Adsorption of humic substances on ferrihydrite affects its use as iron source by plants

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Poorly crystalline Fe oxides are sources of Fe to plants. The adsorption of humic substances (HS) on these oxides alters its reactivity and stability in soils, and thus may affect Fe mobilization and uptake by plants from these compounds. This work aimed at studying how the adsorption of HS on Fe oxides affects its use as Fe source by two plant species with different Fe acquisition strategies, white lupin (Strategy I) and wheat (Strategy II). To this end, two completely randomized experiments, one with each plant, were carried out using a calcareous growing media and involving increasing amounts of HS adsorbed on ferrihydrite (0, 16, 60, and 97 mg C g\(^{-1}\)) which was used as Fe source. The highest HS rate was the only treatment that significantly increased Fe uptake in wheat relative to control without HS. This was related to a decreased concentration of Fe in poorly crystalline oxides in the growing media. On the contrary, HS did not affect significantly Fe uptake by lupin. However, in this crop, the highest HS rate decreased the concentration of Fe in oxides relative to the lowest HS rate, without significant differences with other treatments. Thus, the effect of adsorbed HS on Fe uptake differed in two plants with different Fe acquisition strategies. The increased Fe uptake in wheat at the highest HS rate can be explained at least in part by an increased Fe mobilization from oxides by plant roots. These findings provide new insights on the role of soil organic matter on plant Fe nutrition.

**Key words:** iron oxides, iron nutrition, wheat, lupin, iron acquisition strategy

**Introduction**

Iron (Fe) is an essential micronutrient for humans and plants. Despite being an abundant element in soil, its availability to plants is usually low due to the low solubility and slow dissolution of Fe compounds (Lucena 2003, Kreamer et al. 2006, Lemanceau et al. 2009). Poorly crystalline Fe oxides, such as ferrihydrite, are supposed to be the main Fe sources for plants in soils (de Santiago and Delgado 2006, de Santiago et al. 2009, 2010, 2011). However, many chemical and biological factors in soils, such as pH and the complexation by organic matter, affect the availability to plants of Fe present in oxides (de Santiago et al. 2008a, García-López et al. 2013). In particular, humic substances (HS) enhance Fe availability to plants by preventing its precipitation as Fe oxides (Schwertmann 1966, Olatexea et al. 2018), by formation of soluble complexes (Gerke 1993, Mackowiak et al. 2001, Bocanegra et al. 2006) which can move in the soil and reach the roots increasing Fe transport (Pandeya et al. 1998, Cesco et al. 2000, Garcia-Mina et al. 2004, Chen et al. 2004, Zanin et al. 2019) and by its redox-reactive properties (Kögel-Knabner et al. 2008). Iron complexed to soluble humic substances has proven to be an efficient Fe source for plants grown in nutrient solution (Pinton et al. 1999, Cesco et al. 2002, Bocanegra et al. 2006).

The adsorption of dissolved organic matter by phyllosilicate clays and Fe/Al oxides is a relevant process explaining its mobility, retention and degradation through mineralization (Kothawala et al. 2008, Dwivedi et al. 2017). Iron oxides such as ferrihydrite strongly adsorb organic matter, which explains the accumulation of organic matter in soils (Kaiser et al. 2007). On the other hand, negatively charged functional groups in organic substances (quinones, humic acids) can interact with positively charged minerals such as iron oxides (Fink et al. 2016). This affects the reactivity and stability of Fe oxides in soils (Liu et al. 2007, Zachara et al. 2011, Amstaetter et al. 2012, Zhu et al. 2013). As mentioned above, HS improve solubility and availability of iron to plants. However, little is known about the effect of HS adsorption on the use of poorly crystalline Fe oxides as Fe sources for plants. This is very relevant to fully understand the role of soil organic matter in Fe availability to plants.

Iron oxides are sources of Fe for plants, which have different Fe acquisition strategies, i.e. strategy I (proton and organic ligands release) and strategy II (release of phytosiderophores) (Cesco et al. 2002, Colombo et al. 2012, 2014, Kulikova et al. 2017). The adsorption of HS on Fe oxides seems to alter the capacity of plant roots to
mobilize Fe from these compounds (Och 1996). This may occur because adsorption sites for complexing agents (organic ligands or phytosiderophores) involved in Fe mobilization from oxides are occupied by HS thus decreasing their complexing and mobilization capacity. There is however no evidence of how HS affects Fe availability to plants depending on the Fe acquisition strategy. The effects of adsorption of HS on Fe oxides on Fe availability to plants may be even more relevant in basic pH soils, where the Fe deficiency is a frequent nutritional disorder. It can be hypothesized that adsorption of HS on Fe oxides alter Fe availability to plants, and that this effect may be different depending on the different plant Fe acquisition strategy. This will be relevant for assessing the practical consequences of preserving soil organic matter on plants Fe nutrition, and for defining more sustainable strategies for overcoming Fe deficiency in plants.

The objective of this work was to study how the adsorption of humic substances on ferrihydrite affects its use as Fe source by two species with different Fe acquisition strategy, white lupin (Strategy I) and wheat (Strategy II), grown in a calcareous growing media where Fe availability is supposed to be restricted by the basic pH.

Material and methods

Two pot experiments were performed in a completely randomized design. One experiment was carried out using white lupin (Lupinus albus L.), with five replications and another with wheat (Triticum aestivum L.) with four replications. Both experiments involved one factor, HS adsorbed on ferrihydrite at different rates (0, 16, 60, 97 mg of organic C per g of ferrihydrite). In both experiments, each replication corresponded to a cylindrical pot (polyethylene cylinder: 350 ml, 5.5 cm diameter, 15 cm height) which contained 0.38 kg of growing medium (calcareous [2/3] and siliceous [1/3] sand coated with Fe oxide). One plant of white lupin or wheat was grown in each pot. In a former step, seeds were germinated in perlite. Fifteen days after the germination, plants were transplanted to pots. The experiments were carried out under controlled conditions in a growing chamber (photoperiod of 14 h, a 25/23 °C day/night temperature, 65% relative humidity, and 22 W m⁻² light intensity) for 21 days in the case of lupin and 27 in the case of wheat. A Fe-free Hoagland-type nutrient solution was daily applied during experiment (all concentrations in mmol l⁻¹): MgSO₄(4), Ca(NO₃)₂(5), KNO₃(5), KH₂PO₄(2), H₃BO₃(0.092), MnCl₂(0.0018), CuSO₄(0.0016), ZnSO₄(0.0025), and H₂MoO₄(0.0023). The pH value of the nutrient solution was 6.5.

The growing medium was the same in both experiments, siliceous sand (> 99 % quartz) and calcareous sand (>99.5 % CaCO₃), mixed in order to mimic a calcareous soil. Both materials were sieved between 0.5 and 1 mm in order to get an optimal aeration and hydraulic conductivity in the growing medium. Siliceous sand was washed with Na₂CO₃ before using according to the procedure of de Santiago and Delgado (2006) in order to remove the initial content in Fe oxides. After that, siliceous sand was coated with ferrihydrite as described by Rahmatullah and Torrent (2000). All the siliceous sand used in pots was coated with ferrihydrite in such a way that the final amount of citrate-ascorbate extractable Fe (CA-Fe) in each pot was 70 mg (185 mg kg⁻¹ of growing media; 260 mg kg⁻¹ of citrate-bicarbonate-dithionite extractable Fe in the media). This extraction is specific for the most poorly crystalline Fe oxides and it is usually related to chlorophyll content of plants (de Santiago and Delgado 2006). All CA-Fe was considered to be associated with ferrihydrite. The concentration of Fe in the growing media was selected on the grounds of previous evidences by de Santiago et al. (2009).

Humic substances (HS) were extracted from a commercial liquid mixture of humic and fulvic acids from Leonardite (Solfer hunicos, Valencia, Spain) according to procedure of Stevenson (1994). Extracted HS were dialysed until the EC was < 0.2 dS m⁻¹, and after that pH was adjusted to 5 with HCl. The main properties of HS are described in Table 1. Elemental composition of HS was determined using a Thermo Finnigan CHN elemental analyzer (Thermo Electron, Madrid, Spain). The remaining undetermined mass was assumed as oxygen content. The ash was determined in 50 mg of HS after combustion at 850 °C for 0.5 h. Total acidity of HS was determined according to Sierra et al. (2004). Total Fe in the HS was determined by atomic absorption spectroscopy after digestion with concentrated HNO₃ in closed teflon containers at 130 °C during 15 min in a microwave oven (Milestone, provided by Gomensoro, Madrid, Spain). Spectroscopy study was performed according to Shirshova et al. (2006) in order to characterize HS. This was done by measuring the specific spectral absorbances at 254 nm per unit of dissolved C in 0.1 N NaOH (A), determining the ratio of measured spectral absorbances at 254 nm (E2) and 436 nm (E3), and the ratio of measured spectral absorbances at 465 nm (E4) and 665 nm (E6). E2 can be used as a surrogate measurement for aromaticity and the E4/E6 ratio an indication of molecular size, the ratio decreasing with increasing molecular size (de Santiago et al. 2010). Molecular weight (MW) distribution was studied according to Xu et al. (2006) using ultrafiltration membranes of different pore size. Adsorbed HS in the pots were always below the threshold value for phytotoxic effects (0.06 g kg⁻¹ growing medium, de Santiago et al. 2008b).
Adsorption of HS on ferrihydrite-coated sand was performed as described by Kaiser et al. (2007). To this end, suspensions of ferrihydrite coated sand with concentrations of HS ranging from 10 to 100 mg C l\(^{-1}\) were used to adsorb different quantities of HS on ferrihydrite. The suspension was shaken manually every so often, and its pH fine-tuned to 7.8. Concentration of HS in solution after the sorption process was determined through the relationship between HS concentration and the absorbance at 254 nm, and the adsorbed HS was calculated as the difference of the HS initially present in the suspension and that at the end of the adsorption procedure. It was found that the ultraviolet spectra of HS were not modified after adsorption on Fe oxides (Reiller et al. 2006). Peak around 1380 cm\(^{-1}\) in the infrared spectra of ferrihydrite-coated sand with HS was characteristic of the Fe (III)-humate complexes. This revealed that HS was bound to the oxide surface by carboxilate groups (symmetric stretching in COO\(^{-}\)groups, C-H and OH deformation) (Fu and Quan 2006, Boguta et al. 2019).

Chlorophyll meter readings (Minolta Soil Plant Analysis Development –SPAD– index) were taken at the end of each experiment in triplicate in the last totally expanded leaf of lupins and in the flag leaf of wheat using a Minolta SPAD-502 (Minolta Camera Co., Ltd., Osaka, Japan). Specific correlation between SPAD units and leaf chlorophyll content was previously checked for each plant (Chlorophyll [mg kg\(^{-1}\) fresh weight] = 0.3; SPAD = 0.48; \(R^2\) = 0.85; \(p < 0.001\), \(n = 18\) for lupin, and Chlorophyll = SPAD/136, \(R^2\) = 0.91, \(p < 0.001\), \(n = 22\) for wheat). At the end of the experiments, the apoplastic Fe concentration in roots was analysed according to Bienfait et al. (1985). After that, plants were harvested. Then, shoots and roots of lupins and wheats were separated and dried in a forced-air oven at 65 °C until constant weight (48 h), i.e., dry matter (DM) content was obtained. Dry plant material was milled to pass a 1–mm screen prior to the mineralization of an aliquot of 0.5 g with H\(\text{NO}_3\) in teflon containers in a microwave oven (Milestone, provided by Gomensoro, Madrid, Spain). In the resulting digest, Fe concentration was determined by atomic absorption spectrometry.

Fe was analysed in the growing media at the end of the experiments in order to study its availability and main fractions. To this end, citrate-ascorbate (CA), citrate-bicarbonate-dithionite (CBD), and hydroxylamine extractions (de Santiago and Delgado 2006) were carried out. CA extraction involved the use of 2 M Na citrate + 0.05 M ascorbate (CA-Fe) at pH 6, which dissolves poorly crystalline Fe oxides. CBD extraction was performed to dissolve all the Fe oxides present in the growing media, and involved the use of 0.27 M Na citrate + 0.11 M NaH\(\text{CO}_3\) + 2 % Na dithionite (CBD-Fe) at pH 8.5; in both cases at a growing media:extractant ratio of 1:40. Hydroxylamine extraction was deemed by de Santiago and Delgado (2006) a more accurate Fe availability index than other classical extractions such as those based in the use of complexing agents.

The effect of increasing amounts of adsorbed HS on ferrihydrite on the different measured variables was assessed by analysis of variance (ANOVA), using the General Linear Model (GLM) and regressions, in the software Statgraphics Plus 5.1 (StatPoint 2000). Differences between means were assessed with LSD test at \(p < 0.05\). Normality and homoscedasticity were always previously checked by using the Smirnoff–Kolmogorov and Levene tests, respectively (Acutis et al. 2012), and data transformed when either one or both tests were not passed.

Results

The amount of HS adsorbed on the Fe oxides present in the growing media did not affect chlorophyll content and DM yield of lupins or wheat (Table 2). Total Fe uptake by lupins and wheat was affected by HS adsorbed on the oxides present in the media (Fig. 1). In the case of lupins, total Fe uptake by plants at the highest HS rate (97 mg C g\(^{-1}\)) adsorbed on ferrihydrite was greater than at the lowest rate (16 mg C g\(^{-1}\)); however, non-significant differences were observed with control without HS (Fig. 1). In the case of wheat, the concentration of Fe in roots, and the total amount of Fe in roots and the whole plant were significantly enhanced by HS at the highest rate when compared with other treatments (Table 2).

Table 1. Characteristics of humic substances used in the study

| Molecular weight distribution (KDa)\(^{a}\) | C     | H     | N     | O     | Ash   | Total acidity\(^{b}\) | Total Fe | A\(^{c}\) | E2/E3\(^{b}\) | E4/E6\(^{b}\) | E3/E4\(^{b}\) |\(\frac{E}{E3}\) | E4/E6\(^{b}\) |\(\frac{E}{E3}\) |
|------------------------------------------|-------|-------|-------|-------|-------|-----------------------|----------|---------|--------------|--------------|--------------|-------------|--------------|-------------|
| %                                       | Eq H\(^{+}\) kg\(^{-1}\) | g kg\(^{-1}\) | L g\(^{-1}\) cm\(^{-1}\) | % of total organic C |
|------------------------------------------|-------|-------|-------|-------|-------|-----------------------|----------|---------|--------------|--------------|--------------|-------------|--------------|-------------|
| 41.7                                    | 4.5   | 0.7   | 52.9  | 0.2   | 7.5   | 0.11                  | 117      | 4.5     | 25.3         | 12.5          | 62.2         | 0.7         | 4.5          | 0.7         |

\(a = \) Determined according to Sierra et al. (2004); \(b = \) A, specific spectral absorbances at 254 nm per unit of dissolved C in 0.1 N NaOH; \(E2/E3\), ratio of measured spectral absorbances at 254 nm (E2) and 436 nm (E3), and \(E4/E6\), ratio of measured spectral absorbances at 465 nm (E4) and 665 nm (E6), determined according to Shirshova et al. (2006); \(c = \) Molecular weight distribution determined by ultrafiltration according to Xu et al. (2006); nd = not detectable.

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Adsorbed HS did not affect apoplastic Fe in roots of lupins (Fig. 2). In wheat, apoplastic Fe in the control without HS rate was significantly greater than those observed at all HS rates (Fig. 2).

Table 2. Effect of different amounts of humic substances adsorbed on ferrihydrite on the chlorophyll meter readings (SPAD), dry matter (DM) yields, Fe concentration in aerial parts and roots, and the total amount of Fe in aerial parts and roots of lupins and wheat grown on a calcareous growing medium

| Rate† (mg C g⁻¹) | Lupin | | | | Wheat | | | |
|------------------|------|---|---|---|------|---|---|---|---|
|                  | SPAD | DM | DM | Aerial | Roots | Aerial | Roots | DM | DM | Aerial | Roots | DM | DM | Aerial | Roots |
|                  | Arbitrary | g plant⁻¹ | mg kg⁻¹ | µg plant⁻¹ | Arbitrary | g plant⁻¹ | mg kg⁻¹ | µg plant⁻¹ | | g plant⁻¹ | mg kg⁻¹ | µg plant⁻¹ | g plant⁻¹ | mg kg⁻¹ | µg plant⁻¹ |
| 0                | 48.25 | 1.24 | 0.67 | 111 | 848 | 138 | 579 | 39.50 | 2.03 | 1.32 | 111 | 848 | 138 | 579 | 39.50 | 2.03 | 1.32 |
| 16               | 44.00 | 1.17 | 0.52 | 77 | 756 | 88 | 387 | 40.75 | 1.89 | 1.22 | 170 | 221 b | 339 | 2.77 b | 339 | 2.77 b |
| 60               | 47.50 | 1.32 | 0.70 | 131 | 811 | 174 | 596 | 38.50 | 1.94 | 1.04 | 121 | 342 b | 226 | 4.02 b | 226 | 4.02 b |
| 97               | 52.25 | 1.15 | 0.67 | 301 | 1235 | 306 | 860 | 38.25 | 2.06 | 1.28 | 189 | 7.93 a | 390 | 10.21 a | 390 | 10.21 a |

ANOVA: NS NS NS NS NS NS NS NS NS NS NS NS

† = Rate in mg C per g of ferrihydrite; Means; means in the same column followed by different letters are significantly different according to the LSD test at p < 0.05; DM = dry matter; NS = not significant; * = significant at p < 0.05

Adsorbed HS did not affect apoplastic Fe in roots of lupins (Fig. 2). In wheat, apoplastic Fe in the control without HS rate was significantly greater than those observed at all HS rates (Fig. 2).
Overall, lupins promoted a significant decrease of CA- and CBD-extractable Fe after the experiment (Fig. 3b and 3c) when compared with initial contents (185 and 257 mg Fe kg\(^{-1}\) of CA- and CBD-Fe, respectively). Amounts of Fe mobilized from the media were much higher than those extracted by lupin plants. By contrast, the effect of wheat growth on the contents of extractable Fe was, on average, almost negligible in comparison to initial contents.

For lupin, CBD-Fe significantly decreased at the highest HS rate when compared with control (Fig. 3c). The lowest CBD-Fe was observed in the treatment with the highest Fe uptake by lupins. However, CA-Fe, mostly ascribed to poorly crystalline Fe oxides, was not significantly affected by HS with lupin (Fig. 3b). For wheat, the Fe availability index (hydroxylamine extractable) and CA-Fe was affected by HS adsorption (Fig. 3a). This reveals an effect of HS ascribed only to poorly crystalline Fe oxides in wheat. Citrate-ascorbate extractable Fe after wheat crop was lower at the highest HS rates than with the two lowest rates, which evidences an increased mobilization of Fe from ferrihydrite at the highest rate (Fig. 3b). This is consistent with the observed effect of HS on the total Fe uptake by wheat plants since the lowest CA-Fe was observed in treatment showing the highest Fe uptake by plants.

Fig. 3. Effect of the different rate of Fe-HS on Fe extractable with hydroxylamine in the growing medium (a), on Fe extractable with citrate ascorbate (CA-Fe) in the growing medium (b), and on Fe extractable with citrate bicarbonate dithionite (CBD-Fe) in the growing medium (c) of lupin plants (black columns) and wheat plants (grey columns). One-way ANOVA was performed for each plant independently. Means with different letter were significantly different as per LSD test at \(p < 0.05\). Error bars indicate one standard error.
Discussion

Overall, the adsorption of HS on Fe oxides acting as a Fe source in growing media affected the Fe availability to plants. However, this effect was different depending on the cultivated plant. In the case of lupin, there was not a significant effect of HS on Fe uptake. In contrast, the highest HS rate increased total Fe uptake by wheat when compared with control without HS and the other HS rates (Fig. 1). This effect was mainly ascribed to an increased Fe accumulation in roots with the highest HS rate. Humic substances decreased apoplastic Fe in wheat at the highest rate (Fig. 2). This may reveal an increased transport of Fe from apoplast to symplast with HS adsorbed on Fe oxides. This implies an increased physiological active fraction of absorbed Fe in wheat roots. In this plant, Fe concentration in roots also increased at the highest HS rate relative to the other treatments (Table 2). The increased Fe concentration and accumulation in the roots of wheat at the highest HS rate was partly justified by the increased mobilization of Fe from the medium. This mobilization is evidenced by the decreased hydroxylamine and CA extractable Fe at the highest HS rate (Figs. 3a and 3b). However, Fe concentration and accumulation in aerial parts were not significantly different between treatments in both plants. Thus, the increased Fe uptake by wheat is only reflected in an increased accumulation in roots, without increased translocation to aerial parts. This agrees Cieschi et al. (2019) who found that an increase in iron humate concentration in the rhizosphere might cause a decrease in the transcription level of the genes involved in the iron transport, explaining a decreased transport from roots to shoots. Different plant species have different Fe acquisition mechanisms and response to Fe deficiencies (Bienfait 1988, Brown and Jolley 1989, Shi et al. 1993). It seems possible that these different results in lupin and wheat are due to the differences in the Fe acquisition mechanisms in lupin (Strategy I) and wheat (Strategy II).

Humic substances adsorbed on Fe oxides in the growing media affected the contents of extractable Fe after the experiments. In lupin, the decrease in CA-Fe was roughly equivalent to that of CBD-Fe, thus revealing that most of the oxides dissolved were poorly crystalline (ferrihydrite). These results are consistent with those of other studies and suggest that the exudation of large amounts of organic acids such as citric by lupines roots (Dinkelaker et al. 1989, Egle et al. 2003) is very effective promoting the dissolution of Fe compounds in soil (Gerke 1993, 1997, 2010, Tiping 2002). In lupin, CBD-Fe decreased at the highest HS. It is well known that HS and organic substances produced by plants are involved in the dissolution of Fe minerals (Cesco et al. 2000, Violante et al. 2003, Mimmo et al. 2014). In addition, HS can increase the release of root exudates (Canellas et al. 2019) thus increasing Fe mobilization from soil. Thus, this effect of HS promoting the release of Fe from oxides may improve the response of plants to Fe deprivation. However, this increased mobilization of Fe at the highest HS rate when compared with other HS rates did not result in an increased Fe uptake by lupin. This means that, in addition to Fe mobilization, other factors affecting Fe absorption and transport, such as the functioning of the Fe(III)-chelate reductase in membranes are crucial explaining the effect of HS on Fe uptake by lupin.

Iron requirement for wheat was covered with a very small Fe release from the ferrihydrite present in the growing media as revealed by the small decrease in CA-Fe when compared with initial contents in the growing media. This is consistent with the well-known Fe acquisition strategy of gramineous plants (Strategy II) based on highly selective Fe-complexing compounds (siderophores). Small amounts of Fe released by siderophores are enough to cover plant requirement (Kraemer et al. 2006, Ahmed and Holmström 2014, Poggenburg et al. 2018). Cesco et al. (2000) observed that the acquisition of Fe from ferrihydrite is guaranteed by the siderophore efflux. The stability of the complexes formed between Fe and siderophores are much higher than the stability of the humic-Fe complexes; this makes humic-associated Fe to be an easily available Fe source for Strategy II plant species such as wheat (Gerke 2010). This contributes to explain the increased Fe uptake by wheat at the highest HS rate, which is consistent with the decreased CA-Fe and hydroxylamine extractable Fe at this HS rate.

The effect of the highest rate of HS increasing Fe oxides mobilization with lupin was not only ascribed to poorly crystalline Fe oxides since the significant decrease in extractable Fe was only observed with CBD, which is supposed to dissolve poorly crystalline and crystalline Fe oxides. This effect of HS on extractable Fe is consistent with previous results by Gerke (1993) showing that low molecular weight acids, such those excreted by lupin roots (Dinkelaker et al. 1989), were more effective releasing Fe from Fe oxides bound to HS. The decrease in CBD extractable Fe with lupin was much higher than total Fe in plants. This likely reveal that only a part of mobilized Fe was taken up by plants, and also that a part of Fe initially present in the oxide may precipitate in forms not extractable by the action a strong reductant such as dithionite, or may be lost by leaching if present as soluble Fe-complexes. By contrast, the Fe availability index (hydroxylamine extractable) and CA-Fe decreased with HS adsorption in wheat (Figs. 3a and 3b). This likely reveal a more selective mobilization of Fe from poorly crystalline Fe oxides, to which hydroxylamine extractable Fe is related (de Santiago and Delgado 2006).
Several works have demonstrated iron uptake by plant with the Fe:HS ratio lower than that obtained in our study (Kulikova et al. 2017, Cieschi et al. 2019). Nevertheless, at the highest HS rate, concentration of HS in equilibrium with adsorbed HS must be greater than at other rates. This soluble HS have proved to increase the mobilization of Fe from oxides (Cesco et al. 2000). Also, HS enhance the development and acidification capacity of roots (Trevisan et al. 2010), and Fe-HS complexes are natural substrates for the membrane Fe (III)-chelate reductase and stimulate the proton release promoting the Fe acquisition by strategy I plants (de Santiago and Delgado 2007, Cieschi et al. 2019). An enhanced acidification and reductant capacity of roots can positively contribute to an increased Fe mobilization from oxides which is evidenced by the decrease in different Fe fractions in the growing media after crop. This may explain the increased Fe uptake by wheat with the highest HS rate. In agreement with previous findings, concentration and accumulation of Fe in plants were considered in a nontoxic range and enough for an adequate growth (Marschner 1995). Similar values were reported by Shane et al. (2008) and de Santiago et al. (2010) in previous studies. This may explain the lack of effect of HS treatments on DM yield and SPAD readings.

Conclusions

As hypothesized, the adsorption of HS on ferrihydrite affected the mobilization of Fe from poorly crystalline oxides measured as the decrease of extractable Fe in the growing media after crop. The effect was different for lupin and wheat, with different Fe acquisition mechanisms. The highest HS rate (97 mg C g⁻¹) promoted increased Fe uptake in wheat relative to control and other HS rates. In lupin, the highest HS rate increased Fe mobilization when compared to the lowest rate, without significant differences with the control without HS. These differences were not reflected in differences in Fe uptake by lupin. The effect of HS on Fe uptake by plants can be explained at least in part by an increased mobilization of Fe from oxides by plant roots. These findings enhance our understanding of the role of soil organic matter on plant Fe nutrition.

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