ECOLOGY

Soil organic matter attenuates the efficacy of flavonoid-based plant-microbe communication

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Plant-microbe interactions are mediated by signaling compounds that control vital plant functions, such as nodulation, defense, and allelopathy. While interruption of signaling is typically attributed to biological processes, potential abiotic controls remain less studied. Here, we show that higher organic carbon (OC) contents in soils repress flavonoid signals by up to 70%. Furthermore, the magnitude of repression is differentially dependent on the chemical structure of the signaling molecule, the availability of metal ions, and the source of the plant-derived OC. Up to 63% of the signaling repression occurs between dissolved OC and flavonoids rather than through flavonoid sorption to particulate OC. In plant experiments, OC interrupts the signaling between a legume and a nitrogen-fixing microbial symbiont, resulting in a 75% decrease in nodule formation. Our results suggest that soil OC decreases the lifetime of flavonoids underlying plant-microbe interactions.

INTRODUCTION

Plant-microbe communication relies on the exchange of a wide range of chemical signaling molecules and affects nearly every aspect of plant growth (*1*). In particular, flavonoids, low–molecular mass plant secondary metabolites that exist with a wide range of structures, can serve to enhance or deter plant growth by mediating plant-microbe interactions (*1*). Flavonoids are central to symbioses that enhance nutrient uptake, including the interactions between plants and nitrogen-fixing bacteria (*2*), as well as between plants and arbuscular mycorrhizal fungi (*1*). These signals play an important role in enhancing beneficial rhizosphere communities and could aid in the development of symbiotic relationships in non–nitrogen-fixing crops (*3*). Plants also regulate flavonoid production in response to pests, and these compounds have been shown to participate in the defense against root pathogens by exhibiting antimicrobial and antifungal activity (*4*). Controlling the transmission of these types of chemical signals could be used in pathogen management techniques (*5*). Flavonoids are viewed as encoders of chemical information that affects the outcome of almost all types of biological interactions and thus underlie and regulate large-scale ecological processes, such as community dynamics and nutrient cycling (*6*). Because the expression of flavonoids affects plant fitness, it is important to understand their fate and the environmental feedbacks on their bioavailable concentrations.

The importance of biodegradation on flavonoid signal loss is well established (*7*), but few studies have considered the influence of abiotic environmental parameters on the efficiency of signal transmission through soils. Soil properties vary widely and influence the availability of numerous chemicals including nutrients, metals, and pollutants (*8*, *9*), making it likely that soil physicochemical characteristics play a key role in influencing the bioavailability of flavonoid molecules. Organic carbon (OC) is one abiotic soil property that is particularly important to study (*10*), because OC levels in soil are influenced by the rate of plant growth and of delivery of plant litter into an ecosystem. Furthermore, soil management strategies, including the addition of plant litter or organic amendments (e.g., wood, compost, and pyrolyzed OC), can greatly influence soil OC content (*11*). Greater OC contents are typically thought to enhance plant productivity and symbiotic interactions (*12*), but it is unknown whether OC directly influences the bioavailability of plant-microbe signals. Among the possible mechanisms through which OC and the soil matrix may abiotically influence signal availability, sorption onto particulate OC (POC) and reactions with dissolved OC (DOC) could play roles in modifying plant-microbe signal transmission (*13*). As various flavonoids have distinct chemical properties and functional groups, these mechanisms have the potential to differentially affect flavonoid movement through the soil matrix. However, no direct evidence is available on whether interactions with POC or DOC dominate changes in plant-microbe signaling.

Equally little is known about how variations in flavonoid chemical structure affect signal half-lives in the environment. Studies examining flavonoid persistence in soils have reported a wide range of lifetimes. For example, differences in biodegradation of the flavonoids naringenin and formononetin, which differ in their conjugation and number of functional groups layered onto the phenylchromenone backbone, have been observed on day-long time scales (*7*). Other studies have observed that flavonoids persist in soils through seasons (*14*). However, none of these studies have determined mechanisms of signal loss, making it challenging to develop predictive models of flavonoid persistence. The range of chemical variations to the

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flavonoid backbone has been associated with specific biological functionality (*15*). However, there have been no efforts to investigate how structural differences among flavonoids affect interactions with OC in soils.

In this study, we tested the influence of soil OC contents and organic amendments on the bioavailability of flavonoid signaling molecules using three closely related flavonoids (naringenin, quercetin, and luteolin). Because chemical detectability may not be equivalent to bioavailability, we compared flavonoid concentrations measured by high-performance liquid chromatography (HPLC) with those measured by microbial biosensors. Using these tools, we evaluated signal loss due to sorption and abiotic reactions. We found that flavonoid signal loss is predominately caused by an abiotic reaction mediated by DOC rather than POC, which yields new polyphenolic chemicals with molecular masses consistent with flavonoid heterodimers. We also found that DOC causes a significant decrease in the signal when evaluating flavonoids having three (naringenin) and four (luteolin) hydroxyl groups, but not with a flavonoid having five hydroxyl groups (quercetin). We further quantified the effect of flavonoid signal modulation on the development of nodules in the model symbiosis between *Medicago sativa* and *Ensifer meliloti* to corroborate that flavonoid signal attenuation is sufficient to affect ecological interactions. We found that DOC is able to decrease nodulation, highlighting the relevance of accounting for soil chemistry when evaluating plant-microbe exchanges.

RESULTS

Flavonoid loss in soil increases with OC content

To better understand how soil composition influences both the absolute concentration of flavonoids and their bioavailability, we measured the effects of a series of autoclaved soils with different physicochemical properties on the flavanone naringenin. We chose this flavanone because its core structure is the foundational metabolic unit on which other more complex flavonoids are built (fig. S1A), and it has broad ecological relevance due to its regulation of gene expression in nitrogen-fixing bacteria (*2*), denitrifying bacteria (*16*), and fungal plant pathogens (*1*). Because chemically detectable compounds are not always biologically available, we used two independent techniques for these measurements, including (i) HPLC, a traditional analytical method for measuring flavonoid concentrations (*17*), to monitor the total aqueous concentration of naringenin in each soil and (ii) a genetically modified *Escherichia coli* biosensor that generates an easily detectable output proportional to the bioavailable flavonoid concentration (*18*). The biosensor functions by using a naringenin-dependent transcriptional regulator (FdeR) to control the expression of the cyan fluorescent protein (CFP) reporter. Dose-response curves for both flavonoid detection methods present good fits ($R^2 \geq 0.95$) between 0.08 and 0.31 mM naringenin (Fig. 1, A and B).

We measured naringenin availability after incubation with soils from three different sites and land uses, including agriculture, meadow, and forest (fig. S2A). Following a 24-hour incubation, the naringenin in the supernatant was directly measured using HPLC. In addition, an aliquot of the supernatant was mixed with the biosensor, and the CFP reporter signal was read out following a 24-hour incubation. For both detection methods, we observed inverse linear correlations between total OC in each soil and the concentration of aqueous (R^2 = 0.88) and bioavailable (R^2 = 0.79) naringenin remaining after 24 hours (Fig. 1, C and D). These trends show that soils with high OC content attenuate naringenin concentration up to 40% and bioavailability up to 70%. Weaker linear correlations were observed when comparing the naringenin concentration remaining with other soil properties that follow OC content (fig. S3, A to C), such as water holding capacity (R^2 = 0.74), soil pH (R^2 = 0.35), and total soil nitrogen (R^2 = 0.57). Comparison of naringenin loss with the prevalence of particles having different sizes (sand, silt, and clay) yielded weaker correlations (R^2 = 0.21, 0.02, and 0.29, respectively) (fig. S3, D to F). Because grain size is associated with water diffusion through a soil, these results suggest that the decrease in signal is not related to the signal's physical mobility. However, our experiments were performed under saturated conditions, which is expected to have distinct spatial heterogeneity and signaling dynamics from other hydration conditions.

Plant-derived organic matter causes the largest flavonoid loss

To determine the underlying cause of signal attenuation in soils, we next analyzed the effects of high OC soil amendments on naringenin availability. Four different high OC materials were tested. These materials included maple wood (*Acer sp.*) as plant-derived organic matter (POC_{plant}), two different temperatures of pyrolyzed organic matter (PyOM) made using maple wood, and compost (POC_{comp}). The latter material includes a complex mixture of plant-derived organic matter. After incubating each amendment with naringenin for 24 hours, we removed the amendment and analyzed the level of naringenin remaining using both HPLC and the biosensor (Fig. 1, E and F). POC_{comp} and POC_{plant} decreased the concentrations of both aqueous (by 87 and 98%, respectively) and bioavailable (by 92 and 95%) naringenin [one-way analysis of variance (ANOVA), Dunnett's multiple comparisons test, $P < 0.001$]. In contrast, PyOM had no significant effect on naringenin concentration when analyzed using HPLC. With the biosensor, the high-temperature PyOM caused a 21% decrease in naringenin bioavailability compared with the buffer control (one-way ANOVA, Dunnett's multiple comparisons test, $P < 0.05$).

Previously, we showed that PyOM triggers a significant decrease in the bioavailability of one class of diffusible signaling molecules used for microbe-microbe communication (acyl homoserine lactones) due to both sorption and pH-dependent hydrolysis (*13*). To test whether these effects were specific to maple and compost or other PyOM types enhance flavonoid signal reduction, we evaluated the effects of PyOM derived from three different POC_{plant} types (yard waste, wood waste, and oak wood) on naringenin availability before and after pyrolysis (fig. S4). These different pyrolyzed materials did not alter naringenin levels, while the untreated POC_{plant} decreased aqueous naringenin concentrations (multiple unpaired *t* tests, $P < 0.001$). To assess whether aged PyOM has enhanced reactivity, we performed similar experiments using oxidized PyOM. This material also did not induce significant naringenin attenuation (fig. S5). Because incubation with PyOM (a sorptive and high–surface area material) showed little, if any, signal attenuation, these results suggest that sorption is not the main process driving naringenin signal loss. These results point to a biological or chemical mechanism present in the DOC fraction as the main mechanism behind signal quenching.

Flavonoid signal attenuation is mediated by dissolved organic matter rather than particulate organic matter

To test whether DOC contributes to naringenin signal reduction, we incubated each soil amendment with a buffer solution for 24 hours,

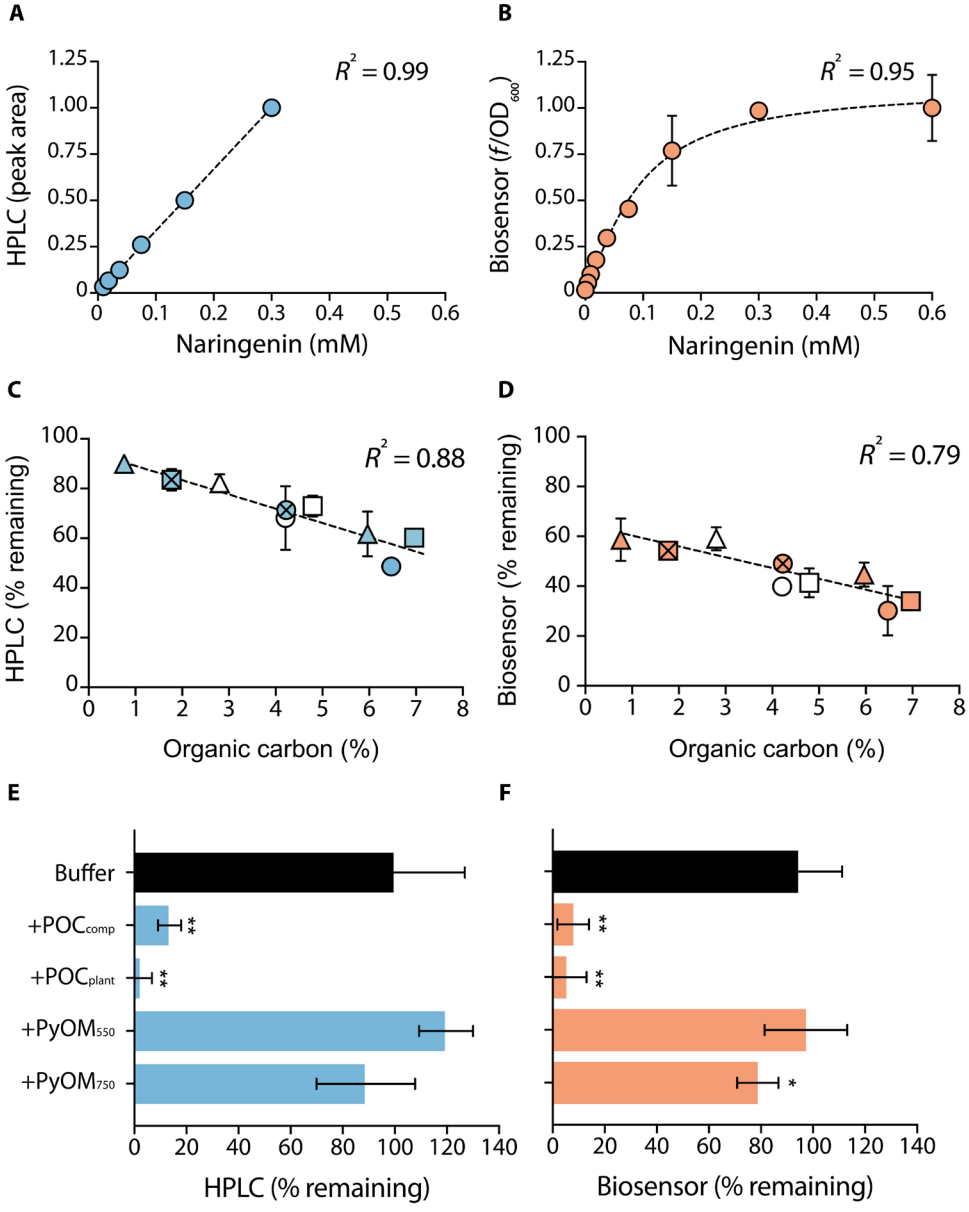

Fig. 1. Naringenin bioavailability changes with the total C content of soils and amendment type. (**A**) HPLC standard curve for naringenin. The dashed line indicates a linear fit. (**B**) Biosensor transfer function for naringenin. The CFP emission (λ_{ex} = 433 nm and λ_{em} = 475 nm) represents background-subtracted signal normalized to cell growth. The dashed line indicates a fit to the Hill function. For both standard curves, the data represent the average from $n = 3$, normalized to the average maximum signal in the absence of soil. (**C** and **D**) Inceptisol soils collected from three different sites (square, triangle, and circle) and three different land uses (agricultural, crossed circle; meadow, open circle; forest, filled circle) were incubated with naringenin for 24 hours, and the amount remaining in the supernatant was analyzed by (C) HPLC or (D) biosensor. With HPLC analysis (75 μM naringenin added), a fit of the data to $y = -0.06172x + 1.022$ yields an R^2 value of 0.88. With the biosensor (0.6 mM naringenin added), a fit of the data to $y = -0.06393x + 0.9427$ yields an R^2 value of 0.79. In both cases, higher OC contents correlate with a decrease in naringenin. (**E** and **F**) Naringenin was incubated with compost (+POC_{comp}), maple wood (+POC_{plant}), and maple wood that had been pyrolyzed at 550°C (+$PyOM_{550}$) or 750°C (+$PyOM_{750}$) for 24 hours. The proportion of naringenin remaining in the supernatant was quantified by (E) HPLC or (F) biosensor. POC_{comp} and POC_{plant} decreased naringenin bioavailability [one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test, $^*P < 0.005$ and $^{**}P < 0.001$]. Error bars represent ±1σ calculated using $n = 3$.

separated the soluble fraction, and mixed the soluble fraction with naringenin (fig. S2B). Following exposure of naringenin to the soluble fraction for 24 hours, we measured naringenin concentration and bioavailability. We found that compost-derived DOC (DOC_{comp}) and plant-derived DOC (DOC_{plant}) both decreased the concentration of naringenin (23 and 43%, respectively) and its bioavailability (24 and 60%, respectively) (one-way ANOVA, Dunnett's multiple comparisons test, $P < 0.001$; Fig. 2, A and B). Comparing the total repression of naringenin caused by the combined effect of POC and DOC, which was experimentally obtained in Fig. 1 (E and F), and the repression as a result of only DOC (Fig. 2, A and B), we estimate that ~37% of the total signaling repression by OC is due to sorption or other reaction with POC, assuming that the POC and DOC effects are additive.

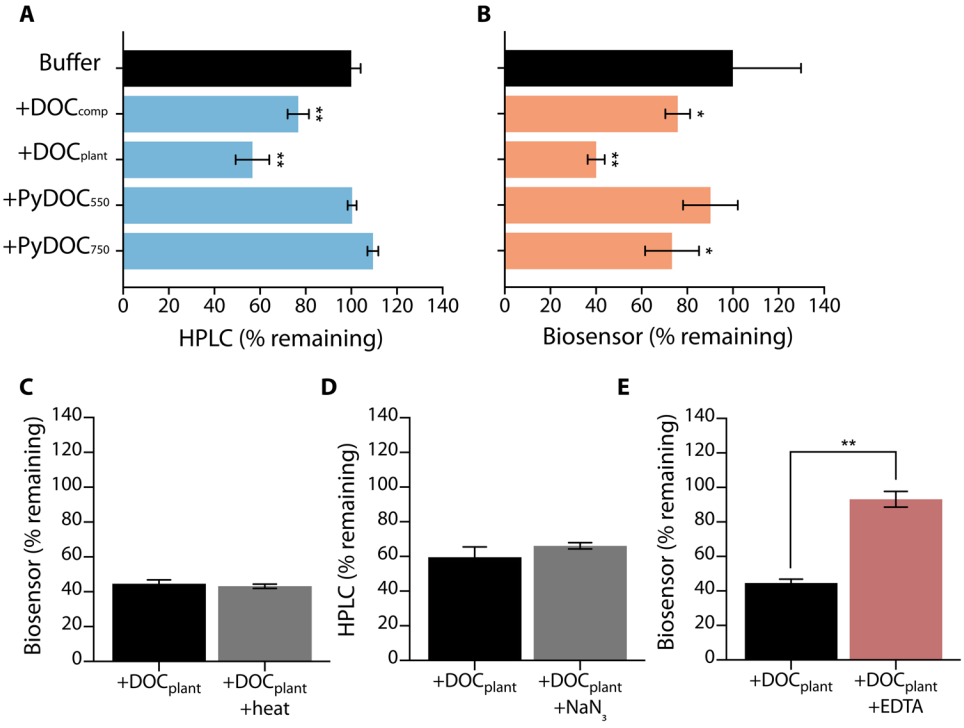

Fig. 2. Naringenin bioavailability is decreased by chemicals in DOC. Each amendment (25 mg ml^{-1}) was incubated in phosphate buffer (10 mM, pH 7) for 24 hours, and DOC_x (x = source) was obtained by centrifugation. Naringenin was incubated in DOC_x for 24 hours and then quantified using (**A**) HPLC (40 μM naringenin added to DOC_x) or (**B**) biosensor (0.6 mM naringenin added to DOC_x). DOC_{plant} and DOC_{comp} decreased naringenin compared with the control (one-way ANOVA with Dunnett's multiple comparisons test, $^*P < 0.005$ and $^{**}P < 0.001$). (**C**) Evaluating the role of DOC enzymes using heat treatment. Untreated (+DOC_{plant}) and heat-treated (+DOC_{plant} +heat) maple wood DOC were mixed with naringenin (0.6 mM), and bioavailability was measured using the biosensor. (**D**) Examining the role of DOC enzymes on naringenin (75 μM) using sodium azide (NaN_3), a laccase inhibitor. HPLC did not detect any difference in naringenin availability between the control (+DOC_{plant}) and azide treatment (+DOC_{plant} +NaN_3). (**E**) Naringenin (0.6 mM) availability before (+DOC_{plant}) and after 1 mM EDTA addition to maple wood DOC (+DOC_{plant} +EDTA). EDTA increased naringenin compared with DOC_{plant} (unpaired t test, $^{**}P < 0.001$), which implicates a metal-mediated reaction as the mechanism for naringenin reduction. Error bars represent ±1σ calculated using $n = 3$.

Flavonoid loss is unlikely to be mediated by enzymes

Although we used autoclaved materials in our previous experiments, it is very difficult to achieve complete sterilization and enzyme inactivation in soils and litter materials (*19*). Thus, one potential explanation for the observed decrease in naringenin after incubation with DOC_{plant} is extracellular enzymes present in the soluble fraction (e.g., laccases and peroxidases) that can chemically modify naringenin (*20*, *21*). To test this hypothesis, we used two different methods to examine biotransformation as the cause for naringenin decrease. First, we compared the effect of heat-treated DOC_{plant} with untreated DOC_{plant} (*22*). To accomplish this, DOC_{plant} was heated at 95°C for 15 min and then rapidly cooled on ice to denature enzymes; untreated DOC_{plant} was kept at 30°C throughout the experiment. If enzymatic reactions were important to naringenin signal reduction, then heat treatment should decrease (or eliminate) any naringenin loss. However, we observed no difference between heat-treated and untreated samples. (unpaired t test, $P > 0.05$; Fig. 2C). Second, we added an inhibitor to DOC_{plant} to block the activity of laccases, a common family of soil enzymes capable of oxidizing flavonoid structures (*23*). Similar to heat treatment, this enzymatic inhibitor did not alter naringenin (unpaired t test, $P > 0.05$; Fig. 2D). Because both treatments did not cause any increase in naringenin concentration compared to the untreated controls, these results suggest that naringenin loss is unlikely to be mediated by enzymatic effects.

Metal ions contribute to DOC interaction with flavonoids

Leaves and tree bark contain high concentrations of polyphenolic chemicals, which can account for up to 40% of their dry weight (*24*). Some of these plant-released polyphenols can bind metal ions and form complexes that detoxify the rhizosphere (*25*). We investigated whether the decrease in naringenin is caused by metal-mediated complexation with other polyphenolic compounds (e.g., tannins) present in DOC_{plant} (*26*). This idea was tested by adding EDTA, a chelator that binds diverse metal cations, to DOC_{plant} before adding naringenin. The metal chelator attenuated the effect of DOC_{plant}, increasing the bioavailability of naringenin (unpaired t test, $P < 0.001$; Fig. 2E), indicating a role for metals in the quenching reaction.

Flavonoid chemical structure controls susceptibility to signal interference

To date, a vast number of flavonoid subclasses have been identified (*1*). The synthesis and release of these diverse plant secondary metabolites depend on several parameters including the environmental conditions and the plant species and developmental stage (*27*). To test whether the decrease in flavonoid signal by DOC_{plant} is specific to the flavanone naringenin, we additionally tested DOC effects on the flavone luteolin (3′,4′,5,7-tetrahydroxyflavone) and the flavonol quercetin (5,7,3′,4′-flavon-3-ol). These flavonoids differ in the number of hydroxyl substituents and C═C double bonds (fig. S1A).

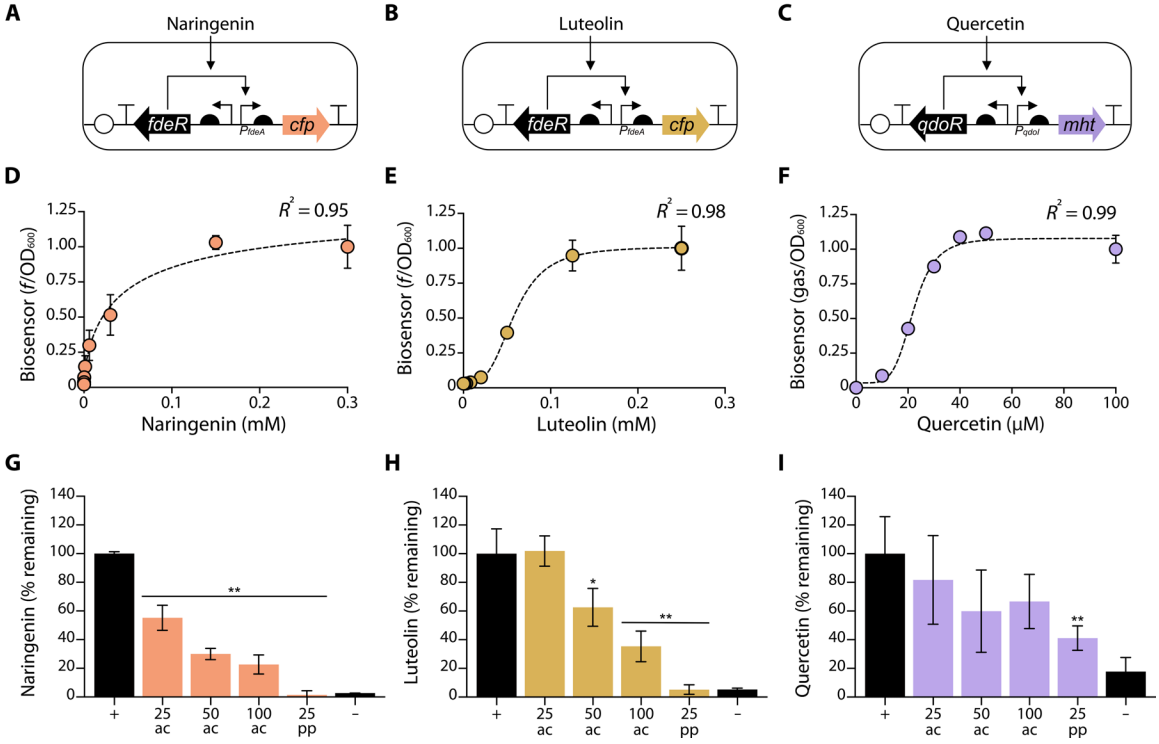

Fig. 3. Plant organic matter has varying effects on the availability of different flavonoids. FdeR activates CFP expression in the presence of (**A**) naringenin or (**B**) luteolin. (**C**) QdoR activates methyl halide transferase (MHT) expression in the presence of quercetin. Biosensor dose-response curves for (**D**) naringenin, (**E**) luteolin, and (**F**) quercetin fit to the Hill function. Standard curves represent background-subtracted signals normalized to the average maximum signal. (**G** to **I**) Varying concentrations of POC_{plant} from maple (ac) and mesquite (pp) were incubated in phosphate buffer (10 mM, pH 7) for 24 hours, including 25 mg ml^{-1} (25_{ac}), 50 mg ml^{-1} (50_{ac}), and 100 mg ml^{-1} (100_{ac}) of maple wood or 25 mg ml^{-1} (25_{pp}) of mesquite wood. Samples were centrifuged to obtain DOC, which was mixed with (G) 0.6 mM naringenin, (H) 0.6 mM luteolin, or (I) 120 μM quercetin. After 24 hours, the biosensor was used to quantify bioavailable flavonoid. *$P < 0.05$ and **$P < 0.001$ indicate statistical significance for one-way ANOVA test with Dunnett's multiple comparisons test between each treatment and the positive control. Positive (buffer and flavonoid only) and negative (buffer only) controls are shown in black. Error bars represent ±1σ calculated using $n = 3$.

To measure bioavailability of luteolin, we used the naringenin-responsive FdeR biosensor (Fig. 3, A and D), which additionally detects luteolin (Fig. 3, B and E). To measure quercetin availability, we engineered a biosensor that uses the transcriptional repressor QdoR (*18*) to regulate the production of an indicator gas (*28*). When QdoR binds quercetin (Fig. 3, C and F), it switches on the production of an enzyme that synthesizes methyl halide as an indicator gas (*28*).

We found that varying concentrations of DOC_{plant} from maple wood (DOC_{ac}; 25, 50, and 100 mg ml^{-1}) and mesquite wood (*Prosopis sp.*) (DOC_{pp}; 25 mg ml^{-1}) attenuate the bioavailability of naringenin and luteolin, while only DOC_{pp} affected the bioavailability of quercetin. The magnitude of the signal attenuation depended on the biomass incubated with the flavonoids. DOC_{pp}, which contains high amounts of polyphenols (*29*), attenuated naringenin to a greater extent than DOC_{ac} (one-way ANOVA, Dunnett's multiple comparisons test, $P < 0.001$; Fig. 3G). Higher concentrations of DOC_{ac} were necessary to decrease the bioavailability of luteolin to the same extent as naringenin (Fig. 3H). The half-maximum response of naringenin was found to be approximately 40 mg ml^{-1} of DOC_{ac}, while about 69 mg ml^{-1} of DOC_{ac} was necessary to decrease luteolin signal by half (nonlinear fit, naringenin $R^2 = 0.97$, luteolin $R^2 = 0.91$). DOC_{pp} was the most effective sample at decreasing the bioavailable level of luteolin (one-way ANOVA, Dunnett's multiple comparisons test). DOC_{ac} had no effect on the bioavailable level of quercetin at any concentration tested (Fig. 3I). DOC_{pp} decreased quercetin (59% of buffer control) to a lesser extent than naringenin (98% of buffer control) and luteolin (94% of buffer control) (one-way ANOVA, Dunnett's multiple comparisons test, $P < 0.001$). Overall, these experiments demonstrate that the different substitutions to the B and C carbon rings decrease the interaction of the flavonoids with two sources of DOC_{plant}.

Metals promote linkages between naringenin and DOC molecules

Our experiments with EDTA suggested a role for metals in the reaction that attenuates flavonoid availability. There are at least two possible ways that metals could participate in this chemistry: metal-mediated complexation or catalytic dimerization. Complexation requires functional groups with an unshared pair of electrons, suggesting that the additional hydroxyl groups in luteolin and quercetin (fig. S1A) should increase their ability to complex metal ions (*26*). However, luteolin and quercetin showed a decrease in reactivity, indicating that the metal-dependent loss of naringenin signal after incubation with DOC_{plant} is unlikely to arise from complexation.

The loss of flavonoids after incubation with DOC_{plant} might arise from a dimerization reaction catalyzed by a transition metal ion and oxygen (*30*). To test this hypothesis, we mixed naringenin with the common flavan-3-ol catechin in the presence of MnO_2 and evaluated

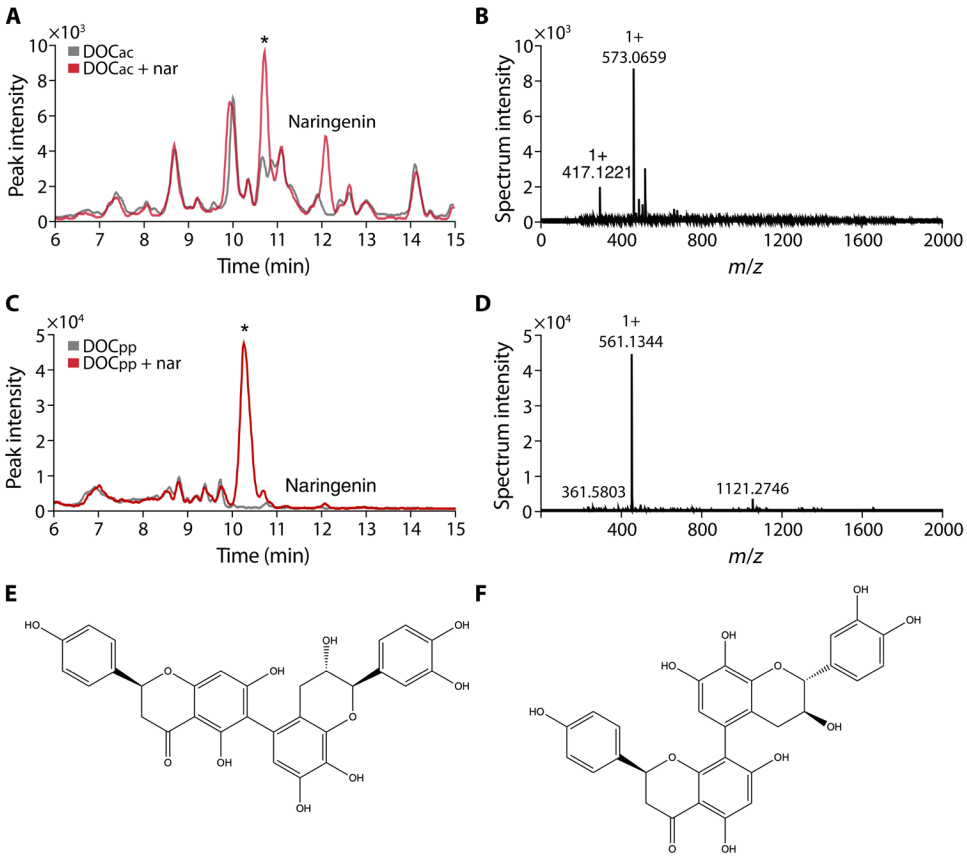

Fig. 4. Chromatograms of DOC show a new peak after naringenin addition. (**A**) Chromatograms of maple DOC before (+DOC_{ac}; gray line) and after (+DOC_{ac} + nar; red line) naringenin addition show that a peak (asterisk) appears with a retention time of 10.7 min that has (**B**) an *m*/*z* of (*M* + 1) = 573. Naringenin has a retention time of 12.1 min under identical conditions. (**C**) Chromatograms of mesquite DOC before (+DOC_{pp}; gray solid line) and after (+DOC_{pp} + nar; red solid line) naringenin addition reveal that a peak (asterisk) appears at a retention time of 10.3 min with (**D**) an *m*/*z* of (*M* + 1) = 561. (**E** and **F**) Structure of the chemical formed in DOC_{pp} following the addition of naringenin, which was determined using NMR spectroscopy. The bonding topologies of isolated products were analyzed (see text S1) and determined to be consistent with the structures of mesquitol-C(5)-C(6)-naringenin and mesquitol-C(5)-C(8)-naringenin heterodimers.

the effect of this reaction on naringenin. We detected a decrease in naringenin bioavailability using the biosensor (unpaired *t* test, $P < 0.001$) (fig. S6). A similar type of reaction could sequester naringenin into covalent conjugates with polyphenols in DOC to yield flavanone-flavonol dimers similar to those observed in *Pinus sp*. bark (*31*) and *Acacia nigrescens* (*32*). To examine whether naringenin interacts with DOC_{plant} to form a dimeric covalent complex, we used LC mass spectrometry (MS) to evaluate how DOC_{ac} affects naringenin. In these chromatograms, naringenin presented a retention time of 12.1 min (figs. S7 and S8) that was distinct from the peaks observed in DOC_{ac} (fig. S9). We incubated naringenin with DOC_{ac} for 24 hours and observed a decrease in the naringenin peak that correlated with the generation of a new peak (573.0659) having a retention time of 10.7 min (Fig. 4A). The mass to charge ratio (*m*/*z*) of this new peak, which represents a +1 species, is greater than that observed for naringenin (273.0659) (Fig. 4B). This finding suggests the formation of a new compound made up of naringenin ($C_{15}H_{12}O_5$) and a second DOC-derived polyphenol or a compound that is being displaced from a polyphenolic complex by naringenin. The *m*/*z* of the compound with this peak is similar to that calculated for compound of molecular formula $C_{29}H_{17}O_{13}$ (573.0663). A wide range of compounds with similar *m*/*z* has been observed in chemical footprints of natural DOC (*33*).

Our previous results (Fig. 2E) showed that the loss of naringenin was suppressed by the addition of EDTA. To confirm that the EDTA mechanism of suppression was through binding to metals in solution and not through binding directly to DOC, we conducted a control experiment where we added EDTA to DOC_{ac} before naringenin and found no change in the chromatogram profile (fig. S10A). This result suggests that EDTA is not reacting with compounds present in DOC_{plant} and that the chemical reaction underlying the formation of the new peak having a retention time of 10.7 min and *m*/*z* of 573.0659 is catalyzed by metal ions in DOC. The addition of EDTA and naringenin to DOC_{ac} halts the formation of the new peak at a retention time of 10.7 min (fig. S10B). Because polyphenols can act as scavengers of free radicals under the aerobic conditions where our experiments were performed, there is also the possibility that the linkage between DOC_{plant} and the flavonoids was catalyzed by a radical coupling reaction activated by reactive oxygen species. For example, this reaction could be initiated by a hydrogen atom transfer or a single electron transfer event (*34*).

Flavonoid and DOC react to form heterodimers

Our understanding of the polyphenol composition of DOC_{ac} remains limited. In contrast, mesquite wood releases DOC with a high proportion of tannins, particularly the flavan-3-ol mesquitol (fig. S1B)

(*29*). To explore how DOC derived from mesquite wood affects naringenin, we conducted a series of LC-MS and nuclear magnetic resonance (NMR) experiments using DOC_{pp}. Because chalcan-flavan-3-ol and flavanone-flavonol dimers have been observed in *Pinus sp.* bark (*31*) and *A. nigrescens* (*32*), we hypothesized that a similar reaction might occur between the mesquitol in DOC_{pp} and naringenin through an oxidative reaction that couples aromatic rings to form a dimer (*30*). When DOC_{pp} was incubated with naringenin (Fig. 4, C and D), we observed the formation of a peak with an *m*/*z* (561.1344) for a +1 species that is similar to that calculated for a compound having the formula $C_{30}H_{25}O_{11}$ (561.1396). To establish the compound that is formed by this reaction, we purified it by its molecular weight and analyzed its structure using one-dimensional (1D) ^{1}H and 2D ^{1}H-^{13}C heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC). This analysis identified two heterodimeric adducts as the products generated under these conditions (Fig. 4, E and F). These results show that the decrease in naringenin bioavailability is caused by a direct metal-mediated reaction with the mesquitol present in DOC_{pp}.

Amendment with plant-derived organic matter inhibits flavonoid-mediated nodulation

Our results suggested that DOC_{plant} might interfere with the flavonoid-driven plant-microbe signaling that underlies nodulation. To test this hypothesis, we inoculated *M. sativa* seedlings with its symbiont *E. meliloti* in the presence and absence of POC_{plant}. This symbiosis is regulated by luteolin, which activates the production of Nod factors in *E. meliloti* (*2*). For *M. sativa* nodulation experiments, we added POC_{plant} to agar slants for each seedling, inoculated the agar with *E. meliloti*, inserted a single seed, and counted nodule numbers after 44 days of growth. When POC_{plant} were added in the absence of added nitrogen, the number of nodules in *M. sativa* plants was 75 ± 35% lower than the number observed in the absence of POC_{plant} (unpaired *t* test, $P < 0.05$; Fig. 5). The number of nodules in the presence of POC_{plant} was not significantly different from plants that had not been inoculated with *E. meliloti* and grown in the presence or absence of added nitrogen.

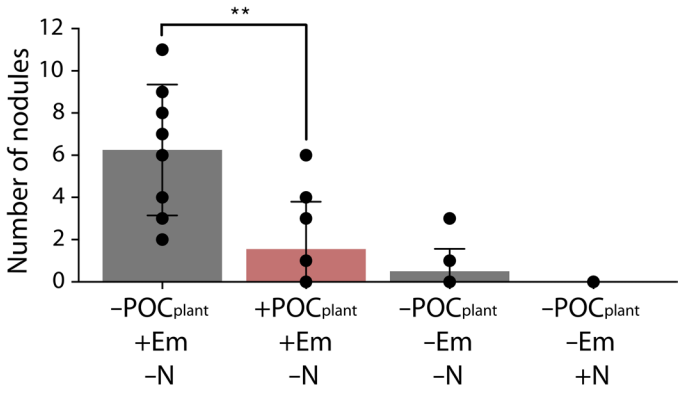

Fig. 5. Maple wood decreases the number of nodules in *M. sativa* plants. The number of nodules in *M. sativa* plants grown on agar slants under different conditions is shown. Inoculations lacking *E. meliloti* (−Em) or containing *E. meliloti* (+Em) and samples lacking ($-POC_{plant}$) or containing maple wood within the agar slants ($+POC_{plant}$) are shown. Slants either contained no nitrogen (−N) or 5 mM NH_4NO_3 (+N). The addition of POC_{plant} significantly decreased the number of nodules in *M. sativa* ($n = 10$; two-tailed *t* test, $P < 0.001$).

DISCUSSION

Reactions with organic matter can attenuate flavonoid bioavailability in soils

Our results provide evidence that as plant-derived organic matter accumulates in soil, it attenuates flavonoid signaling through a metal-mediated reaction between flavonoids and DOC. We demonstrate that this reaction occurs with DOC derived from many plant sources and find that the degree of attenuation is dependent on the chemical structure of the specific flavonoid. Furthermore, we show that flavonoid attenuation by DOC is sufficient to repress signaling critical to plant growth and diminish important ecological processes such as nodulation.

The use of OC-vulnerable signals could be an evolutionary and environmentally dependent adaptation that allows plants to regulate their investment in symbiosis when environmental levels of nutrients change. The abiotic repression of flavonoids is expected to be widely ecologically relevant because oxidative metals are ubiquitously present in the environment and are capable of driving dimerization reactions like the ones we observed in this study. One example of these metals is Mn^{3+}, which is observed at the sites of active plant biomass decay and is an important metal implicated in litter decomposition (*35*). Other heterotrophic bacteria, as well as fungi, can produce exoenzymes that oxidize Mn^{2+}, which can form soluble complexes with DOC that act as oxidants when a fresh input of plant-derived polyphenolic compounds is present in the environment. In future studies, it will be interesting to further explore which metals are responsible for the observed chemistry. Greater insight could be obtained by evaluating the effects of chelators with different metal specificity profiles on the loss of flavonoids.

Flavonoid–organic matter reactions silence plant-microbe communication

We applied concepts from information theory, a field that studies how noise affects the transmission of information between a sender and a receiver, to chemical communication in soils. We found that the interaction of OC with oxidative metals functions as environmental noise, which deamplifies flavonoid-mediated information transfer between organisms and influences the maintenance of host specificity under different environmental conditions. OC can act as a selective pressure to enhance signal selection based on communication efficiency, which then leads to different evolutionary trajectories in a habitat-specific manner. Plant-microbe signaling faces multiple selective pressures from the environment (e.g., temperature and hydration) and pressures related to biotic effects such as pathogen defense and nutrient competition. Previous research has primarily focused on the implications of different flavonoid chemical structures to explain biotic effects, such as host specificity and pathogen defense (*1*). However, the underlying mechanistic causes of these effects are not well understood. Our finding that modifications of flavonoid chemical structures can lead to different interactions with OC highlights the potential importance of the soil chemical environment to the outcome of ecological processes and the evolution of habitat-specific chemical communication (*6*). For example, nodulation factors in various *Rhizobia spp.*, which serve as symbionts for different hosts, are induced by different types of flavonoids (*35*). The extent to which our findings will apply to other flavonoids is

not known because flavonoid production by plants and sensing specificity in different species of bacteria are not well understood. However, our findings suggest that soil and other environmental properties will need to be considered to fully understand this type of cell-cell communication sufficiently to anticipate community behaviors a priori.

Our results showed similar trends using HPLC and biosensors, although the bioavailability was generally lower than the total chemical detectability. To study bioavailability, we applied natural transcriptional regulators, such as FdeR from *Herbaspirillum seropedicae*, which detects both naringenin and luteolin. While our studies focused on using FdeR to detect these two chemicals, soil organisms have evolved other flavonoid sensors with overlapping specificities (*36*). Because genetic elements in soil microorganisms can detect flavonoids with different sensitivities and chemical specificity profiles in the environment, other biosensors could be developed and used in the future to understand how the bioavailability of these molecules controls microbial behaviors across soils.

Soil geochemistry as a mediator of chemical interactions

The OC-flavonoid interactions observed in this study are one example of the general effects of soil properties on plant-mediated chemical signal transduction. Plants interact with diverse beneficial bacteria and fungi. These interactions are also mediated by other plant secondary metabolites secreted into the rhizosphere, including organic acids, strigolactones, phytohormones, and terpenes (*37*, *38*). Studying the impact of abiotic soil properties on the lifetimes of these different chemicals could help us understand the mechanisms by which growth-promoting bacteria function, and the insight obtained from these studies could be used to predict how microbe-plant interactions would change during fluctuating environmental conditions, when soil characteristics can change drastically (*39*). At the same time, some of the same chemicals are used by antagonists (e.g., herbivores and pathogens) to find and attach to a plant host (*37*). The information conflict that results in the same signal being used by mutualists and antagonists has often been described as an ecological cost of chemical signaling (*6*). The level of repression due to OC–chemical signal interaction observed in this study suggests that soil conditions could have strong effects on the outcomes of signal-mediated plant-microbe interactions and the community dynamics in natural and agricultural habitats.

Similarly, soil geochemistry and its interaction with chemical signals could provide opportunities for plants to manipulate interactions with their plant neighbors and affect their competition with proximal plants (*28*). The observed OC-flavonoid quenching observed here might be used by plants under selection to foster OC deposition. Alternatively, plants might secrete chemicals to affect neighbor plant-microbe signaling to limit the accessibility of nutrients. For example, in an ecosystem where nitrogen is limited, plants could leverage their own OC to interfere with the flavonoid signals needed by their rivals to establish symbioses and secure dominance in the ecosystem (*40*).

Overall, the role of OC on the availability of chemical signals that control interactions between organisms can be viewed as an environmental mediator (*6*) whose strength and effect are a function of both biotic processes (signal synthesis, structure, and sensitivity of sensors) and abiotic environmental conditions (sorption to POC and reaction with DOC). As such, this type of chemical-mediated interaction can be expected to be adaptable (*41*), and it could also be purposefully manipulated in the future to gain a fitness advantage for the benefit of a crop plant in agriculture. At the same time, soil-induced alterations of plant secondary metabolites, such as phenolics, will need to be considered because they can alter soil respiration and affect carbon cycling (*42*).

Implications for agricultural intensification and rhizosphere engineering

While plants have been engineered to produce specific root exudates that might favor/deter communication with the rhizosphere, many such strategies have failed because of compounds rapidly degrading in the soil or their bioavailable concentration not being enough to influence microbial behavior in situ (*43*). Information regarding half-lives of plant secondary metabolites under different soil conditions could be intentionally used to engineer more beneficial plant-microbe interactions and design effective soil amendments. Using soil properties to control chemical signaling can be used to boost existing beneficial interactions and/or impede pest growth. Manipulating chemical information to the benefit of a crop plant has been used to control *Striga spp*. weeds and stem boring *Lepidoptera* on maize and sorghum in East Africa (*5*). There, functional intercropping with legumes of the genus *Desmodium* produced strigolactone germination cues for the parasitic plants, while *Desmodium* themselves are not appropriate hosts. The *Striga* weed seedlings germinate but are unable to attach to a suitable host and die (*44*). A functional but false chemical signal mediating an antagonist interaction led to an almost complete control of the weed to the benefit of the crop plant. Our findings suggest that the decrease in signals through DOC enrichment of soils could also affect weed control or that an application of the

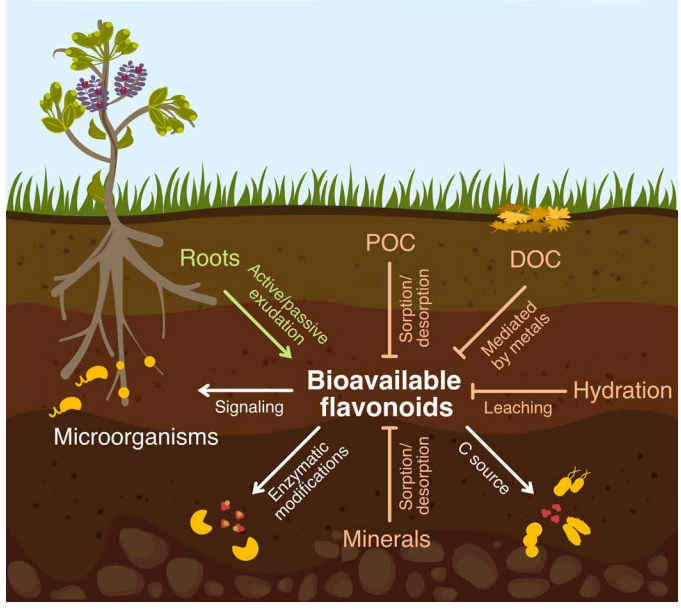

Fig. 6. Environmental properties affecting flavonoid bioavailability in soils. Following the release of flavonoids into the rhizosphere through passive or active exudation (green), these chemicals can interact with the soil microbiome and different soil components. Abiotic attenuation mechanisms (orange) include sorption by minerals and POC, interactions with DOC as described here, and leaching. Leaching enhances the signal dilution through the soil at high hydration conditions because the signal can readily diffuse through the soil column. Biological processes that specifically attenuate flavonoid communication (white) include consumption and biotransformation through the release of exoenzymes.

findings could help optimize the efficiency of this functional intercropping. As such, this knowledge is very important for ecological intensification approaches, such as the push-pull technology (*5*).

In addition, the use of soil amendments with a known impact on signaling processes can be used to help and boost beneficial plant-microbe interactions. For example, naringenin has been shown to incite intercellular colonization of wheat roots by a diazotroph via crack entry (*45*); therefore, understanding naringenin fate in soil could help design soil amendments that provide a competitive advantage to engineered microbes capable of developing synthetic symbiotic relationships with nonfixing crops. Another approach is to enhance nodule-independent nitrogen fixation by manipulating exchange signals to increase the competitive advantage of free-living diazotrophs in the rhizosphere (*3*). Accordingly, a mechanistic understanding of how flavonoids and other PSM (plant secondary metabolites) bioavailability can change depending on soil physicochemical properties will aid the development of mathematical models that help predict how different environmental contexts relate to plant nutrition (Fig. 6).

In this work, we show that the soil matrix can physically and/or chemically render molecules undetectable to microbes (e.g., by sorption or chemical reactions). Because soils are not sterile, the abiotic effects observed here should next be compared with the magnitude and temporal dynamics of microbial modifications known to affect flavonoids within the rhizosphere (*1*). The comparison of these dynamic biotic and abiotic signals will be necessary to develop a full picture of the scope of controls on flavonoid signaling in soils. This includes validating the signaling attenuation using soils that have been subjected to a range of sterilization methods, because the strategy used for sterilization in this study could affect soil properties.

MATERIALS AND METHODS

Soils and organic amendments

A range of Inceptisols was collected from three sites in upstate New York. At each site, soil samples were taken from adjacent areas subjected to different land use, including agricultural, meadow, and forest. Soils were air-dried, stored at room temperature, and autoclaved twice before use. No other methods of sterilization were performed. Soil carbon and nitrogen were measured using a LECO TruMac CNS analyzer (LECO, Saint Joseph, MI). Soil amendments included compost (POC_{comp}), maple wood chips (POC_{plant}) (*46*), and biochar (PyOM) made from the same stock of maple wood chips (*Acer sp.*) at two different temperatures of 550°C ($PyOM_{550}$) and 750°C ($PyOM_{750}$). Maple wood chips were sieved to between 600 and 850 μm. Biochar was made under an argon atmosphere, with a modified muffle furnace at a ramp rate of 2.5°C min^{-1}, a 30-min dwell time at the maximum temperature, and a 3-hour cool time. Biochar was sieved to a particle size between 600 and 850 μm. Maple biochar was pyrolyzed at 550°C and 750°C and contained a moisture content (% air dry weight) of 2.7 and 4.0%, volatiles (% at 105°C dry weight) of 12.8 and 6.6%, and ash (% at 105°C weight) of 1.8 and 1.8%, respectively. Compost peat was from Landscapers Pride, Texas (composted screened pine and other organics). Mesquite wood chips (*Prosopis sp.*) were purchased from H-E-B supermarket, Texas.

Naringenin incubations with soil amendments

To determine the effect of soils and OC amendments on the availability of naringenin, nine different soils (sampled from three sites and with each of three land uses) and four different soil amendments (POC_{comp}, POC_{plant}, $PyOM_{550}$, or $PyOM_{750}$) were incubated separately in centrifuge tubes with different concentrations of naringenin in 10 mM phosphate buffer (pH 7) while shaking at 30°C. Soils and amendments were added at a concentration of 25 mg ml^{-1}, corresponding to saturated water content for all the soils tested. After 24 hours, the samples were centrifuged (10,000 rpm, 10 min), and the supernatant was removed for analysis by either HPLC or the biosensor. Different concentrations of naringenin were used depending on the measurement, because of differences in sensitivity of each approach. For the HPLC experiment, 75 μM naringenin was added to the soils and amendments. The supernatant was then measured directly. For the biosensor experiments, 0.6 mM naringenin was added to the soils and amendments. The supernatants were then collected and diluted 1:1 with the biosensor cells. This lowered the maximum naringenin concentration to 0.3 mM in these measurements, a value within the range of biosensor detection.

Flavonoid incubation with the dissolved fraction of soil amendments

To determine the effect of the soluble fraction of soil amendments on naringenin, 25 mg ml^{-1} of each of the amendments (POC_{comp}, POC_{plant}, $PyOM_{550}$, or $PyOM_{750}$) was incubated for 24 hours in 10 mM phosphate buffer (pH 7) while shaking at 30°C. Samples were centrifuged (10,000 rpm, 10 min), and supernatant from each amendment was transferred to a new tube (DOC_{comp}, DOC_{plant}, $PyDOC_{550}$, or $PyDOC_{750}$). The naringenin concentration added to DOC was different in the HPLC (40 μM) and biosensor (0.6 mM) experiments. The tubes containing DOC and naringenin were incubated for 24 hours at 30°C while shaking at 250 rpm. Following the incubation, the aqueous phase (DOC) was removed for analysis with either HPLC or biosensors. For experiments studying how flavonoid chemical structure relates to susceptibility to DOC (25, 50, and 100 mg ml^{-1}) derived from POC, mesquite or maple POC was first incubated in buffer to obtain DOC from the amendments. After the DOC fraction was separated, 0.6 mM naringenin, 0.6 mM luteolin, or 120 μM quercetin was added to the supernatant and incubated for 24 hours at 30°C before analyzing the amount remaining in solution using the biosensor. The maximum concentrations of flavonoids analyzed by biosensors correspond to half the amount initially added to DOC because mixing with the biosensor resulted in a two-fold dilution of the flavonoid and DOC mixtures.

Incubations to determine enzyme contributions

To determine whether enzymatic degradation altered naringenin availability following sterilization, we compared two conditions: heat treatment, which was designed to denature any remaining active enzymes, and the addition of an oxidase inhibitor. To evaluate the effect of each treatment, naringenin was incubated with (i) DOC_{plant} supernatant that had been heat-treated at 95°C for 15 min and then rapidly cooled on ice for 10 min before naringenin addition; (ii) DOC_{plant} that contained 0.1 mM sodium azide, an oxidase inhibitor; and (iii) untreated DOC_{plant} (control treatment). After heat treatment, naringenin bioavailability was measured with the biosensor, while the concentration following the addition of sodium azide was measured using HPLC because sodium azide is toxic to the biosensor.

HPLC measurements

An Agilent 1100 series HPLC was used to measure the concentration of naringenin in supernatants. To separate naringenin from other

chemicals in soils and amendments, we used a Gemini C18 reversed-phase column (Phenomenex Inc., Torrance, CA) with an injection volume of 15 µl and eluted with 0.25% H_3PO_4 and acetonitrile. The flow rate was 1 ml min^{-1}. During the first 6 min, we used 100% of a 0.25% H_3PO_4 mobile phase. During the next 4 min, acetonitrile was increased using a linear gradient to 12%, then 18% for the next 20 min, and finally 58% for 15 min. Naringenin elution was monitored using absorbance at 280 nm, and concentrations were determined by comparing peak areas to standards prepared in methanol using naringenin from Cayman Chemical. Figure S1B shows the naringenin standard curve.

Plasmid construction

Naringenin and luteolin sensor

A previously described plasmid encoding the naringenin biosensor (*18*) was transformed by heat shock into *E. coli* BL21(DE3). The same biosensor was used to monitor luteolin bioavailability.

Quercetin biosensor

The QdoR transcription regulator under the regulation of its original constitutive promoter from *Bacillus subtilis*, the P_{qdoI} promoter, and a synthetic ribosome binding site (RBS) were synthesized as a gBlock by Integrated DNA Technologies. The synthetic RBS was designed using a thermodynamic model for translation initiation (*47*). Using Gibson assembly (*48*), the gBlock was cloned into a vector containing a pBBR1 origin of replication, kanamycin selection marker, and the *mht* gene as gene expression output. The *mht* gene encodes for a methyl halide transferase enzyme, which uses the common metabolite *S*-adenosyl methionine and halide ions to produce methyl halides, gases that can diffuse out a matrix and be used as a reporter through headspace detection (*28*). The construct was sequence-verified, and this vector was transformed into *E. coli* MG1655 to create the quercetin biosensor.

Naringenin and luteolin biosensor measurements

Naringenin and luteolin biosensors were grown for 18 hours while shaking at 30°C in lysogenic broth (LB) containing kanamycin (50 µg ml^{-1}). Cells were washed twice and resuspended in M63 medium (*28*). Supernatants (100 µl) from the incubation experiments were mixed with an equal volume of biosensor cultures (100 µl) containing cells diluted to an OD_{600} (optical density at 600 nm) of 0.05. This mixture was placed in a transparent U-bottom Falcon 96-well plate, and cells were incubated while shaking at 30°C for 24 hours. Fluorescence (λ_{ex} = 475 nm and λ_{em} = 433 nm) and OD_{600} were measured using a Tecan Infinite M1000 microplate reader. Relative fluorescence was calculated as the ratio of fluorescence to OD_{600}, and these values were normalized to the maximum response.

Quercetin biosensor measurements

The quercetin biosensor was grown for 18 hours while shaking at 30°C in LB containing kanamycin (50 µg ml^{-1}). Cells were washed twice and resuspended in a modified M63 medium containing 100 mM NaBr (*28*). The supernatants (100 µl) from incubation experiments were mixed with cultures of quercetin biosensors (100 µl) at an OD_{600} of 0.1 (8×10^7 cells ml^{-1}), and this mixture was placed in sealed 2-ml Phenomenex glass vials to allow indicator gas accumulation. After incubating the cells while shaking at 30°C for 5 hours, the indicator gas was measured using an Agilent 7820A gas chromatograph and a 5977E mass spectrometer. Headspace gas (50 µl) was injected into a PoraPLOT Q capillary column (24 m; 0.25-mm ID and 8-mm film) at a 50:1 split ratio, and the following oven temperature gradient was used to separate methyl bromide from other gases: 85° to 105°C at 12°C min^{-1}, to 150°C at 65°C min^{-1}, and a final hold for 144 s. MS analysis was performed using selected ion monitoring mode for methyl bromide (molecular weight = 93.9 and 95.9). Agilent MassHunter Workstation Quantitative Analysis software was used to quantify peak area. Immediately after measurement, vials were uncapped and OD_{600} was measured using a Cary 50 spectrophotometer (Varian, Palo Alto, CA). Biosensor signals were reported as the ratio of methyl bromide peak area to OD_{600} normalized to the maximum response.

Manganese dioxide experiments

To test for the possibility of a flavonoid and DOC oxidation reaction catalyzed by metals, naringenin (0.6 mM) was incubated with MnO_2 (1 mg) in the presence or absence of catechin (0.6 mM) for 24 hours at 30°C in 1 ml of phosphate buffer at pH 7. Controls to check for naringenin biosensor reactivity to catechin (+cat-MnO_2), the effect of MnO_2 to cells (–MnO_2), and biosensor leakiness (no treatment) were included. Following incubation, samples were centrifuged for 3 min at 16,000*g* to obtain clarified supernatant, and naringenin was measured using the biosensor.

Liquid chromatography–mass spectrometry

To identify molecular species in DOC_{plant} that are affected by naringenin, we separated the ensemble of chemicals in the DOC chemicals using an HPLC (1200 HPLC, Agilent, Santa Clara, CA) and analyzed their mass using micrOTOF ESI (Bruker, Billerica, MA). Chemicals were bound to a Luna C18 reversed-phase column (Phenomenex Inc., Torrance, CA) by injecting 25 µl of DOC before or after the addition of naringenin or EDTA. The mobile phase consisted of a mixture of 0.1% formic acid in water (pump A) and 0.1% formic acid in acetonitrile (pump B) (*49*). The flow rate was 0.25 ml min^{-1} following the gradient program: 0 to 11 min at 80% A, 11 to 18 min at 30% A, and 18 to 25 min at 80% A. The MS was performed using ion positive mode to scan over the range of 100 to 2000 *m*/*z*. Data were analyzed using Bruker Compass Data Analysis 4.2. Graphs are shown as total ion chromatogram within the *m*/*z* scanning range. Retention time and mass spectrum fragmentation of naringenin and EDTA were compared using naringenin dissolved in ethanol and EDTA prepared in water, with the pH adjusted to 8.0 using 1 M sodium hydroxide.

HPLC purification

To obtain the product arising from naringenin reaction with DOC for NMR analysis, DOC_{pp} (500 ml) was mixed with 0.6 mM naringenin and incubated for 24 hours at 30°C. The crude material was dried by lyophilization. Solutions were then prepared in 10% aqueous dimethyl sulfoxide (DMSO) with 0.1% trifluoroacetic acid (TFA) for purification by reversed-phase HPLC (Shimadzu CBM-20A instrument with a Phenomenex Jupiter 4 µm Proteo 90 Å 250 × 15 mm column for preparative scale purification). The crude material was purified once (25 to 45% acetonitrile/H_2O with 0.1% TFA over 20 min), and a fraction from 15.0 to 16.6 min was collected. The fractions were concentrated under reduced pressure to remove volatile organics and were lyophilized to dryness to obtain purified material for further analysis. The collected material was then dissolved in 10% aqueous DMSO with 0.1% TFA and subjected to a second round of purification (22% acetonitrile/H_2O

with 0.1% TFA over 180 min). Fractions at 44.4 and 48.1 min were collected for structural analysis.

Nuclear magnetic resonance

NMR spectra of the isolated compounds and of genuine naringenin dissolved in DMSO-d_6 were acquired at 800 MHz and 25°C on a Bruker Avance III HD spectrometer equipped with a quadruple resonance inverse cryoprobe at the Baylor College of Medicine NMR and Drug Metabolism Core. The 1D ^{1}H and 2D ^{1}H-^{13}C HSQC and HMBC spectra were acquired without solvent suppression and analyzed to determine the bonding topologies. All expected ^{2}J and ^{3}J ^{1}H-^{13}C HMBC correlations for the inferred structures (and for naringenin) were observed experimentally, as were several ^{4}J "W"-type couplings (see the Supplementary Materials). No unexplained HMBC correlations were observed.

Nodulation experiments

Nodulation experiments were run to test the influence of POC_{plant} on the nodulation of *M. sativa* in the presence of *E. meliloti* Rm1021 (formerly *Sinorhizobium meliloti*) [U.S. Department of Agriculture (USDA) 1033]. *M. sativa* seeds were first submerged in a 30% bleach solution (~2.5% active chlorine concentration) for 5 min. Seeds were rinsed with autoclaved Milli-Q water six times. Seeds were imbibed in sterile water for 1 hour and then transferred to soft water agar plates made with 1% Bacto Agar (BD Biosciences, San Jose, CA). The water agar plates were wrapped in parafilm, inverted, and incubated in the dark at 20°C. Following 2 days of germination, seeds were moved to F medium agar slants. Three types of medium were prepared following a modified Fahräeus medium (*50*) with 1% agar, F medium containing 5 mM NH_4NO_3 (+N) or without extra source of nitrogen. In addition, F medium contained no wood chips (−POC_{plant}), or maple wood chips (10 mg ml^{-1}) were mixed into the agar just before solidification (+POC_{plant}). Ten seedlings were analyzed in each growth condition. Germinated seeds were incubated for 48 hours at 22°C, with 16 hours of light before inoculation. Cultures of *E. meliloti* were grown from glycerol stock to early stationary phase in TY Beringer medium (*51*), and this culture (250 µl) was added to the roots of *Medicago* slants (+Em). *Medicago* slants inoculated with 250 µl of sterile TY Beringer medium were added as a control (−Em). Following inoculation, seedlings were returned to a growth chamber set at 22°C, with 16 hours of light for 44 days before the nodule number was recorded.

Statistical analysis

For each experiment, the proportion of flavonoid remaining was calculated relative to the average buffer control, which was defined as a 100% availability. Raw data were divided by the buffer control to calculate the proportion of naringenin remaining. All results are expressed as means ± SD. The proportion of naringenin attenuated corresponds to the total signal (100%) minus the percentage of naringenin detected after treatment (% remaining). Flavonoid incubations with soils were fit to a linear regression model. The comparison between buffer condition and POC or DOC from different amendments was calculated using an ordinary one-way ANOVA and a Dunnett's multiple comparisons test with a single pooled variance. Differences between different DOC additions or forms and DOC after treatments (e.g., heat) were analyzed using an unpaired two-tailed *t* test. Dose-response curves of the different biosensors were fitted to a hill function model with no constraints. Nodulation experiments were also compared using an unpaired two-tailed *t* test. Variance of the difference was calculated using the delta method. For all experiments, $P < 0.05$ (*) and $P < 0.01$ (**) were considered statistically significant. GraphPad Prism (version 8) was used for all analyses.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/5/eaax8254/DC1

Fig. S1. Structures of flavonoids.
Fig. S2. Experimental protocols for determining the effects of soils and amendments on naringenin.
Fig. S3. Naringenin bioavailability as a function of soil physiochemical properties.
Fig. S4. Naringenin bioavailability is decreased by POC_{plant} but not by PyOM.
Fig. S5. Naringenin bioavailability is not changed by oxidized PyOM.
Fig. S6. Manganese dioxide mediates a reaction between catechin and naringenin.
Fig. S7. Chromatograms of naringenin and EDTA.
Fig. S8. Mass spectrum acquired for naringenin and EDTA.
Fig. S9. Chromatograms of DOC_{ac} and DOC_{pp}.
Fig. S10. Partial chromatograms of DOC_{ac} after the addition of EDTA in the presence or absence of naringenin.
Fig. S11. Bonding topologies of DOC_{pp} and naringenin heterodimers.
Text S1. Structural determination using ^{1}H and ^{13}C NMR spectra.

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Acknowledgments: We thank K. Hanley for help with sample analyses and D. Buckley, S. Barnett, and N. Youngblut for providing soil samples. **Funding:** We are grateful for financial support from the W. M. Keck Foundation, the Rice University, and the Taiwan Ministry of Education Scholarship. Support for this research was also provided by the NSF-BREAD (IOS-0965336; OPP51589), the Department of Energy (Office of Science; DE-SC0016364), the USDA NIFA Carbon Cycles (2014-67003-22069), the Robert A. Welch Foundation (C-1680), and the NSF (CHE-1609654 and CHE-1904865). **Author contributions:** I.D.V. and T.M.W. designed the study. I.D.V. performed biosensor and LC-MS experiments. T.M.W. performed HPLC and plant experiments. M.K.M. and Z.T.B. performed HPLC purification. K.R.M. executed and analyzed NMR data. C.A.M., J.J.S., and J.L. conceptualized and supervised the research. All authors were involved in the interpretation, writing, and editing of the manuscript. **Competing interests:** The authors declare that they have no competing interests. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the donors. **Data and materials availability:** Additional data related to this paper may be requested from the authors. The naringenin biosensor (pG-FdeR plasmid) can be provided by the Technical University of Denmark (DTU), pending scientific review, and a completed material transfer agreement. Requests for the pG-FdeR plasmid should be submitted to J. Maury (jmau@biosustain.dtu.dk).

Submitted 20 May 2019
Accepted 22 November 2019
Published 29 January 2020
10.1126/sciadv.aax8254

Citation: I. Del Valle, T. M. Webster, H.-Y. Cheng, J. E. Thies, A. Kessler, M. K. Miller, Z. T. Ball, K. R. MacKenzie, C. A. Masiello, J. J. Silberg, J. Lehmann, Soil organic matter attenuates the efficacy of flavonoid-based plant-microbe communication. *Sci. Adv.* **6**, eaax8254 (2020).

Science Advances

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Ilenne Del Valle, Tara M. Webster, Hsiao-Ying Cheng, Janice E. Thies, André Kessler, Mary Kaitlyn Miller, Zachary T. Ball, Kevin R. MacKenzie, Caroline A. Masiello, Jonathan J. Silberg and Johannes Lehmann

Sci Adv **6** (5), eaax8254.
DOI: 10.1126/sciadv.aax8254