Rhizosphere modeling reveals spatiotemporal distribution of daidzein shaping soybean rhizosphere bacterial community

[a]Fuki Okutani, [b]Shoichiro Hamamoto*, [c]Yuichi Aoki, [a]Masaru Nakayasu, [b]Naoto Nihei, [b]Taku Nishimura, [a]Kazufumi Yazaki, [a]Akifumi Sugiyama*

[a] Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, 611-0011, Japan

[b] Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8567, Japan

[c] Tohoku Medical Megabank Organization, Tohoku University, Sendai, 980-8573, Japan

*Corresponding authors:

Akifumi Sugiyama, Laboratory of Plant Gene Expression, Research Institute for Sustainable Humanosphere, Kyoto University, Uji 611-0011, Japan. Tel: +81-774-38-3617, fax: +81-774-38-3623, e-mail: akifumi_sugiyama@rish.kyoto-u.ac.jp;

Shoichiro Hamamoto, Laboratory of Soil Physics and Soil Hydrology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan. Tel: +81-3-5841-5351, fax: +81-3-5841-8171, e-mail: shoichi@soil.en.a.u-tokyo.ac.jp

Running title

Daidzein shapes rhizosphere bacterial communities

Abstract

Plant roots nurture a wide variety of microbes via exudation of metabolites, shaping the rhizosphere's microbial community. Despite the importance of plant specialized metabolites in the assemblage and function of microbial communities in the rhizosphere, little is known of how far the effects of these metabolites extend through the soil. We employed a fluid model to simulate the spatiotemporal distribution of daidzein, an isoflavone secreted from soybean roots, and validated using soybeans grown in a rhizobox. We then analyzed how daidzein affects bacterial communities using soils artificially treated with daidzein. Simulation of daidzein distribution showed that it was only present within a few millimeters of root surfaces. After 14 days in a rhizobox, daidzein was only present within 2 mm of root surfaces. Soils with different concentrations of daidzein showed different community composition, with reduced α-diversity in daidzein-treated soils. Bacterial communities of daidzein-treated soils were closer to those of the soybean rhizosphere than those of bulk soils. This study highlighted the limited distribution of daidzein within a few millimeters of root surfaces, and demonstrated a novel role of daidzein in assembling bacterial communities in the rhizosphere by acting as more of a repellant than an attractant.

45 Key words: advection–diffusion equation, bacterial communities, daidzein, rhizosphere
46 modelling, soybean
47
48

Introduction

The rhizosphere, defined as the zone of soil surrounding the root that is affected by it (Hartmann, Rothballer, & Schmid, 2008; Hiltner, 1904), is pivotal in both nutrient uptake and interactions with a diverse range of soil microbes (Bakker, Pieterse, de Jonge, & Berendsen, 2018). Previous studies provided evidence that plant metabolites secreted from roots provoke changes in rhizosphere microbial communities and mediate plant–microbe interactions, from symbiotic to commensal to pathogenic, suggesting the importance of plant metabolites in the rhizosphere for promoting the growth and health of plants (Berendsen, Pieterse, & Bakker, 2012; Massalha, 2017; Sasse, Martinoia, & Northen, 2018). Despite the importance of root-secreted metabolites, little is known of the distribution and fate of these molecules in the rhizosphere (Sugiyama, 2019). Plant metabolites can be divided into primary and secondary, or plant specialized, metabolites (Pichersky & Lewinsohn, 2011). A large portion of primary plant metabolites, which consist of sugars and organic acids, are rapidly metabolized by soil microbes (Gkarmiri et al., 2017; Gunina & Kuzyakov, 2015; Jones & Murphy, 2007). The greater abundance of nutrients near roots produces an environment in which microbes flourish, and microbial abundance is greater in the rhizosphere than in bulk soils (Mendes, Garbeva, & Raaijmakers, 2013; Prashar, Kapoor, & Sachdeva, 2014). In contrast to primary

67 metabolites, the stability of plant specialized metabolites varies depending on their
68 chemical structure and biological activity (Sugiyama & Yazaki, 2014). For example,
69 strigolactones are unstable in soil, making them a signal of living plant roots
70 (Ruyter-Spira, Al-Babili, van der Krol, & Bouwmeester, 2013; Seto, Kameoka,
71 Yamaguchi, & Kyozuka, 2012), whereas flavonoids are relatively stable in soil
72 (Sugiyama, Yamazaki, Hamamoto, Takase, & Yazaki, 2017).
73 Plant specialized metabolites have important ecological functions in the rhizosphere,
74 such as acting as signals for symbiosis, repelling enemies, and modifying microbial
75 communities (Chen et al., 2019; Huang et al., 2019; Massalha, 2017). Volatile
76 compounds are thought to facilitate communication over larger distances (Rasmann et
77 al., 2005; Schulz-Bohm et al., 2018), whereas non-volatile compounds such as
78 coumarins (Stringlis et al., 2018) and flavonoids appear to exert their influences near
79 plant roots. Largely due to the difficulty of analyzing these metabolites in soil, most
80 studies on plant specialized metabolites have been conducted in hydroponic culture or in
81 sterile sand (Oburgera, 2018). However, root structure and metabolite fate differ in soil
82 and under these artificial conditions (Crush J.R., 2005) (Sugiyama & Yazaki, 2014). To
83 gain insight into the functions of plant specialized metabolites in field-grown plants, it is
84 important to analyze rhizosphere plant–microbe interactions using soils from crop fields.

Rhizobox is widely used to analyze the dynamics of proteins, metabolites, and ion in the rhizosphere using soils taken from fields (Kuzyakov & Razavi, 2019). The activities of root-secreted enzymes such as phosphatase and β-glucosidase were visualized using 4-methylumbelliferone-labeled and 7-amino-4-methylcoumarin-labeled fluorogenic substrates to determine the distribution of these enzymes within a few millimeters from root surface (Razavi, Zarebanadkouki, Blagodatskaya, & Kuzyakov, 2016; Zhang, Dippold, Kuzyakov, & Razavi, 2019). Analyses of carbon and nitrogen with autoradiography or Positron Emitting Tracer Imaging System (PETIS) revealed the presence of the rhizodeposits within a few millimeters from root surface as well (Holz, Zarebanadkouki, Kaestner, Kuzyakov, & Carminati, 2018; Kuzyakov & Razavi, 2019). In addition, advection–diffusion (dispersion) equation has been used to simulate the distribution of mineral ions and water contents in soil surrounding roots (Duncan, Daly, Sweeney, & Roose, 2018; Vereecken et al., 2016; Zarebanadkouki, Fink, Benard, & Banfield, 2019; Zarebanadkouki, Kroener, Kaestner, & Carminati, 2014). Although the simulation of the distribution of metabolites could provide valuable insights in the rhizosphere interactions, it has not been employed to analyze the distribution of metabolites in soil, at least partly because of the instability of root-secreted metabolites and the difficulty of the measurement in rhizosphere.

Recent progress in sequencing technologies and the establishment of synthetic communities of culturable bacteria has provided insights into the molecular mechanisms underlying the assemblage and function of rhizosphere microbes (Bulgarelli et al., 2012; Duran et al., 2018; Lundberg et al., 2012); for example, *Arabidopsis* secretes coumarins that encourage assembly of a microbiome adapted to iron deficiency (Stringlis et al., 2018; M. J. E. E. E. Voges, Bai, Schulze-Lefert, & Sattely, 2019). Although these advancements have deepened our understanding of the rhizosphere, a large gap remains between these achievements in microbial ecology and a comprehensive understanding of the rhizosphere in field-grown plants, which are essential for the application of these insights to sustainable agriculture. One of the key limitations is rooted in the definition of the rhizosphere; it is impossible to delineate the area of the rhizosphere in field soils because the zone of root influence varies depending on which metabolites are secreted as well as the environmental or microbial conditions in the field.

Refining our understanding of the spatiotemporal distribution of key specialized metabolites in soils is indispensable for defining the area of the rhizosphere. To better define the extent of the influence of root-secreted specialized metabolites in the rhizosphere, we used daidzein, an isoflavone secreted from soybean (*Glycine max*) roots, as a model metabolite. Isoflavones are a subgroup of flavonoids predominantly found in

Fabaceae (Mazur, Duke, Wahala, Rasku, & Adlercreutz, 1998), and are produced via isoflavone synthase (IFS). The influences of isoflavones to bacterial communities were shown with *IFS*-silenced transgenic hairy roots (White, Ge, Brozel, & Subramanian, 2017) and with the addition of daidzein and genistein to soybean field soils (Guo, Kong, Wang, & Wang, 2011). Soybeans secrete daidzein into the rhizosphere to initiate symbiosis with rhizobia (Kosslak, Bookland, Barkei, Paaren, & Appelbaum, 1987). Although nodulation occurs predominantly during early vegetative stages (Calvert, Pence, Pierce, Malik, & Bauer, 1984), we previously found that daidzein was present in the rhizosphere throughout all growth stages (Sugiyama et al., 2017). These results suggest that daidzein plays additional roles in shaping the rhizosphere microbial communities.

In this report, we simulated the distribution of daidzein in the rhizosphere using the advection–diffusion equation, then validated the daidzein distribution using a rhizobox. To further characterize the effects of daidzein at the concentration measured in the rhizosphere, we set up artificial rhizospheres to analyze changes in bacterial communities. By integrating analyses of the distribution and function of daidzein, we were able to define daidzein's zone of influence in the soybean rhizosphere as the few millimeters surrounding the roots, and to demonstrate spatiotemporal distribution of

daidzein and its influence on microbial community composition.

Materials and Methods

Chemicals and soils

Chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan), unless otherwise stated. Field soil was collected from a soybean field at Kyoto Gakuen University, Japan. Soil chemical and physical properties were analyzed at Tokachi Federation of Agricultural Cooperatives: pH of 6.8, 0.28% total N, 2.5% total C, 4.3% humin, 46.1 ppm NO_3^-, 11 ppm P, 5.7 ppm K, 3.2 ppm S, 443 ppm Ca, 25 ppm Mg, 0.02 ppm B, 0.58 ppm Fe, 0.2 ppm Cu, 0.3 ppm Zn, and 5.51 ppm Mn. Soil particle density was 2.58 g cm^{-3}. Soil texture was light clay (sand: 46%, silt: 23%, clay: 31%). Saturated hydraulic conductivity (Ks) was 9.6×10^{-6} m s^{-1}, as measured by the falling head method. Kaolinite was purchased from The Clay Science Society of Japan, and illite was purchased from Nichika Inc. (Kyoto, Japan).

Dynamics of isoflavones

For daidzein distribution analysis, 30 g of soil was suspended in 300 mL of deionized water. Different amounts of daidzein were added to the soil solution and mixed for 2 h.

157 To determine the distribution coefficient of daidzein on minerals, kaolinite or illite was
158 mixed with water and daidzein, instead of field soils. For decomposition of soil organic
159 matter, 10 g soils were treated with hydrogen peroxide (Gee & Or, 2002). Field soil
160 (10.0 g) was added to 50 mL of ion-exchanged water, then 10 mL of 30% hydrogen
161 peroxide was added. After 60 min, the mixture was heated on a hot plate to 80 °C for 4
162 h. Carbon content was measured by using CN corder (Elementar Vario EL, Elementar,
163 Germany).
164 Soil solutions were centrifuged at 5000 ×*g* for 10 min, and supernatants were
165 filtered through filter paper (Advantech). The pH was adjusted to 3.0 using HCl. The
166 medium was passed through a Sep-pak C18 Plus short cartridge filter (Waters, Milford,
167 MA, USA), as described previously (Sugiyama et al. 2016). The eluant was dried under
168 nitrogen and reconstituted in 95% MeOH with 1% formic acid. The supernatant was
169 filtered through a Minisart 0.45-μm filter (Sartorius, Göttingen, Germany). Isoflavones
170 were analyzed using a HPLC apparatus (LC-10A, Shimadzu, Kyoto, Japan) under the
171 following conditions: column, TSK-gel ODS-80TM (4.6 mm × 250 mm; Toso, Tokyo,
172 Japan); solvent A, 0.3% (v/v) formic acid; solvent B, 0.3% (v/v) formic acid in
173 acetonitrile; detection, 260 nm. Elution was at 0.8 mL min^{-1} with solvent system A
174 (water containing 0.3% (v/v) formic acid) and B (acetonitrile containing 0.3% (v/v)

175 formic acid) with a linear gradient from 15% to 22% B over 30 min, followed by a
176 linear gradient from 22% to 35% B over 20 min, and a linear gradient from 35% to 70%
177 B over 5 min. The adsorption of daidzein on soils and minerals was caluculated by
178 subtracting the amount of daidzein in the aqueous phase from the added amount in the
179 initial solution (Liang et al., 2011). The degradation of daidzein was negligible during
180 adsorption experiments, because half-life of daidzein was calculated to be more than 7
181 days (Sugiyama et al., 2017).
182
183 **Simulation**
184 Movement of daidzein secreted by a single cylindrical root was simulated using a
185 two-dimensional axisymmetric system. The model domain was assumed to be a cylinder
186 of soil with a diameter of 20 cm and depth of 20 cm, with a single root with a diameter
187 of 2 mm and height of 10 cm in the center. The partial differential equation describing
188 transport of the solute (daidzein) had the following general form:
189 $$\frac{\partial(\theta c)}{\partial t}+\frac{\partial(\rho c_a)}{\partial t}=-\nabla\cdot q_w c+\nabla\cdot D\nabla c-kc, \quad (1)$$
190 where c is the solute (daidzein) concentration (kg m^{-3}), θ is the volumetric water content
191 (m^3 m^{-3}), c_a is the daidzein concentration adsorbed onto the solid phase (kg kg^{-1}), ρ is
192 the bulk density (1.34 Mg m^{-3}), q_w is the water flux induced by root uptake (m s^{-1}), D is

193 the solute diffusion coefficient ($m^2\ s^{-1}$), k is the degradation rate constant (s^{-1}), and t is

194 the time (s). When assuming a linear adsorption isotherm, c_a can be written as $k_d\,c$,

195 where k_d is the distribution coefficient ($m^3\ kg^{-1}$).

196 As described in Eq. (1), advective daidzein movement caused by root uptake of water

197 was also considered in the simulation. Water movement in the soil, without considering

198 gravity, can be described by the Richards equation as:

199 $$\frac{\partial\theta}{\partial t} = -\nabla\cdot q_w = \nabla\cdot\left[K\left(\nabla h\right)\right], \qquad (2)$$

200 where h is the water pressure head (m), and K is the unsaturated hydraulic function. The

201 van Genuchten–Mualem soil hydraulic properties model (Millington & Quirk, 1961;

202 van Genuchten, 1980) was adopted as an unsaturated hydraulic function:

203 $$K\left(h\right) = K_s S_e^{\,0.5}\left[1-\left(1-S_e^{\,1/m}\right)^m\right]^2, \qquad (3)$$

204 where K_s is the saturated hydraulic conductivity ($m\ s^{-1}$), m is the van Genuchten

205 parameter, and S_e is the effective water content, described by $(\theta - \theta_r)/(\theta_s - \theta_r)$ where θ_s

206 and θ_r are saturated water content and residual water content, respectively. Note that the

207 van Genuchten water retention model was adopted for the relationship between h and

208 Se) (van Genuchten, 1980).

209 The degradation kinetics of daidzein (k) have previously been reported (Sugiyama et

210 al., 2017; Sugiyama et al., 2016). The solute diffusion coefficient was estimated using

211 the Millington and Quirk model (Millington & Quirk, 1961) as a function of θ. To
212 determine the distribution coefficient (k_d), batch experiments were conducted, in which
213 30 g of dry soils were mixed with 300 mL of daidzein solution with concentrations
214 ranging from 17 to 270 ppb; the soil suspensions were shaken for 2 h. Based on the
215 obtained adsorption isotherm, the distribution coefficient was set as 0.067 m^3 kg^{-1}
216 (Supplementary Fig. 1). Parameters for water retention model and unsaturated hydraulic
217 function were estimated by a pedotransfer function (ROSETTA model) (Schaap, Leij, &
218 van Genuchten, 2001) based on the measured soil texture data, giving θ_s = 0.48, θ_r =
219 0.08, m = 0.28, and α = 1.61 m^{-1} as van Genuchten water retention model parameters.
220 In the simulation, root surface contact with the soil domain was considered as an
221 influx boundary for daidzein secretion and an efflux boundary for water movement due
222 to root uptake. The daidzein secretion rate (i.e., influx boundary at root surface)
223 determined in a previous study was used (Sugiyama et al., 2016). Water uptake rate (i.e.,
224 efflux boundary for water movement) was obtained from the rhizobox experiments
225 (5.43×10^{-8} m s^{-1}). The upper, lower, and outer surfaces of the soil domain were
226 considered as zero flux boundaries for solute and water movement. The initial water
227 content of the soil domain was set as 0.43 m^3 m^{-3}. Eqs. (1)–(3) were solved using the
228 COMSOL Multiphysics 5.4 software (Keisoku Engineering System Co., Ltd., Tokyo,

229 Japan). The parameters used in this study were summarized in Supplementary Table 1.

230

231 **Growth of soybean in the rhizobox and analysis of daidzein**

232 The rhizobox was purchased from Tohoku Materials (Sendai, Japan). Soybeans were
233 grown in a plant box containing vermiculite for 4 days before being transferred to the
234 rhizobox. The water content ratio (mass of soil water / dry soil weight x 100 (%)) of the
235 soil in the rhizobox was 28%. Mixing of soil and water was performed before packing
236 the soil layer. When placing the nylon mesh, a cellulose acetate membrane capable of
237 adsorbing daidzein was placed beyond the nylon mesh on all sides (Supplementary Fig.
238 2). Soybean seedlings were removed from vermiculite, washed with distilled water,
239 dried with a Kimwipe, and sandwiched between two layers of nylon mesh. The top of
240 the rhizobox was covered with an acrylic plate. After 14 days of growth, cellulose
241 acetate membranes and soil layers within 2 mm of plant roots were removed. Extraction
242 of daidzein was performed as described previously (BolanosVasquez & Warner, 1997;
243 Sugiyama et al., 2017). In brief, the cellulose acetate filters were rinsed with distilled
244 water and then extracted with two sequential rinses with 1 ml of methanol for 5 min.
245 Extracts were dried under a nitrogen stream at 50 °C and stored at –30°C prior to HPLC
246 analysis. Soil samples were extracted in 3 × 4 mL of methanol at 50 °C (1 h each) and

centrifuged at 5000 × g for 10 min. The combined supernatant was dried under a nitrogen stream at 50 °C and stored at –30°C prior to HPLC analysis. The detection limit was 0.01 nmol/g soil. Water content ratio was determined by measureing the exact weight before and after drying the sample in an oven at 105 °C for 24 hours (Gardner, 2002).

Analysis of bacterial communities after incubation with daidzein

Soybean field soil was air-dried, and 2 g of soil was mixed with sterilized water to obtain a water content ratio of 30%. To prevent the methanol from affecting the bacterial community, daidzein in methanol was first added to a plastic tube and dried under nitrogen gas. Moistened soils were transferred to a tube containing daidzein (0.04, 0.2, and 1 mM to obtain final concentrations of 10, 50, and 250 nmol, respectively), and mixed thoroughly using a vortex mixer (Supplementary Fig. 3). Daidzein was added every 3 days. The water content ratio was adjusted by addition of water after weighing the soil. After 15 days of culture at 28 °C in the dark, sample tubes were stored at −30 °C until extraction of DNA and daidzein.

DNA was extracted from 0.25 g soil with a Power Soil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer’s protocol and quantified using a

dsDNA HS Assay Kit for the Qubit Quantification Platform (Invitrogen, Carlsbad, CA, USA). PCR amplification of 16S rRNA genes was performed in a 25-μL reaction volume containing 10 ng template DNA, 0.3 μL of KOD FX neo (Toyobo, Japan), 12.5 μL of buffer (provided with the polymerase), 5 μL of dNTPs (2 mM), 0.5 μL of 515F (5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-GGACTACHVGGGTWTCTAAT-3′) primers (10 μM) in triplicate. PCR conditions were as follows: denaturation at 94 °C for 2 min; 20 cycles of 98 °C for 10 s, 50 °C for 30 s, and 68 °C for 30 s. PCR amplicons were purified with Ampure magnetic beads (Beckman Coulter, Danvers, MA, USA) prior to the second round of PCR. To attach MiSeq adaptors, a second round of PCR was performed in a 25-μL reaction volume containing 2.5 μL template DNA, 0.3 μL of KOD FX neo, 12.5 μL of buffer (provided with the polymerase), 5 μL of dNTPs (2 mM), and 0.5 μL of primers provided with Fasmac Co. Ltd. Final PCR products were purified using Ampure magnetic beads, and the concentration of the purified PCR product was measured using a Qubit 2.0 Fluorometer (Life Technologies). PCR products were sent to FASMAC Co. Ltd. for a single sequencing run using a MiSeq reagent kit v3 on a MiSeq platform (Illumina) to obtain 2 × 300-bp paired-end

sequences. Sequence data have been deposited in the DDBJ (DNA Data Bank of Japan) Sequence Read Archive under the accession number DRA008649.

Sequence data for the amplicons were analyzed using the QIIME2 platform, version 2018.11 (Bolyen, 2018). For all paired reads, the first 20 bases of both sequences were trimmed (to remove primer sequences) and the bases after position 200 were truncated (to remove low-quality sequence data), and potential amplicon sequencing errors were corrected using DADA2 (Callahan et al., 2016) to produce an amplicon sequence variant (ASV) dataset. The resultant ASVs were aligned using MAFFT (Katoh & Standley, 2013) and then used to construct a phylogenetic tree with FastTree2 (Price, Dehal, & Arkin, 2010). The α- and β-diversity metrics were estimated from a subsampled ASV dataset, with 45,000 sequences per sample (Supplementary Table 2). Each ASV was identified using a Naïve Bayes classifier trained on 16S rRNA gene sequences from the Greengenes release 13_8 dataset (Bokulich et al., 2018) (Supplementary Table 3). Principal coordinate analysis and other statistical analyses were performed using custom R and Python scripts.

Analysis of rhizosphere bacterial communities of soybean

Field experiments were conducted in an experimental field of Kyoto University of

Advanced Science, Kameoka, Kyoto, Japan (34°99′38″N, 135°55′14″E). Soybean plants (cv. Shintambaguro) were irrigated as needed, and weeds were manually removed throughout the crop season on a weekly basis. Rhizosphere and bulk soil samples were collected at the vegetative stage (V8). Bulk soil, defined as soil that does not adhere to plant roots, was obtained at least 20 cm from the plants. Rhizosphere soil was collected using two methods: traditional dry sampling using brushes, as described previously (Sugiyama, Ueda, Zushi, Takase, & Yazaki, 2014), and fractionation using phosphate buffer with some modification, as follows (Bulgarelli et al., 2012). Roots from five plants were pooled into a 1000-mL glass beaker with 500 mL sterile PBS buffer containing 130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4 (pH 7.0), and 0.02% Silwet L-77, and washed on a shaking platform for 5 min at 100 rpm. Roots were removed, the buffer was centrifuged (5000 ×*g*, 10 min), and the resulting pellet was defined as rhizosphere soil. DNA extraction and PCR amplification for sequencing were performed as described above.

Results

Simulation of daidzein distribution

Metabolites secreted into the rhizosphere are degraded by microbes and adsorbed onto

soil organic matter and clay minerals, depending on the soil type and microbes present (Canarini, Kaiser, Merchant, Richter, & Wanek, 2019; Soma & Soma, 1989; Sugiyama, 2019). In order to gain insight into daidzein distribution in soybean fields, we used gray lowland soils obtained from a soybean farm where soybeans had been cultivated for more than 5 years. Fig. 1 shows a simulated daidzein distribution near a single root. During early stages of vegetative growth (7, 14 days), the predicted daidzein distribution was limited to within a few millimeters of the root surface (Fig. 1A, B). These results were based on the assumption that soybean root secretes equivalent amount of daidzein from all parts of roots, and the growth of roots (i.e., enlargement of daidzein influx area) was not considered. Even during later growth stages (70 days), the predicted daidzein distribution was limited to within 1 cm of the root, as shown in the daidzein concentration profile (Fig. 1C). These results indicate that soil adsorption greatly reduces daidzein's soil mobility. Because the daidzein secretion rate is highest during earlier vegetative growth stages (Sugiyama et al., 2016), the daidzein concentration at the root surface peaked at 28 days. After that time, decomposition and reduced secretion of daidzein decreased the concentration at the root surface.

Adsorption of daidzein to organic matter in gray lowland soil

Possible adsorption sites contained in the gray lowland soil are humic substances and clay minerals. Major structural sites of humic substances include carboxyl groups, hydroxyl groups, and phenolic hydroxyl groups, which are reported to be involved in the formation of complexes with heavy metal ions and organic substances (Gan & Li, 2013). To analyze adsorption of daidzein to humic substances, organic matter in gray lowland soil was decomposed to approximately 65% with hydrogen peroxide treatment (Fig. 2). In these soils, daidzein adsorption was reduced to approximately 40% compared with controls, suggesting that humic substances are involved in adsorption of daidzein (Fig. 2).

Adsorption of daidzein to clay minerals was analyzed using two minerals typical of this lowland gray soil, kaolinite, which is classified as a 1:1 type silicate mineral, and illite, which is classified as 2:1 type silicate mineral. The adsorption data for different concentrations of daidzein were fitted by a linear line based on the Henry's law, and the distribution coefficient was calculated to be 0.0042 (m^3 kg^{-1}) (Supplementary Fig. 4). When daidzein adsorption experiments were carried out with illite, adsorption of daidzein was not observed and distribution coefficients could not be calculated.

Validation of daidzein distribution using a rhizobox

Daidzein distribution was validated using a rhizobox. To grow soybean for 14 days without additional water supply, the water content ratio was set to 28%, which is higher than the normal field condition (24%). After 14 days, the water content ratio was reduced to 24% due to the water absorption by roots (Supplementary Table 4). A cellulose acetate membrane filter was set at 2 mm from the root surface. In HPLC analysis, daidzein could only be detected in the soil layer within 2 mm of the root surface, and could not be detected on the cellulose acetate membrane filter (Fig. 3). In addition, daidzein was not detected in soil layers > 2 mm from the root surface when cellulose acetate membrane filters were not applied (n=4, data not shown).

Effect of daidzein on bacterial community

Daidzein is known to induce the expression of *nod* genes in rhizobia to initiate symbiosis, but our previous observation that the daidzein concentration remained consistent throughout growth stages in field-grown soybean (Sugiyama et al., 2017) suggests that daidzein also performs other functions. To determine the effects of daidzein on soil bacteria, a steady daidzein concentration was maintained in a test tube containing soil collected from the soybean field. After 15 days of incubation, the daidzein concentration varied from 0.81 to 28 nmol g^{-1} soil, depending on the amount

373 added to the soil (Supplementary Fig. 8). The daidzein concentration of soils
374 supplemented with 1250 nmol was within the range of daidzein concentrations observed
375 in the rhizosphere of field-grown soybean (10-20 nmol g^{-1} soil) at the end of incubation.
376 To clarify the daidzein-mediated effects on bacterial diversity, the α-diversity of
377 each sample was calculated using QIIME2 (Bolyen et al., 2018), as observed
378 operational taxonomic units (OTUs) and Shannon diversity. Daidzein treatment reduced
379 the α-diversity of bacterial communities, and observed OTUs were markedly reduced at
380 daidzein concentrations greater than 250 nmol g^{-1} soil (Supplementary Fig. 5),
381 suggesting that daidzein affected the assemblage of the bacterial community. Daidzein
382 possesses antimicrobial activity (Gorniak, Bartoszewski, & Kroliczewski, 2019) and
383 serves as a carbon source for a particular group of bacteria that metabolize daidzein. The
384 reduction of α-diversity is a typical change observed in the bacterial communities in the
385 rhizosphere (Duran et al., 2018).
386 In the principal coordinate analysis (PCoA) of Bray–Curtis similarity (β-diversity),
387 the microbial diversity of untreated soil was clearly distinct from that of
388 daidzein-treated soil (Supplementary Fig. 6A and B), indicating that daidzein had a
389 significant effect on microbiome assemblage. Especially in the weighted Unifrac
390 distance, which compared bacterial community structures using the relative abundance

391 of each bacterial member, the daidzein concentration influenced bacterial community
392 assemblage (Fig. 4A and B).
393 To understand the extent to which daidzein shapes the rhizosphere bacterial
394 communities of soybean in the field, we compared the bacterial communities of
395 daidzein-treated soils (1250 nmol) with those of bulk and soybean rhizosphere soils.
396 Bacterial communities of daidzein-treated soils were closer to those of the soybean
397 rhizosphere than those of bulk field soil (Fig. 5). These communities were then
398 compared at the family level (Fig. 6, Supplementary Fig. 7, Supplementary Table 5 and
399 6). Seven families of microbes were enriched in the daidzein-treated soils, and two of
400 these seven families, Comamonadaceae and Microbacteriaceae, were enriched in both
401 daidzein-treated and soybean rhizosphere soils (Fig. 5B). The relative abundance of
402 Comamonadaceae was positively correlated with the daidzein concentration
403 (Supplementary Fig. 8). In contrast, 17 of the 37 families found to be depleted in
404 daidzein-treated soil were also depleted in rhizosphere soil (Fig. 5B). These results
405 suggest that daidzein functions more as a repellent than an attractant in assemblage of
406 bacterial communities.
407
408 **Discussion**

409 The importance of the rhizosphere for plant growth and sustainable crop production is
410 widely recognized (Berendsen et al., 2012; Vorholt, Vogel, Carlstrom, & Muller, 2017).
411 Since Lorenz Hiltner coined the term rhizosphere more than 100 years ago, researchers
412 have used various methods to define the rhizosphere, including brushes, buffers, or
413 sensors for microbial, mineral, physical, and metabolite analyses (Kuzyakov & Razavi,
414 2019). Unlike heat, ions, and water, other factors that influence soil in the vicinity of
415 plant roots, root-secreted metabolites can be metabolized by soil microbes. This
416 characteristic makes analysis of root-secreted metabolites in the rhizosphere particularly
417 difficult (Oburgera, 2018). Traditionally, most studies of root exudates have been
418 conducted in hydroponic cultures, but the analysis of root-secreted metabolites in field
419 environments has garnered increasing attention over the past decade (Oburger et al.,
420 2013; Sugiyama et al., 2017). To gain a more precise understanding of the rhizosphere
421 in the field, it is essential to define the zone of influence of these metabolites in the soil.
422 In this study, we aimed to define the rhizosphere of soybean, by incorporating soil
423 physical and microbial analysis using daidzein as a model metabolite.
424 Based on simulations using a fluid model, the daidzein distribution was limited to
425 within a few millimeters of the roots, largely due to high adsorption by soil organic
426 matter. In this simulation, the soil water content was constant; however, in actual

soybean fields, the water content changes depending on irrigation and precipitation. In reality, daidzein has a wider distribution under wet conditions and a narrower distribution during dry conditions. Our rhizobox experiments revealed that the daidzein distribution is limited to within 2 mm of the root surface, concordant with the results of our simulation. Incorporating a combination of wet and dry conditions into the simulation would permit even more precise modeling of daidzein concentrations, and would require precise measurement of field water content during soybean growth. The growth of roots and the difference of daidzein secretion rate depending on the part of roots are also to be incorporated into the simulation to predict the daidzein distribution in soybean fields. The distribution of released carbon and nitrogen, as measured using isotopes, and of root-secreted enzymes such as phosphatase and β-glucosidase is limited to within a few millimeters as well (Kuzyakov & Razavi, 2019; Oburger et al., 2013). The distribution and fate of plant metabolites in the rhizosphere had been analyzed in rhizobox or by mass spectrometry imaging (Blossfeld, Gansert, Thiele, Kuhn, & Losch, 2011; Holscher et al., 2014; Veličković & Anderton, 2017). In this report, we demonstrated that simulation based on a fluid model can be used to predict the rhizosphere distribution of plant specialized metabolites. Plant root secretes tremendous varieties of metabolites into the rhizosphere (Massalha et al. 2017). By analyzing the

445 degradation rate and distribution coefficient of each metabolite in field soils, we can
446 simulate the distribution of root exudates in the rhizosphere. Revealing the
447 spatiotemporal distribution of metabolites functioning in the rhizosphere interactions
448 could expand our understanding of the rhizosphere into a 4-dimensional scale. One of
449 the largest limitations of this simulation is that we need different parameters depending
450 on the soil characteristics, for example, the distribution of daidzein could be wider in
451 soils with low organic matters and clay minerals such as sandy soils (Supplementary Fig.
452 4) or in soils with lower daidzein-degradation activity. We also have to appropriately
453 consider actual root growth and their function (e.g., root water uptake) in the field.
454 Recently, disruption of genes involved in synthesis of triterpene, sesterterpene, or
455 coumarin has permitted modulation of the rhizosphere microbiome of *Arabidopsis*
456 (Chen et al., 2019; Huang et al., 2019; Stringlis I.A., 2018; M. J. E. E. E. Voges, Bai, Y.,
457 Schulze-Lefert, P., Sattely, E.S., 2019). In this study, we showed that addition of
458 daidzein at rhizosphere concentrations modulated the bacterial community in vitro and
459 increased the abundance of Comamonadaceae. Rhizobiaceae was enriched in the
460 rhizosphere, but depleted to one-half in daidzein treated soils (Supplementary Table 6).
461 This is probably because daidzein is not suitable carbon source to bacteria in this family,
462 in contrast to other bacteria enriched in daidzein treated soils. When the bacterial

463 communities of daidzein-treated soils were compared with those of soybean fields,
464 daidzein treatment produced bacterial communities more similar to those in soybean
465 rhizosphere soil than those in bulk soil. This finding supports the notion that plant
466 specialized metabolites play key roles in formulating rhizosphere bacterial communities.
467 Daidzein treatment of soil reduced the α-diversity and depleted more bacterial families
468 than it enriched, as in soil from the soybean rhizosphere. These results indicate that
469 daidzein restricts the growth of certain bacteria to shape the rhizosphere bacterial
470 community. Enterobacteria that metabolize food-derived isoflavones have been reported
471 to metabolize daidzein (Feng, Li, Oppong, & Qiu, 2018), but the daidzein-metabolizing
472 enzymes in soil bacteria are still unknown. Bacteria in the Comamonadaceae and/or
473 Microbacteriaceae may possess genes that allow them to use daidzein as a carbon
474 source. Comamonadaceae are common in the soybean rhizosphere (Hamid et al., 2017;
475 White et al., 2017), with >10% relative abundance (Supplementary Fig. 9). This family
476 contains plant growth promoting bacteria such as *Delftia* sp., which enhance nodulation
477 and pulse yield when co-inoculated with *Bradyrhizobium elkanii* (Cagide, Riviezzi,
478 Minteguiaga, Morel, & Castro-Sowinski, 2018), and *Variovorax paradoxus*, a soybean
479 endophyte with characteristics related to plant growth promotion (Lopes,
480 Carpentieri-Pipolo, Oro, Pagliosa, & Degrassi, 2016). Soil type primary influences the

assemblage of rhizosphere microbial communities (Liu et al., 2019; Xiao et al., 2017), and Comamonadaceae was abundant in the rhizosphere of successive soybean-monoculture cropping (Hamid et al., 2017), which is concordant with our findings using soils from soybean field under continuous cropping. It should also be noted that daidzein is not the only metabolite to promote the abundance of Comamonadaceae in the soybean rhizosphere, because the silencing of *IFS* gene in soybean hairy roots resulted in the slight increase of Comamonadaceae in the rhizosphere of IFS-silenced roots (White et al., 2017). A synthetic community-based approach to the daidzein-mediated interaction among soybean, Comamonadaceae and other bacteria could identify the molecular basis of these interactions.

Daidzein was discovered to act as a signaling molecule for initiation of rhizobial symbiosis in the 1980s (Kosslak et al., 1987). Following our previous finding that rhizosphere concentrations of daidzein remain high even after the key period for establishment of rhizobial symbiosis, we found that daidzein has additional functions in shaping the bacterial community in the rhizosphere, answering a long-unresolved question. Plants modulate the rhizosphere microbial community, assembling beneficial microbes to reduce damage from pathogens (Kwak M.-J., 2018; Stringlis I.A., 2018) and enhance uptake of nutrients (Castrillo et al., 2017). It is tempting to speculate that

soybean secretes daidzein into the soil within the vicinity of the root surface, where daidzein helps to assemble a beneficial microbial community by acting as more of a repellant than an attractant.

Acknowledgments

We thank Ms. Yuko Kobayashi and Mr. Takahito Tastuno for technical assistance. We thank Dr. Hisabumi Takase of the Kyoto University of Advanced Science for assistance with soil sampling. We also thank DASH/FBAS, the Research Institute for Sustainable Humanosphere, Kyoto University, and Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Research Center for the soybean seeds. This work was supported in part by JST CREST grant JPMJCR17O2 (to YA and AS), JSPS KAKENHI grants 18H02313 (to SH and AS), and funds from the Research Institute for Sustainable Humanosphere and the Research Unit for Development of Global Sustainability, Kyoto University (to AS).

Author Contributions

Conceived and designed the experiments: SH, AS. Performed the experiments: FO, SH, NN, AS. Analyzed the data: FO, SH, YA, MN, NN, TN, KY, AS. Wrote the paper: FO,

SH, YA, AS, with input from all authors.

References

Bakker, P., Pieterse, C. M. J., de Jonge, R., & Berendsen, R. L. (2018). The Soil-Borne Legacy. *Cell, 172*(6), 1178-1180. doi:10.1016/j.cell.2018.02.024

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

Blossfeld, S., Gansert, D., Thiele, B., Kuhn, A. J., & Losch, R. (2011). The dynamics of oxygen concentration, pH value, and organic acids in the rhizosphere of Juncus spp. *Soil Biology & Biochemistry, 43*(6), 1186-1197. doi:10.1016/j.soilbio.2011.02.007

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., . . . Caporaso, J. G. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2 ' s q2-feature-classifier plugin. *Microbiome, 6*. doi:10.1186/s40168-018-0470-z

BolanosVasquez, M. C., & Warner, D. (1997). Effects of Rhizobium tropici, R-etli, and R-leguminosarum bv phaseoli on nod gene-inducing flavonoids in root exudates of Phaseolus vulgaris. *Molecular Plant-Microbe Interactions, 10*(3), 339-346. doi:10.1094/mpmi.1997.10.3.339

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A. (2018). QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ, preprints.27295v2*

Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E. V. L., Ahmadinejad, N.,

Assenza, F., . . . Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature, 488*(7409), 91-95. doi:10.1038/nature11336

Cagide, C., Riviezzi, B., Minteguiaga, M., Morel, M. A., & Castro-Sowinski, S. (2018). Identification of Plant Compounds Involved in the Microbe-Plant Communication During the Coinoculation of Soybean with Bradyrhizobium elkanii and Delftia sp strain JD2. *Molecular Plant-Microbe Interactions, 31*(11), 1192-1199. doi:10.1094/mpmi-04-18-0080-cr

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581-+. doi:10.1038/nmeth.3869

Calvert, H. E., Pence, M. K., Pierce, M., Malik, N. S. A., & Bauer, W. D. (1984). Anatomical analysis of the development and distribution of Rhizobium infections in soybean roots. *Canadian Journal of Botany-Revue Canadienne De Botanique, 62*(11), 2375-2384. doi:10.1139/b84-324

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root Exudation of Primary Metabolites: Mechanisms and Their Roles in Plant Responses to Environmental Stimuli. *Frontiers in Plant Science, 10*. doi:10.3389/fpls.2019.00157

Castrillo, G., Teixeira, P. J. P. L., Paredes, S. H., Law, T. F., de Lorenzo, L., Feltcher, M. E., . . . Dangl, J. L. (2017). Root microbiota drive direct integration of phosphate stress and immunity. *Nature, 543*(7646), 513-518. doi:10.1038/nature21417

Chen, Q., Jiang, T., Liu, Y.-X., Liu, H., Zhao, T., Liu, Z., . . . Wang, G. (2019). Recently duplicated sesterterpene (C25) gene clusters in Arabidopsis thaliana modulate root microbiota. *Science China. Life sciences*. doi:10.1007/s11427-019-9521-2

566 Crush J.R., C. D. A., Gourdin A., Woodfield D.R. (2005). Root growth media effects on
567 root morphology and architecture in white clover. *New Zealand Journal of*
568 *Agricultural Research, 48*(2), 255-263. doi:10.1080/00288233.2005.9513654
569 Duncan, S. J., Daly, K. R., Sweeney, P., & Roose, T. (2018). Mathematical modelling of
570 water and solute movement in ridged versus flat planting systems. *European*
571 *Journal of Soil Science, 69*(6), 967-979. doi:10.1111/ejss.12711
572 Duran, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., &
573 Hacquard, S. (2018). Microbial Interkingdom Interactions in Roots Promote
574 Arabidopsis Survival. *Cell, 175*(4), 973-983. doi:10.1016/j.cell.2018.10.020
575 Feng, X. C., Li, Y., Oppong, M. B., & Qiu, F. (2018). Insights into the intestinal
576 bacterial metabolism of flavonoids and the bioactivities of their microbe-derived
577 ring cleavage metabolites. *Drug Metabolism Reviews, 50*(3), 343-356.
578 doi:10.1080/03602532.2018.1485691
579 Gan, P. P., & Li, S. F. Y. (2013). Biosorption of Elements. In A. J. Hunt (Ed.), *Element*
580 *Recovery and Sustainability* (pp. 80-113).
581 Gardner, W. H. (2002). Water Content. *Method of Soil Analysis, Part 1* Madison: Soil
582 Science Society of America.
583 Gee, G. W., & Or, D. (2002). Particle Size Analysis. *Method of Soil Analysis, Part 4*
584 Madison: Soils Science Society of America.
585 Gkarmiri, K., Mahmood, S., Ekblad, A., Alstrom, S., Hogberg, N., & Finlay, R. (2017).
586 Identifying the Active Microbiome Associated with Roots and Rhizosphere Soil
587 of Oilseed Rape. *Applied and Environmental Microbiology, 83*(22).
588 doi:10.1128/aem.01938-17
589 Gorniak, I., Bartoszewski, R., & Kroliczewski, J. (2019). Comprehensive review of
590 antimicrobial activities of plant flavonoids. *Phytochemistry Reviews, 18*(1),

241-272. doi:10.1007/s11101-018-9591-z

Gunina, A., & Kuzyakov, Y. (2015). Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. *Soil Biology & Biochemistry, 90*, 87-100. doi:10.1016/j.soilbio.2015.07.021

Guo, Z. Y., Kong, C. H., Wang, J. G., & Wang, Y. F. (2011). Rhizosphere isoflavones (daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped soybean soil in field and controlled conditions. *Soil Biology & Biochemistry, 43*(11), 2257-2264. doi:10.1016/j.soilbio.2011.07.022

Hamid, M. I., Hussain, M., Wu, Y. P., Zhang, X. L., Xiang, M. C., & Liu, X. Z. (2017). Successive soybean-monoculture cropping assembles rhizosphere microbial communities for the soil suppression of soybean cyst nematode. *Fems Microbiology Ecology, 93*(1). doi:10.1093/femsec/fiw222

Hartmann, A., Rothballer, M., & Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil, 312*(1-2), 7-14. doi:10.1007/s11104-007-9514-z

Hiltner, L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache. *Arbeiten der Deutschen Landwirtschaftlichen Gesellschaft, 98*, 59-78.

Holscher, D., Dhakshinamoorthy, S., Alexandrov, T., Becker, M., Bretschneider, T., Buerkert, A., . . . Swennen, R. L. (2014). Phenalenone-type phytoalexins mediate resistance of banana plants (Musa spp.) to the burrowing nematode Radopholus similis. *Proceedings of the National Academy of Sciences of the United States of America, 111*(1), 105-110. doi:10.1073/pnas.1314168110

Holz, M., Zarebanadkouki, M., Kaestner, A., Kuzyakov, Y., & Carminati, A. (2018).

Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content. *Plant and Soil, 423*(1-2), 429-442. doi:10.1007/s11104-017-3522-4

Huang, A. C., Jiang, T., Liu, Y.-X., Bai, Y.-C., Reed, J., Qu, B., . . . Osbourn, A. (2019). A specialized metabolic network selectively modulates Arabidopsis root microbiota. *Science (New York, N.Y.), 364*(6440). doi:10.1126/science.aau6389

Jones, D. L., & Murphy, D. V. (2007). Microbial response time to sugar and amino acid additions to soil. *Soil Biology & Biochemistry, 39*(8), 2178-2182. doi:10.1016/j.soilbio.2007.03.017

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution, 30*(4), 772-780. doi:10.1093/molbev/mst010

Kosslak, R. M., Bookland, R., Barkei, J., Paaren, H. E., & Appelbaum, E. R. (1987). Induction of *Bradyrhizobium japonicum* common nod genes by isoflavones isolated from *Glycine max*. *Proc Natl Acad Sci USA, 84*(21), 7428-7432.

Kuzyakov, Y., & Razavi, B. S. (2019). Rhizosphere size and shape: Temporal dynamics and spatial stationarity. *Soil Biology and Biochemistry, 135*, 343-360. doi:10.1016/j.soilbio.2019.05.011

Kwak M.-J., K. H. G., Choi K., Kwon S.-K., Song J.Y., Lee J., Lee P.A., Choi S.Y., Seo M., Lee H.J., Jung E.J., Park H., Roy N., Kim H., Lee M.M., Rubin E.M., Lee S.-W., Kim J.F. . (2018). Rhizosphere microbiome structure alters to enable wilt resistance in tomato (vol 36, pg 1100, 2018). *Nature Biotechnology, 36*(11), 1117-1117. doi:10.1038/nbt1118-1117

Liang, B., Yang, C. L., Gong, M. B., Zhao, Y. F., Zhang, J., Zhu, C. X., . . . Li, S. P. (2011). Adsorption and degradation of triazophos, chlorpyrifos and their main

641 hydrolytic metabolites in paddy soil from Chaohu Lake, China. *Journal of*
642 *Environmental Management, 92*(9), 2229-2234.
643 doi:10.1016/j.jenvman.2011.04.009
644 Liu, F., Hewezi, T., Lebeis, S. L., Pantalone, V., Grewal, P. S., & Staton, M. E. (2019).
645 Soil indigenous microbiome and plant genotypes cooperatively modify soybean
646 rhizosphere microbiome assembly. *Bmc Microbiology, 19*(1).
647 doi:10.1186/s12866-019-1572-x
648 Lopes, K. B. D., Carpentieri-Pipolo, V., Oro, T. H., Pagliosa, E. S., & Degrassi, G.
649 (2016). Culturable endophytic bacterial communities associated with
650 field-grown soybean. *Journal of Applied Microbiology, 120*(3), 740-755.
651 doi:10.1111/jam.13046
652 Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., . . .
653 Dangl, J. L. (2012). Defining the core Arabidopsis thaliana root microbiome.
654 *Nature, 488*(7409), 86-90. doi:10.1038/nature11237
655 Massalha, H., Korenblum, E., Tholl, D., Aharoni, A. (2017). Small molecules
656 below-ground: the role of specialized metabolites in the rhizosphere. *Plant*
657 *Journal, 90*(4), 788-807. doi:10.1111/tpj.13543
658 Mazur, W. M., Duke, J. A., Wahala, K., Rasku, S., & Adlercreutz, H. (1998).
659 Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans.
660 *Journal of Nutritional Biochemistry, 9*(4), 193-200.
661 doi:10.1016/s0955-2863(97)00184-8
662 Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome:
663 significance of plant beneficial, plant pathogenic, and human pathogenic
664 microorganisms. *Fems Microbiology Reviews, 37*(5), 634-663.
665 doi:10.1111/1574-6976.12028

666 Millington, R., & Quirk, J. P. (1961). Permeability of porous solids. *Transactions of the*
667 *Faraday Society, 57*(8), 1200-1207. doi:10.1039/tf9615701200
668 Oburger, E., Dell'mour, M., Hann, S., Wieshammer, G., Puschenreiter, M., & Wenzel, W.
669 W. (2013). Evaluation of a novel tool for sampling root exudates from
670 soil-grown plants compared to conventional techniques. *Environmental and*
671 *Experimental Botany, 87*, 235-247. doi:10.1016/j.envexpbot.2012.11.007
672 Oburgera, E., Jones, D.L. (2018). Sampling root exudates - Mission impossible?
673 *Rhizosphere, 6*, 116-133. doi:10.1016/j.rhisph.2018.06.004
674 Pichersky, E., & Lewinsohn, E. (2011). Convergent Evolution in Plant Specialized
675 Metabolism. In S. S. Merchant, W. R. Briggs, & D. Ort (Eds.), *Annual Review of*
676 *Plant Biology, Vol 62* (Vol. 62, pp. 549-566). Palo Alto: Annual Reviews.
677 Prashar, P., Kapoor, N., & Sachdeva, S. (2014). Rhizosphere: its structure, bacterial
678 diversity and significance. *Reviews in Environmental Science and*
679 *Bio-Technology, 13*(1), 63-77. doi:10.1007/s11157-013-9317-z
680 Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2-Approximately
681 Maximum-Likelihood Trees for Large Alignments. *PLoS One, 5*(3).
682 doi:10.1371/journal.pone.0009490
683 Rasmann, S., Kollner, T. G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., . . .
684 Turlings, T. C. J. (2005). Recruitment of entomopathogenic nematodes by
685 insect-damaged maize roots. *Nature, 434*(7034), 732-737.
686 doi:10.1038/nature03451
687 Razavi, B. S., Zarebanadkouki, M., Blagodatskaya, E., & Kuzyakov, Y. (2016).
688 Rhizosphere shape of lentil and maize: Spatial distribution of enzyme activities.
689 *Soil Biology & Biochemistry, 96*, 229-237. doi:10.1016/j.soilbio.2016.02.020
690 Ruyter-Spira, C., Al-Babili, S., van der Krol, S., & Bouwmeester, H. (2013). The

biology of strigolactones. *Trends in Plant Science, 18*(2), 72-83. doi:10.1016/j.tplants.2012.10.003

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

Schaap, M. G., Leij, F. J., & van Genuchten, M. T. (2001). ROSETTA: a computer program for estimating soil hydraulic parameters with hierarchical pedotransfer functions. *Journal of Hydrology, 251*(3-4), 163-176. doi:10.1016/s0022-1694(01)00466-8

Schulz-Bohm, K., Gerards, S., Hundscheid, M., Melenhorst, J., de Boer, W., & Garbeva, P. (2018). Calling from distance: attraction of soil bacteria by plant root volatiles. *Isme Journal, 12*(5), 1252-1262. doi:10.1038/s41396-017-0035-3

Seto, Y., Kameoka, H., Yamaguchi, S., & Kyozuka, J. (2012). Recent Advances in Strigolactone Research: Chemical and Biological Aspects. *Plant and Cell Physiology, 53*(11), 1843-1853. doi:10.1093/pcp/pcs142

Soma, Y., & Soma, M. (1989). CHEMICAL-REACTIONS OF ORGANIC-COMPOUNDS ON CLAY SURFACES. *Environmental Health Perspectives, 83*, 205-214. doi:10.2307/3430656

Stringlis I.A., Y. K., Feussner K., de Jonge R., Van Bentum S., Van Verk M.C., Berendsen R.L., Bakker P.A.H.M., Feussner I., Pieterse C.M.J. . (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences of the United States of America, 115*(22), E5213-E5222. doi:10.1073/pnas.1722335115

Sugiyama, A. (2019). The soybean rhizosphere: Metabolites, microbes, and beyond-A review. *Journal of Advanced Research, 19*, 67-73.

doi:10.1016/j.jare.2019.03.005

Sugiyama, A., Ueda, Y., Zushi, T., Takase, H., & Yazaki, K. (2014). Changes in the bacterial community of soybean rhizospheres during growth in the field. *PLoS One*, *9*(6), e100709. doi:10.1371/journal.pone.0100709

Sugiyama, A., Yamazaki, Y., Hamamoto, S., Takase, H., & Yazaki, K. (2017). Synthesis and Secretion of Isoflavones by Field-Grown Soybean. *Plant and Cell Physiology*, *58*(9), 1594-1600. doi:10.1093/pcp/pcx084

Sugiyama, A., Yamazaki, Y., Yamashita, K., Takahashi, S., Nakayama, T., & Yazaki, K. (2016). Developmental and nutritional regulation of isoflavone secretion from soybean roots. *Bioscience Biotechnology and Biochemistry, 80*(1), 89-94. doi:10.1080/09168451.2015.1062714

Sugiyama, A., & Yazaki, K. (2014). Flavonoids in plant rhizospheres: secretion, fate and their effects on biological communication. *Plant Biotechnology, 31*(5), 431-443. doi:10.5511/plantbiotechnology.14.0917a

van Genuchten, M. T. (1980). A Closed-form Equation for Predicting the Hydraulic Conductivity of Unsaturated Soils. *Soil Science Society of America Journal*, *44*(5), 892-898. doi:10.2136/sssaj1980.03615995004400050002x

Veličković, D., & Anderton, C. R. (2017). Mass spectrometry imaging: Towards mapping the elemental and molecular composition of the rhizosphere. *Rhizosphere, 3*(2), 254-258. doi:10.1016/j.rhisph.2017.03.003

Vereecken, H., Schnepf, A., Hopmans, J. W., Javaux, M., Or, D., Roose, D. O. T., . . . Young, I. M. (2016). Modeling Soil Processes: Review, Key Challenges, and New Perspectives. *Vadose Zone Journal*, *15*(5). doi:10.2136/vzj2015.09.0131

Voges, M. J. E. E. E., Bai, Y., Schulze-Lefert, P., & Sattely, E. S. (2019). Plant-derived coumarins shape the composition of an Arabidopsis synthetic root microbiome.

Proceedings of the National Academy of Sciences of the United States of America. doi:10.1073/pnas.1820691116

Vorholt, J. A., Vogel, C., Carlstrom, C. I., & Muller, D. B. (2017). Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. *Cell Host & Microbe, 22*(2), 142-155. doi:10.1016/j.chom.2017.07.004

White, L. J., Ge, X., Brozel, V. S., & Subramanian, S. (2017). Root isoflavonoids and hairy root transformation influence key bacterial taxa in the soybean rhizosphere. . *Environ Microbiol., 19*(4), 1391-1406. doi: 10.1111/1462-2920.13602

Xiao, X., Chen, W., Zong, L., Yang, J., Jiao, S., Lin, Y., . . . Wei, G. (2017). Two cultivated legume plants reveal the enrichment process of the microbiome in the rhizocompartments. *Molecular Ecology, 26*(6), 1641-1651. doi:10.1111/mec.14027

Zarebanadkouki, M., Fink, T., Benard, P., & Banfield, C. C. (2019). Mucilage Facilitates Nutrient Diffusion in the Drying Rhizosphere. *Vadose Zone Journal, 18*(1). doi:10.2136/vzj2019.02.0021

Zarebanadkouki, M., Kroener, E., Kaestner, A., & Carminati, A. (2014). Visualization of Root Water Uptake: Quantification of Deuterated Water Transport in Roots Using Neutron Radiography and Numerical Modeling. *Plant Physiology, 166*(2), 487-499. doi:10.1104/pp.114.243212

Zhang, X., Dippold, M. A., Kuzyakov, Y., & Razavi, B. S. (2019). Spatial pattern of enzyme activities depends on root exudate composition. *Soil Biology & Biochemistry, 133*, 83-93. doi:10.1016/j.soilbio.2019.02.010

766

767 **Disclosure statement**

768 No potential conflict of interest was reported by the authors.

769

770 **Figure legends**

771 Fig. 1 Simulation of daidzein distribution in soil. (A) Daidzein concentrations at 7 days

772 after germination from 0 to 6 mm from root surface. (B) Daidzein concentrations at 14

773 days after germination from 0 to 6 mm from root surface. (C) Daidzein distribution

774 from 7 to 70 days.

775

776 Fig. 2 Daidzein adsorption to soil organic matter. Soils were treated with hydrogen

777 peroxide to remove organic matter. Carbon content was measured by using a CN corder.

778 Adsorption of daidzein by gray lowland soil after hydrogen peroxide treatment to

779 remove organic matter. Values indicate means ± SD. Soil organic carbon was measured

780 in duplicate, and adsorption of daidzein was measured in triplicate. White circle:

781 control; black circle: hydrogen peroxide-treated soil.

782

783 Fig. 3 Daidzein distribution in the rhizobox. Daidzein concentrations in soil within 2

784 mm of the root surface and recovered from the cellulose acetate membrane filter (n = 6).

785

786 Fig. 4 Pairwise comparisons between daidzein-treated and untreated soils. Box plots
787 showing mean and variance of average pair-wise weighted UniFrac distances (A) and
788 unweighted UniFrac distance (B) between daidzein-untreated (0 nmol) and
789 daidzein-treated samples. p-values from Wilcoxon rank-sum test are shown.

790

791 Fig. 5 Comparison of bacterial communities between daidzein-treated soils and soybean
792 field soils. (A) Weighted Unifrac distance between daidzein-treated soils and bulk soils
793 and between daidzein-treated soils and rhizosphere soils. p-value from Wilcoxon
794 rank-sum test is shown. (B) Venn diagrams showing overlap of bacterial families
795 enriched or depleted in soil treated with daidzein and in the soybean rhizosphere.
796 Numbers in the circle represent number of bacterial families.

797

798 Fig. 6 Families enriched or depleted in daidzein-treated soils and soybean rhizosphere.
799 The heatmap represents the fold-changes. Colored stripes indicate whether the family
800 was enriched or depleted in daidzein-treated soils and soybean rhizosphere. Families
801 either enriched or depleted in both soybean rhizosphere and daidzein treatment are

802 shown. Comparisons of other Families are shown in Supplementary Fig. 7.

803

804

805 **Supplementary Figures**

806 Supplementary Fig. 1 Adsorption isotherms of daidzein in gray lowland soil. Initial

807 concentrations ranging from 17 to 270 ppb were used to determine the daidzein

808 concentration in the aqueous phase (n = 2). Cs; adsorbed concentration in soil, Cw;

809 concentration in water in equilibrium with the soil.

810

811 Supplementary Fig. 2 Schematic illustration of the rhizobox. (A) Soybean roots were

812 separated with nylon mesh, and a 2-mm soil layer was placed on both sides of plants.

813 Cellulose acetate membranes were set at 2 mm from the root surface. (B) Representative

814 images of the rhizobox.

815

816 Supplementary Fig. 3 Schematic illustration of daidzein treatment to soils.

817

818 Supplementary Fig. 4 Daidzein adsorption to kaolinite. Daidzein at initial

819 concentrations ranging from 17 to 270 ppb was used to determine the daidzein

concentration in the aqueous phase.

Supplementary Fig. 5 Alpha diversity of bacterial communities of soils augmented with daidzein. Observed operational taxonomic units (OTUs) (A) and Shannon diversity index (B) (n = 4).

Supplementary Fig. 6 Principal coordinate analysis of bacterial communities of soils augmented with daidzein. (A) Unweighted Unifrac and (B) weighted Unifrac analysis. Different colors represent different concentrations of daidzein in soil.

Supplementary Fig. 7 Families enriched or depleted in daidzein-treated soils and soybean rhizosphere. The heatmap represents the fold-changes. Colored stripes indicate whether the family was enriched or depleted in daidzein-treated soils and soybean rhizosphere.

Supplementary Fig. 8 Correlation between daidzein concentration after 15 days of incubation and the relative abundance of Comamonadaceae.

838 Supplementary Fig. 9 (A) Relative abundance of Comamonadaceae in soils treated with
839 different concentrations of daidzein and in bulk and rhizosphere soils of soybean field.
840 (B) Relative abundance of Microbacteriaceae in soils treated with different
841 concentrations of daidzein and in bulk and rhizosphere soils of soybean field.
842
843 Supplementary Table 1. Parameters used in this study
844
845 Supplementary Table 2. Effective Read Number, Observed ASVs, Faith's Phylogenetic
846 Diversity, Shannon's Entropy, Pielou's Evenness of each sample.
847
848 Supplementary Table 3. Number of reads assigned to taxonomy categories.
849
850 Supplementary Table 4. Water content ratio of soils in rhizobox after soybean growth.
851
852 Supplementary Table 5. Fold changes of bacterial taxa in soybean rhizosphere and
853 daidzein-treated soils. Families either enriched or depleted in both soybean rhizosphere
854 and daidzein treatment are shown. P value of Student’s t-test and Q value of false
855 discovery rate for multiple comparison are shown.

Supplementary Table 6. Fold changes of bacterial taxa in soybean rhizosphere and daidzein-treated soils. All Families in Supplementary Fig. 7 are shown. P value of Student’s t-test and Q value of false discovery rate for multiple comparison are shown.