray–Curtis dissimilarities were performed (Fig. 2B and Table S3). The first two axes explained 51.7% of the variance in total (Fig. 2B). Both the duration of monoculture cropping (41.1%) and disease conditions (11.9%) significantly affected the assembly of the soil bacterial community (Table S3). Taken together, these data confirmed that microbial interactions occurred intensively at harvest time.

3.3. L-Arginine and 4-hydroxybenzoic acid may act as indicators for deciphering the disease suppression pattern

We compared the metabolic abilities of 31 carbon sources used by wilt-suppressive soil microbes and wilt-conducive soil microbes at harvest time (Fig. S2). For 14 and 6 carbon sources, utilization rates between suppressive and conducive samples were significantly different ($p < 0.05$; independent-sample *t*-test) for long-term and middle-term cropping fields, respectively. The carbon metabolism of soil microbes in the disease-suppressive or –conducive fields might be shaped into two extreme directions along with the increase of monoculture cropping years. The utilization of amino acids was the most potential character to distinguish the wilt disease pattern considering six functional groups (Table S4). Moreover, L-arginine and 4-hydroxybenzoic acid were both significantly higher in suppressive samples than in conducive samples, regardless of the cropping years (Fig. S2).

We investigated whether the utilization of amino acids and 4-hydroxybenzoic acid can be largely metabolized by the two *R. solanacearum* strains collected from LC and MC fields. Although there were differences in the carbon source metabolism of the two strains, *R. solanacearum* reached the highest population densities when grown with D-glucose compared to amino acids, 4-hydroxybenzoic acid and water (Fig. 3). Moreover, L-threonine increased the growth of *R. solanacearum* during the incubation, while L-arginine and 4-hydroxybenzoic acid could be metabolized only slightly. The observed relationships between pathogen growth and L-arginine could be explained by the bacillaene and macrolactin production of *Bacillus amyloliquefaciens* (Yang et al., 2019). We compared the distribution of genera *Bacillus* and other potential beneficial microbes (Algam et al., 2010; Denison and Kiers, 2004; Lemessa and Zeller, 2007) from different sites. Almost all wilt-suppressive samples shared a relatively higher abundance of beneficial microbes compared with wilt-conducive samples (Fig. S3). Moreover, *Bacillus* in the MS was highly significantly different ($p < 0.001$; independent-sample *t*-test) with MC. This result indicated that there was enrichment of beneficial microbes in the suppressive soils.

Amino acids are reported as the requirement for synthesizing antibiotic production (Handel et al., 2013). However, L-threonine, an amino acid, could increase the growth of *R. solanacearum*, which indicated that the pathogen might also benefit from certain carbon sources. This property has been demonstrated by providing a surplus of carbon sources into soils, thereby stimulating spore germination of pathogens and leading to an increase in disease severity (Chen et al., 1988). Moreover, since the organic acids were supposed to be the major deposits due to the increase of mono-cropping years, the auto-toxicity of

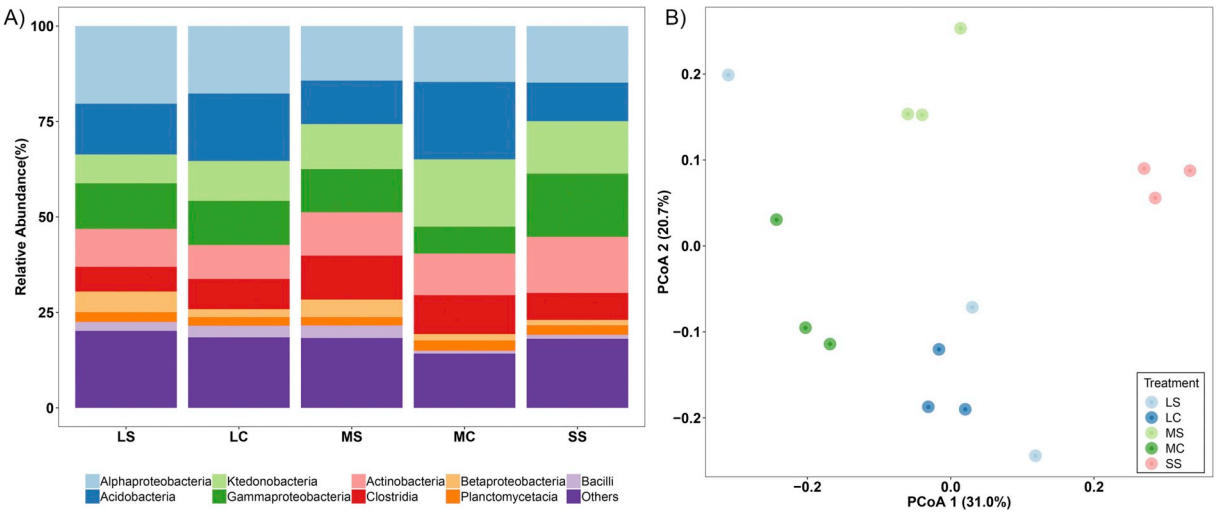

Fig. 2. A) Relative abundance of the top nine most abundant bacterial classes for each sample. B) Principal coordinate plot for all samples generated using the Bray-Curtis distance. Abbreviations as in Fig. 1.

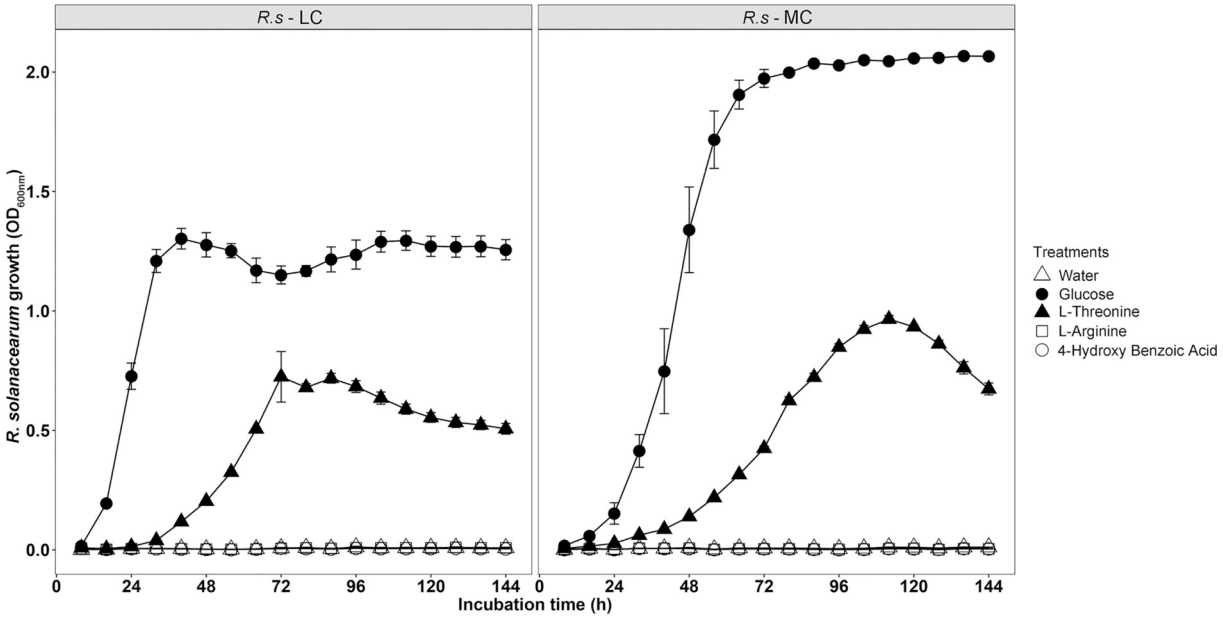

Fig. 3. Effect of different carbon sources on the growth of *R. solanacearum* strains collected in MC and LC fields during 144-h incubation. Abbreviations as in Fig. S1.

4-hydroxybenzoic acid might act as a side effect assisting the disease outbreak (Guo et al., 2015; Huang et al., 2010). This finding also indicated that more suppressive microbes had better metabolism of 4-hydroxybenzoic acid. Therefore, rational choice of carbon source enrichment is important for disease suppression. In practice, this enrichment could be achieved by using certain amino acid (e.g. L-arginine)-induced fertilizers that favour the growth of resident microorganisms, especially beneficial ones. However, due to the complex carbon sources in the soil, more work is thus needed to test the effectivity of sole-carbon-source application and its possible functional redundancies to the microbial community.

In conclusion, the metabolic capabilities of soil microbes with plants were higher than those without plants. Both the duration of monoculture cropping and disease conditions can affect the assembly of the soil microbial community and the generation of microbial carbon metabolism characteristics. L-Arginine may promote the growth of other soil microbes, especially the beneficial microbes, instead of the pathogen. Due to the well-known auto-toxicity of allelochemicals, 4-hydroxybenzoic acid might act as a side effect assisting the disease outbreak but not promoting pathogen growth. Considering the complexity of soil environments, the application of sole carbon source to enrich certain microorganisms should be further investigated.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.07.020.

References

Algam, S.A.E., Xie, G., Li, B., Yu, S., Su, T., Larsen, J., 2010. Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of *Ralstonia* wilt in tomato. J. Plant Pathol. 92, 593–600. https://10.2307/41998847.

Bengtson, P., Sterngren, A.E., Rousk, J., 2012. Archaeal abundance across a pH gradient in an arable soil and its relationship to bacterial and fungal growth rates. Appl. Environ. Microbiol. 78, 5906–5911. https://10.1128/AEM.01476-12.

Chen, W.D., Haj, H., Schmitthenner, A.F., Tuovinen, O.H., 1988. The role of microbial activity in suppression of damping-off caused by *Pythium ultimum*. Phytopathology 78, 314–322. https://10.1094/Phyto-78-314.

Denison, R.F., Kiers, E.T., 2004. Why are most rhizobia beneficial to their plant hosts, rather than parasitic? Microbes & Infection 6, 1235–1239. https://doi.org/10.1016/j.micinf.2004.08.005.

Geisseler, D., Scow, K.M., 2014. Long-term effects of mineral fertilizers on soil microorganisms – a review. Soil Biol. Biochem. 75, 54–63. https://10.1016/j.soilbio.2014.03.023.

Guo, X.-w., Wang, B., Li, K., Liu, Z.-d., Han, X., Xu, S.-j., Guo, Y.-s., Xie, H.-g., 2015. Effect of 4-hydroxybenzoic acid on grape (*Vitis vinifera* L.) soil microbial community structure and functional diversity. Biotechnol Biotec Eq 29, 637–645. https://10.1080/13102818.2015.1037348.

Handel, N., Schuurmans, J.M., Brul, S., ter Kuile, B.H., 2013. Compensation of the metabolic costs of antibiotic resistance by physiological adaptation in *Escherichia coli*. Antimicrob. Agents Chemother. 57, 3752–3762. https://10.1128/AAC.02096-12.

Huang, X.X., Bie, Z.L., Huang, Y., 2010. Identification of autotoxins in rhizosphere soils under the continuous cropping of cowpea. Allelopathy J 25, 383–392. https://10.7666/d.y1805340.

Insam, H., 1997. A new set of substrates proposed for community characterization in environmental samples. In: Microbial Communities: Functional Versus Structural Approaches, pp. 259–260. https://10.1007/978-3-642-60694-6_25.

Joergensen, R.G., Wichern, F., 2018. Alive and kicking: why dormant soil microorganisms matter. Soil Biol. Biochem. 116, 419–430. https://10.1016/j.soilbio.2017.10.022.

Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W., 2007. The myth of nitrogen fertilization for soil carbon sequestration. Journal of Environment Quality 36, 1821. https://10.2134/jeq2007.0099.

Lemanceau, P., Maron, P.-A., Mazurier, S., Mougel, C., Pivato, B., Plassart, P., Ranjard, L., Revellin, C., Tardy, V., Wipf, D., 2014. Understanding and managing soil biodiversity: a major challenge in agroecology. Agron. Sustain. Dev. 35, 67–81. https://10.1007/s13593-014-0247-0.

Lemessa, F., Zeller, W., 2007. Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. Biol. Control 42, 336–344. https://10.1016/j.biocontrol.2007.05.014.

Liu, Y., Wu, L., Wu, X., Li, H., Liao, Q., Zhang, X., Sun, Z., Li, W., 2017. Analysis of microbial diversity in soil under ginger cultivation. Scientifica (Cairo) 2017, 1–4. https://doi.org/10.1155/2017/8256865.

Plener, L., Manfredi, P., Valls, M., Genin, S., 2009. PrhG, a transcriptional regulator responding to growth conditions, is involved in the control of the type III secretion system regulon in *Ralstonia solanacearum*. J. Bacteriol. 192, 1011–1019. https://10.1128/jb.01189-09.

Raaijmakers, J.M., Mazzola, M., 2016. Soil immune responses. Science 352, 1392–1393. https://10.1126/science.aaf3252.

Robert, M., Mercade, M.E., Bosch, M.P., Parra, J.L., Espuny, M.J., Manresa, M.A., Guinea, J., 1989. Effect of the carbon source on biosurfactant production by *Pseudomonas*

aeruginosa 44T1. Biotechnol. Lett. 11, 871–874. https://doi.org/10.1007/Bf01026843.

Ros, M., Goberna, M., Pascual, J.A., Klammer, S., Insam, H., 2008. 16S rDNA analysis reveals low microbial diversity in community level physiological profile assays. J. Microbiol. Methods 72, 221–226. https://10.1016/j.mimet.2008.01.003.

Santhanam, R., Luu, V.T., Weinhold, A., Goldberg, J., Oh, Y., Baldwin, I.T., 2015. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. Proc. Natl. Acad. Sci. U. S. A. 112, E5013–E5020. https://10.1073/pnas.1505765112.

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

Zhang, S., Liu, X., Jiang, Q., Shen, G., Ding, W., 2017. Legacy effects of continuous chloropicrin-fumigation for 3-years on soil microbial community composition and metabolic activity. AMB Express 7, 178. https://doi.org/10.1186/s13568-017-0475-1.