Phosphorus (P) adsorption onto soil minerals decreases its bioavailability as compared to the free, orthophosphate P form in solution (Guzman et al., 1994; Geelhoed et al., 1997). Because both orthophosphate and dissolved organic matter (DOM) predominately adsorb to surface hydroxyl functional groups of metal (oxy)hydroxide soil minerals (Ding et al., 2012; Kramer et al., 2012), it has been suggested that carbon (C)-rich soil amendments may increase soil P bioavailability (Zhu et al., 2018). Crop residues, animal manures, and composts are frequently used to maintain soil quality and organic matter levels, and increasing soil P bioavailability may be a beneficial result of their use in sustainably managed agricultural systems. This effect has been extensively examined in laboratory studies with model organic acid compounds. Seven carboxylic acids increased the P solution concentration in an acidic soil, and some of the organic acids reduced P adsorption to the soil surfaces (Schefe and Tymms, 2013). Wang et al. (2016) reported that oxalic, citric, and malic acids reduce P adsorption through ligand exchange and chelation interactions with iron (Fe) and aluminum (Al) minerals. Higher molecular weight humic substances have also been shown to inhibit P adsorption to soil minerals (Antelo et al., 2007; Fu et al., 2013). Although organic matter can compete with orthophosphate for mineral sorption sites and thus reduce P sorption in laboratory studies, the relevance of this mechanism for increasing P bioavailability in field soils has been questioned. The observed apparent reduction of P adsorption by decomposing organic matter may stem from unaccounted P being supplied directly by the added organic matter (Guppy et al., 2005a,b). In a study that accounted for P contribution by DOM, our group has shown that DOM from crop residues and animal manures can reduce P sorption to soil minerals (Hunt et al., 2007). A study using ultrahigh resolution mass spectrometry and atomic force microscopy showed that aromatic DOM molecules that are greater than 600 Da...
reduced the binding force of orthophosphate to iron (oxy)hydroxide (FeOOH) minerals (Chassé and Ohno, 2016). In the current study, we used a bioassay to directly determine the bioavailability of P adsorbed onto FeOOH minerals that were pre-reacted with DOM from C-rich amendments (crop residues, animal manures, and compost) and P to mimic likely field soil components. Our goal was to use a plant bioassay to determine whether DOM extracted from organic soil amendments can increase P solubility and P uptake by plants.

**Materials and Methods**

**Dissolved Organic Matter Extracts**

Crop residue DOM was obtained from field-grown corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max* (L.) Merr.). Beef cattle and horse manure was sampled from outdoor storage in Texas and Maine, respectively. Food compost was obtained from the composting facility at the University of Maine. The DOM was extracted at a deionized-distilled water (DI–H2O)-to-material ratio of 25:1 (v/w) for the crop residues and beef manure, 10:1 for the horse manure, and 5:1 for the food compost to extract similar quantities of DOM. The mixtures were shaken for 24 h at 4°C on an orbital shaker at 170 rpm. Suspensions were then centrifuged (900 x g) for 30 min before vacuum filtering through 0.45-μm polycarbonate filters. The dissolved organic carbon (DOC) concentration was measured using a Shimadzu 5000 TOC analyzer. The P concentration of the solution was determined using the ascorbic acid method (Murphy and Riley, 1962).

The chemical composition of the DOM was characterized using negative ion mode electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry using a 12 T Bruker instrument. The details of the analysis can be found in Chassé and Ohno (2016). The post-processing of the mass spectra data are described in Ohno and Ohno (2013).

**Dissolved Organic Matter and Phosphorus Loading to Iron (Oxy)hydroxide**

Catalysis-grade (30–50 mesh) FeOOH was purchased from Sigma-Aldrich (#371254) and used after repeated washings with DI–H2O. A DOM adsorption index was obtained by reacting 50 mL of 130 mg C L⁻¹ DOM solution adjusted to pH 6 with 0.40 g of FeOOH in duplicate. The suspensions were shaken on an orbital shaker at 120 rpm for 24 h and then centrifuged, filtered, and analyzed for DOC as described above. The quantity adsorbed was calculated by difference from the initial solution. A P adsorption isotherm was obtained by reacting 150 mL of 0- to 60-mg P L⁻¹ solutions with 0.40 g of FeOOH in duplicate. The suspensions were shaken and filtered as described above, and P in the supernatant analyzed by the molybdovanadate method (Clesceri et al., 1998). The data were fit with the Langmuir model. From the isotherm, an initial P concentration of 2.2 mg P L⁻¹ was selected to obtain a P loading of 10% of the adsorption maxima.

To prepare the FeOOH for the bioassay units, 400 mg of FeOOH and 50 mL of 130 mg DOC L⁻¹ solutions were shaken for 24 h in quadruplicate for each DOM type. Then, the solution was decanted, and the FeOOH was rinsed with DI–H2O. Then 150 mL of 2.2 mg P L⁻¹ solution was added and shaken for 24 h. Iron (oxy)hydroxide with no pre-adsorbed DOM was also treated to serve as reference controls. The P solution was decanted, and the FeOOH was gently rinsed and dried for use in the bioassay study.

**Agar-Culture Bioassay Study**

Nutrient solution (no added P) with Fe supplied by Fe-ethylenediamine-N,N’-bis(2-hydroxyphenylacetic acid) also contained 2-(N-morpholino)-ethanesulfonic acid to buffer to pH 6 (Miyasaka et al., 1988). Agar was added to make a 0.9% agar nutrient solution, and the mixture autoclaved for 15 min. A 315-mL aliquot was poured into each container (6.5 by 6.5 by 9.5 cm), which resulted in a final volume of 300 mL after cooling. When the agar reached 31°C, the FeOOH was added into the agar and mixed in slowly to evenly suspend the FeOOH in the cooled agar. Agar blocks were refrigerated until transplanting (<2 d). Tomato (*Solanum lycopersicum* L. ‘German Johnson’) plants were used as the test species. The seeds were surface-sterilized by treatment with 500 mg L⁻¹ sodium dichloroisocyanuric acid solution for 2 h. They were then sown onto moistened germination paper, wrapped in plastic wrap, and placed in an incubator for 5 d at 25°C in darkness. The seedlings were pulled by the root using a tweezer and transferred into 16 punctured holes in the agar arranged in a 4 x 4 grid. The containers received DI–H2O daily after the first week of growth to avoid dehydrating the agar culture. The shoots and roots were harvested 5 wk after transplantation. The P content of the tissue was determined using H2SO4 and 30% H2O2 digestion. The P content of the solution was determined using the colorimetric molybdenumate method (Clesceri et al., 1998).

**Statistical Analysis**

The DOM adsorption and bioassay results were analyzed by one-way ANOVA using Systat 12. Treatment mean separation used the Tukey’s honestly significant difference test at the p = 0.05 level. The nonlinear fitting of the Langmuir adsorption equation was conducted with MATLAB.

**Results and Discussion**

**Phosphorus and Dissolved Organic Matter Sorption to Iron (Oxy)hydroxide**

The quantity of DOM adsorbed onto FeOOH at a concentration of 130 mg DOC L⁻¹ was used to examine the relative sorption affinities of DOM to FeOOH (Fig. 1). The ANOVA showed that the DOM from three crop residues adsorbed to a significantly (p = 0.001) greater extent than DOM from the two manures and the compost, indicating differences in affinity for adsorption based on the type of DOM. The Langmuir model fit the P adsorption onto DOM-free FeOOH well, and the adsorption maxima (Qmax) value was 10.8 mg P g⁻¹ FeOOH (Fig. 2A). On the basis of the isotherm results, the P loading rate in the bioassay study used a solution initially containing 2.2 mg P L⁻¹ to target a P load-
ing of 10% of the calculated $Q_{\text{max}}$. Phosphorus saturation is a common parameter for predicting water-soluble P based on the soil P content relative to that soil's P sorption capacity (Pautler and Sims, 2000). A study based on 106 soils from the northeastern region of the United States showed a change point at about 15% P saturation, based on oxalate-extractable Al, Fe, and P content, where water-soluble P increases rapidly with increasing P loading (Ohno et al., 2007). The target 10% loading was selected to correspond with reduced risk of adverse environmental risks.

As noted by Guppy et al. (2005a), organic soil amendments release water-soluble P, as well as DOM. We determined the orthophosphate content of the DOM solutions to allow calculation of the amount of inorganic P contributed from the DOM extract. The total P loading (expressed as the quantity of P per gram of FeOOH) of the treatments used in the bioassay was 0.825 mg P added as orthophosphate P plus the contribution from the treatment source (Fig. 2B). The P contributed by the amendment source was roughly equivalent to the quantity supplied by the inorganic P addition for the corn residue, beef manure, and food compost; greater for the horse manure; and less for the wheat and soybean residue. The total P loading remained close to or under the 10% P loading target, except for the horse manure treatment, which reached about 35% P loading (Fig. 2A and B). The DOM on the FeOOH decreased the average added orthophosphate P adsorption to 0.50 ± 0.05 mg P g⁻¹ FeOOH from the 0.63 mg P g⁻¹ FeOOH adsorption in the absence of DOM (Fig. 2B). The ANOVA showed that the 19% decrease in the quantity of P adsorbed by FeOOH caused by the DOM treatment was significant ($p = 0.001$), suggesting that organic matter may be able to compete for adsorption sites.

**Phosphorus Uptake Bioassay**

The ANOVA indicated there was no significant effect of DOM loading on the total plant (shoot and root tissue) bio-

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Fig. 1. Sorption of dissolved organic matter derived from crop residues, animal manures, and food compost. Error bars show standard deviations; means sharing the same letters are not significantly different at the $p = 0.05$ level using Tukey's honestly significant difference test.

Fig. 2. (A) Langmuir isotherm plot of the orthophosphate adsorption to dissolved organic matter–free FeOOH and (B) the total addition of P (experimental P addition plus P contributed from the dissolved organic matter from crop residues, animal manures, and food compost) and the quantity of P adsorption onto FeOOH. OM, organic matter; $Q_{\text{max}}$, adsorption maxima.
mass \((p = 0.58)\) (data not shown). Thus, P bioavailability can be equated to P uptake by the plant (dry matter yield times P concentration for shoots and root). Similar to the biomass data, ANOVA showed no significant effect of DOM on P uptake \((p = 0.931)\) (Fig. 3). The absence of a DOM treatment effect on the bioassay P uptake suggests that the DOM initially leached from crop residues, animal manures, and food compost is not likely to increase P bioavailability. The average coefficient of variation of the P uptake data was 13.5%. While typical for variability found in nutrient uptake studies, this variability may potentially mask any effect that DOM has on P availability.

**Phosphorus–Dissolved Organic Matter Interactions**

Ultrahigh resolution mass spectrometry has recently become the principal method for the characterization of DOM, with its ability to assign unique elemental formulas to the thousands of peaks typically detected in DOM extracts (Sleighter and Hatcher, 2007). The relative content distribution of the van Krevelen diagram–derived chemical classification groups are shown in Fig. 4 for the DOM investigated in this study. Across all six DOM extracts, the lignin-like group was the largest, with an average 47% relative content, followed by the non nitrogen-containing aliphatic group, with an average of 39%. The condensed aromatic molecule content was very low, with an average of 0.6%. The aromatic molecule content encompassed a greater range of 1.5% for the wheat DOM to 11.9% for the food compost DOM (Fig. 4). The reason for the inability of DOM to affect P uptake (Fig. 3) in this study despite this 1.5 to 11.9% range in DOM aromatic molecule content is likely due to the fact that only the content of aromatic molecules with molecular mass >600 was significantly negatively correlated to phosphate binding force as determined by atomic force microscopy (Chassé and Ohno, 2016). Food compost DOM, which had an aromatic content of 11.9%, had only 0.6% of its aromatic molecules...
above the 600 Da threshold (Fig. 4B). Beef manure DOM, which had the second-highest aromatic content of 5.2%, had 0% above the 600 Da threshold.

The reason for the inability of DOM to affect P bioavailability likely results from their different bonding mechanisms to FeOOH. A $^{31}$P nuclear magnetic resonance spectroscopic study has shown that orthophosphate binds to FeOOH through bidentate bonding (Kim et al., 2011). Lindgren and Persson (2010) proposed that aromatic carboxylic acids bond through the formation of numerous hydrogen-bonding interactions between the DOM molecules and FeOOH functional groups. Higher molecular mass aromatic carboxylic acids are likely to contain more acidic carboxylic functional groups, as shown through Kendrick mass defect analysis (Chassé et al., 2015).

**Conclusion**

A number of studies have demonstrated that (i) model organic acids affect the solubility of P in soils and (ii) molecular properties of the organic acids—that is, the number and type of functional groups involved in coordination reactions— Influence the magnitude of their competition with P for soil binding sites. These model organic acids studies have led to the suggestion that natural DOM could affect P availability in soils through the competition between DOM and P for mineral adsorption sites. However, some studies found little or no evidence to support an effect of DOM on the amount of phytoavailable P under realistic soil conditions (Guppy et al., 2005b; Goyne et al., 2008). Our results using a bioassay with DOM isolated from crop residues, animal manures, and compost show that the DOM initially leached or dissolved from these amendments is not likely to outcompete orthophosphate for mineral sorption sites. With orthophosphate bonding to FeOOH through a strong, inner-sphere mechanism, and DOM associating through weaker outer-sphere electrostatic interactions or multiple hydrogen bonds, orthophosphate outcompetes DOM for adsorption sites on mineral surfaces. Our bioassay results support earlier studies showing that only aromatic molecules >600 Da can compete with orthophosphate for mineral adsorption sites.

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