Biochar interferes with kiwifruit Fe-nutrition in calcareous soil

Original Article/Full-length paper

Authors:

Giovambattista Sorrenti^a, g.sorrenti@unibo.it

Caroline A. Masiello^b, masiello@rice.edu

Moreno Toselli^a, moreno.toselli@unibo.it

Author's affiliation and address:

^aDepartment of Agricultural Sciences, University of Bologna, viale G. Fanin 44, 40127
Bologna, Italy

^bDepartments of Earth Science, BioSciences and Chemistry, Rice University, Houston,
Texas 77005, USA

Corresponding author:

Giovambattista Sorrenti

Department of Agricultural Sciences, University of Bologna, viale G. Fanin 44, 40127
Bologna, Italy

E-mail: g.sorrenti@unibo.it

Telephone number: +39 051 2096426

Fax number: +39 051 2096401

Abstract

We combined lab experiments, a 2-year pot experiment, light microscopy and X-ray photoelectron spectroscopic measurements to understand the fate of Fe in biochar-amended soils with, and without, three sustainable Fe-fertilization strategies (siderite, aqueous extract of *Amaranthus retroflexus* either alone or enriched with FeSO₄). In addition, we examined biochar effects on plant-available micronutrients using metal Fe. We also assessed nonlinear interactions between biochar and Fe management strategies, evaluating the effectiveness of sustainable fertilization strategies in improving Fe-nutrition of kiwifruit vines under Fe-stressed conditions. Fe chlorosis symptoms of pot-cropped kiwifruit vines were pronounced in the biochar-amended soils, especially during the first season. However, the biochar rate adopted in this study was relatively high (5 g kg⁻¹ w w⁻¹) while the real impact of this strategy may change at lower rates. In the lab, increasing biochar rates progressively removed dissolved micronutrients from solution. Light microscopy revealed rust-colored patches on the biochar surface exposed to a Fe source in solution and XPS analyses confirmed that the biochar surface had become coated with Fe and O in ratios consistent with the formation of Fe-(hydr)oxides. Although we investigated one specific biochar, our results suggest that some of redox reactions can act to form a micronutrient sink on the biochar surface. This mechanism likely contributed to binding soil Fe (through reactive functional groups on its surface) into plant-unavailable forms, promoting the occurrence of Fe chlorosis symptoms of kiwifruit vines grown in soils amended with biochar. We also confirmed the effectiveness of siderite and *A. retroflexus* extract enriched with FeSO₄ in alleviating the occurrence of Fe-deficiency symptoms in kiwifruit vines.

Keywords: Biochar, calcareous soil, soil extractable Fe, redox reaction, Fe chlorosis

1. INTRODUCTION

Biochar is produced via biomass pyrolysis (heating in the absence of O₂) typically at temperatures below 700°C (Atkinson et al., 2010). Used as a soil conditioner, this C-rich material has been proposed as a potential strategy to mitigate climate change and benefit both soil fertility and crop yield (Atkinson et al., 2010; Jeffery et al., 2011; Laird, 2008; Lehmann, 2007 and references therein). While it is widely accepted that biochar interacts with the soil mineral matrix likely through multiple processes, specific mechanisms driving the biochar-induced interactions are not fully understood (Joseph et al., 2010). Recently it has been suggested that one gram of biochar can take up and release several hundred micromoles of electrons (Klöpfer et al., 2014; Joseph et al., 2015 and literature therein) and participate in redox-mediated reactions in the soil, indicating that biochar could potentially affect biogeochemical cycles in soil, pollutant degradation, contaminant mobilization, abiotic formation of humic structures not only by changing soil physical and chemical properties, but also by mediating electron transfer processes (i.e., as an electron shuttle) (Graber et al., 2014) altering, among others, the iron (Fe) cycle in soils (Kappler et al., 2014).

So far most of the scientific evidence showing benefits from the use of biochar as a soil conditioner comes from tropical and subtropical environments characterized by acid, weathered, and low fertility soils (Jeffery et al., 2011), but alteration of soil Fe cycling is most likely to generate observable plant impacts in alkaline or calcareous soils because Fe solubility is reduced in these systems. Calcareous, high pH soils represent approximately 39% of world soils, and are agriculturally important in a number of areas (Çelik and Katkat, 2010). In these soils Fe in solution precipitates as scarcely soluble Fe-hydroxides, as a consequence of the high pH and the active lime fraction (Römheld

and Nikolic, 2007). In addition, the enzymatic activity of the root Fe^{III} -chelate reductase, responsible for the reduction of the Fe^{III} to Fe^{II} , is dramatically compromised (Susin et al., 1996), limiting the availability of the ionic Fe form absorbable by roots of dicots (Römheld and Marschner, 1986). Limited Fe availability negatively affects leaf chlorophyll (Chl) concentration (Abadía and Abadía, 1993), leaf water relations (Eichert et al., 2010; Fernández et al., 2008), and may reduce yield and fruit quality (Álvarez-Fernández et al., 2006; Sorrenti et al., 2012 and literature therein), including in kiwifruit, which is considered among the most susceptible crops to this disorder (Tagliavini and Rombolà, 2001). Italy is one of the world largest kiwifruit producers. Kiwifruit represents a remunerative crop for growers and in the northern part of the Country, it is grown mainly on alkaline-calcareous soils, thereby plant Fe-nutrition must be carefully managed to avoid significant yield and fruit quality losses. Typically, growers use either soil- or foliar-applied synthetic Fe-chelates to manage plant Fe nutrition. Although effective at preventing or curing Fe-chlorosis (Abadía et al., 2011; Lucena 2006;) these soil chelates induce only a short-lasting re-greening, are expensive (Tagliavini and Rombolà, 2001) and pose environmental concerns (Grčman et al., 2001; Nörtemann, 1999) since they can be easily leached. Because of this, the development of cost-effective and environmentally friendly strategies to overcome Fe-chlorosis is of interest (Pestana et al., 2003). A more environmentally tolerable alternative may be the aqueous vegetal extract of *Amaranthus retroflexus*, which significantly increased the amount of Fe extracted from soil compared to deionized water (DI- H_2O) and improved Fe-nutrition of pear trees (Sorrenti et al., 2011). This response was attributed to natural Fe chelating compounds released by the *Amaranthus* spp. tissues (Matocha and Pennington, 1982; Matocha, 1984). The performance of the

A. retroflexus extract may be improved by exposing it to Fe salts prior to soil amendment (e.g. FeSO_4). On the other hand, when applied alone Fe salts are of little agronomic value since they precipitate into highly insoluble compounds (Tagliavini et al., 2000) becoming inefficient in providing any remediation of Fe-chlorosis. However, natural chelators (like those present in *A. retroflexus* extract) may stabilize Fe in a plant-available form (Matocha and Pennington, 1982).

Another option may be soil-applied synthetic minerals such as vivianite ($\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) and siderite (a Fe(II) carbonate (FeCO_3)), whose slow release of Fe achieved a long-lasting prevention of Fe-chlorosis in different crops (Cañasveras et al., 2014; Rosado et al., 2002; Sánchez-Alcalá et al., 2012a, 2012b;). Reacting with soil carbonate, these minerals form poorly crystalline Fe oxides (Roldán et al., 2002; Sánchez-Alcalá et al., 2012a), which are considered among the main source of Fe to plants (de Santiago and Delgado, 2006).

When biochar is combined with other amendments, results are sometimes not additive (Lecroy et al., 2013) and because of this, it is not possible to extrapolate the response of an ecosystem to combinations of biochar and other amendments based on the ecosystem's response to each amendment individually. Here the aims of this study were to: i) determine mechanisms of interactions between micronutrients and biochar (with emphasis on Fe), ii) investigate the effectiveness of sustainable fertilization strategies in preventing Fe-chlorosis of kiwifruit vines grown in a calcareous soil, and iii) use an improved understanding of Fe-biochar interactions to predict the potential for nonlinear interactions between biochar and Fe management strategies.

2. MATERIALS AND METHODS

2.1 Interaction between biochar and Fe nutrition on kiwifruit vines

2.1.1 Experimental design and growth conditions

We performed a 2-year (2011-12) outdoors experiment at the experimental station of the University of Bologna (located in Cadriano, 44°55' N, 11°40' E, 36 m a.s.l.) on 1-year old micropropagated kiwifruit vines (*Actinidia deliciosa* cv. Hayward) grown in 5.2 L pots filled with a heavy alkaline-calcareous soil (Table 1). Vines were trained as in a single shoot, watered daily (in summer) by microirrigation to return the evapotranspiration (ET_o) rate as estimated by a class A evaporation pan and the specific crop coefficient (K_c) for kiwifruit and covered with shade netting which allowed a light intensity of 73.500 lux (measured under the net in summer at noon on a sunny day). Except for Fe, plant nutrient requirements, nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) were satisfied by periodic supply of a nutrient solution prepared by mineral sources (e.g. NPK complexes, MgNO₃) and distributed by fertigation. During the experiment, we did not spray the canopy with fertilizers or pesticides and we manually removed weeds from pots. The experiment was arranged in a completely randomized factorial experimental design with 6 replicates (single vine) and 2 factors: a) fertilization (5 levels) and b) biochar (2 levels). We compared the following fertilization treatments: a) untreated control; b) commercial Fe-chelate (1.0 g L⁻¹ of commercial Fe-ethylenediamine-N,N'-bis-(o-hydroxyphenylacetic) acid (Fe-EDDHA) with a Fe content of 6% mostly in the ortho-ortho isomer); c) synthetic siderite (1.0 g of suspension per kg of soil); d) aqueous extract of *A. retroflexus* at a rate of 30 g (dry weight -dw) L⁻¹, and e) aqueous extract of *A. retroflexus* at a rate of 30 g (dw) L⁻¹ mixed with Fe(II) sulfate heptahydrate (FeSO₄·7H₂O) at a rate of 2 g L⁻¹. Table 3 summarizes treatments, rates and timing of application.

Synthetic siderite was prepared by mixing 40 g L⁻¹ of K carbonate (K₂CO₃) (Sigma-
 Aldrich) into a pot containing 80 g L⁻¹ of Fe(II) sulfate-heptahydrate (FeSO₄·7H₂O)
 which generated a brownish green suspension containing 34 g L⁻¹ of siderite. The
 solution was continuously stirred and the suspension was mixed into the soil only once
 at planting at a rate of 125 mL per pot in order to apply 1 g of siderite per kg of soil.
 The aqueous *A. retroflexus* extract was prepared and characterized as described by
 Sorrenti et al. (2011). Briefly, the dried powder of *A. retroflexus* was macerated in tap
 water (pH 7.4; Fe < 0.08 mg L⁻¹) at least 24 h before its application, maintaining the
 suspension at room temperature and in the dark. The aqueous *A. retroflexus* vegetal
 extract alone or enriched were applied at weekly intervals 4 and 5 times in the first and
 second season, respectively, starting from bud burst and at a rate of 200 mL plant⁻¹.
 At the same time as the aqueous vegetal extract applications, Fe-chelate treated plants
 received 200 mL plant⁻¹ of Fe-chelate solution while untreated and siderite treated
 plants received the same amount of tap water.
 We did not provide a treatment based on the mere addition of soil-applied FeSO₄
 because it is already known that the supply of inorganic Fe-salts is inefficient in high
 pH (e.g. alkaline-calcareous) soils in enhancing Fe availability, due to the rapid
 transformation of most of the applied Fe into highly insoluble compounds such as
 Fe(III)-hydroxides (Tagliavini et al., 2000).
 Fertilization strategies were either applied to vines grown in: i) unamended soil or ii)
 soil amended with biochar at a rate of 50 g (fresh weight (fw)) kg⁻¹ (w w⁻¹) equal to 52 t
 fw ha⁻¹ (considering a treated area of 2 m wide along vine row in a commercial kiwifruit
 orchard with a 3*5 m spacing (667 vines ha⁻¹), soil incorporation up to 0.20 m depth and
 a specific soil weight of 1.3 t m⁻³). The biochar we used in this experiment consisted of

small chunks obtained from a mixed feedstock of fruit tree wood prunings, with a prevalence of peach (*Prunus persica* L.) and grapevine (*Vitis vinifera* L.) pyrolyzed in a commercial kiln plant at approximately 550°C and atmospheric pressure (Table 2).

2.1.2 Fe-chlorosis incidence, plant nutritional status and plant biomass

We estimated the incidence of Fe-chlorosis periodically by the determination of the leaf Chl concentration. Estimations were performed on the first completely expanded apical leaf per shoot by a hand-held Chl meter (SPAD 502, Minolta Co. LTD, Osaka, Japan). Shoot length was also periodically recorded. In July of the second season, we randomly collected fully expanded leaves from each vine. We removed petioles and then washed leaf laminas in a 0.1 *N* HCl solution supplemented with a surfactant (Tween 20) at a rate of 1 mL L⁻¹. We rinsed samples abundantly in DI-H₂O, and then oven-dried (65 °C) until constant weight and milled them (0.2 mm mesh). We determined leaf macro (N, P, K, calcium (Ca), Mg) and micro (Fe, manganese (Mn), zinc (Zn) and copper (Cu)) nutrient concentration. Total N concentration was determined by the Kjeldahl method (Schuman et al., 1973) by mineralizing 1.5 g of sample with 12 mL of a 95:5 (v v⁻¹) H₂SO₄:H₃PO₃ mixture, at 420 °C, for 180 min and subsequent distillation with 32% (v v⁻¹) NaOH and titration with 0.2 *M* HCl. Phosphorus was spectrophotometrically quantified at 700 nm, through extract mineralization (Saunders and Williams 1955) of 0.5 g of tissue with 96% (v v⁻¹) H₂SO₄ and 35% (v v⁻¹) oxygen peroxide (H₂O₂), and subsequent neutralization with 0.1 *M* NaOH enriched with 0.1 *M* ascorbic acid, 32 *mM* ammonium molybdate, 2.5 *M* sulfuric acid and 3 *mM* potassium antimonyl tartrate trihydrate (Sigma-Aldrich) to develop a phospho-molybdic blue color. Metal concentrations were determined by atomic absorption spectrophotometry (AAS) (Varian

AA200, Mulgrave, Victoria, Australia) after wet digestion according to USEPA Method 3052 (Kingston 1988). To this end, 0.5 g of dry matter were mineralized in an Ethos TC microwave labstation (Milestone, Bergamo, Italy) by adding 8 mL of nitric acid (65%) and 2 mL of H₂O₂ (30%) at 180 °C. Lanthanum chloride (LaCl₃ at 10%) and cesium chloride (CsCl at 5%) solutions were added to the samples at ratios of 20 and 4%, respectively prior to K, Ca and Mg readings. At the end of the second season, vines were harvested and divided into shoots and roots (including stem), oven-dried at 65 °C until constant weight.

2.1.3 Soil pH and soil extractable micronutrients

At the end of the experiment, a soil sample (1 kg) was collected from each pot, oven-dried (105°C), ground (1 mm mesh) and used to determine soil pH and soil diethylenetriamine-pentaacetic-acid (DTPA) (Lindsay and Norwell 1978) extractable Fe, Mn, Cu and Zn concentrations. We also determined soil extractable Fe using DI-H₂O as eluent. For both eluents (DI-H₂O and DTPA), 80 mL were added to glass flasks containing 40 g (dw) of soil, shaken 2 h, decanted and then filtered (Whatman 42). The micronutrient concentration in the supernatant was determined by AAS (Varian AA200, Mulgrave, Victoria, Australia). Soil pH was determined in a soil:DI-H₂O solution at a rate of 1:2.5 (w w⁻¹). 10 g of soil were added to 25 mL of DI-H₂O, then stirred 1 h prior readings under continuous stirring by pH meter (Crison, pH-Meter BasiC 20, Barcelona, Spain).

2.2 Biochar micronutrients release and retention potential

We used the same biochar batch described in the previous experiment. However, we first sieved it to remove ash and impurities and to homogenize the size of the fragments

to a range of 2-7.5 mm. We washed the biochar to reduce ash and tar content by adding 4 L of DI-H₂O to 200 g of biochar (with 3 replicates) and shaking 30 min at 100 rpm with an orbital shaker. After every washing we collected the supernatant and analyzed it for electrical conductivity (EC) (Crison, Conductivity meter 524, Barcelona, Spain) which resulted at the end $51.5 \pm 1.64 \mu\text{S cm}^{-1}$ (avg. $\pm$ SE n=3). These washing steps were necessary since solutions obtained with unwashed biochar, quickly became heavily oily with fine solids floating on, hard to be filtered or analyzed. The biochar fragments were then oven-dried at 30°C. After this, 25 mL of DI-H₂O were added to glass flasks containing 4, 10, 20, 30 and 40 g L⁻¹ of biochar, with 3 replicates. Four additional series of flasks with the same biochar rates (4, 10, 20, 30 and 40 g L⁻¹) with 5 replicates were added to 25 mL solutions containing 10 mg L⁻¹ of one of the following cations: Fe, Mn, Zn or Cu. These solutions were prepared by diluting analytical grade FeCl₃·6H₂O, MnCl₂·4H₂O, ZnCl₂ and CuSO₄ in 0.12 N HCl standard solutions (J.T. Baker), used as a source of micronutrients. The pH of all solutions was adjusted by sodium hydroxide (NaOH) to 7.2 ± 0.1 and then flasks were shaken on an orbital shaker for 120 min at 90 rpm. The supernatant was then filtered (Whatman 42) and analyzed for micronutrients by AAS (Varian AA200, Mulgrave, Victoria, Australia).

2.3 Biochar surface chemistry change as affected by exposure to Fe metal

We added three medium-sized, washed biochar fragments, as described above, to 25 mL of DI-H₂O or to a solution made of DI-H₂O plus 100 mg of Fe dust (> 350 μm , obtained from a commercial Fe bar), in 3 replicates. After one week, fragments were removed

and oven-dried at 65°C. One biochar fragment per replicate was analyzed by X-ray photoelectron spectroscopy (XPS). The top 5 nm of the biochar surface was analyzed for relative Fe, oxygen (O) and C atomic concentration (at%) using a PHI Quantera XPS with aluminum (Al) X-ray source at 1486.6 eV and 49.2 W. The beam diameter was 200.0 µm and the pass energy 26 eV. XPS spectra were analyzed using a nonlinear least-squares curve-fitting program with a Gaussian-Lorentzian mixed function to optimize the spectra which were processed using MultiPak data analysis software (MultiPak V7.0.1, 04 Mar 16, Ulvac-Phi, Inc., 1994-2004).

2.4 Statistical analyses

Pot experiment data were evaluated by analysis of variance according to a factorial randomized block design with 2 factors: fertilization (5 levels) and biochar (2 levels) with 6 replicates. When analysis of variance showed a statistical effect (at $P \leq 0.05$), means were separated by Student-Newman-Keuls Test (SNK); when interaction between fertilization treatment and biochar was significant, 2 times standard error of means (SEM) was used as the minimum difference between two statistically different means (Saville and Rowarth 2008). Coefficient of determination (r^2) between biochar rate and micronutrients concentration was calculated in the lab experiment using linear regression analysis. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC)

3. RESULTS

Generally, our results point to biochar acting as a sink for the micronutrients Fe, Mn, Cu, and Zn, which were effectively removed from the solutions.

3.1 Interaction between biochar and Fe nutrition in kiwifruit

3.1.1 Fe chlorosis incidence, vine nutritional status and plant biomass

Pronounced leaf Fe chlorosis symptoms characterized untreated vines in both seasons (as estimated by the lowest values of leaf Chl content). Without interaction between the two factors (fertilization and biochar) and independently of the amendment with biochar, all fertilization strategies were effective in significantly increasing leaf Chl content except for the aqueous extract of *A. retroflexus* alone (data are available in Table S1 and S2 of the supplementary material).

The Fe-chelate soil application consistently produced the greatest SPAD index. Siderite and *A. retroflexus* enriched with FeSO_4 yielded SPAD values similar to those of the Fe-chelate treated-plants, particularly in the first season, whereas *A. retroflexus* alone was ineffective in preventing Fe chlorosis (data not shown).

Leaf Fe-deficiency symptoms were noticeable in plants grown in biochar amended soil, but only in the first season (Fig. 1).

All control and aqueous extract *A. retroflexus*-alone treated vines died in the summer of the second season, independently of the presence of biochar in soil.

Shoot length was significantly increased by fertigation with aqueous extract of *A.*

retroflexus+ FeSO_4 in the first season (data are available in Table S3 of the

supplementary material) while in the second year, it was promoted mainly by Fe-chelate

and, to a less extent, by *A. retroflexus*+ FeSO_4 and siderite compared to control plants

(data are available in Table S3 of the supplementary material). However, at the end of

the experiment, no significant differences were observed among the remaining

strategies (data are available in Table S3 of the supplementary material).

Independently of the fertilization and without interaction between factors, shoot growth was significantly depressed by the presence of biochar in soil in the measurements of Sep-2011 and Jun-2012 (Fig. 1).

The application of Fe-chelate and *A. retroflexus* + FeSO₄ resulted in higher total plant biomass compared to other strategies (Table 4). However, Fe-chelate significantly promoted shoot biomass while treatment with *A. retroflexus*+FeSO₄ increased root (including stem) biomass compared with siderite at the end of the experiment (Table 4). Nevertheless, no statistical differences were recorded between the highest values (Table 4). The 12 untreated as well as the 12 *A. retroflexus*-alone treated plants died in summer of the second season, and therefore no shoots could be sampled at plant harvest. In addition, a poorly developed root system was recovered from these pots (Table 4). The addition of biochar to the soil did not affect organs or total plant biomass, without interaction between factors (Table 4).

Leaf K concentration was decreased in plants treated with Fe-chelate compared to other treatments while a significant interaction between treatment and biochar occurred for leaf Mg, Fe and Mn (Table 5). When soil was amended with biochar leaf Mg and Fe concentrations were significantly decreased in plants treated with Fe-chelate and the aqueous extract enriched with FeSO₄, but not with siderite (Table 5). Only in unamended soil leaf Mg concentration was significantly increased by Fe-chelate compared to the aqueous extract enriched and siderite (Table 5). The supply of Fe-chelate dramatically increased leaf Fe and decreased leaf Mn concentration compared to other treatments, independently of the substrate (Table 5). Similarly, leaf Fe concentration was higher in plants fertilized with the suspension of siderite in comparison to those fertigated with the aqueous extract enriched (Table 5). The same

trend was observed for leaf Mn, although it increased only in the biochar amended soil (Table 5).

Independently of the fertilization strategy, biochar induced an increase in leaf K and a reduction in leaf Ca concentration (Table 5), while no effects were observed on leaf N, P, Cu and Zn concentration (Table 4).

3.1.2 Soil pH and soil extractable micronutrients

Soil pH was significantly higher in soil amended with biochar (7.56 vs. 7.45) without interaction between biochar and fertilization strategies (Table 6). Among strategies, soil pH was reduced in soils fertilized with Fe chelate, *A. retroflexus* + FeSO₄ and siderite with comparable values among them (Table 6).

Fertilization strategies significantly affected soil extractable micronutrients. Soil extractable Fe was increased only by synthetic Fe treatment when DI-H₂O was used as eluent (Table 6), while with DTPA, the *A. retroflexus* + FeSO₄ induced the highest value, followed by siderite (Table 6). The latter increased DTPA-extractable Fe compared to soils untreated and fertigated with the vegetal extract alone, while intermediate values were obtained by Fe-chelate (Table 6). Compared to other strategies, soil DTPA-extractable Cu was reduced by Fe-chelate application whereas soil DTPA extractable Mn and Zn were not affected by fertilization treatments (Table 6).

Soil amended with biochar significantly decreased the amount of Fe extracted by DI-H₂O while an opposite trend emerged using DTPA as extractor agent (Table 6). A similar response was also measured for soil DTPA extractable Mn, Cu and Zn concentration, without interaction with fertilization strategies (Table 6).

3.2 Biochar micronutrients release and retention potential

The amount of Fe, Mn, Cu and Zn released by biochar in DI-H₂O was negligible, independent of the application rate (data not shown). Values of these metals in solution were lower than 0.015 mg L⁻¹ on average (comparable to those of pure DI-H₂O). Therefore, biochar application rate and released micronutrient concentration were not significantly linked (data not shown). Conversely, when biochar was soaked in 10 mg L⁻¹ solutions of Fe, Mn, Cu and Zn (separately), it significantly reduced the amount of cations in solution as the biochar rate increased (Fig. 2). A significant negative relationship emerged between the concentration of dissolved Fe after the addition of biochar and biochar rate, which best fitted with a polynomial trend ($r^2 = 0.92$) (Fig. 2). Similarly, Mn, Cu and Zn concentrations were negatively and linearly linked with biochar rate and r^2 values were 0.93, 0.93 and 0.88, respectively (Fig. 2).

3.3 Biochar surface chemistry change as affected by exposure to Fe metal

After exposure to Fe, biochar fragments showed diffuse rusty spots distributed over their surface (Fig. 3). The surface relative atomic concentration (at %), as measured by XPS, was 14.9 ± 2.14 , 65 ± 5.21 and 22.6 ± 7.36 (avg. $\pm$ SE n=3) for Fe, O and C, respectively. On the contrary, the biochar surface was not visibly altered in colour after it had been dipped 1 week in DI-H₂O, and its surface atomic concentration was 0.12 ± 0.002 , 14.2 ± 1.62 and 86.1 ± 2.60 (at%, avg. $\pm$ SE n=3) for Fe, O and C, respectively.

4. DISCUSSION

4.1 Biochar hinders kiwifruit Fe nutrition

There are three mechanisms potentially responsible for the negative impact of biochar on plant Fe and Mn uptake: i) a biochar-driven pH increase, which would cause a reduction in the ability of the Fe^{III} chelate reductase enzyme to transport Fe to cells, ii) ionic bonding of Fe and Mn to oxidized groups on the biochar surface, and iii) a redox-driven precipitation of iron oxides on the surface of biochar particles, which would pull any available Fe out of solution. The first mechanism, a soil pH increase, is unlikely to have driven the entire negative impact of biochar on plant Fe and Mn uptake because the observed pH shift was less than 0.1 units. Although we cannot completely rule out a role for this small pH shift, extraction data, microscopic images, and XPS data point to a major role for mechanisms that hold Fe and Mn more tightly to the biochar surface. Chemical extraction data suggest that biochar-amended soils held Fe much more effectively (Table 6). DI-H₂O extraction (a measure of weakly held Fe) extracted 57% less Fe into solution from soils amended with biochar compared to unamended soils (Table 6). On the other hand, a stronger extractant (DTPA) removed more Fe from the biochar-amended soil than the control soil, with the difference reflecting the balance not removed by the first extraction (Table 6). We observed a similar trend for the other micronutrients, suggesting that biochar retained Fe from the soil solution (as well as other cations) and a weak eluent, such as DI-H₂O, was ineffective in solubilizing it. Others have observed a reduction of soil Mn availability upon the addition of biochar to soils (Alling et al., 2014). Although the DI-H₂O and DTPA extractions did not allow us to separate out the relative importance of ionic bonding from redox-driven precipitation reactions, microscopic images show the formation of precipitated Fe oxides on the

surface, making it clear that this third process was active. Therefore, we hypothesize that in potted conditions Fe in soil solution was attracted and retained by biochar (as observed in the second experiment), thereby limiting its availability (in particular in the first season) for plant uptake, accentuating Fe-chlorosis symptoms of kiwifruit vines. After the first season, symptoms of Fe-chlorosis in biochar-amended vines were less evident, suggesting that one growing season was sufficient to saturate the biochar surface. This may be because the surface became loaded with cations or because a sort of cation exchange equilibrium was reached, leading to an increase of Fe availability for plant uptake over time. The fact that Fe was attracted by biochar in soil has been reported also by Lin et al. (2012) who, using transmission electron microscopy (TEM) equipped with energy dispersive spectroscopy (EDS) for elemental analysis, showed that the mineral matter attached to the biochar surfaces on fragments recovered after a 3-month soil incubation experiment included higher concentration of elements such as O, Al, Si, Fe, Ti and trace amounts of other elements such as Mn, Mg, Ca, K, Na, P, and S compared to fresh biochars.

4.2 Micronutrient release from biochar is negligible

In our conditions, biochar did not affect Fe, Mn, Cu and Zn concentration in solution independent of the rate of biochar application (data are available in Fig. S1 of the supplementary material). This response was expected and could be ascribed to: i) the poor micronutrient content in the hardwood feedstock; ii) the scarcity of ash (since biochar was repeatedly washed prior testing) and iii) the retention of micronutrients (i.e. Fe and Mn) in biochar (Amonette and Joseph 2009), thereby transforming them into less soluble chemical forms (Wang et al., 2009), confirming the poor value of biochars

as a direct source of micronutrients (Novak et al., 2009). Although it has been suggested that biochar may represent a potential source of macro- and micronutrients for plants and the soil microbial community (Downie et al., 2009), direct nutrient supply by biochar is often negligible, in particular when plant biomass is used as feedstock (Gaskin et al., 2008; Singh et al., 2010), as it was here. However, since our results were obtained by testing one specific biochar, we cannot exclude that others biochars behave differently.

4.3 Biochar acts as a retaining additive for micronutrients in soil

In our lab experiments, the biochar used in this trial showed a high retention affinity for micronutrients when they were available in solution (Fig. 2). In fact, increasing rates of biochar (and thereby total surface area), progressively reduced the initial content of all micronutrients in solution, suggesting that biochar attracted and retained all the cations we tested (Fig. 2). This phenomenon is consistent with the work of others (e.g. Novak et al., 2009). The affinity of biochar for micronutrients occurred in the order $\text{Fe} > \text{Cu} > \text{Zn} > \text{Mn}$. Biochar completely removed Fe in solution (10 mg L^{-1}) between 20 and 30 g L^{-1} while more biochar was required to completely strip Cu from solution (between 30 and 40 g L^{-1}). The highest biochar concentrations in our solution experiments were insufficient to remove all the Zn and Mn from solution, with ~80% of the Zn and ~50% of the Mn removed at the highest biochar rate (40 g L^{-1}) (Fig. 2). The ability of biochar to sorb and desorb cations and anions has been ascribed to its cation exchange capacity (CEC) and anion exchange capacity (AEC) (Liang et al., 2006).

The chemical interaction of biochar with elements dissolved in solution has been attributed to a range of functional groups identified on its surface, including hydroxyl (-OH), carbonyl (-C=O), carboxylate (-COOH), hydrogen (H) and ether (R-O-R) (Cheng et al., 2008; Mao et al., 2012). Negatively charged carboxyl groups on the biochar surface, whose presence is estimated by the high O:C ratios, are mainly responsible for the high CEC of most biochars (Chan and Xu 2009), since other groups are mostly polar and uncharged (i.e. hydroxyl, carbonyl). However, even uncharged functional groups on the biochar surface contribute to cation retention by cation- π bonding mechanisms (Harvey et al., 2011). This structure allows the sorption of molecules with both polar and non-polar portions on the biochar surface.

The ability of our biochar to retain cations could have been accentuated by the fact that it was 4 years old. In fact, it has been suggested that the CEC of biochar increases with biochar ages as shown by high concentrations of negative charges on the biochar surface due to surface oxidation induced by abiotic processes (Cheng et al., 2006), while AEC is likely to be reduced.

It is also possible that the observed cation retention was due in part to the physical structure of biochar. Its high surface area and porosity likely increased the contact between biochar particles and solution (Major et al., 2009). The nutrient sorption might partly result from dissolved nutrients physically held in the porous structure of biochar before drying and analysis. However, it is unlikely that water absorption completely explains the observed differences since it is doubtful that 100% of the Fe and Cu in solution were physically trapped into biochar pores.

4.4 Fe exposure induces a redox reaction at the biochar surface level

Recently, it has been demonstrated that since biochar is redox-active (Klöpfer et al., 2014; Saquing et al., 2016), it can be involved in abiotic redox reactions (Oh et al., 2013) and, depending on the feedstock and charring temperature, biochar can accept and donate several hundred micromoles of electrons per gram (Klöpfer et al., 2014). Moreover, results from Kappler et al. (2014) suggest that biochar in soil can alter soil biogeochemistry either indirectly by changing the soil structure and chemistry or directly by mediating electron transfer processes, by functioning as an electron shuttle. In our experiment, biochar clearly showed diffuse rusty spots on its surface after exposure to Fe (Fig. 3), and the relative surface atomic concentration significantly changed for Fe, O and C. The relative O and Fe concentration of the biochar surface was much higher than in control fragments, suggesting that Fe was oxidized on the biochar surface. Because Fe metal particles (used as a source of Fe) were larger in size ($>350\text{ }\mu\text{m}$) than biochar pores (mostly $<100\text{ }\mu\text{m}$), it is unlikely that they were physically trapped on the biochar surface. XPS data point to the growth of a Fe_2O_3 film on the surface of the biochar particles. The Fe:O stoichiometric ratio changed from a value that was extremely low (0.00085) to a value of 0.659 after exposure to Fe in the lab, very close to the expected value of 0.667 for pure Fe_2O_3 . Although this evidence shows that the biochar interacted with Fe, we cannot exclude the formation of $\text{Fe}(\text{OH})_3$ as an intermediate product or that Fe simply dissolved into the water precipitated as Fe_2O_3 onto the biochar surface. This evidence helps explain at least one mechanism that occurred in the potted experiment, and consequently helps explain plant responses. Although other reactions cannot be excluded, we hypothesize that Fe interacted with the biochar and a redox

reaction occurred on its surface. The Fe source was the reducing agent (donor of electrons) while water was the oxidizing agent (acceptor of electrons). This process may have been promoted by the presence of biochar functioning as an electron transfer mediator (Klöpfer et al., 2014). The reduction step released Fe^{2+} in solution that was electrostatically attracted to the biochar surface (Kappler et al., 2014) by reactive carboxylic and phenolic functional groups (Lin et al., 2012) and then oxidized. The redox reaction can be summarized as follows:

the product of such reaction was:

Then, the ferric hydroxide ($\text{Fe}(\text{OH})_3$) precipitated as hydrated ferric oxides, resulting in rust as final product observed on the biochar surface:

4.4 Sustainable strategies were effective in preventing Fe-chlorosis

Leaf Fe-chlorosis symptoms (interveinal yellowing starting from youngest leaves) were effectively prevented in both seasons by soil-applied Fe-chelate (the current standard approach), which induced the highest SPAD (data are available in Table S1 and S2 of the supplementary material) values (but see below for negative Mn response).

Symptoms in the first year were prevented to a similar extent by the *A. retroflexus* aqueous extract when enriched with FeSO_4 (but not when applied alone) and siderite, while the latter two treatments were slightly less effective the following year (data are available in Table S1 and S2 of the supplementary material). Untreated and plants

treated with *A. retroflexus* aqueous extract alone died as a consequence of the persistent Fe deficiency induced by the prohibitive growing conditions, confirming the high susceptibility of kiwifruit plants to this disorder and the severe consequences of prolonged Fe-chlorosis.

The beneficial effect on Fe nutrition induced by *A. retroflexus* aqueous extract is likely attributable to the chelating compounds (e.g. organic acids, amino acids, bioregulator-like substances, and siderophores) released by the *A. retroflexus* spp. tissues during either maceration or soil-incorporation (Goos et al., 2001; Matocha and Pennington, 1982; Matocha, 1984; Mostaghimi and Matocha, 1988) which were able to increase Fe-availability in soil. In fact, Sorrenti et al. (2011) measured a 100-fold higher concentration of solubilized Fe from a similar calcareous soil using the aqueous vegetal extract of *A. retroflexus* as Fe-extractant compared to DI-H₂O.

However, in our conditions the *A. retroflexus* aqueous extract alone did not improve Fe nutrition. This may be because the amount of solubilized Fe from a soil naturally poor in Fe was insufficient to sustain plant Fe requirements, while it was enough when the aqueous extract was enriched with an exogenous source of Fe which promoted a weak linkage between Fe and the organic compounds released by the *A. retroflexus* tissues. A similar response was observed in field conditions (Sorrenti et al., 2011), where Fe nutrition of pear trees was improved by the vegetal extract but only when enriched with FeSO₄, suggesting a beneficial effect of the *A. retroflexus* as a natural chelator and its affinity for Fe.

Siderite has been proposed as a long-term slow-release Fe fertilizer able to prevent Fe chlorosis symptoms in olive trees (Sánchez-Alcalá et al., 2012a). The effectiveness of siderite, as also described for synthetic vivianite, is related to its oxidation in calcareous

media resulting in the production of poorly crystalline lepidocrocite and/or goethite (Sánchez-Alcalá et al., 2012b) that, as with other poorly crystalline of nanometer-size Fe-oxides, represent a significant source of Fe for plants (de Santiago and Delgado 2006; Sánchez-Alcalá et al., 2012b). However, the application of siderite (only once at the beginning of the experiment) was more effective in the first than in the second season, suggesting that its effectiveness may not last as long as suggested previously (Sánchez-Alcalá et al., 2012a and literature therein).

The increased leaf K concentration observed in plants treated with siderite and vegetal extract of *A. retroflexus* (Table 5) could be a consequence of the direct supply of this nutrient by siderite (obtained by mixing KCO_3 and $FeSO_4$), while the vegetal extract may have a possible positive effect also on extracting this macronutrient from soil. A similar increase in leaf K concentration was observed in pear trees fertigated with *A. retroflexus* aqueous extract (Sorrenti et al., 2011).

In agreement with literature, Fe-chelate treatments dramatically decreased leaf Mn concentration (Table 5) because of the competitive effect of these synthetic molecules on Mn uptake, as shown in herbaceous as well as in perennial species (Ghasemi Fasaie et al., 2003; Sorrenti et al., 2011; Wallace and Alexander ,1973). These results suggest that to avoid Mn deficiency, supplementary applications of this micronutrient to the canopy should be considered in commercial orchards when Fe-chelates are repeatedly used.

5. CONCLUSION

Although we investigated one specific biochar, in our conditions hardwood derived biochar showed a high potential to remove Fe, Mn, Cu and Zn in solution, likely

through reactive functional groups on its surface while it showed little value as a source of these micronutrients. The affinity of biochar for cations, together with its ability to accept and donate electrons, could trigger redox reactions in soil with significant implications for micronutrient biogeochemical cycling thereby also affecting nutrient forms and availability for plants. In our conditions, the synergic occurrence of these processes in biochar-amended soil, sequestered part of the available micronutrients, limiting their availability for plant uptake.

Incorporating biochar in Fe-limited growing environments (i.e. alkaline-calcareous soils) hindered plant Fe nutrition, and as a consequence worsened the Fe-chlorosis incidence on kiwifruit vines, especially in the first season. This example of biochar-soil interactions must be taken into consideration in the use of biochar as an amendment. Similarly, adequate countermeasures need to be evaluated (e.g. pre-treatment of biochar with Fe). However, given that biochar-induced effects may change with application rates, studies with lower rates compared to that adopted in this study ($5 \text{ g kg}^{-1} \text{ w w}^{-1}$) could elucidate the real impact of this strategy. Likewise, studies are needed to confirm this effect in different soils and determine how aging will affect these properties of biochar.

Finally, our results indicate the potential of innovative and sustainable strategies (aqueous extract of *A. retroflexus* enriched with FeSO_4 and siderite) to alleviate symptoms of Fe chlorosis of kiwifruit vines grown in heavy calcareous soil. These techniques may be improvements on the current standard approach (application of Fe-chelates), which poses plant nutritional and potential environmental challenges.

6. ACKNOWLEDGMENTS

The authors thank Dr. Bo Chen for is valuable assistance with XPS analyses and Dr. Yousif Shamoo for providing microscope access. We also acknowledge funding from the US NSF through grant EAR 0911685. Authors thank three anonymous reviews for their valuable comments which helped to improve the manuscript.

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1 Table 1. Selected chemical and physical characteristics of the soil used in the experiment

Parameter	Value	Unit	Extractant/method
Texture			
Sand	24	%	
Silt	53	%	
Clay	23	%	
Total carbonate (CaCO_3)	78	%	HCl / De Astis method
Active lime (CaCO_3)	19.2	%	Ammonium oxalate (Drouineau, 1942)
Organic matter	0.54	%	Walkley-Black 1919 (Soltner, 1988)
Total N	0.39	‰	Kjeldahl method
Available phosphorus (P)	3	mg kg^{-1}	Olsen (Olsen and Sommers, 1982)
Exchangeable potassium (K)	195	mg kg^{-1}	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable sodium (Na)	186	mg kg^{-1}	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable calcium (Ca)	2611	mg kg^{-1}	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable magnesium (Mg)	47	mg kg^{-1}	Barium chloride (Hendershot and Duquette, 1986)
Available iron (Fe)	2.68	mg kg^{-1}	DTPA (Soltanpour and Schwab, 1977)
Available manganese (Mn)	1	mg kg^{-1}	DTPA (Soltanpour and Schwab, 1977)
Available zinc (Zn)	0.51	mg kg^{-1}	DTPA (Soltanpour and Schwab, 1977)
Available copper (Cu)	2.21	mg kg^{-1}	DTPA (Soltanpour and Schwab, 1977)
Available Boron (B)	0.29	mg kg^{-1}	Calcium chloride (Bingham 1982)
C/N ratio	8.03		
Cation Exch. Capacity (CEC)	14.7	$\text{meq } 100\text{g}^{-1}$	Barium chloride (Hendershot and Duquette, 1986)
pH	8.39		Water/Potentiometric

2 Table 2. Physical and chemical characteristics of the biochar

Parameters	Value	Unit
<i>Physical properties</i>		
Moisture	13.8	%
Bulk density	0.43±0.04	g cm ⁻³
Hydrophobicity	Slightly hydrophobic	
Total porosity	2722	mm ³ g ⁻¹
Transmission pores	318	mm ³ g ⁻¹
Storage pores	1997	mm ³ g ⁻¹
Residuals pores	406	mm ³ g ⁻¹
Max water absorption	4.53	g g ⁻¹ of d.m.
Skeletal density (SD) ^a	1.86±0.04	g cm ⁻³
Envelope density (ED) ^b	0.246±0.006	g cm ⁻³
Porosity (ED/SD)	0.863±0.006	%
Surface area ¹ (BET Brunauer–Emmett–Teller method)	410±6	m ² g ⁻¹
Particle size distribution (w/w) ^c		%
50-20	4.5	%
20-10	12.1	%
10-8	13.1	%
8-4	10.4	%
4-2	19.8	%
2-1	24.2	%
<1	15.9	
<i>Chemical properties</i>		
pH	9.8	-
CEC	101	cmolc kg ⁻¹
Carbon ¹ (C)	778.0	g kg ⁻¹
Total nitrogen (N)	9.1	g kg ⁻¹
C/N	85.5	-
Aluminum (Al)	268	mg kg ⁻¹
Arsenic (As)	0.005	mg kg ⁻¹
Beryllium (Be)	0.001	mg kg ⁻¹
Cadmium (Cd)	0.001	mg kg ⁻¹
Calcium (Ca)	25.0	g kg ⁻¹
Chrome (Cr)	0.002	mg kg ⁻¹
Cobalt (Co)	0.002	mg kg ⁻¹
Copper (Cu)	97	mg kg ⁻¹
Iron (Fe)	333	mg kg ⁻¹
Magnesium (Mg)	28.7	g kg ⁻¹
Manganese (Mn)	84	mg kg ⁻¹
Molybdenum (Mo)	2	mg kg ⁻¹
Phosphorus (P)	23.3	g kg ⁻¹
Potassium (K)	13.9	g kg ⁻¹
Sodium (Na)	11.9	g kg ⁻¹
Sulphur (S)	481	mg kg ⁻¹
Zinc (Zn)	104	mg kg ⁻¹

3 ^aThe skeletal density is the sample mass divided by sample volume occupied by a solid sample,
4 including any pores not accessible to the helium gas. ^bThe envelope density is defined as the sample

5 mass divided by the total sample volume that is measured if an “envelope” would be placed around
6 each individual particle. °data from Baronti et al. (2014) (with permission).

Table 3. Rate and timing of the treatments. Fertilization strategies were applied either to vines grown in unamended soil or to soil amended with biochar as in a factorial experimental design.

Fertilization strategies	Rate	Doses of Fe	Timing
Control		-	-
Soil-applied Fe-chelate (Fe-EDDHA – Fe 6%)	1.0 g L ⁻¹ (200 mL application ⁻¹)	9.2 mg and 11.5 mg Fe kg ⁻¹ soil in the first and second season, respectively	From bud burst, 4 and 5 applications by fertigation in the first and second season, respectively (every 15 days)
Synthetic Siderite (FeCO ₃)	1 g kg ⁻¹	481 mg Fe kg ⁻¹ soil	Single application, at transplanting
Aqueous vegetal extract of <i>Amaranthus retroflexus</i>	30 g L ⁻¹ (200 mL application ⁻¹)	-	From bud burst, 4 and 5 applications by fertigation in the first and second season, respectively (every 15 days)
Aqueous vegetal extract of <i>Amaranthus retroflexus</i> + FeSO ₄	30 g L ⁻¹ + 2 g L ⁻¹ (200 mL application ⁻¹)	61.5 e 76.9 mg Fe kg ⁻¹ soil in the first and second season, respectively	From bud burst, 4 and 5 applications by fertigation in the first and second season, respectively (every 15 days)

19 Table 4. Effect of the fertilization and biochar on plant biomass at the end of the experiment

	Plant biomass at harvest		
	Shoot	Stem+ Root	Total Dry
	Biomass		
	(g dw plant ⁻¹)	(g dw plant ⁻¹)	(g dw plant ⁻¹)
Fertilization			
Control	- ^a	13.1 c	13.1 c
Fe-chelate	18.2 a	50.6 ab	68.8 a
Siderite	12.2 b	42.2 b	54.4 b
<i>A. retroflexus</i>	-	11.2 c	11.2 c
<i>A. retroflexus</i> + FeSO ₄	13.3 ab	51.3 a	64.6 a
Biochar (g kg⁻¹)			
0	15.3	34.9	50.2
50	13.9	31.0	44.9
<i>Significance (p values)</i>			
<i>Fertilization</i>	0.033	<0.0001	<0.0001
<i>Biochar</i>	0.385	0.374	0.292
<i>Fert x Biochar</i>	0.338	0.595	0.684

20 ^aAt the end of the experiment, shoots from the untreated control and *A. retroflexus* alone treated plants
21 were not present because plants had died.
22 When a significant effect occurred, means in the same column followed by the same letter are not
23 statistically different ($p < 0.05$, SNK Test).

24 Table 5. Effect of the fertilization and biochar on leaf macro and micronutrient concentration in July 2012

25

	N	P	K	Ca	Mg		Fe		Mn		Cu	Zn
	g kg ⁻¹						mg kg ⁻¹					
	Biochar rate						Biochar rate		Biochar rate			
	0		50 g kg ⁻¹				0	50 g kg ⁻¹	0	50 g kg ⁻¹		
Fertilization												
Fe-chelate	19.9	2.34	15.2 b	20.0	5.48	3.82	68.3	62.3	2.41	2.92	9.17	22.1
Siderite	22.4	2.44	25.5 a	20.4	3.51	3.80	48.8	49.4	13.3	14.4	8.62	22.3
<i>A. retroflexus</i> + FeSO ₄	19.7	2.15	23.3 a	20.6	4.37	3.52	42.1	39.4	14.1	8.50	8.35	20.0
Biochar (g kg ⁻¹)												
0	21.3	2.23	18.9	22.9								
50	19.6	2.40	23.7	17.8								
Significance (p values)												
Fertilization	0.079	0.478	<0.0001	0.968								

<i>Biochar</i>	0.524	0.387	0.0006	0.016				0.906	0.125	
<i>Fert x Biochar</i>					0.048		0.030	0.002	0.357	0.080
	0.192	0.363	0.095	0.327	(2SEM=0.75)		(2SEM=2.59)	(2SEM=2.41)		

- 26 When a significant effect occurred, means in the same column followed by the same letter are not statistically different ($p < 0.05$, SNK Test) Within the same
- 27 nutrient, values differing by ≥ 2 standard error of means (SEM) are statistically different.

28 Table 6. Effect of the fertilization strategy and biochar on soil pH, soil deionized water (DI-
 29 H₂O) and DTPA extractable Fe, Mn, Cu and Zn at the end of the experiment

Fertilization	Soil pH	DI-H ₂ O		DTPA extractable			
		extractable		Fe	Mn	Cu	Zn
		Fe	Fe				
mg kg ⁻¹ (dw)							
Control	7.52 a	0.25 b	1.94 c	5.20	2.63 a	2.04	
Fe-chelate	7.46 b	0.53 a	2.36 bc	6.22	2.23 b	1.79	
Siderite	7.48 b	0.26 b	2.90 b	6.87	2.72 a	2.03	
<i>A. retroflexus</i>	7.59 a	0.32 b	2.02 c	6.45	2.62 a	2.13	
<i>A. retroflexus</i> + FeSO ₄	7.45 b	0.20 b	3.84 a	5.99	2.43 ab	1.87	
Biochar (g kg⁻¹)							
0	7.45	0.44	2.42	4.86	1.83	0.98	
50	7.56	0.19	2.80	7.46	3.23	2.97	
<i>Significance (p values)</i>							
<i>Fertilization</i>	0.004	0.008	<0.0001	0.079	0.020	0.329	
<i>Biochar</i>	<0.0001	<0.0001	0.014	<0.0001	<0.0001	<0.0001	
<i>Fert x Biochar</i>	0.124	0.537	0.202	0.404	0.350	0.804	

30 When a significant effect occurred, means in the same column followed by the same letter are not
 31 statistically different ($p < 0.05$, SNK Test).

Figure 1

Figure 2

Figure 3

1 **FIGURE CAPTIONS**

2

3 **Fig. 1** Effect of the amendment with biochar on leaf chlorophyll (Chl) content (a) and shoot growth
4 (b) throughout the experiment. Values are averages across fertilization strategies.

5

6 *, ** and *** = effect of the amendment significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

7

8 **Fig. 2** Effect of increasing rates of biochar on Fe (A), Mn (B), Cu (C) and Zn (D) retention potential

9

10 Fe, Mn, Cu and Zn concentration in deionized water (DI-H₂O) was 0.022 ± 0.002 , 0.016 ± 0.01 , $0.0003 \pm$
11 0.0001 mg L^{-1} and $< \text{dl}$ (avg. $\pm$ SE; n=5), respectively. Fe, Mn, Cu and Zn concentration in solution was $9.28 \pm$
12 0.036 , 10.05 ± 0.0 , 9.88 ± 0.0007 and $10.01 \pm 0.0002 \text{ mg L}^{-1}$ (avg. $\pm$ SE; n=5), respectively.

13

14

15 **Fig. 3** Magnification (3.2x) of a biochar fragment recovered after 1-week exposure to a source of Fe.
16 Likely due to redox reactions, typical rusted spots are clearly evident. The biochar surface (top 5 nm)
17 was then scanned by X-ray photoelectron spectroscopy (XPS). Magnification was obtained by an
18 Olympus SXZ16 microscope coupled with an Olympus digital camera