Bioavailability of Iron in Calcareous Soils:

Microbial Reduction and Nanofertilizer Application

Inmaculada Sánchez Alcalá

TÍTULO: Biodisponibilidad de hierro en suelos calcáreos: reducción microbiana y aplicación de nanofertilizantes

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BIOAVAILABILITY OF IRON IN CALCAREOUS SOILS:

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CERTIFICAN:

Que la Tesis Doctoral titulada "**BIODISPONIBILIDAD DE HIERRO EN SUELOS CALCÁREOS: REDUCCIÓN MICROBIANA Y APLICACIÓN DE NANOFERTILIZANTES**" ha sido realizada por la Ingeniera Agrónoma Dña. Inmaculada Sánchez Alcalá en el Departamento de Agronomía de la Universidad de Córdoba bajo su dirección y reúne, a su juicio, las condiciones requeridas para optar al Título de Doctor Ingeniero Agrónomo.

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RESUMEN

La clorosis férrica es uno de los mayores problemas nutricionales de las plantas cultivadas en suelos calcáreos, que abundan en las regiones de clima árido y semiárido del mundo. El pH básico de estos suelos determina que, aunque su contenido total en hierro (Fe) pueda ser moderadamente elevado, dicho elemento se encuentre en formas poco disponibles para la planta. En España y otros países del área mediterránea, la clorosis férrica afecta a cultivos de gran importancia económica como el olivo, la vid y los cítricos.

El primer objetivo general de la tesis era conocer en qué medida la saturación temporal del suelo, con la consiguiente aparición de condiciones reductoras, y su posterior aireación afectan a las formas del hierro y su biodisponibilidad. Para ello se hicieron experimentos de laboratorio y de maceta en un grupo de 24 suelos calcáreos inductores de clorosis férrica del sur de España. La incubación de suspensiones de suelo en condiciones anaerobias durante siete semanas mostró que la población microbiana nativa de estos suelos era capaz de reducir el Fe(III) presente en óxidos de distinta cristalinidad. La movilización del Fe se vio afectada por el carbono orgánico disuelto y por la concentración total de óxidos. La incubación, aireación y secado de las muestras dio como resultado un aumento de la solubilidad de las formas de hierro, rebasándose en muchos casos los niveles críticos de los habituales ensayos de biodisponibilidad del hierro. Experimentos de maceta con cultivos sucesivos de cacahuete y garbanzo corroboraron que la saturación temporal del suelo era eficaz para reducir la incidencia de la clorosis férrica, aunque una segunda saturación no tuvo efectos en la solubilidad del hierro.

Como segundo objetivo general se planteó el desarrollo de fertilizantes alternativos para la corrección de la clorosis férrica. En base a consideraciones teóricas y estudios previos, se seleccionó la siderita (carbonato ferroso) para su estudio en profundidad. La siderita fue fácilmente sintetizada en el laboratorio en forma de partículas nanométricas de alta reactividad. Experimentos en maceta con plantas herbáceas (garbanzo, cacahuete y fresa) y de campo con olivo demostraron que la inyección en el suelo de suspensiones de siderita es eficaz para prevenir y corregir la clorosis férrica durante períodos prolongados. Esta eficacia se atribuye a su rápida oxidación a óxidos de hierro de baja cristalinidad. Las características cristalinas, facilidad de preparación y reacciones de la siderita en el suelo la hacen, además, ambiental y económicamente atractiva para corregir la clorosis férrica.

SUMMARY

Iron (Fe) chlorosis is a major nutritional problem in plants grown in calcareous soils, which abound in arid and semiarid regions of the world. The alkaline pH of these soils reduces the availability of iron to plants, even if the total iron content of the soil is high. In Spain and other Mediterranean countries, iron chlorosis has a considerably adverse impact on economically significant crops including olives, grapes and citrus fruits.

The first general objective of this doctoral work was to determine to what extent seasonal soil saturation, which promotes reducing conditions, and subsequent aeration affect iron forms and their bioavailability. To this end, laboratory and pot experiments were carried out on a group of 24 iron chlorosis-inducing calcareous soils from southern Spain. Incubating soil suspensions under anaerobic conditions for 7 weeks showed the native microbial population of the soils to efficiently reduce Fe(III) in oxides of variable crystallinity. Iron mobilization was affected by dissolved organic carbon and the total concentration of oxides. Incubation, aeration and drying of the samples increased the solubility of iron to levels often exceeding the critical values of tests commonly used to assess bioavailable iron. Pot experiments with successive peanut and chickpea crops confirmed that temporary soil saturation was effective in reducing the incidence of iron chlorosis, but also that further saturation had no effect on the solubility of iron.

The second general objective of this work was to develop alternative fertilizers for correcting iron chlorosis. Based on theoretical considerations and previous studies, siderite [Fe(II) carbonate] was selected for in-depth study. Siderite was easily synthesized in the laboratory in the form of highly reactive nanoparticles. Pot experiments with herbaceous plants (chickpea, peanut and strawberry) and field experiments in olive orchards showed injection of siderite suspensions into the soil to effectively prevent and correct iron chlorosis over long periods. The efficiency of siderite is ascribed to its fast oxidation to poorly crystalline iron oxides. The crystal properties, ease of preparation and reactions of siderite in soil make it an environmentally and economically attractive choice for alleviating iron chlorosis.

GENERAL INDEX

Note:

This current PhD Thesis is written in a bilingual format as a requirement for the International Mention

CHAPTER 1

Introducción General y Objetivos

CLOROSIS FÉRRICA

La deficiencia de hierro (Fe) es uno de los problemas nutricionales más comunes y difíciles de controlar en plantas cultivadas en suelos calcáreos (Tagliavini y Rombolà, 2001; Gruber y Kosegarten 2002; Hansen et al., 2003; Wiersma, 2005). Estos suelos, que se caracterizan por tener una sustancial acumulación de carbonato cálcico secundario, están muy extendidos por todo el área subtropical árida y semiárida de ambos hemisferios, pudiendo llegar su superficie mundial a 1000 millones de hectáreas (FAO, 2001).

El principal síntoma de la deficiencia de Fe en plantas es la clorosis férrica, caraterizada por el amarilleamiento internervial de las hojas más jóvenes (Figs. 1 y 2).

Figura 1. Clorosis internervial en hojas de olivo

Cuando la deficiencia es más grave, el amarilleamiento evoluciona a una decoloración completa de la hoja con aparición de necrosis (Fig. 3), correspondiendo el estadio último a la necrosis de la hoja entera (Champagnol, 1984). Estos síntomas suelen ir acompañados además por una reducción en el crecimiento, inhibición de la formación de hojas nuevas y merma de la calidad del fruto. En el caso del olivo, las aceitunas de los brotes cloróticos adquieren tonos amarillos o verde claros, no llegan a alcanzar el tamaño adecuado y pueden llegar a perder su forma característica (Rosado et al., 2002). En estos casos, la industria no las acepta para aceituna de mesa. En general, con la clorosis disminuye la producción, el rendimiento graso de las aceitunas y empeoran algunos de los parámetros de calidad del aceite (Chova et al., 2000, del Campillo et al., 2000).

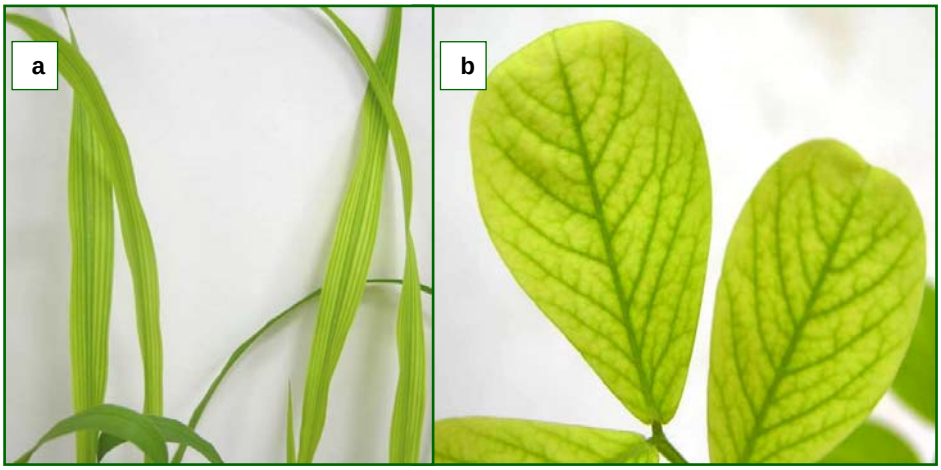

Figura 2. Clorosis férrica severa en plantas de sorgo (a) y cacahuete (b)

Figura 3. Necrosis en plantas de altramuz

Por tanto, las pérdidas económicas son cuantiosas. Por ejemplo, la clorosis férrica afecta en la Cuenca del Ebro a más de 90000 ha de frutales, donde son necesarios tratamientos correctores o preventivos cuyo coste es difícil de calcular, ya que los únicos datos oficiales son los de Sanz et al. (1992) que los estimaron en más de 12 millones de euros anuales.

FACTORES PRINCIPALES QUE INCIDEN EN LA CLOROSIS FÉRRICA

La clorosis férrica raramente está causada por una deficiencia “absoluta” de hierro (Fe), que no es habitual en los suelos calcáreos. El contenido total de Fe en el suelo se encuentra generalmente en el intervalo de 10 – 50 g kg^{-1}. Esta concentración es más que suficiente para satisfacer las necesidades de la mayoría de los cultivos

agrícolas, que son inferiores a 0.5 mg kg^{-1} (Lindsay, 1974). El problema, por tanto, no es de deficiencia de Fe en el suelo, sino de disponibilidad para la planta. La baja disponibilidad de Fe en suelos calcáreos ha sido atribuida a la disminución de la solubilidad del Fe en los suelos con pH alcalino (Miller et al., 1984; Mengel, 1994).

EL HIERRO EN LA PLANTA

La clorosis férrica no afecta en igual grado a todas las plantas ni a distintos cultivares de la misma especie. Se habla de plantas resistentes, que han desarrollado mecanismos de respuesta que en condiciones de deficiencia de Fe en el suelo lo hacen más disponible para la planta (Brown y Jones, 1976; Miller et al., 1984; Jolley et al., 1986; Longnecker y Welch, 1986; Wallace, 1986), y plantas susceptibles, que no han desarrollado estos mecanismos con la misma facilidad que las resistentes. Entre las plantas más afectadas se encuentran frutales como el manzano, naranjo, limonero, mandarino, melocotonero, peral, vid y olivo; cultivos extensivos como el maíz, garbanzo, altramuz, soja y girasol; y cultivos hortícolas como el tomate y fresa (Chen y Barak, 1982; Sanz et al., 1992).

Los mecanismos de respuesta a la carencia de Fe dividen a las plantas en dos grupos: plantas de estrategia I y plantas de estrategia II.

La estrategia I es propia de dicotiledóneas y monocotiledóneas no gramíneas. Estas plantas se caracterizan por la liberación de sustancias reductoras y/o quelantes (Marscher et al., 1986) que producen un incremento en la reducción de Fe(III) a Fe(II) favoreciendo la absorción de Fe (Römheld y Marschner, 1986). Además, las raíces tienen la capacidad de excretar protones que disminuyen el pH de la rizosfera, con lo que se consigue un aumento de la solubilidad del Fe del suelo (Marschner et al, 1986). Los mecanismos de regulación de las respuestas a la deficiencia de Fe implican cambios hormonales (Romera et al., 1992). En este tipo de plantas se producen también cambios morfológicos en la raíz: a nivel macroscópico se produce un engrosamiento de las zonas subapicales y la aparición de pelos radiculares, lo que aumenta la superficie de adsorción (Welkie y Miller, 1993); a nivel microscópico, se observa la presencia de células de transferencia en la zona de engrosamiento en las que se incrementa la superficie de contacto entre la pared celular y el citoplasma (Welkie y Miller, 1993). En las raíces de estas plantas se ha observado acumulación de ácidos orgánicos, principalmente citrato y malato (Rombolà et al., 2002; Abadía et al., 2002).

La estrategia II es desarrollada únicamente por las gramíneas y se caracteriza por la liberación en la zona radicular de unos compuestos quelantes de Fe de bajo peso molecular llamados fitosideróforos por las similitudes que presentan con los sideróforos liberados por los microorganismos (Marschner et al, 1990). Los fitosideróforos son aminoácidos no proteicos que movilizan el Fe(III) inorgánico y favorecen la disponibilidad del nutriente para la planta en condiciones de deficiencia de Fe (Marschner et al., 1986). La reducción de Fe es aquí de muy poca importancia ya que la planta absorbe los fitosideróforos a través de un mecanismo específico ausente en las plantas que utilizan la estrategia I (Marschner et al., 1986).

Una vez en la planta, el Fe(II) se oxida a Fe(III) y es transportado a la parte aérea a través del xilema (plantas que siguen la estrategia I). El Fe(III) forma complejos con sustancias orgánicas (Landsberg, 1984), como citrato (Brown y Tiffin, 1965; Chaney, 1989), malato (Chaney, 1989) y aminoácidos (Cataldo et al., 1988). Una vez en el xilema, el Fe complejado se mueve siguiendo los flujos de transpiración. Cuando el Fe llega a las hojas debe reducirse de nuevo a Fe(II), desestabilizando el complejo Fe(III)-citrato y facilitando la entrada en las células foliares (Brown et al., 1979; Landsberg, 1984; Mengel y Geurtzen, 1986).

La facilidad del Fe para cambiar de estado de oxidación y formar quelatos estables y solubles hace que esté implicado en un gran número de funciones fisiológicas, siendo uno de los micronutrientes esenciales para las plantas. Forma parte de numerosas moléculas e interviene en procesos metabólicos tan importantes como la fotosíntesis, respiración y fijación de N (Clark, 1983).

EL HIERRO EN EL SUELO

El hierro (Fe) es el cuarto elemento más abundante en la corteza terrestre, después del oxígeno, silicio y aluminio (Jackson, 1958). Se encuentra principalmente en forma de minerales primarios de Fe(II) como son los silicatos ferromagnésicos. A partir de la meteorización de los minerales primarios, se libera Fe soluble a la disolución, que puede ser utilizado por los organismos, unirse a distintos ligandos orgánicos, o bien ser transformado en minerales secundarios fundamentalmente óxidos, oxihidróxidos e hidróxidos de Fe(III) (llamados en general óxidos). Estos óxidos se asocian frecuentemente a los minerales de la arcilla y su distinta composición y grados de cristalización son los que básicamente controlan la solubilidad de este elemento en el suelo (Lindsay, 1979; Murad y Fischer, 1988). Solamente una pequeña fracción pasa a

otros minerales secundarios de Fe(III) o es complejada por la materia orgánica del suelo (Chen y Barak, 1982; Schwertmann y Taylor, 1989).

Los óxidos de Fe son muy estables debido a su baja solubilidad; por ello la fracción del Fe en forma soluble es extremadamente baja en comparación con el contenido en Fe total del suelo. Los óxidos de Fe más comunes son goethita (α–FeOOH), hematites (α–Fe_2O_3), ferrihidrita ($Fe_5O_8H•4H_2O$), lepidocrocita (γ–FeOOH), maghemita (γ–Fe_2O_3) y magnetita (Fe_3O_4). La solubilidad del Fe está controlada por el óxido de Fe más soluble y por el pH. Según Lindsay y Schwab (1982) la solubilidad de los óxidos de Fe disminuye en el orden: ferrihidrita > maghemita > lepidocrocita > hematites > goethita. Las reacciones del Fe están influenciadas por el carbonato cálcico, al controlar éste el pH de la solución debido a su poder tampón. Los cálculos termodinámicos (Lindsay, 1979) muestran que las tres formas de Fe más abundantes en el intervalo de pH 7–9 son $Fe(OH)_2^+$, $Fe(OH)_3$ y $Fe(OH)_4^-$, encontrándose los niveles mínimos de solubilidad en el intervalo de pH 7.4–8.5 (Lindsay y Schwab, 1982).

En suelos calcáreos los óxidos de Fe secundarios más comunes son la goethita y la hematites. En menor proporción está la ferrihidrita y, raramente, lepidocrocita y maghemita (Schwertmann, 1991). En suelos del área mediterránea, donde el contenido de materia orgánica es bajo (Torrent, 1995), el Fe aportado por la meteorización forma hematites y goethita, en proporciones que dependen de los factores ambientales (esencialmente temperatura, actividad del agua, pH y diversos,solutos) (Schwertmann, 1985).

La concentración, mineralogía y cristalinidad de los óxidos de Fe del suelo afectan a la disponibilidad de Fe para la planta (Loeppert y Hallmark, 1985). Se ha demostrado la importancia de las formas poco cristalinas de los óxidos de Fe en la prevención y corrección de la clorosis. La ferrihidrita es así la forma de Fe inorgánico más rápidamente movilizable por la planta por su gran superficie específica y solubilidad en comparación con los óxidos más cristalinos (Vempati y Loeppert, 1986).

Los óxidos de Fe poco cristalinos en suelos no calcáreos han sido por lo general cuantificados mediante extracción con una disolución 0.2 *M* de oxalato amónico a pH 3 (Schwertmann, 1964). Sin embargo el pH de esta disolución aumenta en contacto con un suelo calcáreo con lo que la disolución de los óxidos de Fe se reduce drásticamente. Para resolver este inconveniente, Benítez et al. (2002) modificaron el método añadiendo sólo 0.25 g de suelo en 50 cm^3 de extracto con el fin de evitar importantes cambios de pH.

La concentración de hierro extraíble con oxalato amónico ácido (Fe_{ox}) ha sido el indicador que mejor ha predicho la concentración de clorofila en sorgo y soja

(Loeppert et al., 1988; Morris et al., 1990), garbanzo y girasol (del Campillo y Torrent, 1992), melocotonero (Yangüas et al., 1997), olivo (Benítez et al., 2002) y vid (Reyes et al., 2006) cultivados en suelos calcáreos. Ello se debe a que el Fe_{ox} proviene esencialmente de los óxidos de Fe poco cristalinos, de las pequeñas cantidades de Fe complejado por la fracción orgánica y de la disolución parcial de óxidos de Fe menos solubles, como lepidocrocita, maghemita y magnetita (Borggaard, 1982).

Otro método usado para cuantificar los óxidos de Fe poco cristalinos es la extracción con una disolución de citrato/ascorbato a pH 6 (Fe_{ca}) (Reyes y Torrent, 1997). Se observó así una correlación positiva entre la concentración de clorofila en olivo y el Fe_{ca} (Benítez et al., 2002) y vid (Reyes et al., 2006) aunque el Fe_{ca} tuvo menor valor predictivo que el Fe_{ox} y mayor a su vez que el Fe extraído con una disolución de DTPA (ácido dietilentriaminopentaacético) a pH 7.3 (Fe_{DTPA}) que según sus proponentes (Lindsay y Norvell, 1978) extrae el Fe de la disolución y el muy fácilmente soluble de la fase sólida, es decir, las formas “lábiles” de Fe. El papel del Fe_{DTPA} en simular lo que extraen las plantas ha sido puesto de manifiesto en trabajos como el de Viets y Lindsay (1973) y Lindsay y Norvell (1978).

Un reciente estudio ha mostrado como buen predictor de la clorosis férrica en olivo, vid, garbanzo y girasol el Fe extraído con hidroxilamina no tamponada (de Santiago et al., 2008a). La hidroxilamina es efectiva disolviendo óxidos no cristalinos de Fe (McAlister y Smith, 1999), estando su efectividad determinada por la concentración de este reductor, el pH de la solución extractante y la temperatura.

pH, CARBONATOS, BICARBONATOS Y ETILENO

La clorosis férrica se observa con frecuencia en suelos calcáreos, en los que el $CaCO_3$ (en forma de calcita) controla el pH en el intervalo 7.5 – 8.5 (Loeppert, 1988). La solubilidad de los óxidos de Fe está muy relacionada con el pH del suelo, de modo que la solubilidad disminuye 1000 veces por cada unidad que aumenta el pH, reduciéndose la concentración de Fe soluble a valores inferiores a 10^{-20} *M*. La región de mínima solubilidad del Fe corresponde al rango de pH entre 7.5 – 8.5, siendo la concentración de Fe para estos valores de pH de aproximadamente $10^{-10.4}$ *M*, cantidad insuficiente para el crecimiento óptimo de las plantas, que requieren un intervalo de Fe soluble en el medio entre 10^{-9} y 10^{-4} *M* (Guerinot y Yi, 1994). En estas condiciones, es posible que una planta manifieste síntomas de deficiencia de Fe.

Se ha comprobado que la fracción de carbonatos relacionada de forma más directa con el grado de clorosis férrica es aquella capaz de reaccionar con oxalato

amónico a pH neutro, denominada “equivalente de carbonato cálcico activo” (ECCA) o “caliza activa” (Drouineau, 1942). Muchos trabajos han examinado la relación entre el grado de clorosis y las propiedades de los carbonatos, aunque en pocos casos se ha constatado el papel claro de éstos. Yaalon (1957) observó que el grado de clorosis estaba relacionado con el contenido en caliza activa y sugirió que un 10% de caliza activa era el nivel crítico para los cultivos más sensibles. Sin embargo, estudios más recientes han demostrado que el grado de clorosis no está sistemáticamente relacionado con el contenido de caliza activa, aunque ésta ejerce gran influencia en las propiedades del suelo debido a su alta solubilidad, capacidad tampón y basicidad (Clemens, 1990; del Campillo y Torrent, 1992; Yanguas et al., 1997; Benítez et al., 2002; Reyes et al., 2006).

Al estudiar las propiedades del suelo que influyen en la clorosis férrica en girasol y garbanzo, del Campillo y Torrent (1992) propusieron como mejor variable explicativa de la concentración de clorofila en planta el producto $Fe_{ox} \times ECCA^{-1} \times 10^4$, donde Fe_{ox} mide la cantidad de Fe extraíble con oxalato amónico a pH 3 (en g kg^{-1}) y ECCA es el equivalente de carbonato cálcico o caliza activa (en g kg^{-1}). En el trabajo que realizó Reyes et al. (2006) con plantas de vid `Pedro Ximénez / 110 R´, $Fe_{ox} \times ECCA^{-1}$ también resultó ser el mejor predictor de la incidencia de la clorosis férrica.

El anión bicarbonato (HCO_3^-) es uno de los solutos principales en la disolución de los suelos calcáreos, estando su concentración controlada principalmente por la calcita ($CaCO_3$) y por la presión parcial del CO_2 de la atmósfera del suelo (Loeppert, 1986).

El bicarbonato afecta negativamente la solubilidad del Fe en el suelo, debido a que mantiene un pH básico, favoreciendo así la oxidación del Fe(II) y su paso a compuestos de baja solubilidad (Lindsay, 1984; Loeppert, 1986). En todo caso, la concentración de bicarbonato en el suelo está considerada por diferentes investigadores como un factor clave en la inducción de la clorosis férrica en suelos calcáreos y alcalinos (Boxma, 1972; Coulombe et al., 1984a y b; Mengel et al., 1984; Romera et al., 1992; Nikolic y Kastori, 2000; Lucena et al., 2007). Concentraciones altas de bicarbonato se han relacionado con un aumento del pH del apoplasto y un incremento de la cantidad de Fe no disponible fisiológicamente por las células (Mengel, 1994). En 1995, Mengel indicó que el HCO_3^- presente en el apoplasto radicular neutraliza los protones bombeados fuera del citosol y dificulta la absorción de nitratos por el cotransporte H^+/NO_3^-. Esta idea está basada en el hecho de que se han encontrado cantidades mayores de Fe en las hojas cloróticas que en las no cloróticas (la llamada “paradoja de la clorosis”). Sin embargo, Nikolic y Römheld (2002) demostraron que el pH del fluido apoplástico de la hoja no se veía afectado por altas

concentraciones de bicarbonato en el medio radicular (experimento realizado en solución nutritiva con girasol y en viñedo). En estos experimentos se observó que el bicarbonato sí disminuía la absorción y translocación de Fe a la parte aérea, como consecuencia de la inhibición de la capacidad reductora de las raíces (Nikolic et al., 2000; Römheld, 2000; Nikolic y Römheld, 2002). Lucena et al. (2007) mostraron que el bicarbonato podría inducir la clorosis férrica inhibiendo la reductasa férrica y contribuyendo a la inactivación del Fe dentro de la planta.

En los suelos calizos el $CaCO_3$ se encuentra en equilibrio según la reacción:

$$CaCO_3 + CO_2 + H_2O \Leftrightarrow Ca^{2+} + 2\ HCO_3^-$$

La hidrólisis del $CaCO_3$ se ve favorecida en condiciones de alta humedad y presión parcial de CO_2, por lo que los factores que favorecen un aumento de ésta causan aumento de la concentración de HCO_3^- y, en consecuencia, del riesgo de clorosis férrica. Según Chaney (1984) y Loeppert (1986) entre estos factores están un elevado contenido de agua en el suelo, un deficiente drenaje, la compactación, una elevada tasa de respiración de las plantas o de los microorganismos existentes en el suelo o un aumento de la población microbiana al aplicar enmiendas orgánicas.

Aunque en los suelos saturados se acentúa la reducción de Fe(III) a Fe(II), ello no se corresponde necesariamente con una mejora en la asimilación del Fe por la planta. Según Zuo et al. (2007) el exceso de humedad en un suelo calcáreo agravó la clorosis férrica del cacahuete plantado en él como consecuencia del aumento de la concentración de bicarbonato. Estudios realizados con vid (*Vitis labrusca* L.) en campo han arrojado resultados similares (Davenport y Stevens, 2006).

Romera et al. (2002) estudiaron si el bicarbonato y la anaerobiosis alteran las respuestas a la deficiencia de Fe por interacción con el etileno. El etileno participa en la regulación de varias respuestas de estrés por deficiencia de Fe en las plantas de Estrategia I, tales como una mayor actividad reductasa férrica, acidificación de la rizosfera y desarrollo subapical de la raíz. El objetivo de ese trabajo fue estudiar si estos factores actúan sobre la capacidad de reducción de Fe a través de la inhibición de etileno. Los resultados sugieren que la anaerobiosis podría inhibir la capacidad de las plantas de reducir el Fe(III) mediante el bloqueo de la síntesis de etileno mientras que el bicarbonato podría bloquear la acción del etileno.

Un alto grado de compactación del suelo dificulta el intercambio gaseoso de la atmósfera del suelo con el exterior, produciendo un aumento de CO_2 y, consecuentemente, de HCO_3^-. Igualmente, las bajas temperaturas también pueden incrementar la concentración de HCO_3^- en el suelo, ya que al disminuir la temperatura aumenta la solubilidad del CO_2 en agua.

CONDICIONES REDUCTORAS EN SUELOS CALCÁREOS

Los suelos con estancamiento superficial de agua (Fig. 4), denominados con el término de seudogley (Mückenhausen, 1963), sufren condiciones reductoras temporales que alternan con otras en la que prevalece la oxidación. Esta situación puede ocurrir por varios motivos:

(i) Por la existencia de un marcado contraste textural entre un horizonte muy arenoso que repose sobre un horizonte muy arcilloso. La diferente permeabilidad de estos dos horizontes hace que el agua de lluvia o riego quede retenida en el contacto de ambos formando una capa de agua colgada.

(ii) Por períodos de abundantes lluvias o de riegos excesivos.

Un suelo sometido a condiciones reductoras tiene unas características particulares ya que las condiciones hidromórficas del suelo quedan reflejadas en el perfil. Si las condiciones de saturación se mantienen constantes a lo largo del año, el ambiente reductor predomina, el Fe se encuentra en compuestos ferrosos y el perfil es de color gris verde azulado con cromas bajos. Cuando el suelo atraviesa fases de desecación estacionales más o menos largas se origina una alternancia de condiciones oxidantes y reductoras, apareciendo numerosas manchas rojizas debidas a los compuestos férricos, junto a otras zonas grisáceas más o menos verdosas y/o azuladas correspondientes a los compuestos ferrosos, quedando el horizonte abigarrado.

Figura 4. Suelo con estancamiento superficial de agua

Las condiciones de oxidación-reducción (potencial redox) tienen efecto sobre aquellos iones que tienen distintas valencias. Las condiciones redox pueden afectar indirectamente la movilidad de metales. Por ejemplo, en condiciones reductoras el Fe(III) se transforma en Fe(II) mucho más soluble. Así, muchos metales que están asociados o adsorbidos a hidróxidos de Fe y Mn son estables a potenciales redox (Eh)

bajos. La reversibilidad de la reacción oxidación – reducción de Fe juega un papel importante en su comportamiento en los suelos (Schwertmann, 1991). Una vez formados los óxidos de Fe(III) pueden redisolverse por reducción microbiana a Fe(II) o ser complejados por ligandos orgánicos. Los factores ambientales, tales como la temperatura, agua o pH condicionan estas reacciones (Schwertmann, 1985). Los óxidos de Fe que precipitan cuando los suelos son drenados después de una inundación podrían estar más disponibles para los cultivos, pero esta hipótesis no ha sido aún demostrada.

Los procesos de reducción de Fe en el suelo ocurren típicamente en ambientes anaerobios, tales como el fondo de acuíferos y lagos, arrozales y humedales (turberas y pantanos). Estos procesos están mediados por microorganismos que pueden utilizar los óxidos de Fe(III) como aceptores finales de electrones para realizar la descomposición oxidativa de la materia orgánica, lo que da lugar a la reducción del Fe(III) a Fe(II), que es más soluble y facilita la solubilidad de los óxidos (Schwertmann y Taylor, 1989). Pero actualmente la información sobre microorganismos reductores de Fe propios de los suelos calcáreos es aún muy escasa.

Los suelos calcáreos representan más de un tercio de la superficie mundial terrestre (Crowley et al., 1987), localizándose principalmente en climas áridos y semiáridos; por tanto, el estado natural de muchos de estos suelos es aeróbico. Las condiciones reductoras en suelos calcáreos tienden a ocurrir sólo en micrositios sin oxígeno y con alta actividad microbiana, como por ejemplo alrededor de las partículas de materia orgánica o, posiblemente, en la rizosfera de la planta (Crowley et al., 1987). Pero además, los suelos calcáreos, en las condiciones del clima mediterráneo, pueden estar sometidos a inundaciones contínuas y/o cíclicas durante la temporada de lluvias cuando el nivel freático es alto. Según Longoria (1973) y Velázquez et al. (2004), las inundaciones temporales de los suelos calcáreos han demostrado incrementar la biodisponibilidad de Fe. Se especula que la actividad reductora microbiana es capaz de mobilizar el Fe durante dichos episodios. Dependiendo de la gestión del agua y la intensidad de las lluvias, en el área mediterránea pueden ocurrir varios eventos de inundación al año, que pueden durar entre 10 y 30 días cada uno. Sin embargo, las alteraciones provocadas por el exceso de agua en los suelos calcáreos no han sido suficientemente documentadas.

CONTENIDO EN ARCILLA

La arcilla influye en la incidencia de la clorosis férrica, ya que parte de las partículas de los óxidos de Fe amorfos son de tamaño nanométrico y pueden ser adsorbidos en la superficie de las partículas de arcilla o como hidróxidos de Fe intersticiales entre ellas (Loeppert y Hallmark, 1985; del Campillo y Torrent, 1992). Se ha encontrado así una correlación positiva entre el contenido de arcilla y la concentración de clorofila en hojas de sorgo y soja (Loeppert y Hallmark, 1985), garbanzo y girasol (del Campillo y Torrent, 1992) y olivo cv. Hojiblanca, Manzanilla y Picual (Benítez et al., 2002) cultivados en suelos calcáreos. Hay que tener sin embargo en cuenta que la adsorción de Fe por montmorillonita y caolinita ocurre sobre todo en condiciones ácidas así como en medio reductor (Ellis y Knezek, 1972) y que sólo un 2% del peso de algunas arcillas lo constituye el Fe estructural (Carstea et al., 1970). En definitiva: las arcillas pueden ser una fuente adicional de Fe para las plantas que crecen en condiciones de deficiencia de Fe, ya que influyen en la estabilización de los óxidos de Fe amorfos, los más disponibles para las plantas (Vempati y Loeppert, 1986; Golden et al., 1997; Krishnamurti et al., 1998).

Sin embargo, un contenido alto de minerales de arcilla en el suelo puede favorecer, a veces, una alta compactación, lo que dificulta el intercambio gaseoso de la atmósfera del suelo con el exterior, dando lugar a altos niveles de CO_2 y el consecuente incremento de la concentración de HCO_3^- (Mengel et al., 1984).

CONTENIDO EN MATERIA ORGÁNICA Y ACTIVIDAD MICROBIANA

En los suelos cultivados de áreas mediterráneas los contenidos en materia orgánica son, normalmente, bajos (inferiores al 2%) (Torrent, 1995). El contenido de materia orgánica puede reducir la incidencia de la clorosis férrica debido a la formación de complejos estables con los óxidos de Fe poco cristalinos del suelo, que son los más disponibles para la planta (Loeppert y Hallmark, 1985), previniendo así la cristalización de la ferrihidrita (un óxido poco cristalino) en hematites y goethita (Schwertmann, 1964). Además, es capaz de formar quelatos con el Fe(III) (Bloom, 1981). La aplicación de materia orgánica también puede mejorar la actividad microbiana en el suelo, lo que induce una mayor movilidad del Fe por la formación de sideróforos y la excreción de ácidos orgánicos (Loeppert y Hallmark, 1985; Masalha et al., 2000; Crowley, 2001).

En algunos casos las enmiendas orgánicas aumentaron la severidad de la clorosis férrica en dicotiledóneas cultivadas en suelos húmedos (Chaney, 1984). En este caso, el aumento de la actividad microbiana provocado por la enmienda acentúa el consumo de O_2 y la acumulación de CO_2. Los niveles de bicarbonatos de la rizosfera aumentan y agravan la clorosis férrica, tal como se expuso anteriormente. Estos efectos negativos se acentúan en condiciones de alta humedad, compactación y deficiente drenaje (Chaney, 1984; Loeppert, 1986). Por lo tanto, para que los aportes de materia orgánica sean beneficiosos deben ir acompañados de un manejo del suelo que permita una correcta aireación de las raíces y un buen drenaje del suelo.

INTERACCIÓN DEL HIERRO CON OTROS ELEMENTOS

Las interacciones con otros nutrientes pueden dar lugar a problemas de disponibilidad de Fe.

Fósforo

Brown et al., (1955), citaron por primera vez al fosfato como el causante principal de la clorosis férrica. Desde entonces, otros autores (Brown et al., 1959; Brown y Olsen, 1980; Chaney y Coulombe, 1982, Kolesch et al., 1987a y b) han apoyado esta hipótesis. Éstos autores indican la posible inducción de clorosis férrica por la precipitación del Fe del suelo como fosfato de Fe(III), la interferencia en la reducción de Fe(III) en los quelatos (Brown y Olsen, 1980), o la inmovilización de Fe dentro de la planta (Cumbus et al., 1977). Según Brown (1960) (citado por Chaney, 1984), el bicarbonato actúa indirectamente porque incrementa la concentración de fosfato en la disolución del suelo, al que se considera causante de la clorosis férrica.

El fosfato puede absorberse en la superficie de los óxidos de Fe, compitiendo con los agentes quelantes y haciendo al Fe menos disponible para la planta (Mengel y Guertzen, 1986). DeKock (1981) sugirió que las relaciones altas de P/Fe y K/Ca en planta identificarían la clorosis férrica. Maldonado-Torres et al. (2006) encontraron que el grado de clorosis férrica en las hojas de limón mexicano (*Citrus aurantifolia*) disminuyó significativamente cuando las concentraciones de P/Fe y K/Ca eran altas. Sin embargo, según Mengel et al. (1984), elevados niveles de P encontrados en hojas cloróticas son probablemente el resultado de la inhibición del crecimiento y de este modo, son la consecuencia (efecto de la concentración), no la causa de la clorosis férrica.

Así, aunque la deficiencia de Fe pueda ser inducida en cultivos sobre suelos calcáreos con niveles muy altos de fósforo, existe una duda sustancial sobre su responsabilidad en la clorosis férrica inducida bajo condiciones de campo (Marschner, 1986). Además, parece lógico o razonable asumir que en los suelos calcáreos, caracterizados por tener bajas concentraciones de P en la disolución, el fosfato no sea el inductor de la clorosis férrica (Mengel y Kirkby, 2001). Las sales de Fe(III) en presencia de grandes cantidades de P (relación P/Fe = 0.5%) evolucionan a óxidos de Fe poco cristalinos como ferrihidrita y lepidocrocita (Gálvez et al., 1999), ya que el ión fosfato dificulta la cristalización de los óxidos de Fe (Schwertmann y Taylor, 1989).

En los estudios de caracterización de la tolerancia de cultivares de melocotonero a la clorosis férrica de Romera et al. (1991), el fosfato aplicado a la solución nutritiva no indujo clorosis férrica. En ese mismo trabajo, la susceptibilidad a la clorosis férrica inducida por bicarbonato estuvo inversamente correlacionada con el contenido en Fe de las hojas jóvenes y con la capacidad reductora en las raíces, pero no con el contenido en P de las hojas jóvenes. Romera et al. (1992), estudiando el efecto del bicarbonato, el fosfato y pH alto sobre la capacidad reductora del Fe(III) de las raíces de plantas cloróticas, mostraron que un aumento sólo de fosfato no tenía efecto inhibitorio sobre la capacidad reductora, pero sí se incrementó esta inhibición cuando se aplicó conjuntamente con bicarbonato, en plantas de girasol y de pepino.

Ladouceur et al. (2006) estudiaron el efecto de la concentración de P sobre la liberación de fitosideróforos y sobre el nivel de nutrientes minerales en plantas de cebada. Los resultados mostraron que el crecimiento y el índice de clorofila de las plantas fertilizadas con baja concentración de P (0.5, 5 y 50 μm mol L^{-1}) eran mayores que en las plantas control. Igualmente, la cantidad acumulada de nutrientes minerales en la parte aérea fue mayor para K, Fe y Cu en plantas con baja dosis de P que en las plantas control. Ya que en sus experimentos las condiciones de niveles bajos de P aliviaron los síntomas de deficiencia de Fe, estos autores sugirieron que el P compite fisiológicamente con el Fe en los tejidos vegetales.

En este mismo sentido, Samar et al. (2007), estudiaron la relación entre P, Zn y Fe en manzanos. Los resultados mostraron que niveles de P en el suelo de hasta cuatro veces el nivel recomendado, no tuvieron un efecto negativo en el índice de clorofila de la hoja, superficie foliar, peso seco de las hojas jóvenes o viejas y peso de las raíces. Estos autores concluyen su estudio afirmando que la variedad de manzano ensayada (‘*Delicious*’) parece no ser sensible a altos niveles de P disponible en el suelo.

Nitrógeno

En suelos calcáreos el NO_3^- es la forma predominante de N disponible para la planta, ya que el NH_4^+ se transforma en nitrógeno, que es volátil (Mengel y Geurtzen, 1986). La absorción de NO_3^- alcaliniza la rizosfera y potencia la inducción de clorosis férrica, mientras que la absorción de NH_4^+ favorece la secreción de protones (Mengel y Geurtzen, 1986; Lucena, 2000).

Potasio

La interacción entre el K y el Fe presenta aspectos contradictorios. Por una parte, los abonos potásicos pueden incrementar la liberación de H^+, acidificando la rizosfera y favoreciendo la absorción de Fe (Barak y Chen, 1984; Loeppert et al., 1994). Por otra parte, altos contenidos de K^+ pueden ayudar a dispersar las arcillas e influir negativamente en la aireación y compactación del suelo (Loeppert et al., 1994).

Otros elementos

La absorción de Fe también está condicionada por la presencia de otros cationes; así el ión Ca^{2+} puede desplazar al Fe de los compuestos quelantes (Lindsay y Schwab, 1982), lo mismo que el Cu^{2+}, Zn^{2+} y Mn^{2+} (Dekock, 1956).

Existe una competencia del Fe con el resto de metales por su transporte y posterior traslocación (Lucena et al., 2003). El exceso de metales pesados en el suelo puede interferir en los mecanismos de respuesta a la deficiencia de Fe y, por consiguiente, en la absorción y movilización del Fe en la planta, con la consecuente aparición de clorosis férrica (Römheld y Marschner, 1986). En un experimento en cultivo hidropónico con pepino, Alcántara et al. (1994) comprobaron que el Cu^{2+}, Ni^{2+} y Cd^{2+} actuaban inhibiendo severamente la inducción de la reductasa de Fe(III), mientras que el Cu^{2+} y Ni^{2+} inhibían su funcionamiento.

PREVENCIÓN Y CORRECCIÓN DE LA CLOROSIS FÉRRICA EN SUELO

La prevención y corrección de la clorosis férrica en plantas cultivadas sobre suelos calcáreos es relativamente difícil y costosa. A continuación se describen los métodos encaminados a estos fines.

APLICACIÓN DE COMPUESTOS ORGÁNICOS

Quelatos de hierro

Actualmente son los productos más utilizados en la corrección de la clorosis férrica ya que su acción es rápida y eficaz, aunque su efecto es temporal y se tienen que realizar varias aplicaciones al año. El elevado coste de estos fertilizantes de Fe para algunos cultivos también puede ser un inconveniente añadido. Además, al ser altamente solubles, son lixiviados fácilmente pudiendo llegar al subsuelo y contaminar la capa freática. Arizmendi-Galicia, et al., (2011) evaluaron el grado de lixiviación del Fe aplicado a columnas de suelo como quelato Fe-EDDHA y $FeSO_4$. El Fe aplicado en solución en forma de Fe-EDDHA sobre la superficie de suelos calcáreo y no calcáreo, presentó alta lixiviación en ambos suelos a través de una capa de 30 cm de espesor. Cuando el Fe se adicionó como $FeSO_4$ no tuvo lixiviación. También pueden ser inmovilizados por descomposición microbiana o adsorbidos en los minerales de arcilla (Chen y Barak, 1982).

Entre los quelatos existentes, el FeEDTA es poco efectivo en suelos calcáreos, ya que es poco estable por encima de pH 6.5 (Lucena et al., 1987). La aplicación al suelo de FeEDDHA y FeEDDHMA han sido los tratamientos correctores más eficaces y comúnmente usados contra la clorosis férrica en suelos calcáreos (Chen y Barak, 1982; Wallace, 1983) ya que son estables incluso a valores de pH superiores a 9 (Álvarez–Fernández et al., 2002). Sin embargo, uno de sus mayores problemas es el excesivo costo para un uso general. Reed et al., (1988) mostraron que la aplicación al suelo de FeEDDHMA fue, al menos, tan eficaz como la aplicación de FeEDDHA en melocotoneros (*Prunis persica* L.), mientras que la aplicación de $FeSO_4$ y citrato de hierro no fue eficaz en la corrección de la clorosis.

Aunque la dosis de los quelatos aplicados al suelo depende del producto, como orientación, Reynier (2005) recomienda para el viñedo que la aplicación se efectúe alrededor de mayo a razón de 7 a 15 g por cepa en función de la gravedad de la clorosis. Los quelatos aplicados al suelo no son efectivos cuando se aplican demasiado pronto en primavera y la temperatura es muy baja o cuando la absorción del Fe se dificulta por exceso de humedad (Tagliavini y Rombolà, 2001). Recientemente, se ha propuesto el uso de quelatos de Fe biodegradables como el IDHA (Villén et al., 2007).

Aplicación de sustancias húmicas

Existen evidencias del incremento en la disponibilidad de Fe debido a la presencia de compuestos orgánicos en el suelo. Compuestos secretados por las raíces (fitosideróforos), al complejar el Fe, favorecen la disolución de los óxidos de Fe y la absorción de este elemento (Marschner et al., 1989; Gerke, 1993). También se ha observado que las sustancias húmicas naturales presentes en el suelo incrementan la difusión del Fe a las raíces (Pandeya et al., 1998; Pinton et al., 1999; Cesco et al., 2002).

Las evidencias en cuanto al efecto que diferentes compuestos orgánicos tienen sobre la disponibilidad de Fe han llevado a ensayar con éxito mezclas de sales de Fe(II) y ácidos húmicos y fúlvicos. La aplicación conjunta de vivianita o sulfato ferroso (1 g kg^{-1} de arena calcárea) con una mezcla dializada de ácidos húmicos y fúlvicos (0.06 g kg^{-1} de arena calcárea) incrementó la eficiencia de estas fuentes de Fe (contenido de clorofila y materia seca) (de Santiago y Delgado, 2007). En este experimento la mezcla de compuestos húmicos y sales de Fe resultaron ser tanto o más efectivas que la aplicación de FeEDDHA en la prevención de la clorosis férrica de altramuz cultivado en arena calcárea.

El efecto de los ácidos húmicos y fúlvicos junto a las sales de Fe parece estar asociado a una inhibición de la cristalización de óxidos de Fe (Delgado et al., 2002a y b) más que a un incremento en la disponibilidad asociado a la complejación del Fe por la materia orgánica. El incremento de la eficiencia en la corrección de la clorosis férrica de sulfato ferroso o vivianita cuando se aplican junto con ácidos húmicos y fúlvicos puede permitir reducir las dosis de dichas sales, facilitando su aplicación en campo. La eficiencia conseguida con el sulfato ferroso podría permitir pensar en la aplicación de esta sal y de los ácidos húmicos y fúlvicos mediante fertirrigación, lo que solventaría una de las mayores limitaciones en la búsqueda de alternativas a la aplicación de quelato de Fe.

APLICACIÓN DE COMPUESTOS INORGÁNICOS

Los compuestos inorgánicos que han mostrado eficacia en la prevención de la clorosis férrica incluyen el sulfato de Fe(II) y el fosfato de Fe(II).

El grado de eficacia de las sales de Fe para prevenir la clorosis férrica cuando se mezclan con el suelo depende de la facilidad con la que generen los óxidos de Fe de baja cristalinidad, que constituyen la principal fuente de Fe para las plantas

cultivadas en suelos calcáreos (Loeppert y Halmark, 1985; Schwertmann y Fitzpatrick, 1992).

Sulfato ferroso

El sulfato ferroso ha sido uno de los tratamientos más empleados para prevenir la clorosis férrica y todavía hoy es ampliamente utilizado por los agricultores de los países en desarrollo gracias a su bajo coste (Tagliavini y Rombolà, 2001). Para el cultivo de la vid, la aplicación de sulfato de Fe al suelo puede realizarse enterrando de 2 a 5 t ha^{-1} antes de la plantación o cada 2 ó 3 años con una labor de subsolado (Reynier, 2005). Este tratamiento tiene poco efecto residual ya que el Fe precipita como óxidos de Fe poco solubles en suelos calcáreos y alcalinos (Loeppert, 1988). A pesar de esto, son más eficaces si se aplican con fertilizantes orgánicos como el estiércol (Hagstrom, 1984). En vid ha sido mucho más eficaz la incorporación de sulfato ferroso en disolución con una riqueza entre 2.5 y 10% en agua, a razón de 20 a 50 m^3 ha^{-1}, ya que se ha comprobado que se extiende mejor en el suelo y se pone al alcance de las raíces antes de ser oxidado (Hidalgo, 2002).

Fosfato de hierro (vivianita)

Para la mayoría de los cultivos, la prevención o corrección de la clorosis férrica no resulta rentable debido al elevado coste de las dosis a las que los productos aplicados son eficaces, como es el caso de los quelatos de Fe, o al escaso efecto residual que presenta el tratamiento con sulfato ferroso. Por este motivo, en la Unidad de Edafología de la Universidad de Córdoba (Grupo de Investigación AGR-165) desarrollaron productos alternativos de bajo coste y capaces de liberar el Fe lentamente para satisfacer las necesidades de la planta en un periodo más o menos prolongado. Se tuvo además en cuenta que estos productos no tuvieran efectos negativos sobre el ambiente.

Entre los productos desarrollados, el fosfato ferroso octahidratado [$Fe_3(PO_4)_2 \cdot 8H_2O$] análogo al mineral llamado vivianita, (solicitud de patente española ES2035766) presenta una serie de ventajas sobre otras sales inorgánicas de Fe dado que tiene un alto contenido en Fe (>32%) y que el producto expuesto al aire tiene >25% en forma de Fe(II). Además, la hidrólisis del Fe en presencia del ion fosfato favorece la precipitación de compuestos poco cristalinos como la lepidocrocita de pequeño tamaño de partícula, completamente soluble en oxalato amónico a pH 3 (Roldán et al., 2002), lo que explica su eficacia en la prevención de la clorosis férrica.

El hecho de que las partículas de vivianita tengan un tamaño comprendido entre 5 y 10 µm y baja solubilidad al pH de los suelos calcáreos, favorece la lenta liberación de Fe a la disolución. Esto permite que una aplicación de vivianita en el suelo tenga un efecto prolongado en el tiempo como se ha observado durante tres años en olivar (Benitez et al., 2002) cinco en peral (del Campillo et al., 1998) y tres en viñedo. En suelos calcáreos la dosis de 1 g kg^{-1} ha sido eficaz en la prevención de la clorosis férrica en girasol, altramuz y garbanzo en ensayos realizados en macetas en condiciones controladas (Eynard et al., 1992), y en olivo (Rosado et al., 2002). En kiwi la dosis de 1.8 kg $árbol^{-1}$ fue efectiva en campo (Rombolà et al., 2003). La producción de materia seca de altramuz fue significativamente mayor cuando se aplicó la mezcla de 1 g vivianita con 0.06 g de compuestos húmicos (ácidos húmicos y fúlvicos) por kg de suelo que cuando se aplicó sola, lo que permitiría reducir la cantidad de vivianita aplicada (Patente española ES2245253).

La vivianita se puede sintetizar fácil y rápidamente en campo y en laboratorio a partir de productos baratos y de uso frecuente por los agricultores (sales de Fe como $FeSO_4 \cdot 7H_2O$ y de P como $NH_4H_2PO_4$ ó $(NH_4)_2HPO_4$). Para su aplicación en campo es necesario una cuba en constante agitación para evitar que las partículas de vivianita se depositen en el fondo y un inyector manual con el que el operario realice las inyecciones en el suelo hasta aplicar la cantidad de vivianita acordada, resultando una operación lenta e incómoda. Para solucionar el problema de aplicación en campo se ha diseñado un prototipo de máquina para la inyección de líquidos al suelo (Fernández, 2002).

La presencia de fósforo en estos productos eleva su valor como fertilizante frente a otros cuyo único nutriente es el Fe. Es posible, además, enriquecer estos fertilizantes con otros nutrientes, como Mg, Mn o Zn. Esto puede ser práctico, debido a que muchas veces la deficiencia de Fe se presenta junto a la de estos microelementos (Eynard et al., 1992).

La solubilidad de los fosfatos de Fe al pH de los suelos que normalmente ocasionan clorosis férrica es baja, por lo que pueden persistir en el suelo (fertilizantes de liberación lenta). Por tanto, la vivianita no puede considerarse un fertilizante de acción rápida ya que sus efectos son más lentos que los del quelato de Fe pero, sin embargo, su efecto es mucho más persistente.

OBJETIVOS

A pesar de que los estudios sobre la clorosis férrica comenzaron con Molz en 1907, más de 100 años después todavía no se entiende completamente este problema, y los medios disponibles para evitarlo no son del todo satisfactorios.

En este contexto se pretende profundizar en el conocimiento de las condiciones del suelo que influyen sobre la biodisponibilidad del Fe, como medida de prevención de la clorosis férrica, planteando el primer objetivo general de la tesis:

1. Estudiar los efectos de las inundaciones temporales de los suelos calcáreos sobre la biodisponibilidad de Fe en relación a la actividad microbiana:

El Capítulo 2 describe la incubación *in vitro* de suelos bajo condiciones de anoxia para examinar la reducción del Fe(III) mediante la actividad microbiana, identificando las propiedades del suelo implicadas. Los efectos de los ácidos orgánicos como estimulantes en la reducción de Fe(III) y los cambios en las formas Fe del suelo son discutidos en este capítulo.

En el Capítulo 3 se determina si la inundación del suelo antes de cultivar incrementa la fitodisponibilidad de Fe sin efectos nocivos para las plantas. Incluye experimentos con distintos cultivos en maceta con el objetivo de validar un método indicador fácil, rápido y efectivo para predecir el potencial de los suelos calcáreos en la prevención de la clorosis férrica tras un periodo de inundación. También se trata de determinar cómo evolucionan las formas de Fe reducidas en la inundación cuando son reoxidadas.

Por otra parte, la necesidad de desarrollar nuevos fertilizantes de Fe que sirvan para corregir la clorosis férrica en suelos calcáreos, como alternativa a los existentes, mediante productos eficaces, persistentes, sostenibles y económicos, ha llevado a plantear el segundo objetivo general:

2. Estudiar el uso de otra sal de Fe(II), como la siderita ($FeCO_3$) sintética, bajo la hipótesis de que su oxidación en medio calcáreo pueda favorecer la formación de compuestos que sean fuente de Fe para las plantas en suelos calcáreos:

El Capítulo 4 incluye la caracterización de la siderita sintética, tanto pura como dopada con fosfato. Se evalúa la efectividad y persistencia de estas sideritas como fertilizantes de Fe utilizando especies herbáceas sensibles a la clorosis férrica como indicadores, cultivadas en macetas en condiciones controladas.

En el Capítulo 5 se presentan los resultados de varios ensayos en campos de olivar. En estos ensayos, que se prolongaron por un período de tres años, se compararon las sideritas sintéticas con otros fertilizantes de Fe.

CHAPTER 2

Iron(III) reduction in anaerobically incubated suspensions of highly calcareous agricultural soils

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RESUMEN

La clorosis férrica en plantas cultivadas en suelos calcáreos está frecuentemente influida por las formas de Fe del suelo y su contenido. Estudios previos sugieren que la inundación temporal del suelo puede aumentar la biodisponibilidad del Fe. Para estudiar el efecto de la inundación en la actividad microbiana, se incubaron suspensiones de suelo bajo condiciones de anoxia en el laboratorio, examinándose los cambios en la mineralogía del Fe mediante extracciones químicas y espectroscopía de reflectancia difusa. Se eligieron al efecto veinticuatro suelos calcáreos de propiedades muy variables del sur de España. Las suspensiones de suelos esterilizados y nativos se compararon con las de los suelos nativos con diferentes enmiendas. Tras seis semanas de incubación la mayoría de las suspensiones de los suelos nativos liberaron una cantidad de Fe(II) a la solución sustancialmente mayor que la de los controles estériles; además, la concentración de Fe(II) estuvo correlacionada significativamente con el contenido en carbono orgánico disuelto del suelo. De hecho, la adición de ácidos orgánicos típicos de los exudados de las raíces incrementó pronunciadamente la producción de Fe(II), observándose un efecto similar en suspensiones de suelos inoculadas con *Geobacter sulfurreducens*, una conocida bacteria reductora de Fe(III). La reducción microbiana de Fe(III) afectó a óxidos de Fe cristalinos y poco cristalinos. El valor crítico de Fe extraíble necesario para la nutrición férrica de las plantas tolerantes se superó en 18 suspensiones de suelos nativos y en 22 de suelos nativos enmendados con ácidos orgánicos. La inundación temporal parece, pues, estimular la reducción microbiana de Fe(III) (especialmente en presencia de carbono orgánico fácilmente disponible), con el consiguiente aumento de la biodisponibilidad del Fe en suelos calcáreos.

ABSTRACT

The frequent presence of Fe chlorosis in plants grown on calcareous soils is influenced by the forms of soil Fe present and their contents. Previous studies suggest that temporary soil flooding may increase Fe phytoavailability. To study flooding effects in relation to microbial activity in greater depth, we incubated soil slurries in the laboratory under anoxic conditions and monitored changes in Fe mineralogy using wet chemical extractions and diffuse reflectance spectroscopy. Twenty-four calcareous soils from southern Spain ranging widely in their properties were chosen for this purpose. Slurries of sterilized and native soils were compared with those of native soils with different amendments. In contrast to the sterilized controls, most of the slurries containing native soils released substantially increased amounts of Fe(II) to the solution after six weeks of incubation; also, the extent of Fe(II) production correlated well with native contents in dissolved organic carbon. Indeed, the addition of organic acids typically found in root exudates resulted in a pronounced increase in Fe(II) production, and a similar effect was observed in soil slurries additionally inoculated with *Geobacter sulfurreducens*, a well-known Fe(III)-reducing bacterium. Microbial Fe(III) reduction mobilized poorly crystalline and crystalline Fe oxides. The critical extractable Fe value required for Fe nutrition of tolerant plants was reached in 18 of the slurries of native soils and in 22 of the native soils amended with organic acids. Temporary flooding seems to stimulate microbial Fe(III) reduction (especially in the presence of readily available organic carbon), thereby effectively increasing Fe phytoavailability in calcareous soils.

INTRODUCTION

The occurrence of iron (Fe) chlorosis in plants grown on calcareous soils is strongly influenced by the content and reactivity of carbonate, and, especially, the content and properties of Fe oxides in the soil (Loeppert and Hallmark, 1985). Because the solubility of Fe oxides (a term used here to designate Fe oxides, hydroxides and oxyhydroxides) in aerobic soils is low (Lindsay, 1979), only poorly crystalline oxides (basically ferrihydrite) contribute significantly to Fe nutrition in plants. This assumption is supported by the negative correlation between the degree of chlorosis and the content in acid ammonium oxalate- or citrate/ascorbate-extractable Fe of soil, which provides an estimate of the concentration of Fe present as poorly crystalline oxides (Benítez et al., 2002).

Iron oxides in waterlogged soils undergo reductive dissolution and release Fe(II) into the soil solution (Ponnamperuma, 1972; Schaffer et al., 2006). In theory, this could alleviate Fe chlorosis; however, the opposite is usually the case owing to the Fe chlorosis-inducing effect of bicarbonate accumulating in the solution of saturated calcareous soils (Chaney, 1984) or to root asphyxia. However, flooding the soil temporarily before cultivation occasionally alleviates Fe chlorosis, as reported by Longoria (1973) for sugarcane and sorghum grown on calcareous Vertisols of Mexico. Velázquez et al. (2004) observed in this respect that Fe chlorosis was alleviated in lupine and strawberry pot-grown on calcareous Inceptisols of Spain that had never undergone flooding in the field but were flooded in the pots for 30 days before cropping. Significant increase in the oxalate-extractable Fe content of the soils in these experiments indicate that extractable iron relates to chlorosis in temporarily flooded soils.

According to Larson et al. (1991), annual, cyclic flooding may have the potential for alleviating crop deficiencies: Concentrations of extractable Mn and Fe were increased significantly during 7 weeks of flooding in two different calcareous soils. It is therefore plausible to assume that the redox changes brought about by temporary flooding affect the nature of the more labile soil Fe species and their contents and hence that the soil has lost Fe chlorosis-inducing capacity.

The observed reductive dissolution of iron oxides in waterlogged soils is most likely the consequence of microbial activities. Phylogenetically diverse bacterial and archaeal species have the ability to reduce Fe(III) during fermentation or respiration (reviewed e.g. by Lovley, 1991; Lovley et al., 2004). For bacteria such as Clostridium pasteurianum, Lactobacillus lactis, or Lactococcus lactis that grow by fermentation of organic substrates (e.g. glucose, citrate, malate), Fe(III) reduction is only a minor pathway for electron

disposal and it is unclear whether this process yields energy for growth (e.g. Lovley, 1991; Straub and Schink, 2004a). In contrast, dissimilatory Fe(III)-reducing prokaryotes grow with Fe(III) as terminal electron acceptor, i.e. they gain respiratory energy by coupling the oxidation of electron donors (e.g. hydrogen, acetate, ethanol, malate) to the reduction of Fe(III). The majority of known Fe(III)-reducing prokaryotes were isolated from freshwater and marine sediments, wetlands, aquifers, and the deep subsurface (reviewed by Lovley et al., 2004); amongst them, *Geobacter sulfurreducens* is one of the most intensively studied representatives (Caccavo et al., 1994).

Fe(III)-reducing prokaryotes that thrive at pH values above 3 have to cope with a poorly soluble electron acceptor and apparently developed different strategies for the transfer of electrons: (A) Physical contact between cell and mineral surfaces allows for direct delivery of electrons; (B) Iron chelators increase the solubility of ferric iron; (C) Electron-shuttling compounds accomplish the transfer of electrons from the cell to the mineral (reviewed e.g. by Lovley et al., 2004; Kappler and Straub, 2005). Naturally occurring electron-shuttling compounds which were shown to sustain prokaryotic growth include humic substances (e.g. Lovley et al., 1996) and sulphur compounds (e.g. Straub and Schink, 2004b). Note that prokaryotes which reduce Fe(III) only indirectly via naturally occurring electron-shuttling compounds are no Fe(III)-reducing prokaryotes in a strict sense as their actual electron acceptor is the shuttling molecule and not Fe(III).

Available information on microorganisms with Fe(III)-reducing capabilities in calcareous soils is scant. Valencia-Cantero et al. (2007) isolated bacterial strains with Fe(III)-reducing capabilities from the rhizospheres of bean and maize: Strains of *Arthrobacter* sp., *Bacillus megaterium*, and *Stenotrophomonas maltophilia* stimulated plant growth in calcareous soils presumably through their Fe(III)-reducing activity in the plant rhizosphere.

For the present study, soil slurries were incubated under anoxic conditions to examine the reduction of Fe(III) by indigenous microbial populations in a group of highly calcareous agricultural soils of southern Spain. The specific purposes were as follows: (i) to identify the soil properties affecting the extent of microbial Fe(III) reduction and the Fe forms involved; (ii) to explore whether microbial activities are limited by an inadequate supply of macro and/or micro nutrients; (iii) to assess the stimulating effect of rhizospheric organic acids that may serve as substrates for microbial fermentation and/or respiration; (iv) to study Fe mobilization of soil iron minerals by known Fe(III)-reducing bacteria; and (v) to examine the changes in soil Fe caused by anoxic incubation in relation to the tests commonly used to estimate the risk of Fe chlorosis.

MATERIALS AND METHODS

SOILS AND BASIC SOIL ANALYSIS

Samples were collected from the surface horizon (0-30 cm) of twenty-four calcareous soils (21 Inceptisols and 4 Alfisols) in vineyards and olive orchards from southern Spain from January to March 2008. The studied soils were developed on Tertiary limestones, marls and calcareous sandstones. The samples were air-dried and ground to pass through a 2-mm sieve and analyzed, in duplicate, in the laboratory for the content in clay-sized particles (pipette method following dispersion with Na hexametaphosphate), pH (potentiometric measurement in a 1:2.5 mass soil : volume water suspension), cation exchange properties (extraction with 1 *M* NH_4OAc buffered at pH 7), total $CaCO_3$ equivalent (CCE) [determined from the weight loss upon treatment with 6 *M* HCl (van Wesemael, 1955)], electrical conductivity (EC) (in a 1:5 soil:water suspension with a conductivity meter), organic carbon (OC) (rapid dichromate oxidation), and available P [extraction with 0.5 *M* $NaHCO_3$ buffered at pH 8.5 (Olsen et al., 1954)]. Dissolved organic carbon (DOC) was determined in the filtrate (0.22 μm) of a 1:10 soil:water suspension according to APHA (2005).

SOIL IRON FORMS

Citrate/bicarbonate/dithionite-extractable Fe (Fe_d) was determined according to Mehra and Jackson (1960) except that extraction was carried out at 298 K for 16 h. NH_4 oxalate-extractable Fe (Fe_{ox}) at pH 3 was determined according to Schwertmann (1964) except that the soil:solution ratio was 1:200 in order to prevent significant pH change due to dissolution of soil carbonates during extraction (amendment proposed by Benítez et al., 2002). Citrate/ascorbate-extractable Fe (Fe_{ca}) was determined according to Reyes and Torrent (1997) and diethylenetriaminepentacetic acid-extractable Fe (Fe_{DTPA}) according to Lindsay and Norvell (1978). All extractions were performed at room temperature (298 K), using 50 mL polyethylene centrifuge flasks that were shaken at 3 Hz in an end-over-end shaker. After extraction, the suspensions were centrifuged at 104 m s−2 for 15 min and supernatants analyzed for total Fe with the *o*-phenanthroline colorimetric method (Olson and Ellis, 1982). Atomic absorption spectrophotometry was used for the DTPA extracts because this reagent prevents color development when the the *o*-phenanthroline method is used.

COLONY FORMING UNITS

Colony forming units (CFUs) of aerobic heterotrophic bacteria were determined with the plate count method. A suspension of 3 g of soil in 27 mL of 9 g L^{-1} NaCl in an Erlenmeyer flask was incubated at 293 K on a rotary shaker at 3.33 Hz for 60 min and then diluted 10-fold with 9 g L^{-1} NaCl. Aliquots of the resulting dilutions were spread over agar plates (in triplicate for each dilution) and incubated at 295 K for 3 days. For statistical reasons, only those agar plates containing 30—300 colonies were considered.

ANAEROBIC CULTIVATION OF MICROORGANISMS

The methods used to prepare media and cultivating the microorganisms under anoxic conditions are described in detail elsewhere (Widdel and Bak, 1992) (Chapter 8, Fig. A1.A). *Geobacter sulfurreducens* and *Lactococcus lactis* were routinely cultivated in anoxic bicarbonate-buffered freshwater medium as described elsewhere (Straub and Schink, 2004a). Both strains were cultivated in an anoxic 0.005 *M* $CaCl_2$ solution that was amended with macro and micro nutrients: 0.4 m*M* K_2HPO_4 + KH_2PO_4 adjusted to pH 7, 0.1 m*M* $MgSO_4$, 0.5 m*M* NH_4Cl, non-chelated trace elements (7.5 µ*M* $FeSO_4$, 0.5 µ*M* H_3BO_3, 0.5 µ*M* $MnCl_2$, 0.8 µ*M* $CoCl_2$, 0.1 µ*M* $NiCl_2$, 0.01 µ*M* $CuCl_2$, 0.5 µ*M* $ZnSO_4$, 0.15 µ*M* Na_2MoO_4), 0.02 µ*M* selenite and 0.02 µ*M* tungstate (Widdel and Bak, 1992). *G. sulfurreducens* was grown with acetate (5 m*M*) as electron donor and carbon source, fumarate (20 m*M*) or soil iron minerals as electron acceptors. In experiments excluding viable soil microorganisms, cysteine (2 m*M*) was used as reductant. *L. lactis* was grown on 10 m*M* citrate plus 1 g L^{-1} yeast extract. *G. sulfurreducens* (DSM 12127) was obtained from D.R. Lovley (Amherst, USA) and *L. lactis* ssp. *lactis* (DSM 20481) from D. Schmitt-Wagner (Konstanz, Germany).

SOIL SLURRY EXPERIMENTS

For the soil slurry experiments, an amount of 5 g of soil was weighed into sterile glass tubes, suspended in 10 mL of anoxic 0.005 *M* $CaCl_2$ solution and sealed with butyl rubber stoppers, the headspace of the tubes being immediately replaced with a N_2 atmosphere (Chapter 8, Fig. A1.B). Tubes were incubated horizontally at 298 ± 0.5 K in the dark and gently shaken by hand every other day.

Sterilized controls, which were used to test the hypothesis that reduction of Fe(III) is mainly due to microbial activity and not to abiotic processes, were obtained by autoclaving completed tubes at 394 K for 20 min twice. Nutrients (0.4 m*M* potassium phosphate, 0.1 m*M* magnesium sulphate, 0.5 m*M* ammonium chloride, non-chelated trace elements, selenite and tungstate [Widdel and Bak, 1992]), organic acids (5 m*M* sodium acetate, 5 m*M* sodium citrate and 5 m*M* sodium malate), or a combination of nutrients plus organic acids, were added to subsets of soil slurries. Further subsets of slurries were inoculated with 2% (vol/vol) *G. sulfurreducens* or 2% (vol/vol) *L. lactis* and amended with their respective growth substrates (see “Anaerobic cultivation of microorganisms”). All experiments were conducted up in triplicate.

Samples from the supernatants were taken after 1 day and then on a weekly basis until the end of the sixth week of the experiments. Tubes were incubated vertically for 24 h prior to withdrawing the supernatants with sterile syringes without filtration. Previous control tests with 0.2 µm filters showed that this procedure ensured adequate sedimentation of soil particles. To prevent oxidation of Fe(II), samples were immediately transferred to a 1 *M* HCl solution. Dissolved Fe(II) was then determined with the ferrozine method (Stookey, 1970).

CHARACTERISATION OF SOILS FROM THE SLURRY EXPERIMENTS

After seven weeks of incubation, tubes were opened to measure the solution pH. A portion of the suspension from each tube, taken under continuous stirring, was immediately frozen in liquid N_2, freeze-dried and analyzed for Fe_{ox} and Fe_{ca}. HCl-extractable Fe(II) [$Fe(II)_{HCl}$] and total Fe (Fe_{HCl}) were determined by transferring 0.1 g of freeze-dried soil to 3 mL of 1 *M* HCl in a glass vial which was then vigorously shaken for 30 s in a vortex mixer and allowed to stand at room temperature for 30 min. After centrifuging, the supernatant was analyzed for Fe(II) and total Fe with the *o*-phenanthroline method (Olson and Ellis, 1982). With this procedure, the final proton concentration of the solution was always 0.5 to 0.8 *M.* Under these conditions, one can expect exchangeable Fe(II) and Fe(II) in hydroxides formed following reduction of Fe in a calcareous medium to be released to the solution; Fe(II) substituting for Ca in calcite can also make a significant, albeit small contribution to HCl-extractable Fe(II) (Jackson, 2005). Total Fe in solution was also expected to include Fe from partial dissolution of ferrihydrite, which is partially soluble in dilute HCl (Schwertmann, 1991).

Soil color parameters for finely ground freeze-dried soil samples were obtained from their visible diffuse reflectance spectra, which were acquired from 400 to 700 nm at 0.5 nm steps on a Cary 5000 UV-Vis-NIR spectrophotometer, using $BaSO_4$ as a white standard (Merck DIN 5033). The powdered samples were firmly pressed by hand into the 8 × 17 mm rectangular holes of white plastic holders. The CIE 1931 color-matching functions weighted by the relative spectral radiant power distribution of CIE Standard Illuminant C were used to calculate the tristimulus values (X, Y, Z) from the reflectance values. Calculations were based on the tabulated data of Wyszecki and Stiles (1982), which were taken at 5 nm intervals in the 380–770 nm range. Then, the chromaticity coordinates were converted into CIE 1976 *L* a* b** coordinates with the aid of the 2008 version of software that can be downloaded (currently, the 2010 version) from htpp://www.wallkillcolor.com. In this color notation system, *L** is lightness, the positive *a** value the degree of "redness", and the positive *b** value the degree of "yellowness".

STATISTICAL ANALYSIS

One-way analysis of variance for data not conforming to a normal distribution was performed by using the nonparametric Kruskal−Wallis method as implemented in Statistix 8 (Analytical Software, Tallahassee, FL). Significance was determined at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

PROPERTIES OF THE NATIVE SOILS

Table 1 shows the values for selected properties of the native soils. All soil samples were rich in calcium carbonate (300−925 g kg^{-1}), which is consistent with their alkaline pH values (8.1−8.6). Clay-sized particles contents ranged widely (125−415 g kg^{-1}), and so did cation exchange capacity (CEC) (7.3−28.4 cmolc kg^{-1}). The content of soluble salts was small [electrical conductivity (EC) values in the 1:5 soil:water extract were <0.33 dS m^{-1}].

Table 1. Selected soil properties

Soil	Latitude	Longitude	Crop	Clay	$CaCO_3$	OC[a]	DOC[b]	pH	CEC[c]	Mg	Na	K	EC[d]	CFUs[e]	Fe_d	Fe_{ox}	Fe_{ca}	Fe_{DTPA}	P_{Olsen}
				—— g kg^{-1}——			mg kg^{-1}		—— $cmol_{(c)}$ kg^{-1}——				dS m^{-1}	g^{-1}	——— g kg^{-1}———			mg kg^{-1}	mg kg^{-1}
1	37º 34´ 26´´ N	4º 38´ 25´´ W	Vineyard	210	490	1.5	330	8.7	8.3	0.6	0.6	0.2	0.15	7.0×105	1.8	0.2	1.4	2.2	4.1
2	37º 34´ 26´´ N	4º 38´ 25´´ W	Vineyard	270	555	4.5	505	8.5	20.0	1.5	0.8	0.5	0.10	3.8×106	3.7	0.5	1.5	3.5	18.6
3	39º 04´ 23´´ N	3º 04´ 35´´ W	Vineyard	190	560	4.8	305	8.6	13.1	0.8	0.9	0.4	0.11	2.5×106	4.4	0.2	0.4	2.0	19.7
4	37º 18´ 17´´ N	6º 33´ 17´´ W	Vineyard	195	335	6.6	525	8.4	10.5	0.8	0.7	0.3	0.19	2.8×106	3.6	0.3	0.6	2.3	10.1
5	37º 21´ 48´´ N	6º 31´ 29´´ W	Vineyard	125	300	3.3	395	8.6	7.3	0.6	0.5	0.1	0.08	2.0×106	2.5	0.2	0.4	1.8	5.7
6	36º 42´ 04´´ N	6º 13´ 48´´ W	Vineyard	300	595	11.0	785	8.2	23.0	1.9	0.9	1.3	0.27	1.2×107	1.6	0.5	1.0	3.7	75.2
7	36º 41´ 42´´ N	6º 13´ 10´´ W	Vineyard	335	630	6.8	450	8.4	22.0	1.4	0.7	0.6	0.13	7.8×106	1.2	0.3	0.8	3.0	37.5
8	36º 42´ 43´´ N	6º 12´ 04´´ W	Vineyard	410	525	9.0	505	8.3	27.2	2.2	0.9	1.0	0.14	1.1×107	1.7	0.5	1.2	3.7	45.9
9	37º 09´ 32´´ N	4º 42´ 53´´ W	Olive trees	200	675	14.0	1280	8.4	14.8	2.4	0.8	2.1	0.33	1.2×107	3.7	0.3	0.8	3.8	32.9
10	37º 14´ 29´´ N	4º 54´ 58´´ W	Olive trees	335	490	13.0	710	8.1	28.4	1.7	0.8	1.2	0.18	1.1×107	2.1	0.5	1.0	2.3	52.5
11	37º 17´ 23´´ N	4º 54´ 50´´ W	Olive trees	320	925	6.7	535	8.4	12.7	0.6	0.7	0.3	0.12	7.3×106	1.1	0.3	1.4	2.6	22.6
12	37º 17´ 59´´ N	4º 51´ 43´´ W	Olive trees	270	830	9.3	530	8.5	17.2	0.8	1.0	0.4	0.11	3.8×106	1.3	0.4	1.0	2.5	31.5
13	37º 36´ 54´´ N	4º 42´ 18´´ W	Olive trees	255	605	5.3	645	8.4	10.4	0.7	0.6	0.1	0.17	3.1×106	2.0	0.2	1.0	2.5	5.3
14	37º 36´ 51´´ N	4º 42´ 19´´ W	Olive trees	190	430	5.7	630	8.4	10.5	0.8	0.8	0.2	0.26	2.6×106	2.4	0.3	0.9	3.0	6.6
15	37º 38´ 49´´ N	4º 42´ 18´´ W	Vineyard	125	465	3.9	440	8.5	9.4	0.6	0.7	0.2	0.08	4.3×106	2.6	0.2	0.7	2.3	22.7
16	37º 38´ 50´´ N	4º 41´ 19´´ W	Vineyard	415	490	3.6	580	8.5	28.0	2.0	0.9	1.1	0.16	4.1×106	2.3	0.4	0.8	2.6	17.2
17	38º 10´ 11´´ N	3º 11´ 27´´ W	Olive trees	245	595	5.5	800	8.5	18.7	1.5	0.8	0.5	0.09	2.2×106	2.2	0.4	1.2	3.2	5.3
18	37º 59´ 41´´ N	3º 13´ 37´´ W	Olive trees	300	380	1.6	600	8.4	19.5	3.0	0.9	0.8	0.08	1.3×106	4.4	0.7	1.2	4.0	2.4
19	37º 56´ 38´´ N	3º 20´ 20´´ W	Olive trees	365	695	1.0	445	8.6	21.0	2.9	0.8	0.9	0.08	2.2×106	0.7	0.3	0.8	2.6	5.4
20	37º 51´ 12´´ N	3º 20´ 03´´ W	Olive trees	170	735	5.9	630	8.6	11.8	4.4	1.2	1.2	0.17	7.6×106	3.5	0.3	0.8	2.0	62.1
21	37º 42´ 01´´ N	4º 36´ 42´´ W	Olive trees	305	440	5.2	480	8.6	27.2	2.3	0.8	3.6	0.10	7.6×106	1.6	0.5	1.0	3.2	36.3
22	37º 38´ 12´´ N	4º 18´ 49´´ W	Olive trees	220	700	5.6	630	8.6	18.3	1.8	0.8	0.4	0.08	5.6×106	1.4	0.3	1.1	2.5	23.8
23	37º 36´ 50´´ N	4º 32´ 56´´ W	Olive trees	205	570	5.3	385	8.6	21.7	1.8	0.9	1.9	0.07	4.4×106	1.6	0.5	1.1	3.1	42.1
24	37º 34´ 53´´ N	4º 35´ 26´´ W	Olive trees	290	655	6.3	690	8.5	26.9	1.5	0.7	0.5	0.08	4.9×106	3.5	0.5	1.3	5.5	30.7

[a] OC= organic carbon; [b] DOC= dissolved organic carbon; [c] CEC= cation exchange capacity ; [d] EC= electrical conductivity in the extract 1:2.5; [e] CFUs=colony forming units per g dry weight of soil

Organic carbon (OC) ranged from 1.0 to 14.1 g kg^{-1}, and dissolved organic carbon (DOC) from 300 to 1280 mg kg^{-1}; both were positively correlated with one another (R^2 = 0.45***).

The soils exhibited large differences in their contents in various extractable Fe forms; thus, Fe_d ranged from 0.7 to 4.4 g kg^{-1}, Fe_{ca} from 0.37 to 1.49 g kg^{-1}, Fe_{ox} from 0.17 to 0.71 g kg^{-1}, and Fe_{DTPA} from 1.8 to 5.5 g kg^{-1}. As discussed in greater detail later on, most of soils exhibited contents in extractable Fe forms below the critical threshold where plants that are moderately tolerant to low Fe availability are likely to develop chlorosis. For instance, in thirteen soils Fe_{ox} was < 0.35 g kg^{-1}, an average critical level for some olive cultivars (Benítez et al., 2002).

The number of viable aerobic heterotrophic cells in the soils was determined as a very general parameter for the viability of the microbial soil populations and ranged from 7 × 10^5 to 1.2 × 10^7 CFUs g^{-1} dry weight of soil.

SOIL SLURRIES INOCULATED WITH *G. sulfurreducens* OR *L. lactis*

A growing number of microorganisms are known to utilize Fe minerals to obtain energy. Dissimilatory Fe(III)-reducing microorganisms such as *G. sulfurreducens* use Fe(III) as a terminal electron acceptor in anaerobic respiration, whereas other bacteria such as *L. lactis* are capable of reducing small amounts of synthetically produced ferrihydrite during fermentative growth on organic substrates (Lovley 1991; Straub and Schink, 2004a). Initial growth experiments without soils showed that both strains, *G. sulfurreducens* and *L. lactis*, grew successfully in a 0.005 *M* $CaCl_2$ solution amended with nutrients and organic growth substrates. In order to study the potential reduction of soil iron minerals by microbial respiration or fermentation, we studied soil slurries prepared from six sterilized soils (1, 3, 4, 6, 11, 21; Table 1) that were inoculated with either *G. sulfurreducens* or *L. lactis*. Both sterilized uninoculated controls and sterilized samples inoculated with *L. lactis* exhibited no Fe(II) production during 8 weeks of incubation. These results exclude the presence of abiotic or nonspecific Fe(III) reduction reactions and suggest that soil iron minerals were unavailable for *L. lactis*. As previously shown for acetate, citrate and malate (Lovley et al., 1991), the presence of an organic acid did not suffice to reduce iron minerals chemically. By contrast, *G. sulfurreducens* produced Fe(II) in a time-dependent manner from soil iron minerals in all soils studied (by way of example, see soils 1 and 11 in Fig. 1). Overall, these results indicate that the experimental conditions used facilitate microbial reduction of soil iron minerals provided viable microorganisms with appropriate physiological capabilities are present.

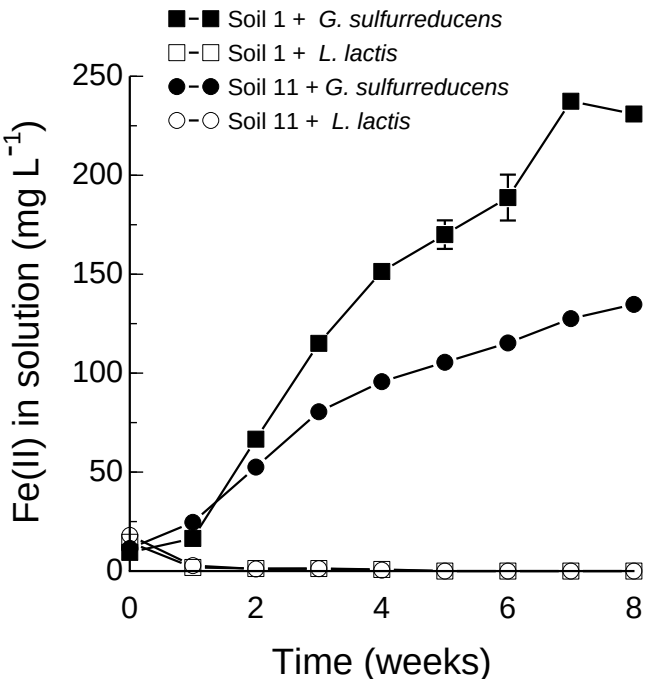

Figure 1. Production of Fe(II) in solution in slurries from sterilized soils (Soils 1 or 11) inoculated with *Geobacter sulfurreducens* or *Lactococcus lactis*.

INFLUENCE OF NUTRIENTS AND ORGANIC ACIDS ON THE MICROBIAL REDUCTION OF SOIL IRON MINERALS

Microbial activity can be limited by a variety of factors including an inadequate supply of (macro and micro) nutrients and/or energy substrates. We therefore performed slurry experiments with six native soils (1, 3, 4, 6, 11, 21; Table 1) that were supplied with: (1) macro and micro nutrients; (2) organic acids; and (3) macro and micro nutrients plus organic acids. Relative to the slurries containing no amendments, the addition of nutrients had no significant effect on Fe(II) production, probably because the samples were collected from the top horizon of agricultural soils that were fertilized on a more or less regular basis. By contrast, the addition of organic acids substantially enhanced microbial Fe(III) reduction as shown in detail below. No accessory effect was observed from the addition of nutrients to these samples also supplied with organic acids (data not shown).

RELEASE OF IRON(II) TO THE SOLUTION IN SOIL SLURRIES

The above described results obtained from slurries of six soils markedly differing in their properties were used to design a factorial experiment with twenty-four different soils (Table 1) and four treatments (Table 2).

Table 2. Four types of soil slurry experiments were carried out in triplicate with soil samples from twenty-four different locations in southern Spain

Treatment	Soil microorganisms	Additions
Sterilized soils	Killed	None
Native soils	Viable	None
Native soils plus organic acids	Viable	Acetate, citrate, malate
Positive control	Viable	Macro and micro nutrients, acetate, citrate, malate, *G. sulfurreducens*

Comparing the results for sterilized soil samples (“Sterilized soils” treatment) with those for native soils (“Native soils” treatment) should allow us to discriminate between abiotic and biotic reduction reactions. The treatment with organic acids (“Native soils plus organic acids” treatment) was included because organic acids such as acetate, citrate and malate are major constituents of root exudates that may promote microbial iron reduction by acting as substrates (for respiration or fermentation) and/or increasing iron availability. In the fourth treatment, slurries were amended with macro and micro nutrients, organic acids, and *G. sulfurreducens*. Because inoculation with *G. sulfurreducens* was found to substantially enhance Fe(II) production and thus provide somehow an estimate of the potentially microbially reducible Fe, this treatment was referred to as the “Positive control” (Table 2). It should be noted that the growing understanding of the biochemistry and genetics of microbial Fe(III) reduction and of the microbial diversity involved has to date provided no readily available molecular tool for monitoring microbial Fe(III) reduction reactions specifically. We did not identify specific microbial processes behind the reduction of soil iron minerals in our soil slurries. The term microbial Fe(III) reduction is therefore used here in a very general sense and may include direct and indirect Fe(III) reduction by respiration or fermentation (reviewed e.g. by Lovley et al., 2004).

As in the previous soil slurry experiments [which were based on only six soils (1, 3, 4, 6, 11, 21)], the experiment with the twenty-four soils showed that the concentration of

Fe(II) in solution did not significantly increase with time in any sterilized soil as exemplified by soils 6, 19 and 24 (Fig. 2 A, B, C) (see also Chapter 8, Fig. A2). This again excludes the possibility of nonspecific abiotic reduction of Fe(III) induced by the experimental conditions.

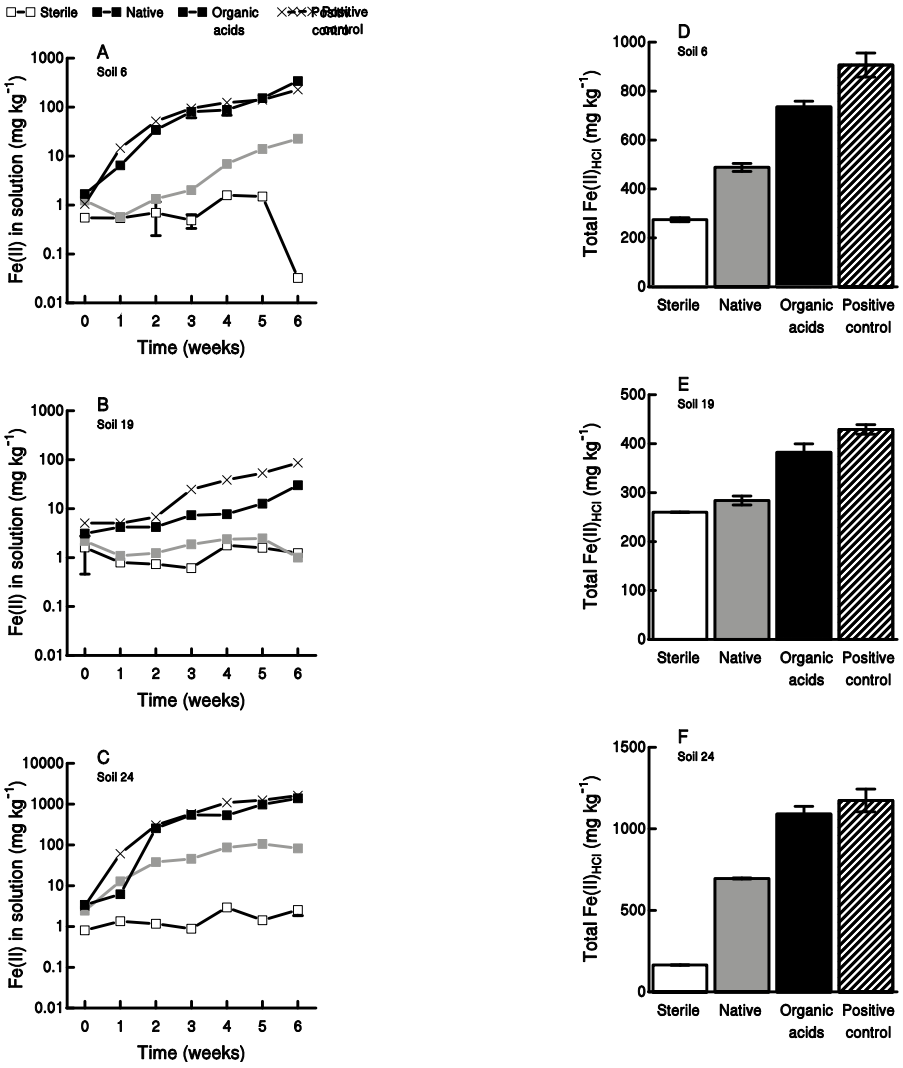

Figure 2. Production of Fe(II) in slurries: comparison of Fe(II) in solution from Soils (A) 6, (B) 19, and (C) 24, using a logarithmic scale for better spread of data, and 1 *M* HCl-extractable Fe [$Fe(II)_{HCl}$] in freeze-dried samples of Soils (D) 6, (E) 19, and (F) 24 taken at the end of soil incubation, showing the mean ± standard error (sterilized soils, white symbols and bars; native soils, gray symbols and bars; native soils plus organic acids, black symbols and bars; positive controls, x and hatched bars).

The concentration of Fe(II) in solution increased in most slurries of native soils by effect of anaerobic microbial activity during 6 weeks of incubation. The time-dependent

production of Fe(II) observed is exemplified by three soils; thus, soil 6 exhibited a moderate increase, soil 19 a small increase and soil 24 a large increase in Fe(II) in solution (Fig. 2 A, B, C) (see data for the 24 soils in Chapter 8, Fig. A2). There were large differences in the amounts of Fe(II) released to the solution at the end of the incubation period. Thus, Fe(II) concentrations ranged from 0.8 to 2.5 mg kg^{-1} soil for sterile soils, 0.05 to 82.5 mg kg^{-1} for native soils, 30 to 1390 mg kg^{-1} for native soils amended with organic acids and 86 to 1625 mg kg^{-1} for positive controls. In contrast to the sterilized samples, slurries containing native soils from 18 different locations released significantly more Fe(II) to the solution; the opposite was true for soil 1 and no significant differences were observed in five incubated soils (4, 5, 7,19, 21). These results emphasise the role of viable microorganisms in the reduction of soil iron minerals (Fig. 2 A, B, C).

The concentrations of Fe(II) in solution at the end of the incubation period correlated with Fe_{DTPA} only (R^2 = 0.224; P < 0.05) among extractable Fe forms, and more significantly with the soil content in OC (R^2 = 0.248; P < 0.05) and DOC (R^2 = 0.374; P < 0.01; Fig. 3 A). The latter correlations suggest that microbial Fe(III) reduction in the slurries was mainly limited by the content in readily available organic carbon. Therefore, one can then hypothesize that the extent of Fe(II) production depends largely on soil properties and on the availability of organic carbon.

Indeed, the addition of organic acids to native soils generally increased the Fe(II) concentration in solution by more than one order of magnitude. This indicates that the microbial Fe(III)-reducing activity was limited by the supply of readily available organic substrates. However, the concentration of Fe(II) in solution for those soils was only correlated to Fe_d (R^2 = 0.186; P < 0.05). This suggests that the production of Fe(II) in solution in the presence of microbially available carbon was mainly controlled by the total concentration of Fe oxides (as estimated by Fe_d) rather than by that of the poorly crystalline Fe forms (as estimated by Fe_{ox} or Fe_{ca}); in fact, the amount of Fe(II) released to the solution during anaerobic incubation was generally of the same order of magnitude or even greater than Fe_{ox} in the respective intact soils.

For 16 soils, the concentrations of Fe(II) in solution were significantly higher in positive controls than in native soils amended with organic acids; the opposite was true for four soils (6, 10, 13, 14), and no significant differences were found in the other four (7, 9, 15, 17). These differences are rather difficult to explain here owing to the complexity of the positive controls (including varying soil matrix properties and complex microbial communities).

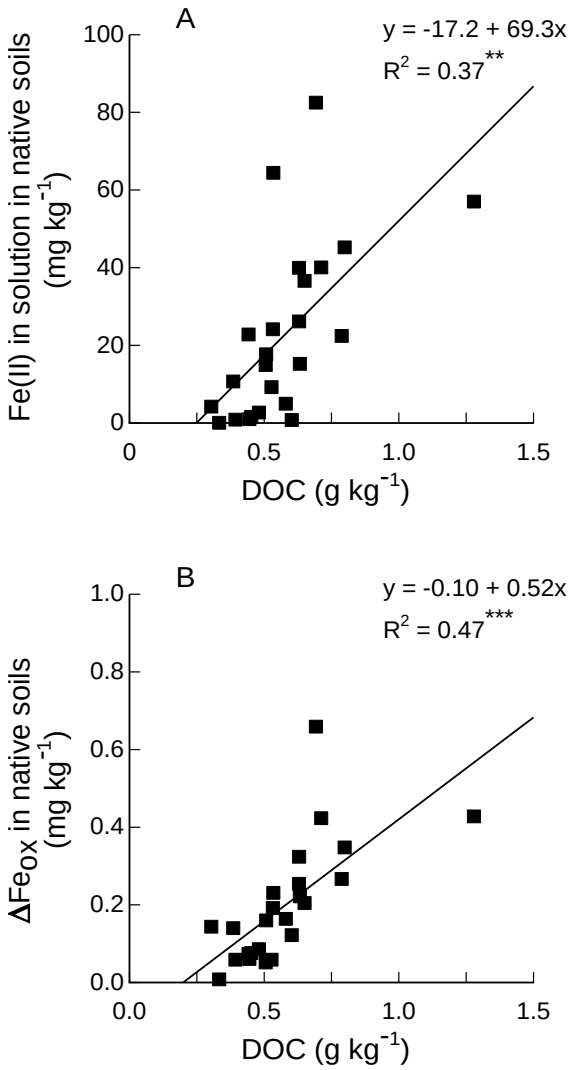

Figure 3. (A) Relationship between concentrations of Fe(II) in solution released from native soils during 7 weeks of anaerobic incubation and concentrations of dissolved organic carbon (DOC) in native soils; and (B) increase in oxalate-extractable Fe (ΔFe_{ox}) following incubation of native soils as a function of their DOC content.

The average pH values of the solutions at week seven were 7.80, 6.90, 6.15 and 6.40 for sterilized controls, native soils, native soils amended with organic acids and positive controls, respectively (i.e. pH decreased with increasing iron reduction). The decrease, which can be ascribed to the production of CO_2 by microbial respiration or fermentation, is a common occurrence in flooded calcareous soils (Larson et al., 1991).

IRON IN FREEZE-DRIED SAMPLES FROM THE SLURRIES

The increase in $Fe(II)_{HCl}$ in the freeze-dried samples observed at the end of slurry experiments generally paralleled the increase in Fe(II) in solution. Incubation of the native soils induced a moderate increase, whereas the addition of organic acids resulted in a pronounced increase in Fe(II) and the inoculation with *G. sulfurreducens* had no additional effect on Fe(II) production of the addition of organic acids, in general (Fig. 2). In all soils, the concentration of Fe(II) in solution was typically one order of magnitude lower than that of $Fe(II)_{HCl}$ in the corresponding freeze-dried samples (Chapter 8, Table A1). The difference can be ascribed to HCl extracting not only soluble, but also exchangeable Fe(II) and Fe(II) from solid phases such as Fe(II, III) hydroxides, Fe(II)-bearing clays and carbonates (calcite, dolomite) in the freeze-dried samples. Because some of these Fe(II) sources occur in concentrations significantly higher than that of Fe(II) in solution, or are not significantly affected by reduction, the differences in $Fe(II)_{HCl}$ between soil slurries were less marked than those in Fe(II) in solution.

Oxalate-extractable Fe was not significantly affected by double sterilization followed by incubation under sterile conditions and freeze-drying, as supported by the approximately 1:1 relationship between Fe_{ox} in intact soils and Fe_{ox} in sterilized soils:

[$Fe_{ox,\ original}$ (in mg kg^{-1}) = 0.96 $Fe_{ox,\ sterile}$ – 16.4; R^2 = 0.91; P < 0.001].

Therefore, it can be safely assumed that changes in Fe_{ox} are basically caused by reduction-related chemical alterations occurring during soil incubation. Incubation under non-sterile conditions significantly affected Fe_{ox}, which ranged from 0.17 to 1.12 g kg^{-1} for native soils, 0.25 to 1.29 g kg^{-1} for native soils amended with organic acids and 0.32 to 1.24 g kg^{-1} for positive controls against 0.11 to 0.72 g kg^{-1} for sterilized soils. Generally, the increase in Fe_{ox} caused by incubation was greater for native soils amended with organic acids and positive controls than for the corresponding native soils subjected to incubation (Fig. 4).

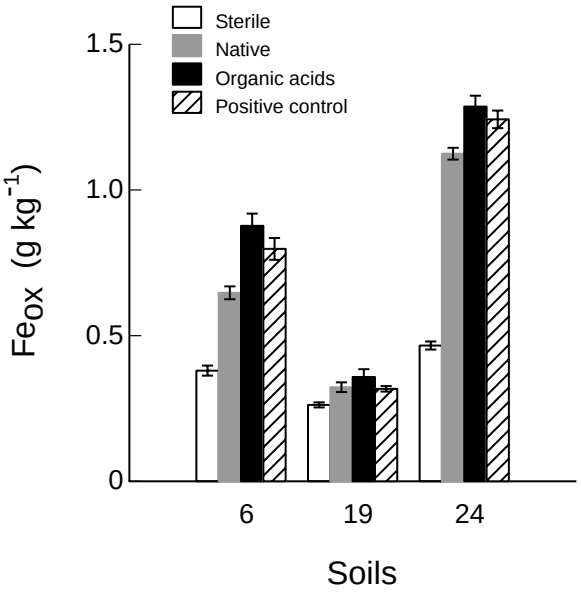

Figure 4. Oxalate-extractable Fe (Fe_{ox}) (mean ± standard error) at the end of the experiments with slurries from Soils 6, 19 or 24.

The differences between the positive control and the native soils amended with organic acids were significant only in eight soils: in five and three soils, Fe_{ox} was higher and lower, respectively, for the positive control than for the native soil amended with organic acids. This suggests that, in principle, the indigenous microbial community in each soil was capable of mobilizing soil Fe forms other than those that are oxalate-extractable (poorly crystalline Fe oxides and organically complexed Fe mainly). Apparently, more crystalline Fe oxides might have been microbially mobilised to some extent. Microbial activity was not analyzed in depth. It is therefore unclear whether more crystalline Fe oxides were reduced directly via microbial activity or indirectly as a result of the well-known catalytic effect of Fe^{2+} on the dissolution of crystalline Fe oxides by oxalate (Cornell and Schwertmann, 2003; van Oorschot et al., 2002). The increase in Fe_{ox} (ΔFe_{ox}) brought about by incubation was apparently limited by the supply of labile carbon compounds, an idea supported by the good relationship between ΔFe_{ox} for the native soils and DOC (Fig. 3 B).

Concentrations of citrate/ascorbate-extractable Fe provide another measure of poorly crystalline Fe phases in soils (Reyes and Torrent, 1997). In accordance with general observations in calcareous soils, the Fe_{ca} concentrations measured in the soil slurries were greater than the corresponding Fe_{ox} concentrations, which is usually the case

for calcareous soils (Benítez et al., 2002). Fe_{ca} concentrations ranged from 0.27 to 1.16 g kg^{-1} in sterilized soils, 0.22 to 1.78 g kg^{-1} in native soils, 0.34 to 1.80 g kg^{-1} in soils amended with organic acids, and 0.56 to 1.56 g kg^{-1} in positive controls. This supports the aforementioned assumption that microorganisms in the native soil successfully mobilized soil Fe forms more stable than the poorly crystalline Fe oxides. By contrast, no significant relationship between the increase in Fe_{ca} in slurries from the native soils and soil DOC was observed.

Although the number of colony forming units of the 24 soils was not correlated with any other data, we must be aware that this information is only a very general measure of the viability of soil microorganisms.

The Fe chlorosis-inducing capacity of the dried slurries was not directly measured in this study; however, the substantial increase in the concentrations of those forms of extractable Fe that provide a measure of the content in poorly crystalline Fe oxides indicate that this capacity was substantially attenuated by microbial activity during anoxic incubation. In fact, only nine intact soils exhibited an Fe_{ox} value exceeding the critical value above which moderately tolerant plants will exhibit no Fe deficiency symptoms (~0.35 g kg^{-1}; Benítez et al., 2002). This value was in fact exceeded in 18 and 22 soils after incubation without and with organic acids, respectively (Fig. 5). In four soils, the Fe_{ox} after incubation with organic acids was higher than the critical level for Fe chlorosis sensitive plants (1 g kg^{-1}; Yanguas et al., 1997). Similar results were reported by Larson et al. (1991) for two southern Florida soils in which 7 weeks of continuous flooding resulted in a 15- to 30-fold increase in Fe_{ox}. It should be noted that part of Fe_{ox} in the incubated soil suspensions (which were freeze-dried immediately after opening of the tubes and measurement of pH) was present as Fe(II) or Fe(II, III) phases, the phytoavailability of which might differ from that of the initial Fe_{ox} forms in the intact soil. However, reoxidation of these Fe(II)-containing phases in both redoximorphic soils and in experiments with different Fe(II) salts (Roldán et al., 2002) typically results in the formation of ferrihydrite or poorly crystalline lepidocrocite, both of which are oxalate-soluble and readily phytoavailable.

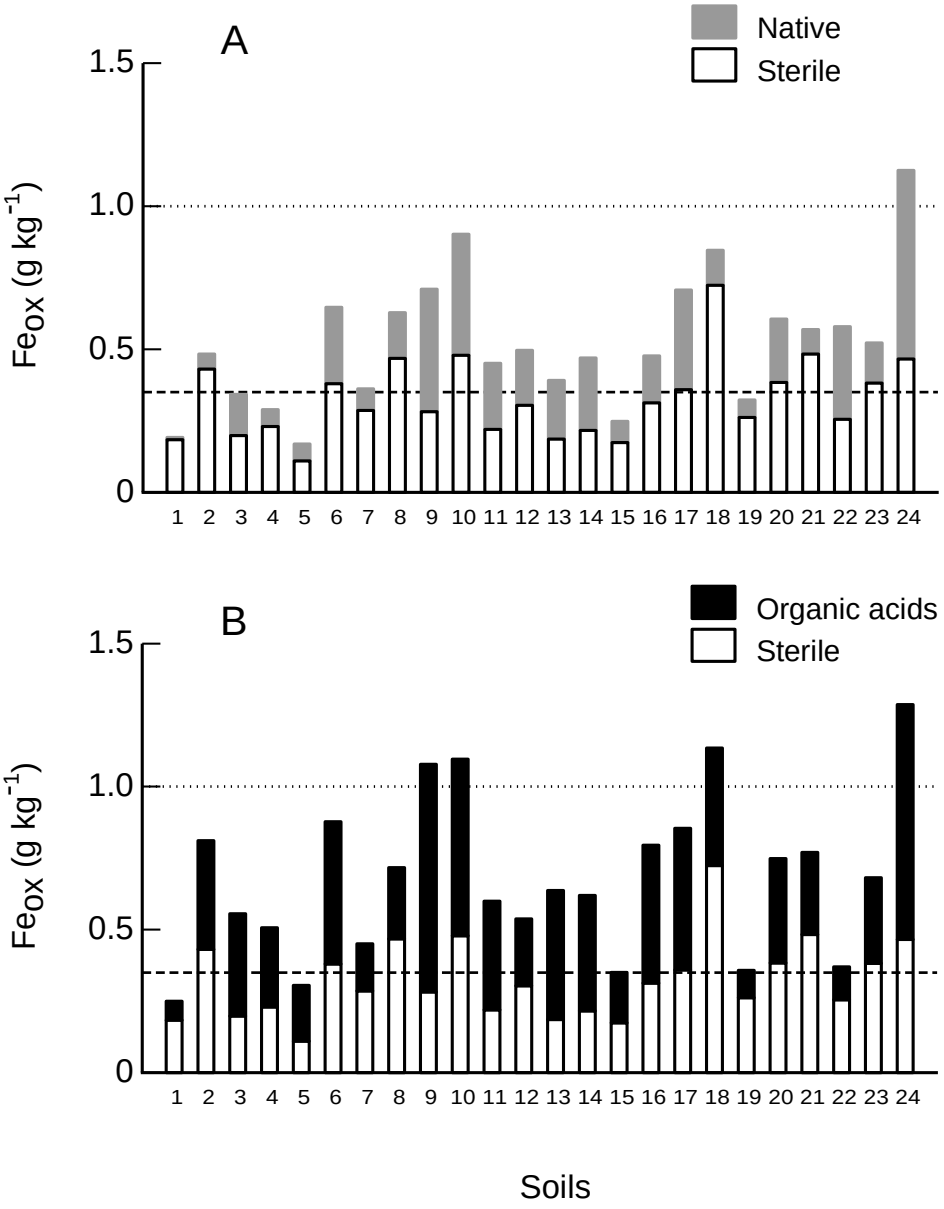

Figure 5. Concentrations of oxalate-extractable Fe (Fe_{ox}) at the end of incubation: sterile soils with (A) the increase in experiments with native soils and (B) the increase in experiments with native soils plus organic acids. The Fe_{ox} levels in sterile soils correspond to Fe_{ox} levels in untreated soils. The critical Fe_{ox} levels for tolerant (dashed line) and sensitive plants (dotted line) are included.

COLOR CHANGES INDUCED BY MICROBIAL REDUCTION

The changes in the CIE 1976 *L* a* b** coordinates (*L**= lightness; positive *a** value = degree of 'redness'; positive *b** value = degree of 'yellowness') of soils from all slurry experiments were recorded. The *L** values increased in the soils incubated under

nonsterile conditions, probably because the soil components undergoing reduction (Fe and Mn oxides mostly) were darker than the common soil silicates and carbonates. On the other hand, *a** and *b** decreased significantly in all soils incubated in the presence of viable microorganisms, especially when supplied with organic acids, as exemplified by Soils 6, 19, and 24 (Fig. 6 A, B, C). The changes in *a** and *b** were significantly negatively correlated with the amount of Fe(II) released during incubation (Chapter 8, Fig. A1.C, Table A2). This is consistent with the assumption that Fe oxides, which impart red and yellow hues to soils, were significantly reduced during incubation.

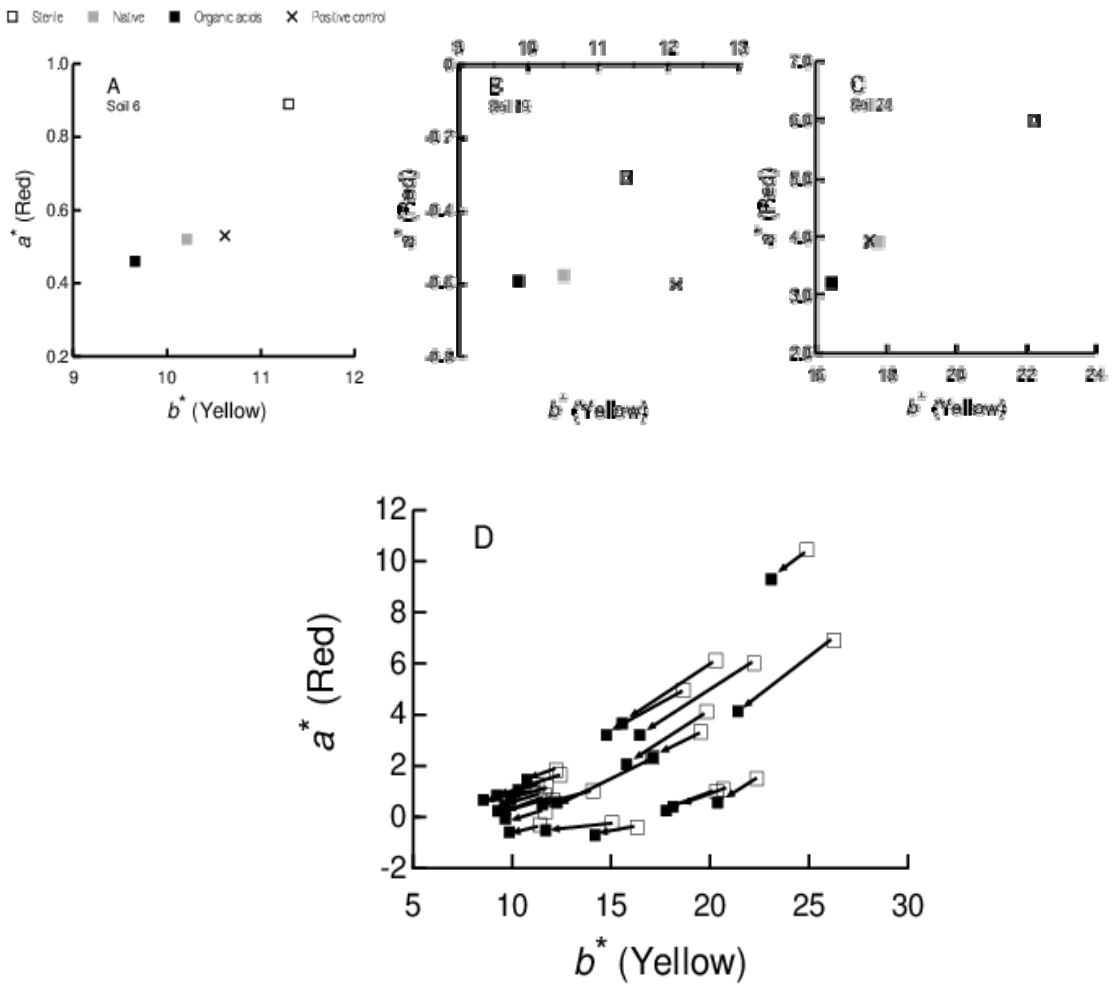

Figure 6. The degree of redness (*a**) and yellowness (*b**) color coordinates for freeze-dried soil suspensions in all four incubation experiments with Soils (A) 6, (B) 19, or (C) 24 and (D) changes in *a** and *b** for all soils from sterile soils to native soils plus organic acids.

Irrespective of the original soil color, the *a**/*b** ratio decreased in all soils incubated in the presence of viable microorganisms. This is illustrated in Fig. 6 D, where the arrow

connecting the data point for the sterilized soil with that for the native soil amended with organic acids points to the negative *a** axis in all cases. This shift in redness/yellowness ratios can be ascribed to preferential reduction of reddish brown-colored varieties of ferrihydrite and limited dissolution of red-colored hematite, yellow-colored goethite remaining relatively unaltered (Schwertmann, 1991; Scheinost and Schwertmann, 1999).

CONCLUSIONS

Experiments with anaerobic soil slurries showed that the indigenous microbial population of each soil was capable to reduce Fe(III). The extent of microbial Fe(III) reduction was apparently limited by the natural DOC concentrations. The supply of organic acids as carbon and energy sources stimulated the direct or indirect microbial reduction of, mainly, poorly crystalline iron oxides, but also of small amounts of crystalline iron oxides such as hematite. Additional inoculation with *Geobacter sulfurreducens* resulted only in a modest additional increase in Fe(II) production in most soil slurries, which suggests that the indigenous microbial communities were effective in reducing Fe(III). Incubation of native soils or native soils amended with organic acids generally resulted in a substantial increase in Fe_{ox} to values exceeding the critical levels for Fe chlorosis-tolerant plants. Therefore, temporary flooding before cropping appears to be a promising practice towards reducing Fe chlorosis in plants grown in highly calcareous soils; also, the effect may be boosted by the addition of organic acids. However, further studies are needed to determine how these solubilised Fe forms evolve when the soil is re-aerated, and to ascertain whether flooding before cropping can increase phytoavailability of Fe without undesirable effects on plants.

ACKNOWLEDGEMENTS

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CHAPTER 3

Pot evaluation of pre-flooding effects on iron extractability and phytoavailability in highly calcareous soils

RESUMEN

Experimentos previos de cultivo en maceta e incubación en laboratorio son congruentes con observaciones de campo que muestran que la inundación temporal del suelo antes del cultivo puede aumentar la disponibilidad del hierro para las plantas. En este trabajo se examinó el efecto de la inundación temporal del suelo sobre los tests de extracción de hierro y su fitodisponibilidad en 24 suelos altamente calcáreos inductores de clorosis férrica. Se llevó a cabo un experimento en maceta donde, después de 30 días de inundación y posterior aireación del suelo, se cultivaron sucesivamente cacahuete y garbanzo. Al final del experimento, las muestras de suelo previamente inundadas mostraron mayores concentraciones de Fe extraíble con oxalato amónico, citrato/ascorbato y DTPA (ácido dietilentriaminopentaacético) (Fe_{ox}, Fe_{ca} y Fe_{DTPA}, respectivamente) que las muestras control (no inundadas). También se observó que los valores de Fe_{ox} y Fe_{ca} no cambiaron significativamente cuando los suelos cultivados volvieron a inundarse ni tampoco cuando suspensiones de suelos incubados anaeróbicamente en viales durante varias semanas se liofilizaron y sometieron a tres ciclos de humedecimiento y secado. La concentración de clorofila en hoja (CCH), tanto en cacahuete como en garbanzo, se incrementó en gran medida como consecuencia de la inundación. El mejor predictor de la CCH fue el Fe_{ox}, seguido del Fe_{ca} y Fe_{DTPA}. Las relaciones entre la CCH y el Fe extraíble sugirieron que las formas de Fe extraídas por oxalato y citrato/ascorbato de suelos previamente inundados eran más biodisponibles que las de los suelos control. Este aumento en la biodisponibilidad de Fe parecía estar limitada a la primera cosecha (cacahuete). La inundación dio lugar a aumentos notables del Fe_{DTPA}. Sin embargo, estos altos valores de Fe_{DTPA} no se tradujeron en altos valores de la CCH, sobre todo para la segunda cosecha, lo que hace de esta prueba un mediocre predictor de la severidad de la clorosis férrica en suelos previamente inundados.

ABSTRACT

Previous pot cropping and laboratory incubation experiments were consistent with field observations showing that temporary flooding before cropping can increase the availability of soil Fe to plants. In this work, we examined the effect of temporary flooding on changes in soil Fe phytoavailability by using 24 highly calcareous, Fe chlorosis-inducing soils to carry out a pot experiment where peanut and chickpea were successively grown after flooding for 30 days and aeration. At the end of the cropping experiment, the pre-flooded soil samples exhibited higher concentrations of oxalate-, citrate/ascorbate- and diethylenetriaminepentacetic acid (DTPA)-extractable Fe (Fe_{ox}, Fe_{ca} and Fe_{DTPA}, respectively) than the control (unflooded) samples. Also, Fe_{ox} and Fe_{ca} exhibited no change by effect of re-flooding of the cropped soils or three wetting-drying cycles in freeze-dried slurries of soils previously incubated anaerobically for several weeks. Leaf chlorophyll concentration (LCC) in both peanut and chickpea was greatly increased by pre-flooding. The best predictor for LCC was Fe_{ox}, followed by Fe_{ca} and Fe_{DTPA}. The LCC−soil Fe relationships found suggest that the Fe species extracted by oxalate and citrate/ascorbate from pre-flooded soils were more phytoavailable than those extracted from control soils. However, the increased phytoavailability of extractable Fe forms was seemingly limited to the first crop (peanut). Flooding dramatically increased Fe_{DTPA}; however, high Fe_{DTPA} levels did not result in high LCC values, particularly in the second crop. Therefore, this test is a poor predictor of the severity of Fe chlorosis in pre-flooded soils.

INTRODUCTION

Soils are often characterized by heterogeneous physicochemical environments, where the availability of oxygen can fluctuate over short temporal scales (hours to days) and small spatial scales (centimeters to meters) at certain times of the year. Periodic depletion of O_2 coupled with inputs of labile C are likely to make these ecosystems suitable for bacterial Fe(III) reduction. Thus, incubation of slurries of 24 calcareous soils under anaerobic conditions showed the indigenous microbial population to be capable to reduce Fe(III) (Sánchez-Alcalá et al., 2011). Anaerobic incubation generally resulted in substantially increased contents in the more labile Fe forms (particularly, poorly crystalline Fe oxides). This was indicated by significantly increased concentrations of acid NH_4 ammonium oxalate-extractable Fe (Fe_{ox}) (Schwertmann, 1964), which provides a useful measure of phytoavailable Fe (Loeppert and Hallmark, 1985; Benítez et al., 2002). Fe_{ox} after incubation exceeded the critical levels for plants susceptible to Fe deficiency chlorosis in most soils.

The results of the above-described experiments were in line with field observations that temporary flooding before cropping increases the phytoavailability of soil Fe and can thus alleviate Fe chlorosis in plants growing in calcareous soils. These effects were observed in sugarcane and sorghum crops grown on Mexican Vertisols (Longoria, 1973), subtropical and tropical fruit trees in Florida (Larson et al., 1991; Schaffer et al., 2006), and lupine and strawberry pot-grown on Spanish Inceptisols which had never undergone flooding in the field (Velázquez et al., 2004).

Changes in the contents and nature of the more labile soil Fe species were appreciable after only a few weeks of flooding (Sánchez-Alcalá et al., 2011). However, little is known about whether these changes persist over time after the soil is re-aerated or eventually re-flooded and how they can affect the future performance of Fe chlorosis sensitive species. In this work, we used a group of 24 Fe chlorosis-inducing calcareous soils to investigate: (i) the effect of pre-flooding followed by aeration on Fe forms and availability to pot-grown Fe chlorosis-sensitive species; (ii) the usefulness of the various Fe extraction tests for predicting the performance of sensitive species; and (iii) the effects of wetting−drying cycles and re-flooding on Fe forms.

MATERIALS AND METHODS

SOILS AND BASIC SOIL ANALYSES

The 24 soil samples used in this study, which were previously characterized by Sánchez-Alcalá et al. (2011), were collected from the surface horizon of 3 Alfisols and 21 Inceptisols in vineyards and olive orchards in southern Spain. The soils developed mainly on limestones, marls and calcareous sandstones, in areas where the mean annual temperature ranges from 15 to 18 °C, the mean annual rainfall from 300 to 700 mm, and the moisture regime is xeric (Soil Survey Staff, 1999).

The samples were air-dried, passed through a 2-mm sieve and analyzed in the laboratory for clay-sized particles (pipette method), pH (potentiometric measurement in a 1:2.5 soil:water suspension), cation-exchange properties (extraction with 1 *M* NH_4OAc buffered at pH 7), calcium carbonate equivalent (CCE) [via weight loss upon treatment with 6 *M* HCl (van Wesemael, 1955)], "active lime" or active calcium carbonate equivalent (designated as ACCE) (Drouineau, 1942), electrical conductivity (EC) of the 1:5 soil:water suspension (conductivity meter), organic carbon (OC) (rapid dichromate oxidation), and Olsen P [extraction with 0.5 *M* $NaHCO_3$ buffered at pH 8.5 (Olsen et al., 1954)]. Dissolved organic carbon (DOC) was determined in the filtrate (0.22 µm) of a 1:10 soil:water suspension according to APHA (2005). Citrate/bicarbonate/dithionite-extractable Fe (Fe_d) was determined according to Mehra and Jackson (1960) except that extraction was carried out at 25 ºC for 16 h.

POT EXPERIMENTS

Flooding

Cylindrical PVC pots 13 cm high and 5.5 cm in diameter with a small hole at the bottom were filled with 220 g of soil and slowly immersed in a tray filled with deionized water so that the final water level was 2 cm above the soil surface. Then, the hole at the bottom was plugged with a rubber stopper to avoid drainage and the pot was covered with parafilm to prevent water losses by evaporation before keeping it in the dark at 25 °C for 30 days. After this flooding period, the supernatant in each pot was removed with a pipette and pots were allowed to drain for 3 days so that the water content was close to field capacity at the time cropping was started. A control treatment using unflooded soils was also performed. Three replicates per soil were prepared.

After the cropping experiment described below, the soil in the pots was re-flooded for 30 days as described above. Finally, the pots were allowed to drain and the soil was air-dried before analysis.

Cropping and plant analysis

Peanut (*Arachis hypogaea* L.) and chickpea (*Cicer arietinum* L., cv. ICC 11224) were grown in a growth chamber with a photoperiod of 14 h, a light intensity of 270 µmol m^{-2} s^{-1}, a temperature of 25 ± 3 °C and a relatively humidity of 45−55%. Seeds were germinated on moistened paper towel for 4−6 days. Two seedlings of peanut and chickpea were then transplanted to the pots and, after one week, only one plant was left. After the first crop (peanut), the pot was emptied and the air-dried soil from the three replicates mixed before filling each pot with 220 g of soil prior to the second cropping (chickpea). For each crop and pot, 30 mL of a pH 6, Fe-free modified Hoagland nutrient solution [2.5m*M* $Ca(NO_3)_2$, 2.5m*M* KNO_3, 1m*M* $MgSO_4$, 1m*M* KH_2PO_4, 0.1m*M* KCl, 50 µ*M* H_3BO_3, 4 µ*M* $MnSO_4$, 4 µ*M* $ZnSO_4 \cdot 7H_2O$, 1 µ*M* $CuSO_4 \cdot 5H_2O$, and 0.1 µ*M* $(NH_4)_6Mo_7O_{24}$] was applied over 31 days for peanut and 24 days for chickpea. The pots were irrigated with deionized water every day to keep soil moisture near field capacity and randomly arranged to minimize the influence of position in the growth chamber.

The relative chlorophyll contents of the youngest fully developed leaves were estimated from the SPAD values acquired with a SPAD 502 Portable Chlorophyll Meter (Minolta Camera Co., Osaka, Japan). SPAD readings were taken at 10, 17, 24 and 30 days after transplanting for peanut, and 10, 17 and 24 days after transplanting for chickpea. After the last SPAD reading, and immediately before harvesting, the youngest fully expanded leaf in each plant was cut, its surface area measured and its chlorophyll extracted with 96% ethanol. The chlorophyll concentration was determined according to Wintermas and de Mots (1965). The rest of the plant was dried at 65 °C for at least 72 h and weighed. A sample of dry matter was digested in nitric/perchloric acid (Zazoski and Burau, 1977) and the resulting solution analyzed for Ca, Mg, Fe, Mn, Cu and Zn by atomic absorption spectrophotometry, K by flame emission, and P with the Molybdenum Blue method of Murphy and Riley (1962).

Analysis of Fe forms and colour measurements

After cropping, acid NH_4 oxalate-extractable Fe (Fe_{ox}) was determined according to Schwertmann (1964) except that the soil:solution ratio was 1:200 in order to prevent a significant pH change through dissolution of soil carbonates during extraction (Benítez et al., 2002). Citrate/ascorbate-extractable Fe (Fe_{ca}) was determined according to Reyes and Torrent (1997) and diethylenetriaminepentacetic acid (DTPA)-extractable Fe (Fe_{DTPA}) following the method of Lindsay and Norvell (1978). HCl-extractable Fe(II) [$Fe(II)_{HCl}$] was determined by transferring 0.1 g of soil to 3 mL of 1 *M* HCl in a glass vial which was then vigorously shaken for 30 s in a vortex mixer and allowed to stand at room temperature for 30 min. After centrifuging the vial, the supernatant was analyzed for Fe(II) with the *o*-phenanthroline method (Olson and Ellis, 1982).

The colour of finely ground soil samples was determined from visible diffuse reflectance spectra (380–770 nm, 0.5-nm steps), using a Cary 5000 UV-Vis-NIR spectrophotometer with halon powder (polytetrafluoroethylene, PTFE) as a white standard (Merck DIN 5033). The CIE 1931 color-matching functions weighted by the relative spectral radiant power distribution of CIE Standard Illuminant C were used to calculate the tristimulus values (X, Y, Z) from reflectance values. Calculations were based on the tabulated data of Wyszecki and Stiles (1982), which were taken at 5 nm intervals over the 380–770 nm range. The chromaticity coordinates were converted into the Munsell notation with the aid of the 2008 version (currently, the 2012 version) of software that can be downloaded from htpp://www.wallkillcolor.com.

WETTING−DRYING EXPERIMENT

This experiment was conducted on samples of soil slurries incubated anaerobically for 7 weeks according to Sánchez-Alcalá et al. (2011). After opening the vials containing the slurry, a 2-g sample was freeze-dried and subjected to three wetting−drying cycles. In each cycle, the sample was placed in a small Petri dish, sprayed with water to saturation, and allowed to dry for 5 days in a room with circulating air at 25 ± 1 ºC. The concentrations of Fe_{ox}, Fe_{ca} and $Fe(II)_{HCl}$ were determined before and after the wetting−drying cycles —Fe_{DTPA} was not determined because of limitations in the sample size.

STATISTICAL ANALYSES

The analysis of variance (ANOVA) was performed with Statistix 9.0 (analytical Software, Tallahassee, Florida, USA). Means were separated via the LSD test. Unless otherwise stated, the word ‘‘significant’’ is used here to indicate significance at the $P < 0.05$ level. One, two and three asterisks mean significance at the $P < 0.05$, 0.01 and 0.001 significance level, respectively. Comparisons of goodness of fit for the different models describing the relationships between SPAD and extractable Fe forms were made by using the Akaike Information Criterion (AIC) (Burnham and Anderson, 2002).

RESULTS AND DISCUSSION

PROPERTIES OF THE NATIVE SOILS

The studied soils varied widely in properties (particularly in those that may significantly affect the risk of Fe chlorosis, Table 1).

Table 1. Selected soil properties[a]

	Maximum	Minimum	Mean	Median
Clay (g kg^{-1})	415	125	260	263
$CaCO_3$ (g kg^{-1})	925	300	570	565
ACCE (g kg^{-1})	341	69	219	227
OC (g kg^{-1})	14.1	1.0	6.1	5.6
DOC (mg kg^{-1})	1280	305	580	533
pH	8.65	8.05	8.45	8.45
CEC ($cmol_{(c)}$ kg^{-1})	28.4	7.3	17.8	18.5
Mg ($cmol_{(c)}$ kg^{-1})	4.4	0.6	1.6	1.5
Na ($cmol_{(c)}$ kg^{-1})	1.23	0.54	0.79	0.77
K ($cmol_{(c)}$ kg^{-1})	3.55	0.14	0.81	0.50
EC (dS m^{-1})	0.33	0.07	0.14	0.12
Olsen P (mg kg^{-1})	75.2	2.4	25.7	22.7
Fe_d (g kg^{-1})	4.40	0.70	2.37	2.15

[a] ACCE, active calcium carbonate equivalent; OC, organic carbon; DOC, dissolved organic carbon; CEC, cation exchange capacity; EC, electrical conductivity of the 1:5 soil:water extract; Fe_d, citrate/bicarbonate/dithionite-extractable Fe

Soils were rich in calcium carbonate equivalent (CCE) (300 − 925 g kg^{-1}) and active calcium carbonate equivalent (ACCE) (69 − 341 g kg^{-1}), consistent with a mean pH of 8.5, and poor in organic carbon (OC) (1.0 – 14.1 g kg^{-1}) and dissolved organic carbon (DOC) (305 – 1280 mg kg^{-1}). OC and DOC were positively correlated (R^2 = 0.45***). The contents in clay-sized particles ranged widely (125 − 415 g kg^{-1}), and so did the

cation exchange capacity (CEC) (7.3 − 28.4 cmolc kg^{-1}). The content in soluble salts was low [electrical conductivity (EC) values in the 1:5 soil:water extract were <0.33 dS m^{-1}]. The total concentration of Fe present in the form of Fe oxides, as estimated by Fe_d, was also low (0.7 − 4.4 g kg^{-1}).

SOIL PROPERTIES AFTER CROPPING

A t-paired test showed that the pre-flooded soil samples exhibited higher concentrations of Fe_{ox}, Fe_{ca}, $Fe(II)_{HCl}$ and Fe_{DTPA} than the control samples at the end of the cropping experiment (Fig. 1) (Chapter 8, Table A3).

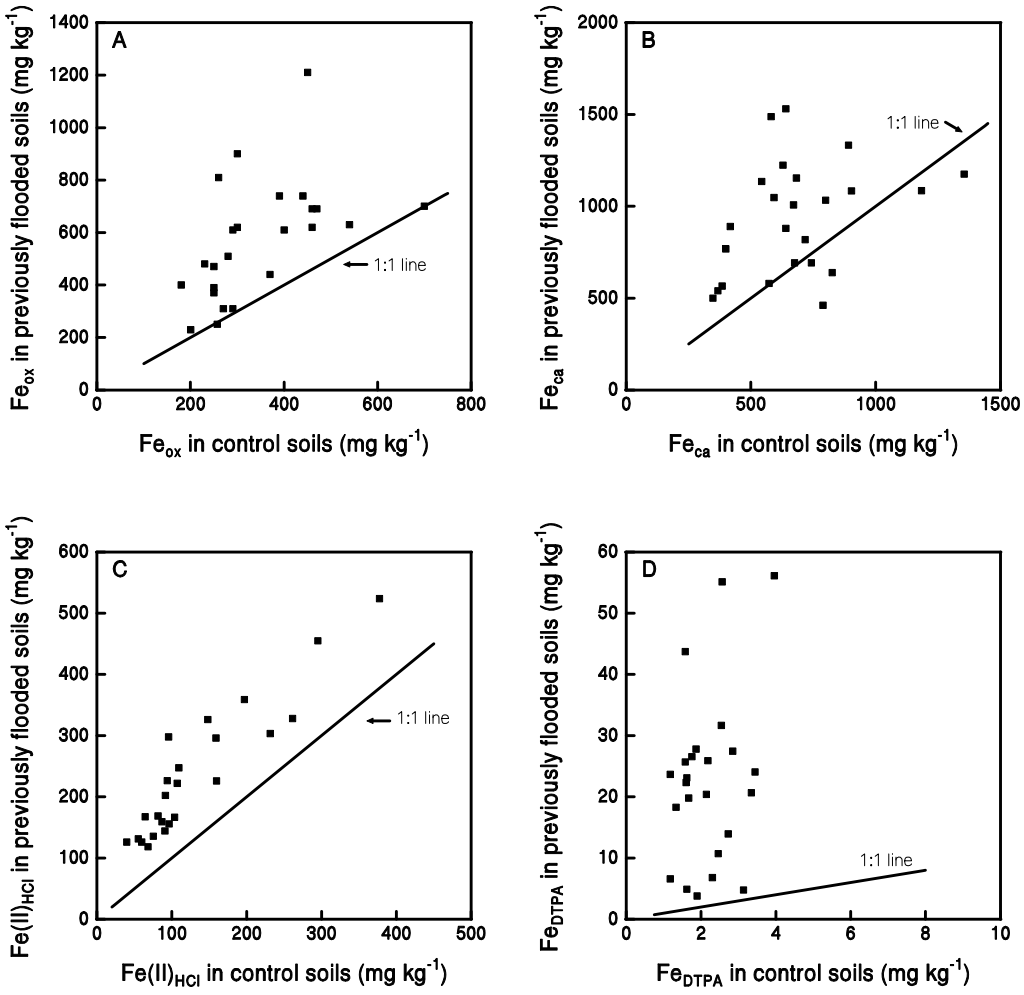

Figure 1. Relationships between the extractable Fe forms in pre-flooded and control soils after the cropping experiment: (a) oxalate-extractable Fe (Fe_{ox}); (b) citrate/ascorbate-extractable Fe (Fe_{ca}); (c) HCl-extractable Fe(II) [$Fe(II)_{HCl}$]; and (d) diethylenetriaminepentacetic acid (DTPA)-extractable Fe (Fe_{DTPA}).

Thus, Fe_{ox} ranged from 230 to 1210 mg kg^{-1} in the pre-flooded samples versus 180 to 700 mg kg^{-1} in the control samples; there was thus an average increase of 70% (Fig 1a). By contrast, Fe_{ca} ranged from 460 to 1530 mg kg^{-1} in the pre-flooded samples and from 350 to 1350 mg kg^{-1} in the control samples, with a modest average increase (about 10%, Fig 1b). Fe_{ox} and Fe_{ca} were significantly correlated (R^2 = 0.34*** for the control samples and R^2 = 0.82*** for the pre-flooded samples) —in fact, both provide a measure of Fe present in the form of poorly crystalline oxides (Reyes and Torrent, 1997). However, oxalate seems to be more sensitive to changes in soil Fe forms caused by flooding. Part of this effect could be due to the presence of some residual Fe(II) in pre-flooded soils since ferrous iron is known to enhance the dissolution of Fe oxides by oxalate (Cornell and Schindler, 1987).

$Fe(II)_{HCl}$ ranged from 120 to 520 mg kg^{-1} in the pre-flooded soils versus 40 to 380 mg kg^{-1} in the control soils (Fig 1c), as expected from the anaerobic conditions of incubation and the active role native microorganisms play in Fe reduction under these conditions (Sánchez-Alcalá et al., 2011). Flooding resulted in a dramatic increase in Fe_{DTPA}: 4 − 56 mg kg^{-1} in the pre-flooded soils versus 1 − 4 mg kg^{-1} in the control soils (Fig 1d). Such a marked increase was also previously observed by Velázquez et al. (2004). Fe_{DTPA} and Fe_{ox} were significantly correlated (R^2 = 0.67*** and 0.64*** for the pre-flooded and control soils, respectively).

Figure 2 shows the effect of pre-flooding on the Munsell notation of the soils after cropping. The increase in value (Fig. 2b) caused by pre-flooding can be ascribed to reduction of Fe and Mn oxides, which are darker than the soil minerals insensitive to reduction (silicates and carbonates, mainly). In fact, differences in value between the pre-flooded and control soils (DC) were significantly correlated with Fe_d (R^2 = 0.44***). This suggests that reductive dissolution may have affected Fe oxides of higher crystallinity than those dissolved by oxalate. The observation in the microcosm anaerobic incubation experiments of Sánchez Alcalá et al. (2011) that the concentration of Fe(II) in solution was positively correlated with Fe_d, but not with Fe_{ox} or Fe_{ca}, provides support for this assumption.

The hue turned yellower and the chroma decreased by effect of pre-flooding (Figs. 2a and 2c, respectively). This is consistent not only with the reductive dissolution of Fe oxides (which have hues in the yellow−red range), but also with the preferential disappearance of species with a more reddish hue (ferrihydrite and/or hematite).

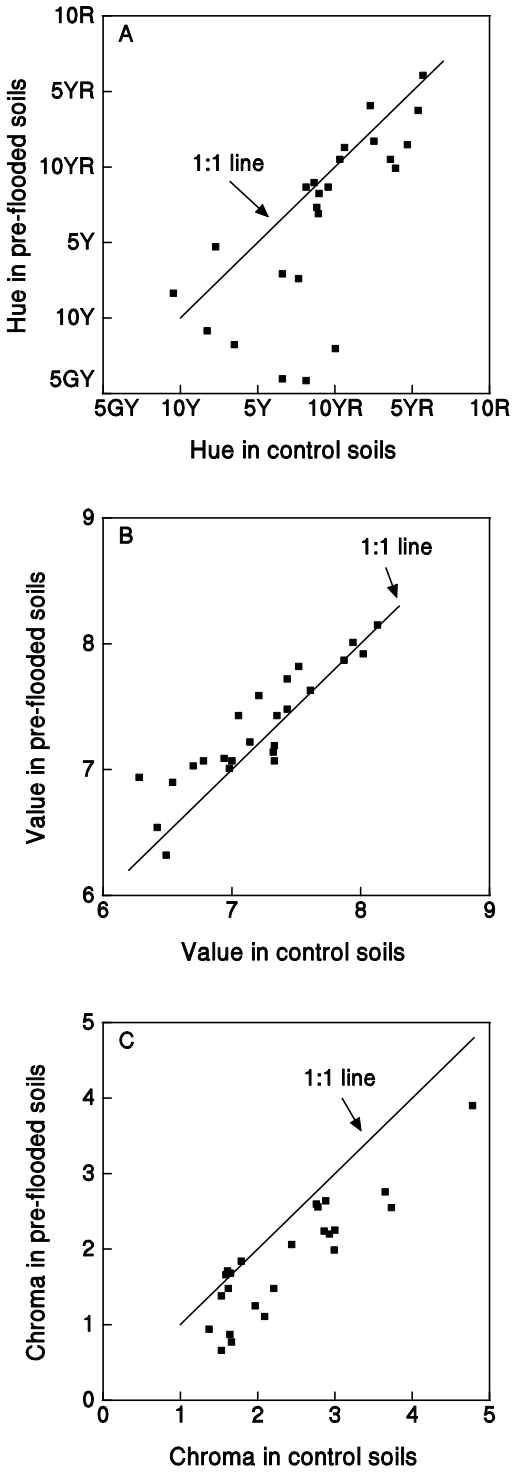

Figure 2. Relationships between the Munsell notation in pre-flooded and control soils after the cropping experiment: (a) hue; (b) value; (c) chroma.

EFFECT OF WETTING−DRYING CYCLES, CROPPING AND RE-FLOODING ON EXTRACTABLE FE FORMS

Subjecting the freeze dried slurries previously incubated anaerobically for 7 weeks by Sánchez-Alcalá et al. (2011) to three wetting−drying cycles had no significant effect on Fe_{ox} or Fe_{ca}, but decreased $Fe(II)_{HCl}$ significantly (22% on average), which suggests oxidation of Fe(II)-containing species forming during the anaerobic treatment (Table 2, Chapter 8, Tables A4 and A5). The Munsell value decreased whereas the chroma increased and the hue turned redder after the wetting−drying cycles (Table 2). In summary, there were changes in the degree of oxidation and other properties of the labile, poorly crystalline Fe moieties, but not in their extractability by oxalate or citrate/ascorbate.

Table 2. Oxalate-extractable Fe (Fe_{ox}), citrate/ascorbate-extractable Fe (Fe_{ca}), HCl-extractable Fe(II) [$Fe(II)_{HCl}$], and Munsell notation of freeze dried slurries of soil anaerobically incubated for 7 weeks before and after three wetting–drying cycles

	Fe_{ox}	Fe_{ca}	$Fe(II)_{HCl}$	Hue	Value	Chroma
Before wetting and drying	459 a	752 a	327 a	0.54Y a	6.97 a	2.21 b
After 3 wetting–drying cycles	468 a	726 a	255 b	0.52Y b	6.81 b	2.30 a
Treatment × soil interaction			ns	ns	ns	ns

It should be noted that Fe_{ox} in the pre-flooded, pot-cropped soil samples was highly correlated, in a virtually 1:1 relationship, with Fe_{ox} in the slurries subjected to wetting and drying [Fe_{ox}(pots) = 0.97 × Fe_{ox}(slurries) + 120; R^2 = 0.73***]. A similar result was obtained for Fe_{ca} [Fe_{ca}(pots) = 1.04 × Fe_{ca}(slurries) + 180; R^2 = 0.78***], but the pot treatment decreased $Fe(II)_{HCl}$ more markedly than the in vitro slurry treatment [$Fe(II)_{HCl}$(pots) = 0.74 × $Fe(II)_{HCl}$(slurries) + 40; R^2 = 0.69***]. In vitro experiments involving anaerobic incubation of slurries followed by wetting−drying cycles are thus useful with a view to predicting changes in Fe forms following temporary flooding, aeration and cropping under field conditions for several weeks. On the other hand, taken together, the present results suggest that, once the soil has been aerated after flooding, further changes in those Fe tests that provide a quantitative measure of the more labile and phytoavailable Fe forms are relatively small, even if the soil undergoes repeated wetting and drying or cropping.

Re-flooding pre-flooded,cropped soils generally resulted in a small increase or decrease in Fe_{ox} (Fig. 3a). Thus, one episode of flooding sufficed in most cases to mobilize most of the pool of Fe species that can eventually be transformed into the labile Fe forms measured by this test. A lack of significant changes in Fe_{ox} was also previously observed in Mexican Vertisols flooded in the field and re-flooded in a pot experiment (Velázquez et al., 2004). In contrast to the oxalate test, re-flooding resulted in substantial increases in Fe_{DTPA} in almost half of the soils (Fig. 3b). The reasons why DTPA seems more sensitive than oxalate to changes in Fe forms induced by redox processes are uncertain. In contrast to oxalate, which extracts poorly crystalline Fe forms and organic Fe complexes quantitatively (Schwertmann, 1964), DTPA, a complexing agent, is more sensitive to changes in specific surface than to the total volume of poorly crystalline Fe phases formed in redox processes.

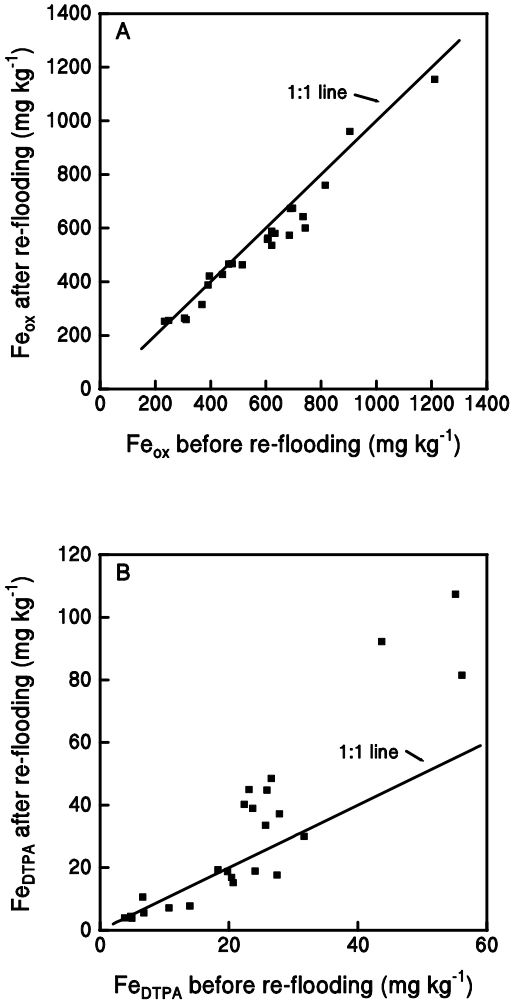

Figure 3. (a) Oxalate-extractable Fe (Fe_{ox}), and (b) DTPA-extractable Fe (Fe_{DTPA}) before and after re-flooding of the cropped soils

PLANT PERFORMANCE IN THE CROPPING EXPERIMENTS

The ethanol-extractable chlorophyll concentration (in μg cm^{-2} leaf surface) was linearly correlated with SPAD for peanut (SPAD = 1.57 × Chlorophyll + 9.4, R^2 = 0.96***) and chickpea (SPAD = 0.51 × Chlorophyll + 9.4, R^2 = 0.84***). For this reason, SPAD readings were deemed to provide an effective non-destructive method for measuring Fe chlorosis development during cropping.

Figure 4 shows the time course of SPAD (mean of 24 soils) for peanut and chickpea in pre-flooded and control soils (see all DPAD data in Chapter 8, Tables A6 and A7). Based on a paired t-test, differences in SPAD between treatments were not significant 10 days after planting, although values for plants grown in pre-flooded soils were higher than those for control plants in 15 (peanut) and 19 soils (chickpea) —few control plants exhibited clear visual symptoms of chlorosis, however. SPAD for plants grown in pre-flooded soils was significantly higher than it was for the control plants 17, 24 and 30 days (peanut) and 17 and 24 days (chickpea) after planting with the exception of 4 soils in the chickpea cropping. Also, the mean difference between the SPAD values increased with time for both crops (Fig. 4). Flooding was thus effective to alleviate Fe chlorosis across crops and soils (Chapter 8, Fig. A3).

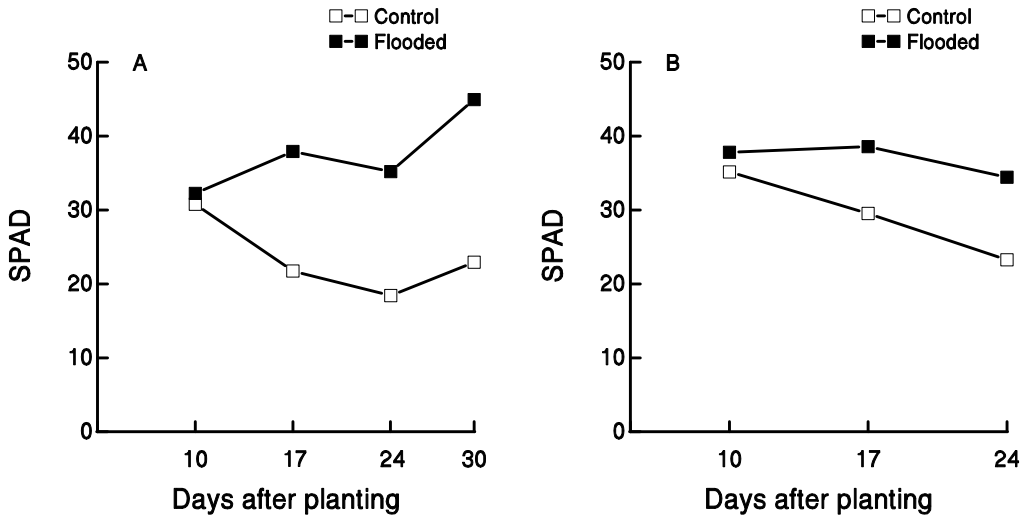

Figure 4. Time course of the SPAD value in plants grown on pre-flooded (filled squares) and control soils (empty squares) in the successive crops of (a) peanut and (b) chickpea

Based on the criteria of Benton Jones et al. (1991) and Robinson et al. (1997), our measured mineral concentrations (Table 3) were above the sufficiency level (Chapter 8, Tables A8 and A9). The concentrations of several macronutrients were higher in the

plants grown in pre-flooded soils than in those in the control soils for both crops, the differences being significant for P, Mg and Ca. In particular, an increase in dissolved P after flooding is often observed (Ponnamperuma, 1972; Patrick and Khalid, 1974; de Mello et al., 1998) that is usually ascribed to reductive dissolution of Fe oxides causing the release of phosphate sorbed on or occluded in them (Willet and Higgins, 1978). Flooding can also enhance the mineralization of organic P and its subsequent uptake by plants (de Mello et al., 1988).

Table 3. Dry matter and mineral element concentrations in the successive peanut and chickpea crops[a]

	Dry matter	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	g	g kg^{-1}				mg kg^{-1}			
Peanut									
Control	0.49 b	3.08 b	13.28 a	22.36 b	2.82 b	67.93 b	14.95 a	72.13 a	37.58 b
Pre-flooded	0.65 a	3.48 a	13.95 a	25.73 a	3.46 a	83.28 a	15.49 a	64.70 b	41.55 a
Interaction[b]	ns	ns	ns	*	ns	*	ns	ns	***
Chickpea									
Control	0.11 b	2.80 b	24.58 a	32.96 b	2.96 b	51.51 a	18.87 a	74.53 a	27.53 a
Pre-flooded	0.12 a	3.16 a	26.01 a	34.42 a	3.32 a	50.13 a	17.26 b	58.53 b	29.88 a
Interaction[b]	ns	ns	*	*	ns	***	*	*	*

[a] Values followed by the same letter in the same column and crop, are not significantly different ($P < 0.05$; LSD test)

[b] Treatment × soil

The iron concentration in peanut was significantly higher for plants grown on pre-flooded soils than on control soils. No differences in this respect were observed in chickpea. The iron concentration in plants was correlated with SPAD only for peanut grown in the pre-flooded soils (R^2 = 0.16*). Dry matter was uncorrelated with the concentration of Fe in plant, as is often the case with Fe chlorosis-affected crops. The concentration of Mn was significantly greater in the control plants than in those grown on pre-flooded soils, probably because the increased release of protons and reducing capacity in dicotiledoneous species induced by Fe deficiency increased Mn solubility and uptake by plants (Venkatraju and Marschner, 1981; Marschner, 1995; Moraghan and Freeman 1978). This effect apparently offset the increase in Mn availability

resulting from the reductive dissolution of Mn oxides to Mn(II) in the flooded soils. Similar results were reported in studies of Fe chlorosis in vineyard (Díaz et al., 2009), olive (Sánchez-Alcalá et al., 2012b), and peanut and chickpea (Sánchez-Alcalá et al., 2012a). Differences in Zn and Cu were significant, but not consistently dependent on soil treatment or crop.

Differences in nutrient concentrations resulted in significant differences in dry matter weight (Table 3). However, it is difficult to ascertain whether the differences were caused by flooding-induced changes in nutrient availability or soil physical properties affecting root development.

RELATIONSHIP BETWEEN FE CHLOROSIS AND EXTRACTABLE FE FORMS

Leaf chlorophyll concentration (LCC) was uncorrelated with alkalinity-related soil properties (CCE, ACCE, pH). By contrast, significant correlations were found between LCC and the concentrations of the different extractable soil Fe forms. The relationships between LCC at harvest and the amounts of Fe extracted in the three commonly used soil Fe tests (Fe_{ox}, Fe_{ca}, Fe_{DTPA}) for the two crops and two soil treatments are shown in Fig. 5. Based on the AIC, non-linear regression models (e.g. the logarithmic model) were inferior or not clearly superior to the linear model, so the latter was adopted for data fitting (Fig. 5). For peanut (Figs. 5a, c, e), the regression lines fitted to the data points for pre-flooded and control soils were significantly different in the three tests; for chickpea and the three tests, the regression lines were not significantly different, so only the joint regression lines are plotted in Figs. 5b, d and f.

The best LCC predictor was Fe_{ox}, followed by Fe_{ca} and Fe_{DTPA}. The better predictive value of Fe_{ox} and Fe_{ca} relative to Fe_{DTPA} is usually ascribed to the ability of both extractants to quantitatively dissolve poorly crystalline Fe oxides, which constitute the most labile soil Fe pool (Loeppert and Hallmark, 1985; del Campillo and Torrent, 1992; Benítez et al., 2002; Reyes et al., 2006; de Santiago et al., 2008; Díaz et al., 2010). Based on the Cate−Nelson graphical method (Nelson and Anderson, 1977), the critical levels, i.e. those above which the probability of a response to the addition of fertilizer Fe to the soil is small (marked with a dashed line), were (i) ~600 mg kg^{-1} for Fe_{ox} (both crops), which is similar in magnitude to those for pot-grown soybean (Morris et al., 1990), chickpea and sunflower (del Campillo and Torrent, 1992); (ii) ~1000 mg kg^{-1} for Fe_{ca} (peanut crop), ~1100 mg kg^{-1} for Fe_{ca} (chickpea crop); and (iii) ~7 mg kg^{-1} for Fe_{DTPA} (peanut crop). It should be noted that no clear-cut critical level could be established for Fe_{DTPA} in the chickpea crop.

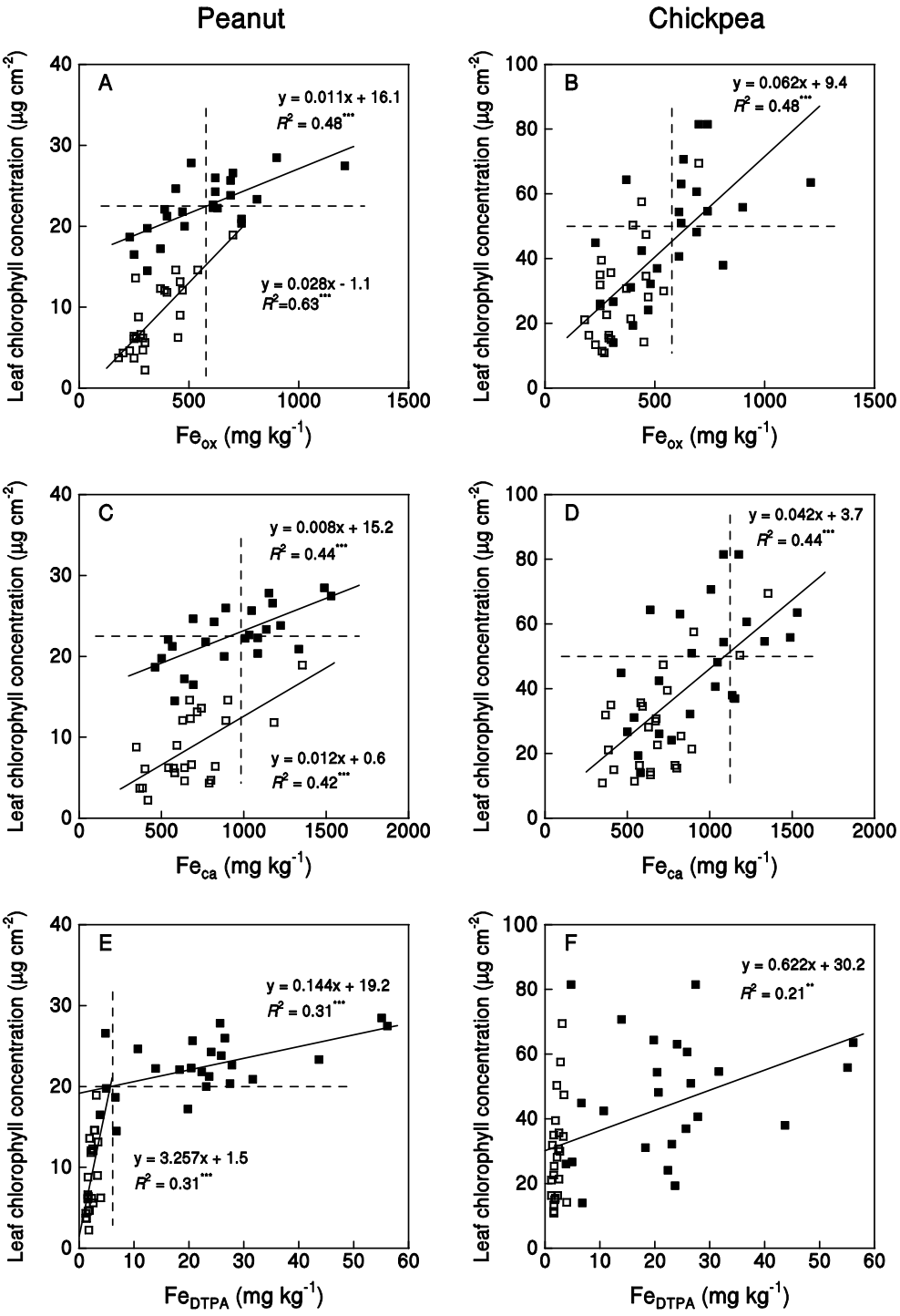

Figure 5. Relationships between leaf chlorophyll concentration in peanut and chickpea and (a, b) oxalate-extractable Fe (Fe_{ox}), (c, d) citrate/ascorbate-extractable Fe (Fe_{ca}), and (e, f) DTPA-extractable Fe (Fe_{DTPA}). Critical levels drawn according to the Cate-Nelson graphical method are indicated with a dashed line. Filled squares represent pre-flooded soils and empty squares control soils

The fact that the regression line relating LCC in peanut to Fe_{ox} in pre-flooded soils lay significantly above that for the control soils (Fig. 5a) is intriguing. It can be hypothesized that, upon flooding and re-oxidation, new oxalate-soluble Fe forms are produced in most soils that are more soluble and thus more phytoavailable than the oxalate-extractable forms present in the native (control) soil; this would result in a LCC-Fe_{ox} response curve lying above that for the control soil. Because a single regression line fitted the data points for both pre-flooded and control soils in the LCC-Fe_{ox} relationship for chickpea (Fig. 5b), it can be speculated that the more soluble Fe forms resulting from flooding evolve to forms similar in solubility and phytoavailability to those in the control soils. In summary, the Fe_{ox} test tends to overestimate the performance of plants growing in pre-flooded soils relative to those growing in native soils; however, this overestimation is seemingly temporary. In any case, a conservative critical level can be established to provide for this loss of Fe availability. A similar conclusion can be drawn for Fe_{ca} in view of the data point distribution of Figs. 5c and 5d.

Flooding resulted in a dramatic increase in Fe_{DTPA}; thus, nearly all data points fell well above the widely adopted critical level of 4.5 mg kg^{-1} (Lindsay and Norvell, 1978). This increase resulted in a highly significant increase in LCC in the first crop (Fig. 5e) but not in the second (Fig. 5f). Therefore, Fe_{DTPA} appears to include Fe forms whose phytoavailability decreases with time, as is the case —albeit to a much lesser extent— with Fe_{ox} and Fe_{ca}. This makes Fe_{DTPA} less useful than either Fe_{ox} or Fe_{ca} for predictive purposes.

ACKNOWLEDGEMENTS

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CHAPTER 4

Pot evaluation of synthetic nanosiderite for the prevention of iron chlorosis

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RESUMEN

La clorosis férrica es un problema que afecta a las plantas cultivadas en suelos calcáreos. En este trabajo se evaluó la eficacia de una suspensión de siderita ($FeCO_3$) de tamaño de partícula nanométrico para prevenir la clorosis férrica, siendo la hipótesis subyacente que los productos de oxidación de la siderita en el suelo son óxidos de hierro poco cristalinos y, por lo tanto, disponibles para la planta. La siderita nanométrica se preparó mezclando disoluciones de $FeSO_4$ y K_2CO_3, bien pura o dopada con fosfato (sideritas SID y SIDP, respectivamente). La superficie específica era de ~140 $m^2\ g^{-1}$ para SID y ~220 $m^2\ g^{-1}$ para SIDP. La oxidación experimental de la siderita en una suspensión de calcita produjo goethita (para SID) o una mezcla de lepidocrocita y goethita (para SIDP). En dos experimentos en macetas, en los que se fertilizó un suelo calcáreo con una suspensión de SID o SIDP a razón de ~2 g Fe kg^{-1} suelo, la nanosiderita fue eficaz para prevenir la clorosis férrica en garbanzo. En otro experimento en macetas con cinco cultivos sucesivos, una aplicación inicial de ~0.7 g Fe kg^{-1} suelo en forma de SID o SIDP fue tan eficaz como el FeEDDHA para prevenir la clorosis férrica. El efecto residual de la nanosiderita aplicada sólo al primer cultivo superó claramente al efecto residual del FeEDDHA. Las suspensiones de nanosiderita son, pues, eficaces en la prevención de la clorosis férrica y tienen un gran efecto residual.

ABSTRACT

Iron chlorosis is a problem that affects crops grown on calcareous soils. In this work, we assessed the effectiveness of nanosized siderite ($FeCO_3$) to prevent iron chlorosis, the underlying hypothesis being that the oxidation products of siderite in soil are poorly crystalline, and hence plant-available, iron oxides. Nanosized siderite was prepared by mixing $FeSO_4$ and K_2CO_3 solutions, either pure or doped with phosphate (siderite SID and SIDP, respectively). The average specific surface area was ~140 $m^2\ g^{-1}$ for SID and ~220 $m^2\ g^{-1}$ for SIDP. Experimental oxidation in a calcite suspension yielded goethite for SID and a mixture of lepidocrocite and goethite for SIDP. Two pot experiments in which a SID or SIDP suspension was applied to a calcareous soil at a rate of ~2 g Fe kg^{-1} showed nanosiderite to prevent iron chlorosis in chickpea. In a pot experiment with five successive crops, one initial application of ~0.7 g Fe kg^{-1} soil in the form of SID or SIDP was as effective as FeEDDHA in preventing Fe chlorosis. The residual effect of nanosiderite when applied to the first crop alone clearly exceeded that of FeEDDHA. Nanosiderite suspensions applied at rates of ~0.7 Fe kg^{-1} soil were highly effective in preventing iron chlorosis and have a great residual effect.

INTRODUCTION

Iron (Fe) chlorosis, which is characterised by internervial yellowing of young leaves, is the morphological evidence of an Fe nutrition problem that affects many crops grown on calcareous soils. The problem is commonplace in the Mediterranean region and other areas with a semiarid or arid climate, where significant reductions in crop yield and quality lead to economical losses in agriculture (Fernández-Escobar et al., 1993; Tagliavini and Rombolà, 2001).

Iron chlorosis has been related to the total calcium carbonate content (Inskeep and Bloom, 1987), the content in "active lime" (i.e. the most reactive calcium carbonate fraction) (Drouineau, 1942; Yaalon, 1957), and the surface area of soil carbonates (Loeppert et al., 1988). However, the individual soil property best correlating with the severity of Fe chlorosis is the content in poorly crystalline Fe oxides as estimated with various extractants (particularly acid ammonium oxalate) (Schwertmann, 1964). Such correlation has been observed in soybean (Loeppert and Hallmark, 1985), chickpea and sunflower (del Campillo and Torrent, 1992), peach (Yangüas et al., 1997), olive (Benítez et al., 2002), lupin (de Santiago and Delgado, 2006), and grapevine (Díaz et al., 2010), among other crops. Therefore, one possible way of solving the problem is by increasing the content in poorly crystalline Fe oxides of soil either by direct application or by addition of precursor Fe compounds. The latter approach proved successful in experiments where synthetic ferrous phosphate [an analogue of the mineral vivianite, $Fe_3(PO_4)_2 \cdot 8H_2O$] was applied to soil (Eynard et al., 1992; del Campillo et al., 1998; Rosado et al., 2002; Díaz et al., 2009). The effectiveness of vivianite was explained by Roldán et al. (2002), who found oxidation and incongruent dissolution of vivianite in an artificial calcareous medium to result in the production of nanosized (<2 nm thick) lepidocrocite particles that were 100% soluble in acid oxalate. When a suspension of vivianite is injected into soil, its transformation into lepidocrite is evidenced by the appearance of the characteristic orange yellow colour of this mineral in the soil volume affected by the injection. In contrast to soluble Fe fertilisers (Fe chelates), lepidocrocite particles are not leached from soil, which explains the long-lasting Fe-fertiliser effect of vivianite (Rosado et al., 2002; Díaz et al., 2009).

In this work, we explored the use of synthetic Fe(II) carbonate, an analogue of the mineral siderite ($FeCO_3$), as an Fe amendment for Fe chlorosis-inducing soils. The underlying hypothesis was that the oxidation products of siderite would be poorly crystalline Fe oxides capable of supplying Fe to plants. For this purpose, various siderites of nanometre size were synthesised and their effectiveness and persistence as Fe fertilisers studied in pot experiments under controlled conditions. Synthetic rather

than natural siderite was used because the latter rarely occurs in masses of small particles thus requiring vigorous grinding before use.

MATERIALS AND METHODS

SIDERITE SYNTHESIS

Siderite suspensions SID1, SID2, SID3 were obtained by mixing 200 mL of 1*M* $FeSO_4$ with 200 mL of 1*M* K_2CO_3 and adding 200 mL of deionised H_2O. Siderites suspensions SIDP1, SIDP2 and SIDP3 were obtained similarly to the "SID" suspensions except that 100 mL of 0.1 *M* $H_2NH_4PO_4$ was added to the initial $FeSO_4$ solution (P/Fe atomic ratio = 0.05). The reason for adding phosphate was its strong effect on the nature and properties of Fe oxides formed by precipitation/hydrolysis of various Fe salts (Gálvez et al., 1999; Cumplido et al., 2000; Barrón et al., 2006). Salt-free suspensions of siderites designated SID1w, SID2w, SID3w, SIDP1w, SIDP2w and SIDP3w were obtained by centrifuging the corresponding initial SID or SIDP suspensions, decanting the supernatant, washing the sediment with deionised water until the electrical conductivity of the supernantant solution was <0.50 dS m^{-1}, and resuspending it in a volume of water equal to that of the initial siderite suspension. No specific action was taken to prevent partial oxidation of siderite in the fresh or salt-free suspensions, which were applied to the pots immediately after preparation. A portion of each siderite suspension was freeze-dried and stored in a polyethylene tube for subsequent analysis. Note that numbers 1, 2, 3 in each series correspond to siderites prepared in the same way but at different times (corresponding to Experiments 1, 2 and 3 described below).

CHEMICAL ANALYSES OF THE SIDERITE SUSPENSIONS

Total Fe (Fe_t) and Fe(II) were determined by mixing 0.25 mL of the siderite suspension with 4.1 mL of 12.1 *M* HCl in a glass vial. After 15 minutes, the supernatant was analysed for Fe(II) and total Fe with the *o*-phenanthroline method (Olson and Ellis, 1982). Oxalate-extractable Fe (Fe_{ox}) was determined by using 0.2*M* ammonium oxalate at pH 3 according to Schwertmann (1964). Phosphate in solution was determined with the method of Murphy and Riley(1962). All determinations were performed in duplicate or triplicate.

SIDERITE OXIDATION EXPERIMENTS

A 150-mL portion of a siderite suspension prepared as described above was transferred to a bottle of 250 mL and 5 g of 0.2−0.5 mm $CaCO_3$ sand was added to adjust the pH to a value similar to that of calcareous soils, the bottle then being sealed with punctured parafilm to allow air exchange but no significant water loss. The suspension was then magnetically stirred in a dark room at 25 °C for 96 h. At preset times, a portion was withdrawn to determine total Fe and Fe(II). At the end of the experiment oxalate-extractable Fe was determined and a portion of the suspension freeze-dried for powder X-ray diffraction (XRD) analysis. The siderite oxidation experiment was performed in triplicate.

MINERALOGICAL ANALYSES

The powder XRD patterns of the freeze-dried products were obtained on a Siemens D5000 diffractometer (Siemens, Munich, Germany) using Co*Kα* radiation and a graphite monochromator. The proportions of the different minerals in the mixtures were semiquantitatively estimated by using the software PowderCell (BAM, Berlin, Germany) (Kraus and Nolze, 1996). Micrographs of the products were obtained by using a JEOL JEM 2010 transmission electron microscope (JEOL, Tokyo, Japan) after dispersing small portions of the powder in water and depositing small drops of the suspension on a carbon copper grid. Specific surface area was determined with a Micromeritics ASAP 2010 surface area analyzer (Norcross, GA, USA), using N_2 as adsorbate [the Brunauer, Emmett and Teller (BET) method].

SOIL SELECTION AND ANALYSIS

The soil used in this study was collected in an area in southern Spain where olive trees and vines exhibit the typical symptoms of Fe chlorosis. Its clay content (Gee and Bauder, 1986) was 180 g kg^{-1}, organic carbon (Walkley and Black, 1934) was 5 g kg^{-1}, pH (water) was 8.6, calcium carbonate equivalent (CCE) (van Wesemael, 1955) was 570 g kg^{-1}, active lime content (Drouineau, 1942) was 210 g kg^{-1}, Olsen P (Olsen et al., 1954) was 16 mg kg^{-1}, and available K (Soil Survey Staff, 1984) was 160 mg kg^{-1}. The diethylenetriaminepentacetic acid-extractable Fe content (Fe_{DTPA}) (Lindsay and Norvell, 1978) was 1.7 mg $kg^{-1}$and the acid oxalate-extractable Fe content (Fe_{ox}) (Schwertmann, 1964) was 0.12 g kg^{-1}.

POT EXPERIMENTS: DESIGN

Three growth experiments were carried out in PVC cylindrical pots (diameter, 5.5 cm; height, 13 cm). In Experiment 1, two fresh (SID1, SIDP1) and two salt-free (SID1w, SIDP1w) siderite suspensions were diluted to one half with water, spread and thoroughly mixed with 220 g of soil at a rate of 4.2 g siderite (~2 g Fe) kg^{-1} soil. Two chickpea crops were then successively grown. Experiment 2 was like Experiment 1 but involved other siderite suspensions (SID2, SIDP2, SID2w, and SIDP2w) and only one chickpea crop was grown. In Experiment 3, suspensions of siderites SID3 and SIDP3 were mixed with 220 g of soil at a rate of 0.24, 0.46, 0.93 and 1.40 g siderite (0.12, 0.22, 0.45, and 0.67 g Fe) kg^{-1} soil, and five crops [chickpea (twice), peanut (twice) and strawberry] were successively grown. In all experiments, a control (no Fe applied) and a 'positive' control supplied with Fe chelate [Ethylenediamine-N, N'-bis (2-hydroxyphenylacetic acid) (FeEDDHA)] over the growth period of each crop were included. Furthermore, a treatment involving the application of FeEDDHA to the first crop only was included in Experiment 3 in order to assess the residual effect of this Fe fertiliser. Each treatment was replicated four times.

POT EXPERIMENTS: PLANT MATERIAL, GROWTH CONDITIONS AND PLANT ANALYSIS

Chickpea (*Cicer arietinum* L., cv. ICC 11224) and peanut (*Arachis hypogaea* L.) seeds were germinated on a moistened paper towel for 4−6 days. Two seedlings of each chickpea and peanut were transplanted to the pots and, after one week, only one plant was left. In Experiment 3, one 30-day-old strawberry plant (*Fragaria* × *ananassa* Guedès cv. Camarosa) per plot was planted. Pots were randomly arranged in a growth chamber with a photoperiod of 14 h, a light intensity of 270 µmol m^{-2} s^{-1}, a temperature of 25 ± 3 °C and a relatively humidity of 45−55%.

For each crop and pot, a total of 30 mL of a pH 6, Fe-free modified Hoagland nutrient solution [2.5m*M* $Ca(NO_3)_2$, 2.5m*M* KNO_3, 1m*M* $MgSO_4$, 1m*M* KH_2PO_4, 0.1m*M* KCl, 50 µ*M* H_3BO_3, 4 µ*M* $MnSO_4$, 4 µ*M* $ZnSO_4$ $7H_2O$, 1 µ*M* $CuSO_4$ $5H_2O$, and 0.1 µ*M* $(NH_4)_6Mo_7O_{24}$] was distributed in several applications over the growth period shown in Fig. 1. FeEDDHA (6% Fe, 4.8% Fe ortho–ortho isomer, Laboratorio Jaer S.A., Barcelona, Spain) was applied at a total rate of 20 mg per crop and pot over the growth period. (This dose had previously shown to be effective to prevent leaf chlorosis). Besides the nutrient solution, deionised water was applied on a daily basis to keep soil

moisture near field capacity and re-randomised to minimise the influence of location in the growth chamber.

The chlorophyll content of the youngest leaves was estimated from the SPAD value (SPAD 502 portable chlorophyll meter, Minolta Camera Co., Osaka, Japan), which was measured twice (at 10 and 20 days, SPAD10 and SPAD20, respectively), or three times for chickpea (10, 17 and 24 days, SPAD10, SPAD17 and SPAD24, respectively), three times for peanut (SPAD10, SPAD17 and SPAD24), and five times for strawberry (SPAD10, SPAD17, SPAD24, SPAD 31 and SPAD 38). After the last SPAD reading, and immediately before harvest, the youngest fully expanded leaf in each plant was cut, its surface area measured and its chlorophyll extracted with 96 wt% ethanol. Chlorophyll concentrations were determined according to Wintermas and de Mots (1965). Plants were dried at 65°C for at least 72 h, weighed, digested in nitric/perchloric acid (Zazoski and Burau, 1977) and the resulting solution analysed for Ca, Mg, Fe, Mn, Cu and Zn by atomic absorption spectrophotometry, K by flame emission, and P with the molybdenum blue color method of Murphy and Riley (1962).

STATISTICAL ANALYSIS

The analysis of variance (ANOVA) was performed with Statistix 9.0 (Analytical Software, Tallahassee, FL, USA). Unless otherwise stated, the word ‘significant’ is used here to indicate significance at the $P < 0.05$ level. Means were separated via the LSD test. In those figures showing the time course of the SPAD value and mineral element content, the mean and standard error for each treatment and time are shown.

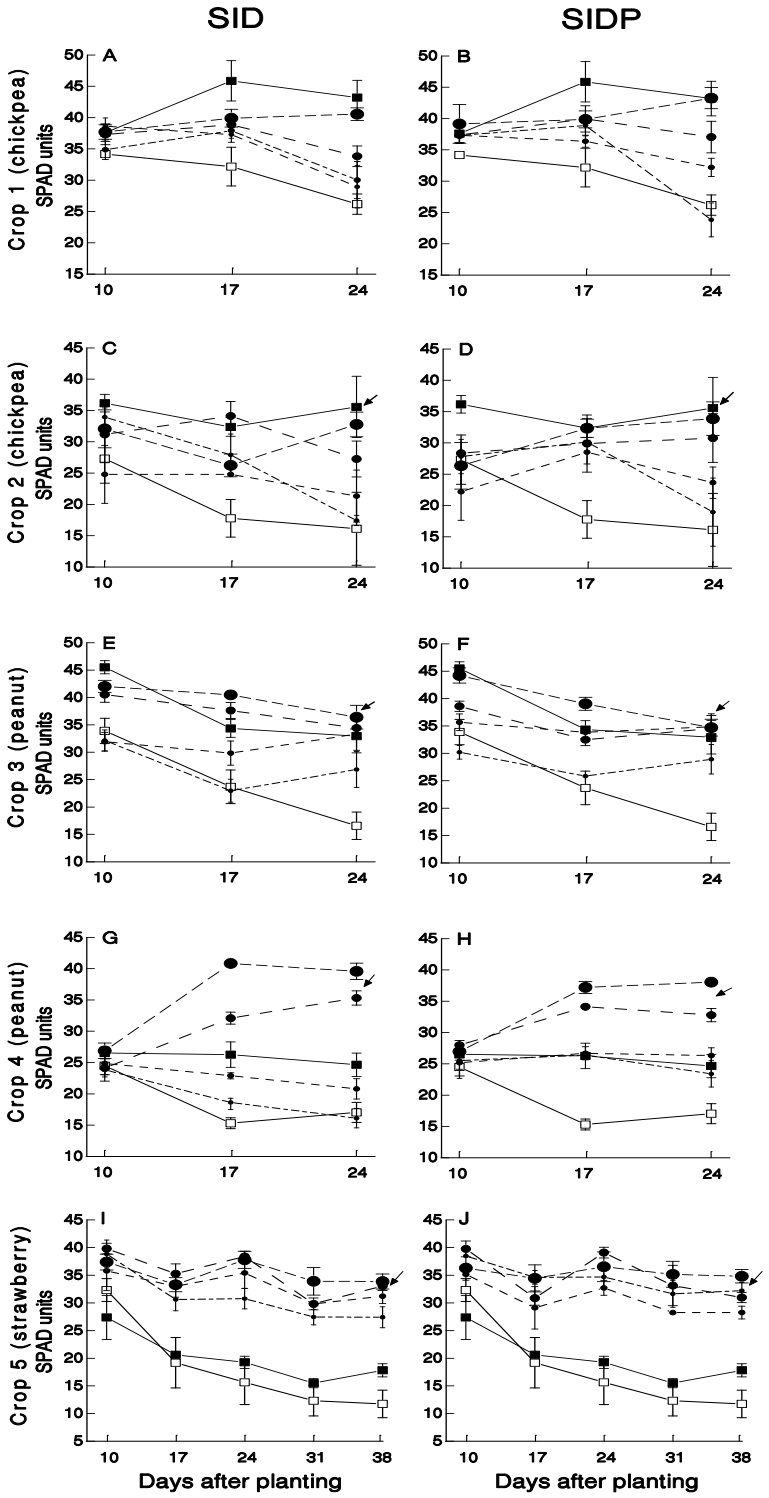

Figure 1. SPAD values for the five successive crops of Experiment 3. The SPAD values for the pure siderite (left) and siderite prepared in the presence of phosphate (right) with rates of 0.12, 0.23, 0.45, and 0.67 g Fe kg^{-1} soil are symbolised by black circles of increasing radius. The SPAD valuesfor the control plants are symbolised by white squares and those for the plants growing in pots where FeEDDHA was applied only to the first crop by black squares. An arrow marks the SPAD value at harvest for the 'positive' control treatment (FeEDDHA applied to each crop).

RESULTS AND DISCUSSION

PROPERTIES OF THE SYNTHETIC SIDERITES AND THEIR OXIDATION PRODUCTS

Selected properties of the synthetic siderite samples are shown in Table 1. About 90% Fe was in ferrous form in the freshly prepared suspensions of samples SID and SIDP; this suggests that Fe(II) was partly oxidised during preparation. This was consistent with a change in colour from pale to brownish dark green within a few minutes after siderite precipitation. Judging from the XRD patterns, the SID samples contained goethite and traces of lepidocrocite (only in SID2 and SID3) in addition to siderite; by contrast, the SIDP samples were pure siderite (Fig. 2).

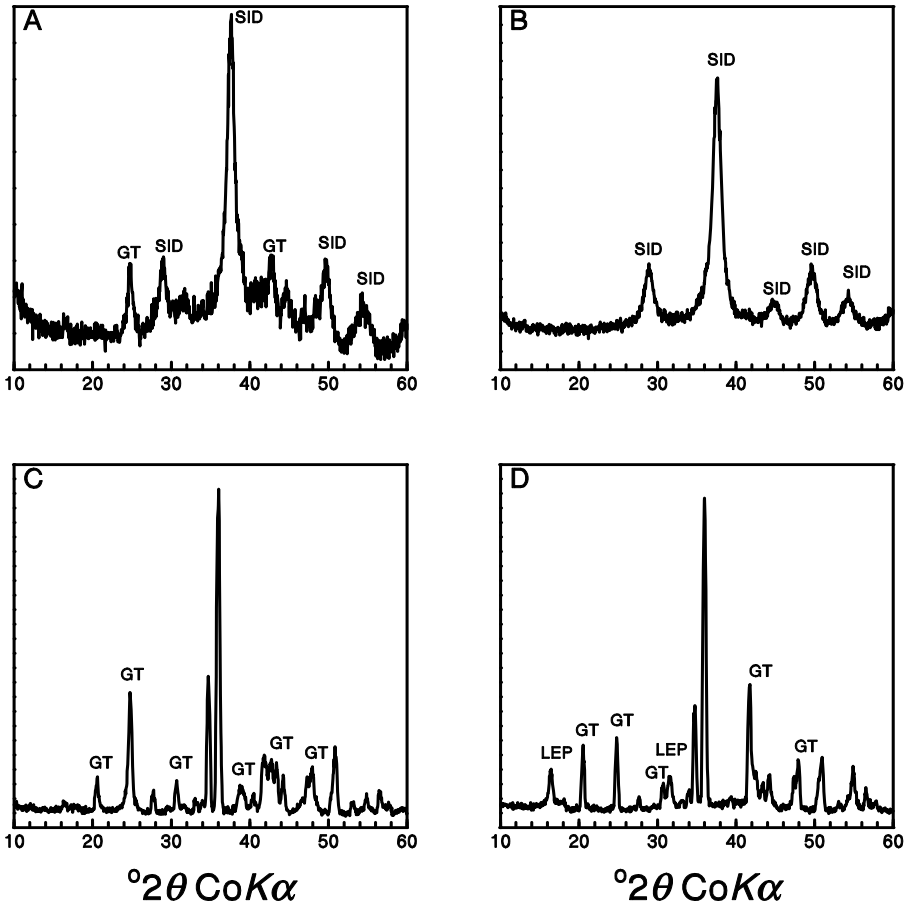

Figure 2. X-ray diffraction patterns of: (A) slightly oxidised pure siderite; (B) slightly oxidised siderite prepared in the presence of phosphate; (C) and (D) oxidation products of (A) and (B), respectively, for 96 h in the presence of CaCO3. SID, siderite; GT, goethite; LEP, lepidocrocite. Reflections not marked correspond to calcite and other salts.

The SID and SIDP samples differed in particle shape and size: the former consisted of 300−500 nm long, ~100 nm wide needles whereas the latter were equi-dimensional to plate-like and about 120 nm on average in size (Fig. 3). These differences in shape and size reflected in differences in specific surface area (SSA) of the partly oxidised, salt-free samples: 126−153 m^2 g^{-1} for the SID and 186−235 m^2 g^{-1} for the SIDP samples. These results clearly suggest that phosphate hindered growth of the siderite crystals and their oxidation products, probably through preferential adsorption on some crystal faces. In fact, growth of calcite, an isomorph of siderite, is known to be delayed by the adsorption of phosphate (House, 1987; Dove and Hochella, 1993); on the other hand, siderite is known to strongly adsorb arsenate, a chemical analogue of phosphate (Guo et al., 2007). The ability of the siderites studied here (and their initial oxidation products) to adsorb phosphate was clearly supported by the small change in phosphate concentration of the siderite suspension upon removal of salts (from 0.44−0.49 to 0.36−0.44 g P L^{-1}; Table 1).

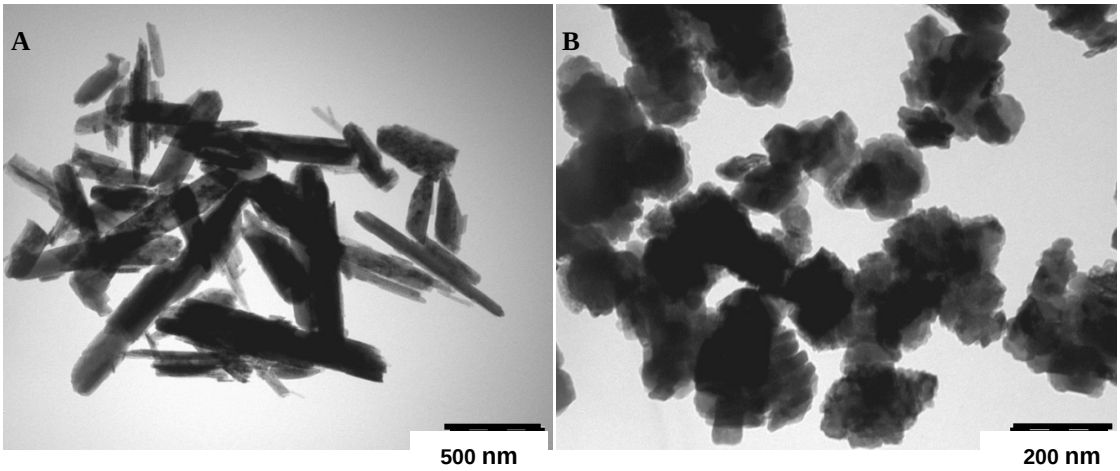

Figure 3. Transmission electron micrographs of slightly oxidised (A) pure siderite, and (B) siderite prepared in the presence of phosphate

Washing the sediment of the initial suspension several times with water to eliminate salts (K_2SO_4) in the presence of air resulted in substantial Fe(II) oxidation [Fe(II)/Fe_t decreased from 0.88−0.95 to 0.58−0.70 (Table 1)] and the concomitant formation of magnetite in significant proportions (Table 1). The XRD patterns of the washed samples (not shown) suggested that the SID samples contained higher proportions of magnetite than the SIDP samples, probably because phosphate hinders the growth of Fe oxides (Jurado et al., 2003).

Stirring the initial suspensions with calcite sand for 96 h caused nearly complete oxidation of siderite and production of goethite in the SID suspensions, and goethite

and lepidocrocite in the SIDP suspensions (Table 1, Fig. 2). These results are consistent with a strong influence of phosphate on the transformation of Fe(II) salts; for instance, phosphate favoured lepidocrocite over goethite when Fe oxides were prepared by oxidizing green rust (Cumplido et al., 2000). Whereas goethite from the oxidation of the SID siderites was poorly soluble in oxalate (Fe_{ox}/Fe_t < 0.11; Table 1), the mixture of goethite and lepidocrocite from the SIDP siderites was highly oxalate soluble (Fe_{ox}/Fe_t > 0.77; Table 1). Because the proportion of lepidocrocite in these samples never exceeded 30%, their high solubility in oxalate should be ascribed not only to the increased solubility of this mineral (Reyes and Torrent, 1997), but also to the high solubility of paragenetic goethite by virtue of its structural defects or small crystal size. In summary, oxidation of the siderites prepared in the presence of phosphate yielded Fe oxides of moderate crystallinity and high solubility, which, as noted earlier, are a good source of Fe to the plants in calcareous soils (Benítez et al., 2002; Díaz et al., 2010).

Table 1. Selected properties of the synthetic siderites

Sample[a]	Fe(II)/Fe_t[a]	Fe_{ox}/Fe_t[a]	Phosphorus[b] g L^{-1}	SSA[a] m^2 g^{-1}	pH	EC[a] dS m^{-1}	XRD Patterns[a]	Oxidation experiment Fe(II)/Fet (2h)	Fe(II)/Fet (96 h)	Fe_{ox}/Fet (96 h)	XRD patterns[a]
SID1	0.91	0.90	-	-	7.3	43	SID,	0.90	0.02	0.10	GT
SID1w	0.68	0.83	-	126	7.3	0.23	SID, MT	-	-	-	
SID2	0.94	0.89	-	-	7.2	44	SID, LEP	0.88	0.02	0.07	GT
SID2w	0.60	0.81	-	148	7.1	0.14	SID, MT	-	-	-	
SID3	0.95	0.88	-	-	7.2	43	SID, LEP	0.84	0.02	0.06	GT
SID3w	0.70	0.81	-	153	7.3	0.21	SID, MT	-	-	-	
SIDP1	0.88	0.86	0.44	-	7.3	44	SID	0.79	0.02	0.78	GT, LEP
SIDP1w	0.67	0.80	0.40	235	7.5	0.29	SID, MT	-	-	-	
SIDP2	0.90	0.87	0.49	-	7.4	43	SID	0.81	0.01	0.81	GT, LEP
SIDP2w	0.64	0.80	0.36	186	7.5	0.27	SID, MT	-	-	-	
SIDP3	0.91	0.88	0.46	-	7.4	43	SID	0.80	0.02	0.81	GT, LEP
SIDP3w	0.58	0.84	0.44	232	7.3	0.29	SID, MT	-	-	-	

[a]Abbreviations: subscript w, salt-free suspensions; Fe_t, total Fe; Fe_{ox}, oxalate extractable Fe; SSA, specific surface area (BET method); EC, electrical conductivity of the suspension; SID, siderite; MT, magnetite; GT, goethite, LEP, lepidocrocite.

[b]Total phosphorus concentration in the suspension.

POT GROWTH EXPERIMENTS

The ethanol extractable chlorophyll content per unit leaf area was highly correlated with the SPAD readings for the first chickpea (y = 0.94x + 10, R^2 = 0.91***), second peanut (y = 0.93x + 7.0, R^2 = 0.83***) and strawberry crop (y = 1.6x + 5.9, R^2 = 0.79***). For this reason, we used SPAD as a measure of Fe chlorosis.

Experiments 1 and 2

In Experiment 1, the control plants for both chickpea crops were those exhibiting the lowest SPAD 10 days after transplanting (SPAD10; Table 2). This was to be expected in view of the small amount of available Fe present in the soil (Fe_{DTPA} = 1.7 mg kg^{-1}, Fe_{ox} = 0.12 g kg^{-1}), given that Fe_{ox} levels below 0.65 g kg^{-1} are likely to induce Fe chlorosis in sensitive chickpea cultivars (del Campillo and Torrent, 1992). At all times, in both crops, the plants fertilised with Fe exhibited higher SPAD values than the control ones, differences being significant with the only exception of the plants treated with one of the salt-free suspensions (SID1w). In addition, the SPAD values at harvest time of plants fertilised with any of the different siderites were not significantly different from those of plants fertilised weekly with FeEDDHA. As can be seen from Table 2, the salt-free siderite suspensions were generally less effective in preventing Fe chorosis than were their freshly prepared counterparts, although differences were not significant. Nor were there any significant differences in SPAD between plants treated with SID and SIDP siderites.

Dry matter weight (DM) was greatest for the siderites SIDP1 and SID1, and smallest for the plants fertilised with FeEDDHA. The highest values of the SPAD × DM product, an indicator of the total amount of chlorophyll produced, were for those plants supplied with the freshly prepared siderites and the lowest for the control (Chickpea 1) or the FeEDDHA-supplied plants (Chickpea 2).

The differences between treatments were much less marked in Experiment 2 (Table 2) than in Experiment 1, the most salient features being the low SPAD value for the control and the low DM value for the FeEDDHA-supplied plants.

Table 2. Chickpea SPAD, dry matter yield (DM), and SPAD × DM in Experiments 1 and 2

Experiment 1					
Treatment	SPAD10	SPAD17	SPAD24	DM (g)	SPAD×DM
First crop					
Control	19.3 c	17.6 b		0.113 ab	1.95 c
SID1	32.0 ab	36.0 a		0.139 a	5.10 a
SID1w	26.7 bc	31.8 a		0.104 b	3.30 bc
SIDP1	30.5 ab	33.4 a		0.128 ab	4.30 ab
SIDP1w	28.5 ab	32.6 a		0.103 b	3.39 bc
FeEDDHA	36.1 a	39.6 a		0.098 b	3.90 ab
Second crop					
Control	32.9 c	27.9 c	34.1 b	0.149 ab	5.15 c
SID1	38.2 ab	37.6 ab	48.6 a	0.149 ab	7.24 ab
SID1w	36.3 bc	36.8 ab	48.7 a	0.121 bc	5.88 bc
SIDP1	40.6 ab	36.2 ab	50.1 a	0.168 a	8.52 a
SIDP1w	37.8 abc	35.9 b	45.3 a	0.124 bc	5.61 bc
FeEDDHA	42.1 a	41.5 a	46.9 a	0.099 c	4.64 c

Experiment 2				
Treatment	SPAD10	SPAD20	DM (g)	SPAD×DM
Control	32.7 b	28.7 c	0.143 a	4.19 a
SID2	34.1 b	34.2 bc	0.144 a	4.95 a
SID2w	34.9 b	45.9 a	0.126 a	5.83 a
SIDP2	33.1 b	37.7 b	0.142 a	5.35 a
SIDP2w	35.4 b	37.3 b	0.121 ab	4.53 a
FeEDDHA	45.0 a	47.3 a	0.086 b	4.09 a

[a] Different letters indicate significant differences ($P < 0.05$, LSD test) between treatments.

Experiment 3

Experiments 1 and 2 were used as a preliminary test to examine the effectiveness of siderite in preventing Fe chlorosis; for this reason, the dose used (>4 g siderite kg^{-1} soil) was substantially higher than that known to be effective for vivianite (1 g vivianite

kg^{-1} soil) (Eynard et al., 1992; Rosado et al., 2002). In Experiment 3, the effect of siderite was examined at a much lower dose (0.24 ─ 1.4 g siderite kg^{-1} soil).

Figure 1 shows the time course of SPAD for the five consecutive crops [chickpea (twice), peanut (twice) and strawberry] of Experiment 3 as a function of siderite rate; the SPAD values for the plants supplied with FeEDDHA, which was applied to the first crop (Chickpea 1) only or to all crops, are also shown. These results can be summarised as follows: (i) After 17 days (SPAD17), control plants exhibited clear Fe chorosis symptoms and, at harvest (24 or 38 days), SPAD for the control plants was in most cases significantly lower than the values of the plants Fe-supplied; (ii) SPAD for the plants fertilised with FeEDDHA (positive control) was in most instances higher than the values for the siderite-fertilised plants; (iii) the residual effect of FeEDDHA applied to the first crop only decreased markedly with time and was barely appreciable in the fifth crop (strawberry); (iv) based on the SPAD values obtained, SID3 and SIDP3 were similarly effective; (v) for all crops, times and siderite types, SPAD tended to systematically increase with increasing siderite dose; (vi) at harvest, SPAD for the plants fertilised with the highest siderite dose (1.40 g kg^{-1}) did not differ significantly from, or was significantly higher than that of FeEDDHA-fertilised plants. (Chapter 8, Fig. A4 and Fig. A5).

Table 3 shows the dry matter weights (DMs) for the different crops harvested in this experiment. Increasing the siderite rate (i) had no significant effect on DM for the chickpea crops, and (ii) resulted in a linear increase in DM for the next three crops (except for strawberry supplied with SIDP3, where the relationship was quadratic).

The application of FeEDDHA had generally a negative effect on plant growth (Tables 2 and 3); in some instances, the DM of the FeEDDHA-treated plants was even lower than the DM of the control plants. One possible explanation is that the application of Fe chelate, i.e. a highly soluble Fe form, has a negative impact on the plant root response to Fe deficiency. One other hypothesis is that chelation of soil Mn by EDDHA affects the uptake of Mn by the plant. In fact, the concentration of Mn in plants treated with FeEDDHA was significantly lower than that in plants treated with siderite (detailed results not shown); however, no Mn deficiency symptons were observed in any plant during the experiments.

Figure 4 shows the concentrations of mineral elements at harvest for each crop and Fe fertiliser. The data points for siderite represent the means for all SID3 and SIDP3 doses, and those for FeEDDHA the values for the positive control (i.e. FeEDDHA applied to all crops) (Chapter 8, Tables A10, A11, A12, A13 and A14). The nutrient contents of the plants supplied with siderite exceeded reported critical levels (Benton Jones et al., 1991; Reuter et al., 1997; Robinson et al., 1997) and, for most

nutrients, they fell in between those for control and FeEDDHA-supplied plants. Generally, the Fe concentration in plant was uncorrelated with SPAD, unaffected by the source of Fe, and not significantly correlated with dry matter, as is often the case with Fe chorosis-affected crops (Díaz et al., 2010). The K content of the plants fertilised with siderite tended to be higher, and the Ca and Mg contents lower, than those of the control plants; this can be ascribed to the presence of K^+ in the siderite suspension (because K_2CO_3 was used in the synthesis) and to the effect of this cation on the uptake of competing cations (Mg^{2+}, Ca^{2+}) (Kurvits and Kirkby, 1980; Marschner, 1986). The higher concentrations of Mn in the control plants relative to the Fe-fertilised plants can be ascribed to the increased proton release and reducing capacity of root cells in chlorotic plants (Venkatraju and Marschner, 1981; Moraghan, 1991).

Table 3. Dry matter yields in Experiment 3

		Dry matter (g)				
Treatment	Siderite rate (g kg^{-1} soil)	Crop 1 (chickpea)	Crop 2 (chickpea)	Crop 3 (peanut)	Crop 4 (peanut)	Crop 5 (strawberry)
Control	0.00	0.100 ± 0.014	0.185 ± 0.025	0.708 ± 0.027	0.505 ± 0.020	0.390 ± 0.034
SID3	0.24	0.106 ± 0.014	0.169 ± 0.018	0.808 ± 0.007	0.502 ± 0.004	0.533 ± 0.033
SID3	0.46	0.100 ± 0.013	0.244 ± 0.016	0.868 ± 0.013	0.661 ± 0.013	0.482 ± 0.016
SID3	0.93	0.105 ± 0.026	0.204 ± 0.014	0.856 ± 0.024	0.689 ± 0.010	0.579 ± 0.050
SID3	1.40	0.092 ± 0.007	0.190 ± 0.013	0.945 ± 0.028	0.726 ± 0.014	0.554 ± 0.029
Significance		NS	NS	L**	L***	L**
CV (%)		14.5	18	11.8	5	13.4
Control	0.00	0.100 ± 0.014	0.185 ± 0.025	0.708 ± 0.027	0.505 ± 0.020	0.390 ± 0.034
SIDP3	0.24	0.096 ± 0.024	0.231 ± 0.022	0.812 ± 0.012	0.789 ± 0.012	0.571 ± 0.017
SIDP3	0.46	0.104 ± 0.016	0.235 ± 0.020	0.913 ± 0.030	0.774 ± 0.011	0.431 ± 0.032
SIDP3	0.93	0.119 ± 0.027	0.227± 0.012	0.844 ± 0.013	0.658 ± 0.010	0.518 ± 0.056
SIDP3	1.40	0.105 ± 0.012	0.223 ± 0.014	0.876 ± 0.007	0.958 ± 0.020	0.428 ± 0.018
Significance		NS	NS	L*	L***	Q*
CV (%)		18.4	17.4	11	5.6	14.7
FeEDDHA	0.00	0.076 ± 0.024	0.176 ± 0.020 a	0.774 ± 0.018 a	0.487 ± 0.010 a	0.519 ± 0.040 a
FeEDDHA	0.00		0.185 ± 0.006 a	0.758 ± 0.037 a	0.491 ± 0.043 a	0.414 ± 0.025 a

Abbreviations and symbols: L, linear; Q, quadratic; *, ** and ***, significant at the 0.05, 0.01 and 0.001 probability level, respectively; NS, not significant; CV, coefficient of variation for all plants. For the FeEDDHA treatments, different letters indicate significant differences at $P < 0.05$ (LSD

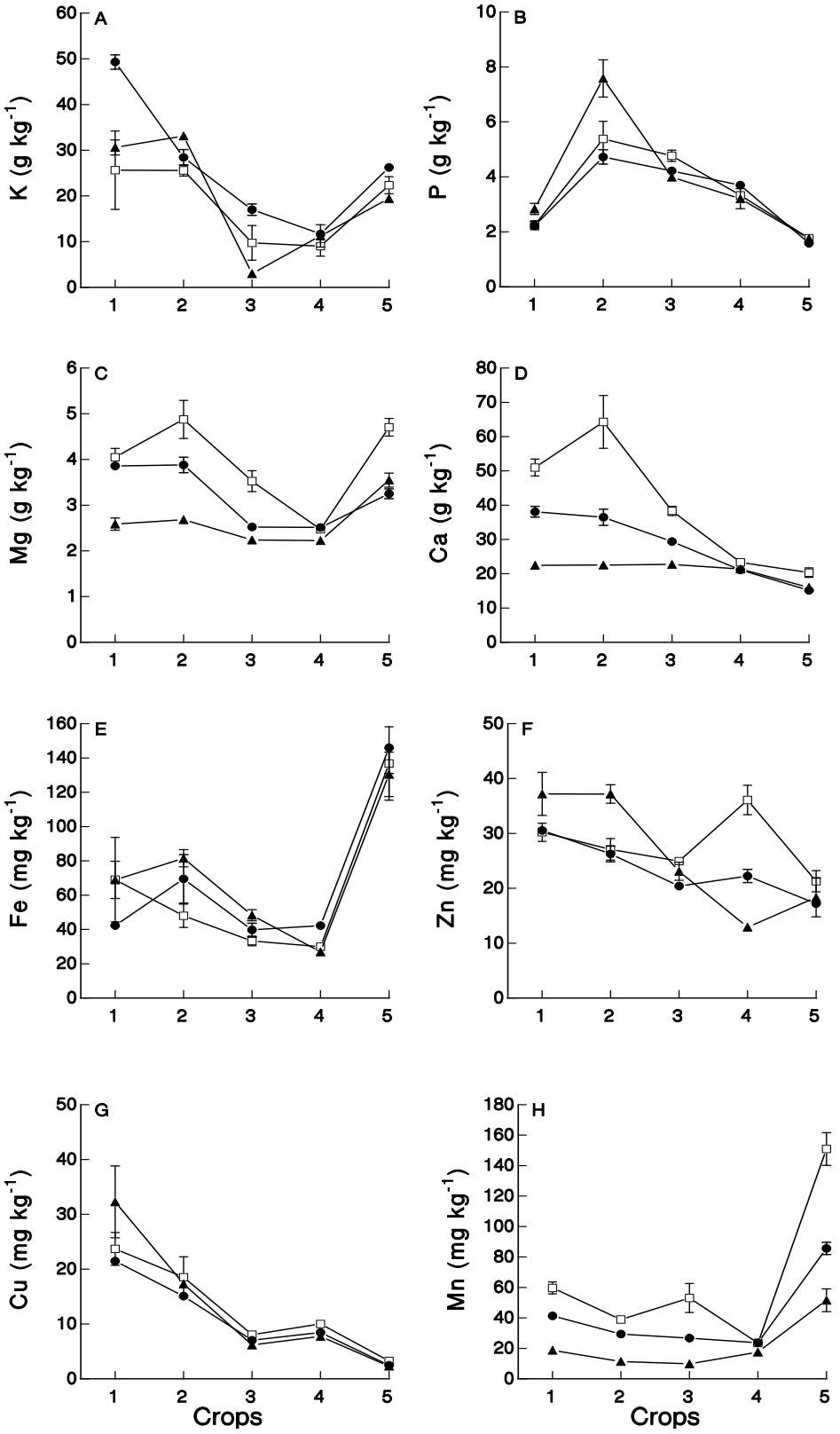

Figure 4. Element concentrations at harvest for the five crops of Experiment 3. White squares: control plants; black circles: plants supplied with siderite (average values for all rates and types of siderite); black triangles: FeEDDHA-supplied plants ('positive' control).

EFFECTIVENESS OF SIDERITE IN PREVENTING FE CHLOROSIS

The three experiments showed a single application of siderite to be effective in preventing or alleviating Fe chlorosis in various crops (Tables 2 and 3, Fig. 1). Experiment 3 showed that the residual effect of siderite substantially exceeded that of FeEDDHA, whose effectiveness decreased markedly after the second crop (Fig. 1). This experiment also showed the positive effect of siderite to increase with increasing rate up to 1.4 g siderite kg^{-1} soil, where the effect of siderite on SPAD was not significantly different from that of continuously applied FeEDDHA (Fig. 1).

The freshly prepared suspensions of siderite tended to be slightly more effective in preventing Fe chlorosis than their salt-free siderite counterparts, especially in terms of the SPAD × DM index (Table 2). This might be a result of the loss of nanosized siderite particles when the fresh suspension was washed to remove salts, and of their reducing the rate of Fe actually applied to the soil, or to the fact that the washed siderites were partly oxidised and transformed into magnetite (Table 1), which, based on the XRD patterns (data not shown), exhibited good crystallinity and was thus expected to be largely unavailable to plant roots. The lower effectiveness of the salt-free relative to the freshly prepared siderite suspensions is consistent with the Fe_{ox} values of the soils at the end of Experiments 1 and 2: a lower proportion of the added Fe was recovered as Fe_{ox} from the soils supplied with the salt-free than from those supplied with freshly prepared siderite suspensions (Table 4).

Table 4. Oxalate-extractable Fe (Fe_{ox}) in soils after Experiments 1 and 2[a]

Experiment 1		Experiment 2	
Treatment[b]	Fe_{ox} (mg kg^{-1})	Treatment[b]	Fe_{ox} (mg kg^{-1})
Control	258 e	Control	244 d
SID1	2330 a	SID2	2376 a
SID1w	1567 d	SID2w	1654 c
SIDP1	2156 b	SIDP2	2274 a
SIDP1w	1889 c	SIDP2w	1926 b
FeEDDHA	246 e	FeEDDHA	240 d

[a] Values in each column, followed by the same letter are not significantly different ($P < 0.05$; LSD test).

[b] Abbreviations: SID, pure siderite; SIDP, siderite prepared in the presence of phosphate; w, salt-free siderite.

Based on the results of the oxidation experiments (Table 1, Fig. 2) we hypothesised that the siderites prepared in the presence of phosphate (SIDPs) should be more effective than those that were not, basically because the former yielded lepidocrocite, which is more oxalate soluble, hence more available to plant roots, than is goethite (Schwertmann, 1991). However, the plant growth experiments (Tables 2 and 3, Figs. 1 and 4) indicated no significant differences in Fe chlorosis prevention between the two types of siderite. In addition, the Fe_{ox} values of the soils at the end of Experiment 3 were a function of the rate, but not of the type, of siderite supplied to the soil (Fig. 5); in other words, the slope of the regression line of Fe_{ox} against Fe applied to soil in the form of siderite was not significantly influenced by siderite type. Such discrepancy between the *in vitro* and pot results may have resulted from the oxidation and incongruent dissolution of pure siderite to form Fe oxides being influenced by the adsorption of phosphate and other ions present in soil, which can hinder crystallization of the more stable and less soluble Fe oxides (particularly goethite). In fact, the slope for the common regression line of Fe_{ox} against siderite-Fe applied (Fig. 5) was 0.82, which indicates that most siderite Fe was transformed into oxalate-soluble forms.

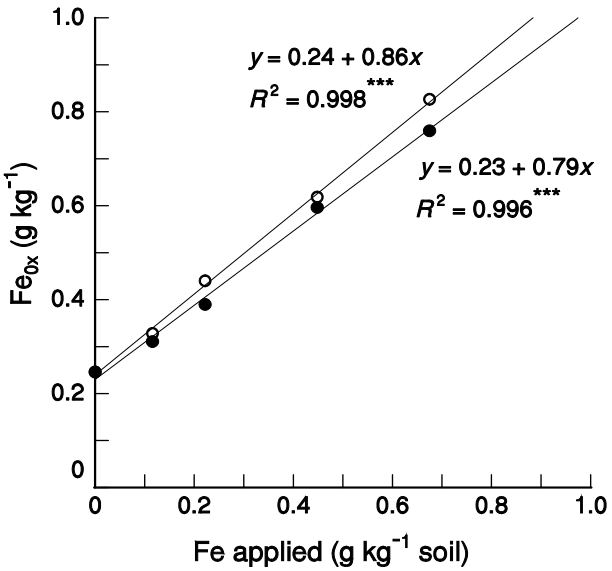

Figure 5. Oxalate-extractable Fe (Fe_{ox}) in soil after the fifth cropping of Experiment 3 as a function of Fe applied in the form of pure siderite (white circles) or siderite prepared in the presence of phosphate (black circles).

Besides its long-term effectiveness as a source of Fe to plants, siderite suspensions are not toxic and can be readily prepared in the field in a way similar to that described in this work by using an appropriate stirred tank. Ongoing experiments

involving injection of a siderite suspension into highly calcareous soils (results not shown) have confirmed its effectiveness in preventing Fe chlorosis in olive and the absence of detrimental effects due to high local concentrations of salts (K_2SO_4); in addition, the presence of K provided additional fertiliser value to siderite suspensions prepared as described above. In practice, the decision to adopt this method of preventing Fe chlorosis is highly dependent on the cost of the raw materials and, because of the low solubility and mobility of siderite, on those involved in properly distributing the suspension in soil zones with a high root density.

ACKNOWLEDGEMENTS

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CHAPTER 5

Application of synthetic siderite ($FeCO_3$) to the soil is capable of alleviating iron chlorosis in olive trees

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RESUMEN

La clorosis por deficiencia de hierro (Fe) se observa comúnmente en olivos cultivados en suelos calcáreos del sur de España. El propósito de este estudio fue evaluar la eficacia de la siderita ($FeCO_3$) sintética en la prevención de la clorosis férrica en olivo. La hipótesis subyacente era que la siderita se oxida y disuelve incongruentemente en el suelo produciendo óxidos de Fe poco cristalinos que pueden actuar como fertilizantes de Fe de liberación lenta. Experimentos de tres años de duración (2008−2011) se llevaron a cabo en tres olivares en suelos muy calcáreos situados en las provincias de Jaén, Córdoba y Sevilla y con olivos 'Picual' de 25 años, 'Picudo' de 11 años y 'Lechín de Sevilla' de 20 años, respectivamente. Los experimentos, iniciados en la primavera de 2008, incluían siempre un control (ninguna adición de Fe al suelo) y tratamientos consistentes en una única inyección al suelo de suspensiones de siderita pura y siderita preparada en presencia de fosfato. En las parcelas de Córdoba y Sevilla se incluyeron también tratamientos con suspensiones de vivianita (un eficaz fertilizante de Fe de liberación lenta) y de vivianita más ácidos húmicos así como de disoluciones de un quelato de Fe (FeEDDHA). La concentración de clorofila de la hoja, estimada mediante el valor SPAD, fue en general significativamente mayor en los árboles fertilizados con Fe que en los árboles control, observándose la mejor respuesta a la fertilización en el olivar de Córdoba y la más débil en el de Sevilla. El peso de las hojas aumentó significativamente con la fertilización con Fe en casi todos los muestreos realizados, excepto en el olivar de Sevilla. El efecto de la fertilización de Fe sobre la producción de aceituna fue en general positivo, pero sólo significativo para el tratamiento con siderita en el olivar de Jaén. La eficacia de los diversos tratamientos con siderita y vivianita fue similar y se mantuvo durante tres años. Por el contrario, el efecto residual de FeEDDHA disminuyó notablemente después del segundo año. Las suspensiones de siderita no sólo son eficaces fertilizantes de Fe de liberación lenta, sino que también son fáciles de preparar en el campo y carecen de toxicidad, constituyendo, por tanto, una buena alternativa a otros fertilizantes de Fe.

ABSTRACT

Iron (Fe) deficiency chlorosis is commonly observed in olive trees cultivated in calcareous soils of southern Spain. The purpose of this study was to assess the efficacy of synthetic siderite ($FeCO_3$) in preventing Fe chlorosis in olive trees. The underlying hypothesis was that siderite can be easily oxidized and incongruently dissolved in soil to give poorly crystalline Fe oxides that can act as slow-release Fe fertilizers. Three-year (2008−2011) experiments were carried out in three orchards with highly calcareous soils located in the provinces of Jaén, Córdoba and Seville, and cropped with 25-year-old 'Picual', 11-year-old 'Picudo' and 20-year-old 'Lechín de Sevilla' olive trees, respectively. The experiments involved a control (no Fe) treatment, and treatments consisting of a single injection into the soil of suspensions of (i) pure siderite and (ii) siderite prepared in the presence of phosphate ("P-siderite") in the spring of 2008. In the Córdoba and Seville orchards, suspensions of vivianite (an effective slow-release Fe fertilizer) and vivianite plus humic acids, in addition to a solution of Fe chelate (FeEDDHA) were also applied. Leaf chorophyll concentration as estimated via SPAD was significantly higher in the Fe-fertilized trees than in the control trees at nearly all times, the strongest response to Fe fertilization being that for the Córdoba and the weakest that for the Seville orchard. Leaf weight increased significantly with Fe fertilization at nearly all times except in the Seville orchard. The effect of Fe fertilization on yield was generally positive, but significant only for siderite in the Jaén orchard. The effectiveness of the two siderite and two vivianite treatments was similar and persisted over three years; by contrast, the residual effect of FeEDDHA decreased markedly after the second year. Siderite suspensions are not only effective slow-release Fe fertilizers, but also easy to prepare in the field and nontoxic; therefore, they constitute a good alternative to other Fe fertilizers.

INTRODUCTION

Iron (Fe) deficiency chlorosis is commonly observed in olive trees cultivated in highly calcareous soils of southern Spain. Iron chlorosis can be easily recognised by the typical interveinal yellowing of young leaves and is generally accompanied by yield reduction, yellowing of the olives (which makes them of little commercial value for direct consumption), and decreased stability and carotenoids concentration of the resulting virgin olive oil. This has nutritional significance, since carotenoids are anticarcinogenic and antiulcer agents (Mínguez-Mosquera et al., 1991).

Iron chelates [essentially Fe(III) chelates] are effective sources of Fe and thus widely used to control the problem (Hernández-Apaolaza, et al., 1997; Álvarez-Fernández et al., 2004; Lucena, 2006). However, their use has significant limitations because they are expensive and have little residual effect due to their high solubility and eventual leaching; chelates may thus require more than one application during the crop growing season.

Studies over the past twenty years have shown the usefulness of some poorly soluble, slow–release Fe fertilizers to prevent Fe chlorosis. In particular, suspensions of synthetic vivianite [$(Fe_3(PO_4)_2 \cdot 8H_2O)$] injected into soil at rates of about 1 g kg^{-1} were found to be as effective as Fe chelate (FeEDDHA) to prevent Fe chlorosis in pot-grown chickpea (Eynard et al.,1992), 'Picual' olive trees (Marta, 1999) and grapevine (Díaz et al., 2010). Field experiments showed that vivianite was effective in correcting Fe chlorosis for more than three seasons in different grapevine rootstock/varieties (Díaz et al., 2009); two seasons in 'Hojiblanco', 'Manzanillo' and 'Picual' olive trees (Rosado et al., 2002); and five in pear (del Campillo et al., 1998). The effectiveness of vivianite is ascribed to its high Fe content (about 30%), and its oxidation and incongruent dissolution in calcareous media to give poorly crystalline lepidocrocite (Roldán et al., 2002), which, as with other poorly crystalline Fe oxides, can constitute a good source of Fe for plants (Loeppert and Halmark 1985; Vempati and Loeppert, 1986, del Campillo and Torrent, 1992; Yanguas et al., 1997; de Santiago and Delgado, 2006). Indeed, the severity of Fe chlorosis is negatively correlated with the soil content in poorly crystalline Fe oxides as measured by extraction with acid oxalate-extractable Fe (Fe_{ox}; Schwertmann, 1964), or citrate/ascorbate-extractable Fe (Fe_{ca}; Reyes and Torrent, 1997), as shown by a number of studies (Benítez et al, 2002, and references therein). In this respect, it should be noted that the effectiveness of mixtures of Fe salts with humic compounds is likely associated with inhibited crystallization of the Fe oxides formed rather than with complexation of Fe by organic matter (de Santiago et al., 2008b).

The purpose of this work was to ascertain whether synthetic Fe(II) carbonate, an analogue of the mineral siderite ($FeCO_3$), was capable of correcting Fe chlorosis under field conditions. The rationale for using siderite was that, by analogy with vivianite, its artificial oxidation in a calcareous medium should result in its tranformation into poorly crystalline lepidocrocite and/or goethite. Field experiments were carried out in three different olive cultivars during three growing seasons by testing the effectiveness of suspensions of siderite prepared by a procedure usable by olive growers.

MATERIALS AND METHODS

EXPERIMENTAL ORCHARDS AND SOIL ANALYSES

Three olive orchards located in Andalusia, southern Spain, were selected for study (Chapter 8, Fig. A6). The location of the orchards, the local climate in the 2007−2008, 2008−2009 and 2009−2010 growing seasons, and the characteristics of the plant material are shown in Table 1. In all orchards, trees had shown interveinal yellowing of the youngest leaves before 2008. Only the Jaén field was irrigated.

Table 1. The experimental orchards

Province	Jaén	Córdoba	Seville
Nearest town	Mancha Real	Baena	Estepa
Coordinates			
Latitude	37º 49´ N	37º 38´ N	37º 19´ N
Longitude	3º 31´ W	4º 15´ W	4º 55´ W
Altitude (m)	663	477	309
Rainfall (mm)[a]			
2007/2008	428	404	408
2008/2009	470	419	462
2009/2010	495	744	738
Mean temperature (ºC)[a]			
2007/2008	17.1	17.4	16.4
2008/2009	17.9	17.1	17.2
2009/2010	18.4	17.9	18.1
Cultivar	'Picual'	'Picudo'	'Lechín de Sevilla'
Age (years)	25	11	20
Plantation frame (m × m)	10 × 10	8 × 8	8 × 8
Irrigation	Drip irrigation	Rainfed	Rainfed
Tillage	No	No	No

[a] 1 October to 30 September.

Composite samples from the topsoil (0–15 cm) and the subsoil horizon with maximum root density (generally at a depth of 15–35 cm) were collected in each orchard. Each composite sample was the combination of four subsamples. Soil samples were air-dried and passed through a 2 mm sieve before analysis. Organic matter was determined by dichromate oxidation (Walkley and Black, 1934), the content in clay-sized particles by the pipette method following dispersion with Na hexametaphosphate, and cation exchange capacity (CEC) in 1 *M* NH_4OAc buffered at pH 7. Soil pH was measured in a 1:2.5 soil:water mixture and electrical conductivity (EC) in a 1:5 soil:water extract. The total $CaCO_3$ equivalent (CCE) was determined from the weight loss observed after treating 2 g of sample with 6 *M* HCl. The "active lime" or active calcium carbonate equivalent (ACCE) was determined with NH_4-oxalate according to Drouineau (1942). Citrate/bicarbonate/dithionite-extractable Fe (Fe_d) was determined according to Mehra and Jackson (1960) except that extraction was carried out at 25 ºC for 16 h. Acid oxalate-extractable Fe (Fe_{ox}) at pH 3 was determined according to Schwertmann (1964) with the exception that the soil:solution ratio was 1:200 in order to prevent significant pH changes through dissolution of soil carbonates during extraction (a modification proposed by Benítez et al., 2002). Diethylenetriaminepentacetic acid (DTPA)-extractable Fe (Fe_{DTPA}) was determined according to Lindsay and Norvell (1978).

EXPERIMENTAL DESIGN AND DESCRIPTION OF TREATMENTS

Thirty-six trees exhibiting Fe deficiency were selected in each orchard. The plantation frame was 10 m ×10 m in the Jaén orchard (2−3 trunk olive trees), and those in the Córdoba (one-trunk olive trees) (Chapter 8, Fig. A7) and Seville (2−3 trunk olive trees) orchards were 8 m × 8 m. In the Jaén orchard, a Latin square design was used with three treatments [control (no Fe fertilizer), siderite, and siderite prepared in the presence of phosphate (henceforward designated "P-siderite")], with three replications and four trees per plot. In the Córdoba and Seville orchards, the experimental design was also a Latin square with six treatments [control (no Fe), siderite, P-siderite, vivianite, vivianite plus humic acid, and Fe chelate (FeEDDHA)], with six replicates and one tree per plot. Treatments and doses differed in each orchard depending on the particular tree size (Table 2). Note that the dose of siderite was selected on the basis of the Fe contained in the dose of vivianite that proved to be effective to correct Fe chlorosis. In field experiments, 1 kg vivianite $tree^{-1}$ was effective to alleviate Fe chlorosis in <20 years old 'Hojiblanco'and 'Manzanillo' olives trees; in >20 years old

'Picual' trees, 2 kg vivianite tree^{-1} were needed because their root system extended to a greater soil volume (Rosado et al., 2002). The olive trees in the Córdoba field (Chapter 8, Fig. A7) were younger and smaller than those in the other two fields; thus, the siderite dose was halved.

Table 2. Fe and K doses for the different experimental orchards and treatments.

Treatment	Jaén		Córdoba		Seville	
	Fe	K	Fe	K	Fe	K
	(kg tree^{-1})		(kg tree^{-1})		(kg tree^{-1})	
Control	0	0.22	0	0.11	0	0.22
Siderite	0.4	0.56	0.2	0.28	0.4	0.56
P-Siderite	0.4	0.56	0.2	0.28	0.4	0.56
Vivianite	Not applied		0.2	0	0.4	0
Vivianite + humic	Not applied		0.2	0	0.4	0
FeEDDHA	Not applied		0.0015	0.11	0.0024	0.22

The experiment was started in spring 2008, when suspensions or solutions of the different fertilizers were prepared in situ and injected into the soil by using a T-shaped injector connected to a stirred 100-dm^3 tank of a petrol engine-powered injecting equipment. Injections were made at 10–20 points regularly distributed below the tree canopy at a depth of 25–35 cm (where active root density was high) when the soil moisture content was significantly below field capacity. Between 0.5 and 2 dm^3 of suspension was injected at each point. The pressure and the volume of suspension applied at each point were controlled with the injector. No more injections of any Fe fertilizer were applied to the soil during the experiment.

In the Córdoba and Seville experiments, siderite was synthesized by successively dissolving 4 kg of $FeSO_4 \cdot 7H_2O$ and 2 kg of K_2CO_3 in 100 dm^3 of water, which provided a pale brownish green suspension containing about 1.7 kg of siderite. In the Jaén orchard, 6 kg of $FeSO_4 \cdot 7H_2O$ and 3 kg of K_2CO_3 were used, and about 2.5 kg of siderite suspended in 100 dm^3 of water was obtained as a result. P-siderite was synthesized as described above, but adding 82 g (Córdoba and Seville) and 123 g (Jaén) of $(NH_4)H_2PO_4$ to the solution after $FeSO_4 \cdot 7H_2O$ was dissolved. In each field, the volume of the siderite suspension applied to the tree was that corresponding to the Fe dose shown in Table 2. Previous synthetic experiments following the above described procedures in the laboratory showed that (i) the solid phase in the suspension exhibited the typical X-ray diffraction pattern for siderite, and (ii) the

crystals of pure siderite were 300−500 nm long, 100 nm wide needles, whereas those of P-siderite were equidimensional to plate-like with an average size of about 100 nm (Sánchez-Alcalá et al., 2012). It should be noted that this nanometric size implies large specific surface areas and hence a high reactivity in the siderites. In all cases, samples of the siderite suspensions were taken immediately after preparation and kept in tightly closed polyethylene bottles that were taken to the laboratory and stored at 4 ºC. The pH and the electrical conductivity of the siderite suspensions were measured with a pH meter and a conductivimeter, respectively, within the next 24 h. A portion of each suspension was digested with 6 *M* HCl to determine total Fe (Fe_t) and Fe(II) in the resulting solution with the *o*-phenanthroline method (Olson and Ellis, 1982).

Vivianite was synthesized by successively dissolving 1.340 kg of $(NH_4)H_2PO_4$ and 4 kg of $FeSO_4 \cdot 7H_2O$ in 100 dm^3 of water in a continuously stirred tank in the injecting equipment. After a few minutes, a pale blue-green color suspension containing about 2.6 kg of vivianite was ready for use. For the “vivianite + humic acids” treatment, 0.660 dm^3 of a solution containing 15.5% w/w total humic extract (11% humic acids, 4% fulvic acids, 0.5% humic extract), with density 1.16 g cm^{-3} and pH 11 (from Solfer Húmicos®, Valencia, Spain) was added to the vivianite suspension.

Because application of the siderite suspension involved the addition of substantial amounts of K and some of the soils had available K levels only slightly above the critical values for this element, water (in the control treatment) and the FeEDDHA solution (in the FeEDDHA treatment) were enriched with K_2SO_4 at the rates shown in Table 2. This largely ensured eliminating soil K level as a confounding variable. However, no K was applied with the vivianite treatments to check whether the extra fertilization with K in the other treatment had a significant effect on the leaf K concentration. In addition, the local olive growers applied NPK fertilizer each year according to their usual practice.

PLANT MEASUREMENTS AND ANALYSES

The trunk perimeter 50 cm above the ground, which was used as an indicator of tree growth homogeneity during the observation period, was measured at the start (spring 2008) and end of the experiments. In the Jaén and Seville orchards, the mean perimeter of two or three trunks was recorded.

The youngest fully expanded leaf of 30 (Seville and Jaén) or 20 (Córdoba) randomly picked shoots per tree at about 1.60 m from the soil surface was taken before the start of the experiments and in the spring, summer and autumn of 2008, 2009 and

2010. The leaves were kept at 4–5 ºC for no more than two days before their chlorophyll content index was estimated with a Minolta 502 apparatus (Minolta Co., Ltd., Osaka, Japan). This apparatus provides readings of so-called “SPAD”, which is strongly correlated with the chlorophyll content per unit surface area (e.g. Benítez et al., 2002). Leaf mineral element concentrations were determined in one leaf from the central part of 30 (Jaén and Seville) or 20 (Córdoba) randomly selected shoots of the year taken every year in July. The leaves from each tree were combined, washed with distilled water and Tween 20, dried in a circulating air oven at 65 ºC for 72 h, and ground in an IKA A10 laboratory mill. Nutrient concentrations were determined after acid digestion (nitric−perchloric method; Zasoski and Burau, 1977). P in the solution was determined with the colorimetric method of Murphy and Riley (1962), K by flame emission, and Ca, Mg, Fe, Cu, Mn and Zn by atomic absorption spectrophotometry. The N concentration was determined by direct combustion of the plant material, using an EuroVector EA-3000 Elemental Analyzer (EuroVector SpA, Milan, Italy).

The olive yield from each tree was recorded annually. Olives were harvested in (i) December 2008, January 2010, and December 2010 in the ‘Picual’ orchard (Jaén); (ii) November 2008, November 2009 and January 2011 in the ‘Picudo’ orchard (Córdoba); and (iii) November 2008 and November 2010 in the ‘Lechín de Sevilla’ orchard (Seville). In order to check whether the different fertilizers affected oil concentration and quality, a representative sample of olives from each tree was taken and its oil content determined by nuclear magnetic resonance (NMR) spectroscopy (calibrated against Soxhlet extraction), and the total polyphenol concentration in oil [an indicator of the stability of olive oil against oxidation (Papadopoulos et al., 1991; Baldioli et al., 1996)], using the spectrophotometric method of Vázquez et al. (1973) and expressed as mg kg^{-1} oil. These two determinations were conducted in the Laboratorio Agroalimentario de Córdoba (Consejería de Agricultura y Pesca, Junta de Andalucía).

STATISTICAL ANALYSIS

Regression analyses were performed with Statistix 9.0 (Analytical Software, Tallahassee, FL, USA). Unless otherwise stated, the word ‘‘significant’’ is used here to indicate significance at the $P < 0.05$ level. Means were separated via the LSD test.

RESULTS

SOIL PROPERTIES

Table 3 shows selected properties of the soils in the experimental orchards. The organic carbon content ranged from 5 to 12 g kg^{-1}, the clay content from 220 to 403 g kg^{-1}, CCE from 673 and 789 g kg^{-1} and ACCE from 239 to 280 g kg^{-1}, values consistent with a pH range of 8.0–8.5. The salt content was low (EC in the 1:5 extract < 0.3 dS m^{-1}) and CEC ranged from 13.8 to 24.1 $cmol_c$ kg^{-1}. Ca was the dominant exchangeable cation as a result of the high carbonate content of the soil. Olsen P was, except for the subsoil sample of the Seville orchard, above 10 mg kg^{-1}, a value for which no P deficiency in olive has been observed in the region.

Fe_{DTPA} ranged from 2.4 to 5.3 mg kg^{-1}, with only the topsoil in the Córdoba orchard exceeding 4.5 mg kg^{-1} (the critical level proposed by Lindsay and Norvell, 1978). Fe_{ox} ranged from 0.25 to 0.40 g kg^{-1}, the highest value also corresponding to the topsoil in the Córdoba orchard. According to the work of Benítez et al., (2002), the Fe_{ox} critical level for 'Hojiblanca', 'Manzanilla' and 'Picual' olive cultivars is ~0.35 g kg^{-1}. Fe_{ox} in the subsoil, where most active roots accumulate, ranged from 0.25 to 0.29 g kg^{-1}, consistent with the strong Fe chlorosis-inducing capacity of these soils. The Fe_{ox}/Fe_d ratio, which is a measure of the poorly crystalline to total Fe oxides ratio, averaged at 0.17, which is a low value typical of soils in the Mediterranean regions (Torrent, 1995).

Table 3. Selected properties[a] of the soils in the three experimental orchards

Field	Depth	OC	Clay	CCE	ACCE	pH	EC (1:5)	CEC	Mg	Na	K	P	Fe_{DTPA}	Fe_{ox}	Fe_d
	cm	g kg^{-1}				1:2.5	dS m^{-1}	$cmol_{(c)}$ kg^{-1}				mg kg^{-1}		g kg^{-1}	
Jaén	0 − 15	10	248	743	239	8.5	0.12	18.9	1.9	1.0	0.6	23	3.6	0.38	2.4
	15 − 35	6	308	789	263	8.5	0.12	17.9	1.4	0.8	0.3	10	2.9	0.29	1.9
Córdoba	0 − 15	7	220	673	248	8.2	0.23	14.8	1.2	1.4	0.6	64	5.3	0.40	1.8
	15 − 35	5	231	677	253	8.0	0.27	13.8	1.3	1.6	0.4	19	2.4	0.25	1.4
Seville	0 −15	12	316	750	259	8.3	0.19	21.7	2.3	0.7	1.4	66	3.7	0.33	2.0
	15 − 35	5	403	773	280	8.5	0.14	24.1	2.1	0.9	0.4	6	2.7	0.26	1.6

[a] Abbreviations: OC, organic carbon; CCE, calcium carbonate equivalent; ACCE, active calcium carbonate equivalent (active lime); EC, electrical conductivity in the 1:5 extract; CEC, cation exchange capacity; P, Olsen P; Fe_{DTPA}, DTPA-extractable Fe; Fe_{ox}, oxalate-extractable Fe; Fe_d, citrate/bicarbonate/dithionite-extractable Fe.

PROPERTIES OF THE SIDERITE SUSPENSIONS

The pH of the siderite suspensions ranged from 6.8 and 7.1 and the electrical conductivity reached values of up to ~24 dS m^{-1}, values similar to those of the suspensions of vivianite. Although these values were high, precipitation of sulphate as gypsum in these highly calcareous soils was expected to lower the electrical conductivity of the suspension upon injection into the soil. However, the volume of soil where the siderite suspension was applied constituted only a small fraction of the soil volume occupied by roots, likely limiting the adverse effects of salinity. Indeed, Rosado (2001) observed no salinity effects on olive upon application of vivianite suspensions with electrical conductivity values up to 20 dS m^{-1} to some orchards.

The Fe(II)/total Fe ratio in suspension ranged from 0.7 to 0.95, consistent with the high susceptibility of siderite to oxidation. Experiments in the laboratory (Sánchez-Alcalá et al., 2012) showed the initial oxidation of siderite suspensions to result in the formation of small amounts of Fe oxides (goethite and lepidocrocite). When a portion of the siderite suspension fell on dry soil, its initial brownish green hue changed to a brownish yellow (for siderite) or orange brown (for P-siderite). These colours are consistent with the dominance of goethite and lepidocrocite in the oxidation products of siderite and P-siderite, respectively, and also with the results of previous laboratory experiments of oxidation of siderite in a calcareous medium (Sánchez-Alcalá et al., 2012).

OLIVE RESPONSES TO THE Fe FERTILIZER TREATMENTS

Figure 1 shows the time course of SPAD as a function of treatment in the trees of the three orchards. All SPAD data are shown in Chapter 8, Tables A15, A16 and A17. Some small, albeit significant differences in SPAD were found between treatments before the start of the experiments (specifically between the siderite and P-siderite treatments in Jaén, and between the FeEDDHA and the P-siderite treatments in Seville), even though the control trees exhibited SPAD values not significantly different from those under other treatments in both orchards. Control trees showed visual symptoms of Fe chlorosis throughout the sampling period, particularly in the Jaén and Córdoba orchards. However, SPAD for chlorotic leaves changed during the growing season, generally increasing from spring to autumn (partly a result of leaves becoming older and thicker).

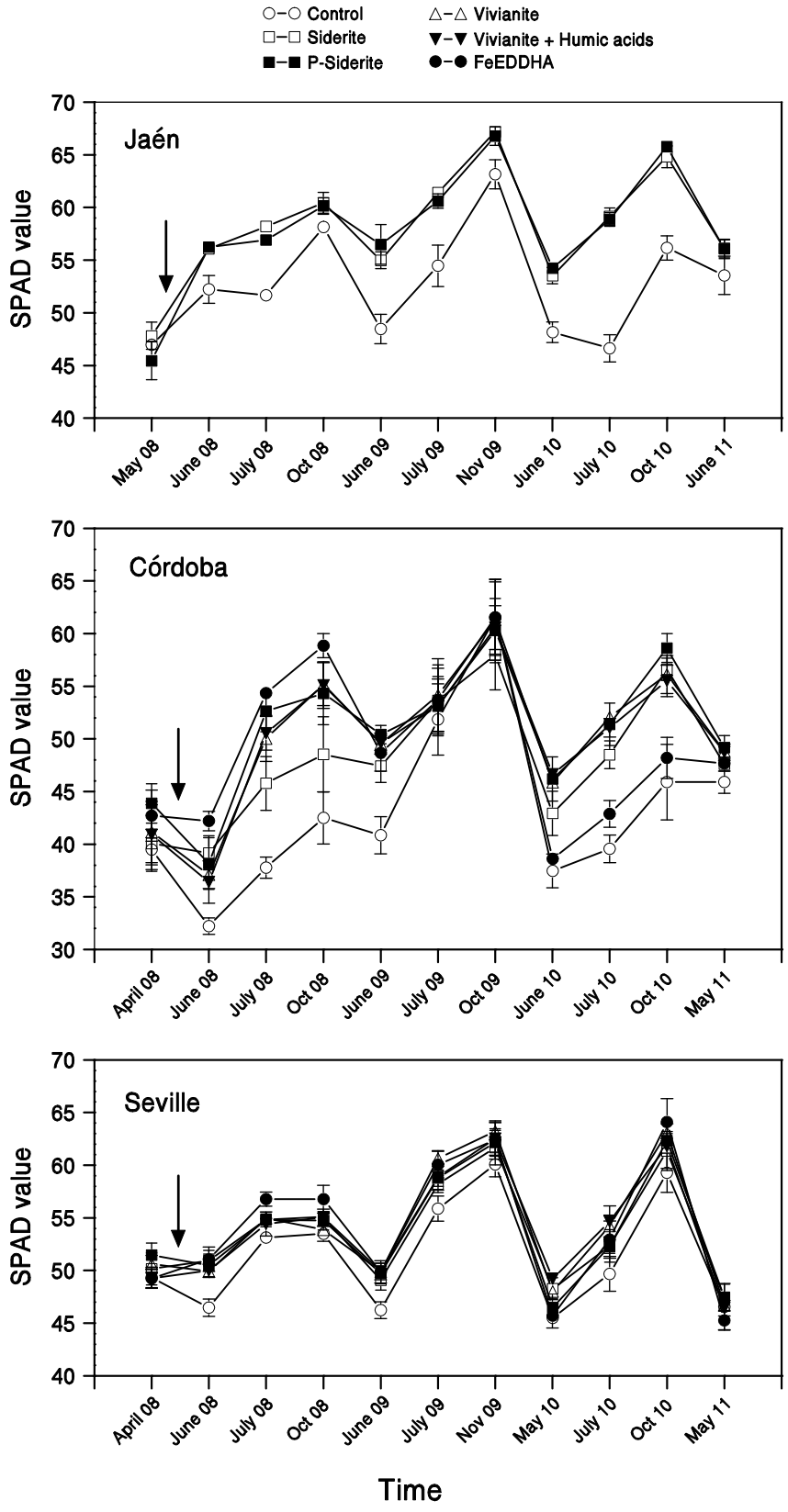

Figure 1. Time course of the SPAD value (mean ± standard error) for the different Fe treatments in the three olive orchards. The arrow indicates the time when the Fe fertilizer was applied.

The SPAD values for the trees fertilized with siderite or P-siderite were significantly higher than those for the control trees at nearly all times over the three years. The times in which differences were not significant (October 2008 in Jaén, and October 2008, November 2009 and October 2010 in Seville) did not match periods of fast vegetative growth. There were no significant differences in SPAD between the trees fertilized with siderite and P-siderite except for the Córdoba orchard, where the latter fertilizer proved more effective than the former.

Generally, no significant differences in SPAD were observed between the vivianite, vivianite + humic acids and P-siderite treatments. However, the vivianite treatments were significantly more effective than the siderite treatment at several sampling times in the Córdoba orchard. In contrast to the results of de Santiago et al. (2008b), who grew white lupin in pots, the addition of humic substances failed to significantly increase the effectiveness of vivianite in preventing leaf chlorosis in olive trees grown in highly calcareous soils.

In the first year (2008), the SPAD values for the trees supplied with FeEDDHA (in the Córdoba and Seville orchards) were significantly higher than those for the trees treated with vivianite or siderite. However, this trend was largely reversed after June 2009 in the Córdoba orchard, where SPAD for the FeEDDHA-supplied trees was only slightly higher than for the control trees after June 2010. In the Seville orchard, FeEDDHA had a residual effect in 2009 and 2010, but weaker than that observed in 2008. In summary, the residual value of FeEDDHA was lower than that of either siderite or vivianite, as found in previous studies (Rosado et al., 2002; Sánchez-Alcalá et al., 2012).

As can be seen in Table 4, the mean leaf weight for the control trees was, except for the Seville orchard in May 2011, lower than that for the Fe-fertilized trees, although the differences were only significant in July and November 2009 in Jaén; October 2009, October 2010 and May 2011 in Córdoba; and October 2010 in Seville. Reduced leaf growth and internodal length are commonplace in olive trees showing severe leaf chlorosis and result in extreme cases in what local olive growers call a “mousy shoot”. At the end of the experiment, however, no significant differences between treatments in terms of relative increase in trunk perimeter were observed in any of the experimental orchards (Chapter 8, Tables A18 and A19).

Table 4. Leaf weight (mg leaf^{-1}).

Treatments	Jaén			Córdoba			Seville			
	July 2009	November 2009	June 2011	October 2009	October 2010	May 2011	November 2009	July 2010	October 2010	May 2011
Control	68 b	70 b	124 a	89 c	93 c	120 b	119 a	100 a	82 b	108 a
Siderite	78 a	82 a	135 a	106 a	120 a	140 a	125 a	105 a	93 a	90 a
P-Siderite	78 a	83 a	137 a	96 bc	119 ab	142 a	120 a	105 a	90 ab	90 a
Vivianite				104 ab	116 ab	141 a	127 a	104 a	90 ab	87 a
Vivianite + humic acids				101 ab	121 a	140 a	127 a	104 a	89 ab	97 a
FeEDDHA				106 a	107 b	147 a	120 a	103 a	91 a	105 a

Different letters in the same column indicate significant differences ($P < 0.05$, LSD test) between treatments.

Table 5 shows the yield (olive and oil weight) for the years in which olives were harvested. The yields were much higher in Jaén than in the other two provinces. This can be partly ascribed to the older age (and larger canopy size) of the trees in Jaén and to the fact that alternate bearing strongly affected the trees in the other two provinces. Except for the siderite treatment in Seville, fertilization with Fe resulted in increased cumulative yield and oil weight. However, the increase was only significant in Jaén for the siderite treatment in 2010 and in Córdoba for the FeEDDHA treatment in 2009. It should be noted that the effect of Fe fertilizers on yield was never significant when yields were very low. Fertilization with Fe had no significant effect on the polyphenol content of the oil in any of the orchards (Chapter 8, Tables A20, A21 and A22).

Table 5. Olive and oil yields (kg tree^{-1})[a].

	2008		2009		2010		Cumulative	
	Olive	Oil	Olive	Oil	Olive	Oil	Olive	Oil
Jaén								
Control	42.8 ± 2.4 b	10.6 ± 0.5 a	71.8 ± 4.3 a	16.9 ± 0.3 a	37.8 ± 4.3 b	9.1 ± 1.3 b	152.5 ± 7.5 b	36.6 ± 1.1 b
Siderite	49.2 ± 3.0 a	11.3 ± 0.4 a	80.4 ± 4.3 a	17.6 ± 0.3 a	57.8 ± 4.3 a	13.9 ± 0.7 a	187.4 ± 14.0 a	42.8 ± 0.9 a
Siderite+P	42.9 ± 2.7 b	10.1 ± 0.5 a	75.9 ± 4.0 a	16.5 ± 0.5 a	54.6 ± 4.0 a	13.2 ± 0.6 a	173.5 ± 10.5 ab	39.8 ± 1.3 ab
Córdoba								
Control	2.5 ± 0.9 a	0.5 ± 0.2 a	15.8 ± 1.8 b	4.1 ± 0.4 b	8.8 ± 2.1 a		27.1 ± 1.9 a	
Siderite	3.8 ± 1.8 a	1.5 ± 0.5 a	19.0 ± 1.4 ab	4.7 ± 0.4 ab	10.6 ± 3.3 a		33.5 ± 5.1 a	
Siderite+P	1.1 ± 0.4 a	0.6 ± 0.1 a	20.1 ± 2.0 ab	4.8 ± 0.5 ab	5.4 ± 1.5 a		26.6 ± 1.2 a	
Vivianite	1.5 ± 1.1 a	1.2 ± 0.3 a	19.1 ± 3.2 ab	4.6 ± 0.7 ab	5.6 ± 3.3 a		26.2 ± 3.5 a	
Vivianite+humic acids	1.3 ± 0.6 a	0.5 ± 0.2 a	20.0 ± 1.2 ab	4.9 ± 0.3 ab	4.4 ± 1.7 a		25.7 ± 2.5 a	
Fe chelate	3.7 ± 2.6 a	1.4 ± 0.6 a	22.8 ± 1.7 a	5.6 ± 0.4 a	5.4 ± 1.8 a		32.0 ± 3.4 a	
Seville			Not harvested					
Control	0.7 ± 0.3 a				20.0 ± 4.4 a	4.3 ± 1.3 a	20.8 ± 4.6 a	
Siderite	1.6 ± 0.2 a				13.9 ± 2.5 a	3.1 ± 0.8 a	14.3 ± 2.7 a	
Siderite+P	0.4 ± 0.2 a				20.3 ± 4.4 a	5.0 ± 1.1 a	20.7 ± 4.5 a	
Vivianite	0.1 ± 0.1 a				18.0 ± 2.0 a	4.4 ± 0.5 a	18.1 ± 2.1 a	
Vivianite+humic acids	4.0 ± 4.0 a				20.8 ± 7.1 a	4.7 ± 1.6 a	24.8 ± 10.4 a	
Fe chelate	2.2 ± 1.9 a				22.8 ± 9.3 a	4.1 ± 2.3 a	24.9 ± 11.2 a	

[a] Oil was not measured when yield was < 5 kg olive tree^{-1}.

Table 6 shows the mean mineral element concentrations in July leaves for each year and orchard. Some significant differences between treatments were recorded for some elements but they were inconsistent over time and are not reported in detail. All concentrations were always above the critical levels compiled by Fernández-Escobar (2008). In particular, the values of K in leaf were clearly above the critical level even for the vivianite treatments, which, in contrast to other treatments, involved no fertilization with K; this means that the effectiveness of these treatments was not impaired by a low availability of this element.

The concentration of Mn in the control trees in the second and third year of the experiment was significantly higher than that in trees fertilized with Fe because Fe-deficient conditions increase the outflow of protons and the reducing capacity of root cells, thereby contributing to the reduction and dissolution not only of Fe, but also of Mn (Moraghan and Freeman, 1978; Marschner, 1995). In fact, negative linear relationships were found between the July 2010 SPAD value and the leaf Mn concentration in the Córdoba (y = 60.3 − 1.1x; R^2 = 0.49***) and Seville (y = 66.0 − 0.3x; R^2 = 0.29***) orchards. A positive linear relationship was also found between SPAD and the leaf Fe concentration in July 2008 (y = 18.6 + 0.9x; R^2 = 0.40***). However, leaf Fe concentrations are known to be poor indicators of Fe deficiency because this element can accumulate in leaves even under conditions of deficiency (a fact known as the “chlorosis paradox") (Morales et al., 1998; Römheld, 2000).

Table 6. Mineral element concentrations[a] in July leaves.

	Jaén		Córdoba		Seville	
Element	2008	2010	2008	2010	2008	2010
N (g kg^{-1})	18.0 ± 0.5	14.0 ± 0.3	17.0 ± 0.3	14.0 ± 0.3	15.0 ± 0.1	14.0 ± 0.4
P (g kg^{-1})	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	0.8 ± 0.0
K (g kg^{-1})	6.3 ± 0.3	9.8 ± 0.3	8.4 ± 0.5	10.0 ± 0.3	8.3 ± 0.2	9.9 ± 0.2
Ca (g kg^{-1})	12.0 ± 0.1	6.0 ± 0.1	12.0 ± 0.3	18.0 ± 0.5	25.0 ± 0.4	9.0 ± 0.3
Mg (g kg^{-1})	1.8 ± 0.0	1.7 ± 0.0	1.4 ± 0.0	1.2 ± 0.0	1.5 ± 0.0	1.9 ± 0.0
Fe (mg kg^{-1})	30.0 ± 0.5	24.0 ± 0.8	34.0 ± 1.0	27.0 ± 2.1	34.0 ± 0.8	69.0 ± 1.0
Cu (mg kg^{-1})	6.0 ± 0.1	7.0 ± 0.2	7.0 ± 0.8	6.0 ± 0.2	162.0 ± 5.4	35.0 ± 0.9
Mn (mg kg^{-1})	31.0 ± 0.0	20.0 ± 2.0	21.0 ± 0.4	12.0 ± 1.4	33.0 ± 0.9	51.0 ± 1.8
Zn (mg kg^{-1})	15.0 ± 0.2	18.0 ± 0.3	16.0 ± 0.3	17.0 ± 0.3	16.0 ± 0.5	14.0 ± 0.2

[a] Mean ± standard error.

GENERAL DISCUSSION AND CONCLUSIONS

A single application of siderite proved to be effective in alleviating Fe chlorosis in 'Picual', 'Picudo' and 'Lechín de Sevilla' olive trees over three growing seasons. The effectiveness of siderite was similar to that of vivianite, which, as stated before, has proved to be a successful slow-release Fe fertilizer for different crops. As for vivianite, the long-term effectiveness of siderite is attributed to the nanometric size of the Fe oxide particles that result from its alteration (Sánchez-Alcalá et al., 2012). In contrast, in the long term siderite was superior to one single application of FeEDDHA at the beginning of the experiment. This can be partly ascribed to the latter fertilizer being soluble and thus easily washed from the soil.

Our results do not allow one to assess whether the two types of siderite differ in their effectiveness against Fe chlorosis, even though laboratory experiments and field observations indicate that the products of the oxidation and incongruent dissolution of siderites and P-siderites differ (basically, the lepidocrocite/goethite ratio is higher for P-siderite). It can be speculated in this respect that, after the suspension is injected into and mixed with the soil, pure siderite particles adsorb phosphate from the soil, which can alter them being similarly to P-siderite particles.

The increase in leaf weight caused by the different Fe fertilizers (Table 4) was concomitant with a leaf greening effect (Figure 1). However, the increase was only partly translated into increases in yield. One possible reason is that olive is a perennial species strongly affected by alternate bearing, which results in complex changes in yield. On the other hand, one can expect a delayed response to fertilizers in trees having a large mass.

Siderite suspensions have some advantages as Fe fertilizers. They can be readily prepared in a stirred tank in the field and injected with a variety of devices. Also, they are nontoxic and the simple method of preparation proposed in this paper work involves applying K to the soil, which increases their fertilizing value with no apparent adverse salinity effects. Above all, their capacity to prevent Fe chlorosis seems to last several years, which may suppress the need for yearly additions of Fe fertilizers.

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CHAPTER 6

Conclusions

Las conclusiones más importantes de esta tesis sobre la biodisponibilidad de hierro en los suelos calcáreos subrayan:

1. La población microbiana indígena de los suelos calcáreos estudiados fue capaz en condiciones anaeróbicas de incubación de reducir el Fe(III) con igual eficacia que bien conocidas bacterias reductoras de Fe. El grado de reducción estuvo condicionado, entre otros factores, por el carbono orgánico disuelto y la concentración total de óxidos de Fe del suelo.

2. Las concentraciones de Fe soluble al oxalato, citrato/ascorbato y DTPA, así como del Fe(II) soluble al ácido clorhídrico se incrementaron tras un período de siete semanas de incubación anaeróbica de una suspensión del suelo. Ensayos de maceta demostraron que la saturación previa del suelo fue eficaz para aliviar la clorosis férrica en cacahuete y garbanzo

3. La aireación del suelo después del período de saturación causó pocos cambios en las formas más lábiles y biodisponibles de Fe. Posteriores ciclos de humectación y desecación o un nuevo período de saturación del suelo tampoco tuvieron efectos significativos en dichas formas.

4. Siderita (carbonato ferroso) de tamaño nanométrico y alta reactividad fue preparada con facilidad en el laboratorio e in situ en el campo a partir de disoluciones de sulfato ferroso y carbonato potásico. La siderita preparada en presencia de pequeñas cantidades de fosfato demostró tener mayor reactividad que la siderita pura.

5. Inyecciones al suelo de distintas suspensiones de sideritas sintéticas demostraron ser eficaces para prevenir o reducir la incidencia de la clorosis férrica en distintas plantas herbáceas cultivadas en maceta y olivo en campo sin efectos adversos por salinidad u otras causas. Esta efectividad se atribuye al hecho de que las partículas de óxido de Fe resultantes de la oxidación y disolución incongruente de la siderita son poco cristalinas y de alta superficie específica y solubilidad. No se observaron diferencias de efectividad entre las sideritas puras y las preparadas en presencia de fosfato, quizá porque las primeras adsorben fosfato al entrar en contacto con la solución del suelo.

6. La efectividad de la siderita demostró ser prolongada, lo que unido a la ausencia de efectos ambientales negativos, facilidad de preparación y coste moderado la hacen una buena alternativa a otros fertilizantes de Fe.

CHAPTER 7

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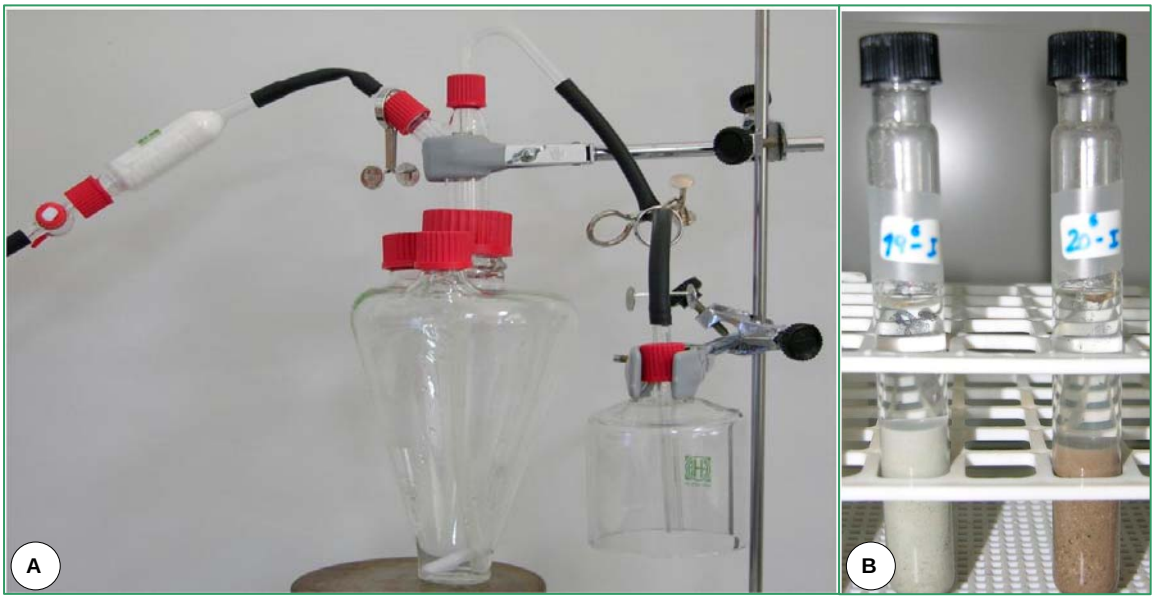
CHAPTER 8

Annexes

ANNEX 1

Chapter 2



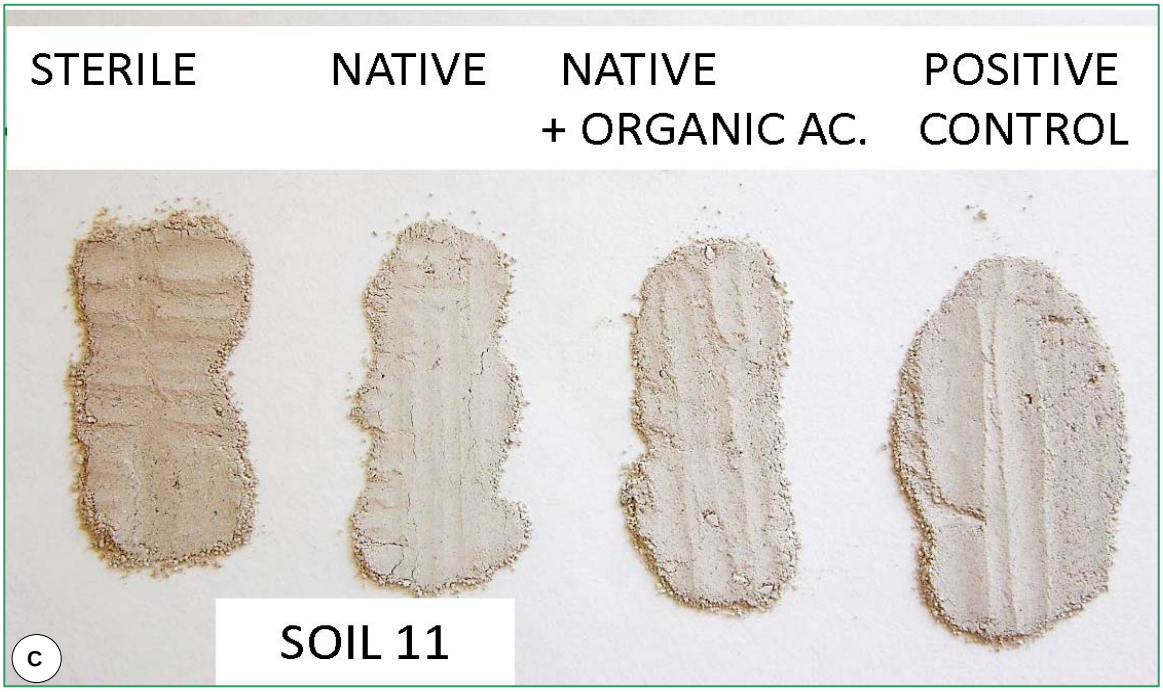


Figure A1. Widdel and Bak flask to prepare media under anoxic conditions (A); tubes of soil slurry experiments (Soils 19 and 20) (B); example of color changes induced by microbial reduction and treatments (Soil 11) (C).

Table A1. Fe(II) in solution after six weeks, and HCl-extratable Fe(II), oxalate-extractable Fe, and citrate/ascorbate-extractable Fe in the freeze-dried soil suspension after seven weeks of anaerobic incubation under different conditions for the 24 studied soils. Different letters in the column corresponding to one soil and one Fe form indicate significant differences according to the Kruskal−Wallis nonparametric test ($P < 0.05$).

	SOIL																								
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Fe(II) solution mg kg^{-1}																								
S	1.26 b	2 a	1.2 a	1.9 a	1.34 a	2.22 a	1.6 a	2.12 a	1.93 a	1.86 a	0.72 a	1.27 a	1.33 a	1.23 a	0.81 a	1.13 a	1.89 a	1.9 a	1.2 a	1.5 a	1.28 a	0.95 a	1.51 a	2.54 a
N	0.05 a	14.95 b	4.2 b	9.2 a	0.86 a	22.4 b	1.6 a	17.7 b	57.0 b	40.1 b	64.4 b	24.1 b	36.6 b	40 b	22.8 b	5 b	45.2 b	0.78 b	1 a	15.2 b	2.72 a	26.2 b	10.7 b	82.5 b
OA	663 c	857 c	449 c	720 b	384 b	339 d	293 b	316 c	1038 c	268 d	838 c	205 c	1231 d	1034 d	1015 c	600 c	783 c	660 c	30 b	214 c	153 c	284 c	166 c	1390 c
PC	1022 d	1556 d	845 d	926 c	911 c	226 c	317 b	391 d	1046 c	108 c	1010 d	323 d	933 c	915 c	1070 c	834 d	691 c	930 d	86 c	299 d	256 d	387 d	183 d	1625 d
$Fe(II)_{HCl}$ mg kg^{-1}																								
S	754 a	516 a	141 a	310 a	226 a	316 a	342 a	358 a	298 a	348 a	589 a	585 a	359 a	281 a	223 a	341 a	915 a	438 a	216 a	740 a	256 a	747 a	278 a	324 a
N	817 a	1091 b	778 b	1061 b	578 b	1047 b	1328 b	1130 b	1600 b	1259 b	1375 b	1302 b	1201 b	1158 b	802 b	896 b	1895 b	961 b	735 b	1482 b	908 b	2249 b	805 b	1511 b
OA	2260 b	2708 c	997 c	1375 b	1043 c	2238 c	1531 c	1412 c	2900 c	2956 c	2687 c	2286 d	1956 c	1726 c	1114 c	2331 c	4370 d	3462 c	1515 d	3191 c	2496 d	3089 d	2593 d	2312 d
PC	2310 b	3326 c	1013 c	1471 b	1111 c	2136 c	1684 c	1537 c	2832 c	2994 c	2542 c	1960 c	1894 c	1861 c	1087 c	2191 c	3437 c	3942 c	1222 c	2983 c	2162 c	2794 c	1961 c	1978 c
Fe_{ox} mg kg^{-1}																								
S	184 a	431 a	198 a	230 a	110 a	380 a	286 a	468 a	282 a	479 a	220 a	304 a	186 a	216 a	174 a	313 a	359 a	724 a	262 a	384 a	483 a	255 a	382 a	466 a
N	192 a	483 a	342 b	289 a	169 b	647 b	362 b	628 b	710 b	902 b	451 b	496 b	391 b	470 b	248 b	477 b	707 b	846 b	323 b	606 ab	569 b	579 d	522 b	1125 b
OA	250 b	811 b	556 c	507 b	305 c	877 c	451 c	717 b	1078 d	1096 c	600 c	538 b	637 c	620 c	351 c	795 c	854 d	1135 c	358 b	749 b	770 c	370 b	682 d	1287 c
PC	332 c	1037 c	615 c	555 b	386 c	798 c	490 c	786 c	890 c	1177 d	604 c	539 b	557 c	679 c	350 c	849 c	765 c	1179 c	317 b	738 b	793 c	475 c	599 c	1242 c
Fe_{ca} mg kg^{-1}																								
S	859 a	789 a	345 a	444 a	270 a	673 a	1012 b	718 ab	626 a	981 a	780 a	907 a	724 a	495 a	312 a	839 a	1164 a	1136 a	915 a	763 a	1006 ab	933 a	836 a	914 a
N	735 a	834 a	304 a	673 ab	224 a	659 a	854 ab	540 a	1094 ab	1145 b	1783 b	1420 b	1243 b	684 ab	1031 b	820 a	1309 ab	1070 a	823 a	1456 a	721 a	1417 b	1116 b	1462 b
OA	690 a	975 ab	610 b	686 b	336 a	800 a	689 a	576 ab	1797 b	965 a	1226 b	867 a	951 a	747 b	546 b	1093 a	1319 bc	1431 a	698 a	1356 a	667 a	836 a	665 a	1698 b
PC	806 a	1460 b	1038 c	946 c	559 b	839 a	842 ab	1106 b	1531 b	1473 b	1420 b	1024 ab	887 a	1169 c	794 b	1391 b	1498 c	1354 a	908 a	1279 a	1277 b	1401 b	1062 b	1561 b

Treatments S: sterile; N: native; OA: organic acids; PC: positive control

Table A2. *L* a* b** colour coordinates of the freeze-dried soil suspension after 7 weeks of anaerobic incubation under different conditions for the 24 studied soils. Different letters in the column corresponding to one soil and one Fe form indicate significant differences according to the Kruskal−Wallis nonparametric test ($P < 0.05$).

	SOIL																								
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
*L**																								
S	71 a	72 a	61 a	68 a	73 a	77 b	83 b	72 b	65 a	72 ab	80 ab	69 a	76 a	69 a	69 a	80 b	75 b	78 b	85 b	66 a	65 a	71 b	62 a	65 b
N	75 b	72 a	63 a	68 a	75 b	77 b	85 b	75 b	70 b	73 b	80 ab	70 a	75 a	69 a	70 ab	82 b	76 b	80 c	85 b	70 b	68 b	76 d	63 a	66 a
OA	73 b	73 a	63 a	67 a	74 ab	77 a	84 b	74 b	69 b	74 b	82 c	74 b	79 b	71 b	72 b	81 b	76 b	80 c	86 a	70 b	68 b	73 c	64 b	69 b
PC	73 b	73 a	62 a	67 a	73 ab	75 a	81 a	71 a	67 b	70 a	79 a	70 a	76 a	69 a	69 a	76 a	73 a	77 a	80 c	67 a	65 a	70 a	62 a	62 a
*a**																								
S	1.51 b	1 c	10.5 a	3.32 b	1.09 b	0.89 c	0.22 c	1.18 a	6.13 b	0.84 c	0.66 c	1.1 b	2.34 c	4.12 c	6.9 c	-0.23 c	1.02 c	-0.4 c	-0.31 b	4.96 a	1.25 c	1.64 d	1.83 c	6 c
N	1.02 a	0.67 b	9.68 a	3.41 b	0.69 a	0.52 b	0.03 b	0.87 a	3.49 a	0.49 b	0.35 b	0.82 a	1.52 b	2.99 b	5.74 b	-0.49 a	0.52 a	-0.51 b	-0.58 a	3.85 a	0.97 b	0.84 a	1.49 a	3.92 b
OA	0.58 a	0.25 a	9.29 a	2.34 a	0.4 a	0.46 a	-0.07 a	0.74 a	3.66 a	0.29 a	0.25 a	0.67 a	0.58 a	2.06 a	4.14 a	-0.51 a	0.5 a	-0.7 a	-0.59 a	3.21 a	0.84 a	1.07 b	1.47 a	3.2 a
PC	0.62 a	0.2 c	9.17 a	2.28 a	0.38 a	0.53 b	-0.05 c	0.88 a	4 a	0.42 b	0.41 b	0.8 a	1 b	2.13 a	4.84 a	-0.39 b	0.76 b	-0.61 a	-0.6 a	3.53 a	1.01 b	1.21 c	1.56 b	3.94 b
*b**																								
S	22.4 b	20.3 a	24.9 a	19.5 c	20.7 b	11.3 d	11.7 b	11.7 b	20.3 c	11.6 c	12.1 c	11 c	17.1 d	19.8 c	26.3 c	15 c	14.1 c	16.3 c	11.4 b	18.7 b	11.3 c	12.4 c	12.2 c	22.2 c
N	20.8 a	20.5 b	24.3 a	18.3 b	19.6 a	10.2 b	10.4 ab	10.9 ab	17.1 bc	10.5 ab	10.7 ab	9.7 b	15.5 c	18 b	24.3 b	13.2 b	11.9 a	15.9 bc	10.5 ab	16.2 ab	10 b	9.4 a	10.9 ab	17.8 b
OA	20.4 a	17.8 a	23.1 a	17.1 a	18.1 a	9.7 a	9.7 a	9.9 a	15.6 a	9.6 a	9.3 a	8.6 a	12.3 a	15.8 a	21.4 a	11.7 a	11.5 a	14.2 a	9.9 a	14.8 a	9.2 a	10.3 b	10.8 a	16.4 a
PC	20.4 a	18 a	22.8 a	17.9 b	19 a	10.6 c	11.2 ab	10.8 a	16.6 b	10.5 b	10.3 b	9.3 b	14 b	16.1 a	23 a	13 b	12.8 b	15.5 b	12.1 c	15.4 ab	10.1 b	10.7 b	11.1 b	17.5 b
a/b**																								
S	0.07 b	0.05 c	0.42 b	0.17 b	0.05 c	0.08 d	0.02 c	0.10 b	0.30 b	0.07 c	0.05 c	0.10 b	0.14 d	0.21 c	0.26 c	-0.02 c	0.07 c	-0.03 d	-0.03 c	0.27 c	0.11 c	0.13 d	0.15 c	0.27 c
N	0.05 a	0.03 b	0.40 a	0.19 b	0.04 b	0.05 c	0.00 b	0.08 a	0.21 a	0.05 b	0.03 a	0.08 a	0.10 c	0.17 b	0.24 b	-0.04 a	0.04 a	-0.03 c	-0.06 ab	0.24 bc	0.10 ab	0.09 a	0.14 a	0.22 b
OA	0.03 a	0.01 a	0.40 a	0.14 a	0.02 ab	0.05 b	-0.01 a	0.07 a	0.23 a	0.03 b	0.03 a	0.08 a	0.05 a	0.13 a	0.19 a	-0.04 a	0.04 a	-0.05 a	-0.06 a	0.22 a	0.09 a	0.10 b	0.14 a	0.20 a
PC	0.03 a	0.01 a	0.40 a	0.13 a	0.02 a	0.05 a	-0.01 ab	0.08 a	0.24 a	0.04 a	0.04 b	0.09 a	0.07 b	0.13 a	0.21 a	-0.03 b	0.06 b	-0.04 b	-0.05 b	0.23 bc	0.10 b	0.11 c	0.14 b	0.23 b

Treatments S: sterile; N: native; OA: organic acids; PC: positive control

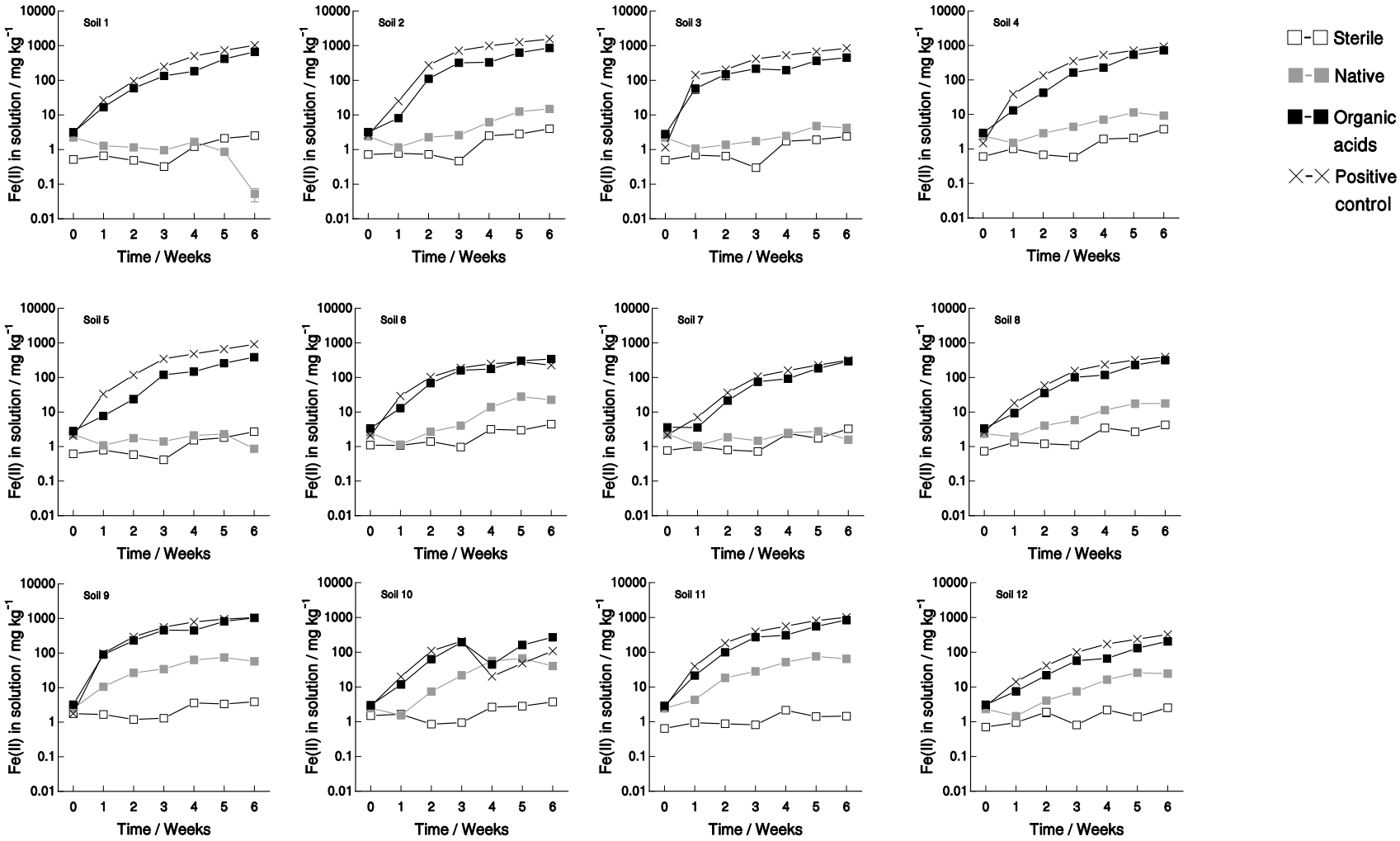


Figure A2. (A) Time course of Fe(II) in solution in the different anaerobic incubation treatments for Soils 1- 12. The mean ± standard error is shown.

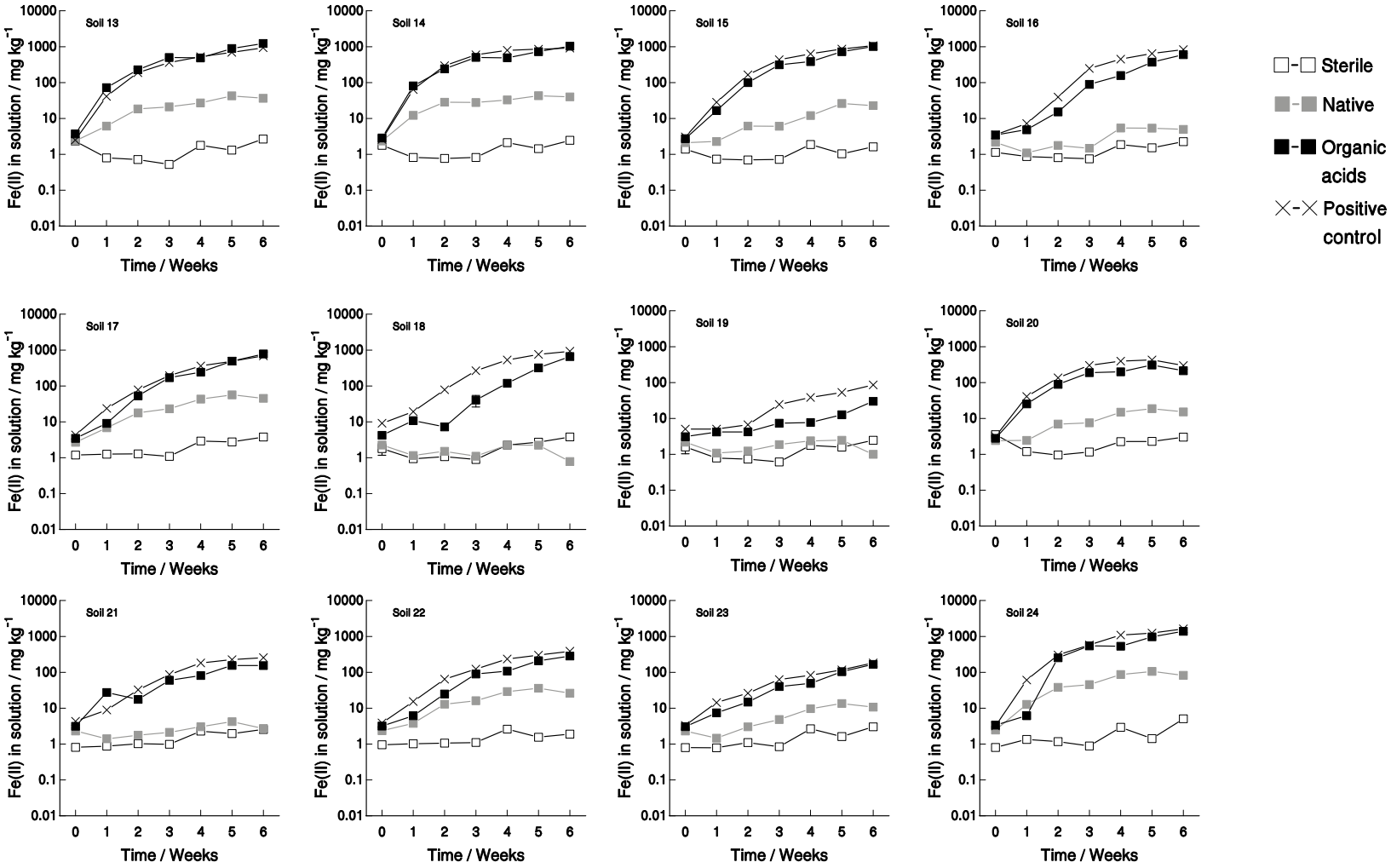


Figure A2. (B) Time course of Fe(II) in solution in the different anaerobic incubation treatments for Soils 13-24. The mean ± standard error is shown.

ANNEX 2

Chapter 3

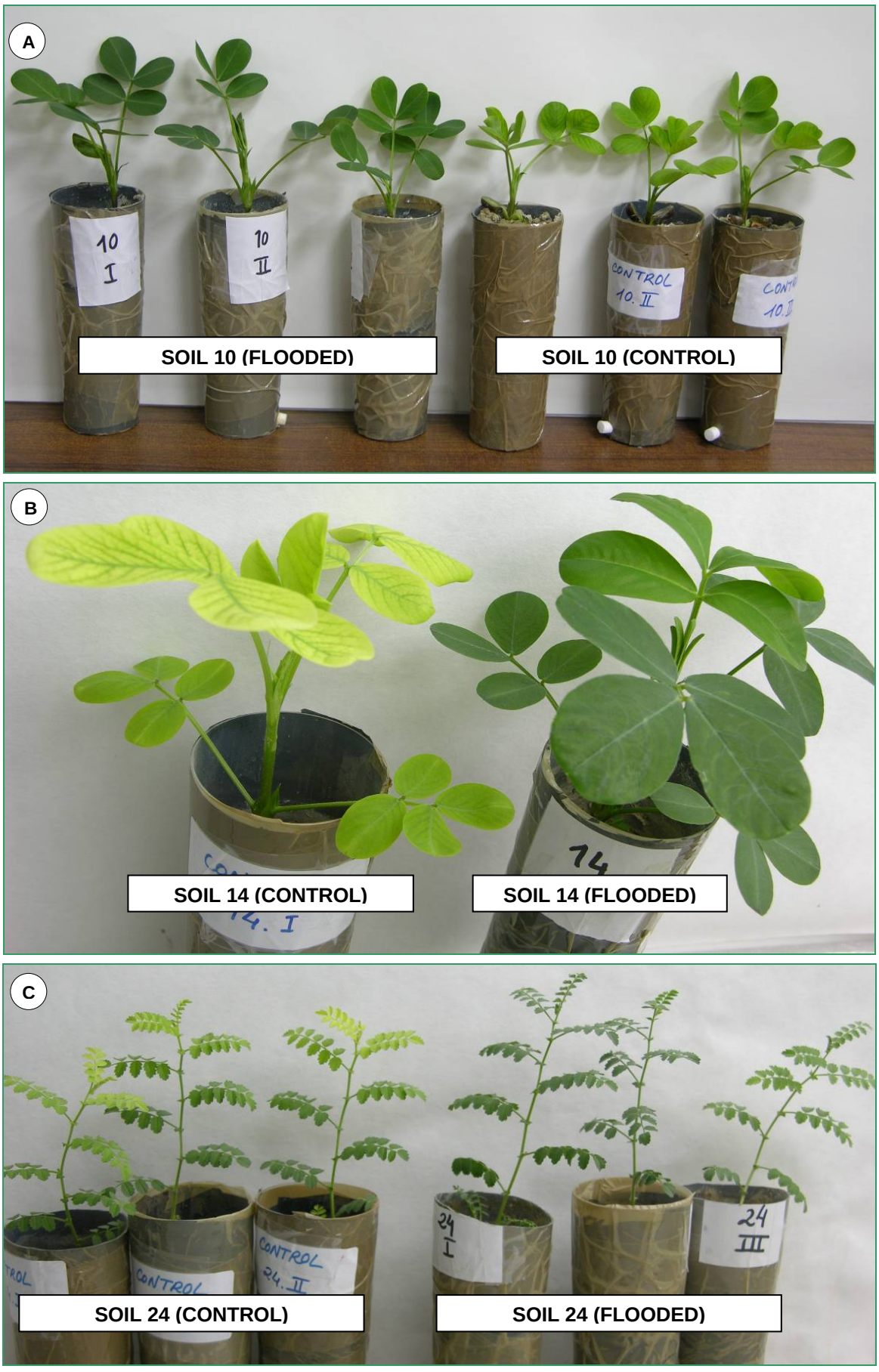


Figure A3. A) Peanut plants grown in soil 10, B) Peanut plants grown in soil 14, C) Chickpea plants grown in soil 24.

Table A3. Analysis of Fe forms in soils from pots.

Treatment	Soil	Fe_{DTPA}	Fe_{ox}	F_{eca}	$Fe(II)_{HCl}$	Total Fe_{HCl}
		mg kg^{-1}	g kg^{-1}			
Flooded	1	3.79	0.25	0.69	0.23	0.49
Flooded	2	27.45	0.74	1.08	0.36	0.79
Flooded	3	18.29	0.39	0.54	0.13	0.42
Flooded	4	19.79	0.37	0.64	0.13	0.32
Flooded	5	6.60	0.23	0.46	0.13	0.26
Flooded	6	24.06	0.62	0.82	0.22	0.77
Flooded	7	6.79	0.31	0.58	0.16	0.39
Flooded	8	20.65	0.69	1.05	0.17	0.68
Flooded	9	55.12	0.90	1.49	0.30	0.93
Flooded	10	25.90	0.69	1.22	0.20	0.79
Flooded	11	23.12	0.48	0.88	0.33	0.58
Flooded	12	27.83	0.61	1.03	0.30	0.60
Flooded	13	22.34	0.47	0.77	0.23	0.47
Flooded	14	26.55	0.62	0.89	0.17	0.56
Flooded	15	23.67	0.40	0.57	0.12	0.31
Flooded	16	10.69	0.44	0.69	0.30	0.54
Flooded	17	31.65	0.74	1.33	0.52	1.05
Flooded	18	4.76	0.70	1.18	0.14	0.77
Flooded	19	4.92	0.31	0.50	0.14	0.37
Flooded	20	43.71	0.81	1.14	0.33	0.77
Flooded	21	13.94	0.63	1.01	0.16	0.69
Flooded	22	25.67	0.51	1.15	0.45	0.74
Flooded	23	20.39	0.61	1.08	0.17	0.69
Flooded	24	56.12	1.21	1.53	0.25	0.98
Control	1	1.90	0.26	0.74	0.16	0.40
Control	2	2.84	0.44	0.90	0.20	0.48
Control	3	1.33	0.25	0.37	0.04	0.13
Control	4	1.67	0.25	0.83	0.06	0.18
Control	5	1.18	0.20	0.79	0.06	0.15
Control	6	3.44	0.46	0.72	0.11	0.42
Control	7	2.30	0.29	0.57	0.09	0.27
Control	8	3.34	0.46	0.59	0.08	0.35
Control	9	2.57	0.30	0.58	0.10	0.20
Control	10	2.18	0.47	0.63	0.09	0.51
Control	11	1.62	0.23	0.64	0.26	0.36
Control	12	1.86	0.29	0.80	0.23	0.42
Control	13	1.60	0.25	0.40	0.09	0.23
Control	14	1.76	0.30	0.42	0.06	0.23
Control	15	1.18	0.18	0.38	0.07	0.17
Control	16	2.46	0.37	0.68	0.16	0.43
Control	17	2.55	0.39	0.89	0.38	0.66
Control	18	3.13	0.70	1.35	0.09	0.80
Control	19	1.62	0.27	0.35	0.08	0.34
Control	20	1.58	0.26	0.54	0.15	0.30
Control	21	2.72	0.54	0.67	0.10	0.57
Control	22	1.58	0.28	0.68	0.30	0.47
Control	23	2.14	0.40	1.18	0.10	0.53
Control	24	3.96	0.45	0.64	0.11	0.26

Table A4. Concentrations of Fe_{ox} and Fe_{ca} in Wetting−Drying Experiment

Soil	Reoxidized soil samples				Control soil samples			
	Steril	Native	Organic acids	Positive control	Steril	Native	Organic acids	Positive control
	Fe_{ox} (mg kg^{-1})				Fe_{ox} (mg kg^{-1})			
1	187	161	184	333	161	172	220	259
2	450	447	687	916	396	518	735	810
3	187	235	352	435	217	252	394	431
4	181	332	385	436	191	332	417	467
5	144	131	228	323	139	151	235	287
6	406	556	659	707	470	564	725	723
7	296	318	344	439	318	330	320	422
8	476	571	602	720	454	588	579	676
9	279	536	786	718	295	521	797	869
10	475	831	893	1030	437	779	980	895
11	206	394	441	499	191	372	522	476
12	312	454	429	540	276	484	445	432
13	170	422	511	457	215	321	533	567
14	214	561	508	581	254	403	555	489
15	134	292	297	314	132	201	300	426
16	369	445	621	684	295	382	661	645
17	367	620	654	587	298	693	686	694
18	735	762	972	1031	735	739	970	731
19	269	243	261	288	303	225	320	445
20	349	468	549	681	376	462	617	607
21	475	517	577	679	402	500	634	446
22	258	460	323	453	240	475	407	638
23	412	506	467	512	390	544	529	1018
24	440	972	1029	1080	400	998	1115	1299
Mean	325 d	468 c	532 b	602 a	316 d	459 c	571 b	615 a
	Fe_{ca} (mg kg^{-1})				Fe_{ca} (mg kg^{-1})			
1	633	662	541	716	544	581	529	688
2	795	770	1043	1473	856	917	946	
3	253	281	481	751	207	296	490	720
4	366	603	566	739	344	582	591	690
5	302	296	311	545	270	239	415	767
6	754	676	846	889	549	747	783	1433
7	421	576	496	608	454	1221	456	919
8	534	662	716	762	679	871	622	818
9	481	807	1219	1284	324	814	1063	1309
10	725	1073	1087	1399	734	1073	1129	2301
11	540	775	905	1078	513	794	829	1060
12	603	667	654	833	590	728	728	876
13	358	533	530	825	274	564	694	771
14	458	717	591	881	452	576	659	885
15	285	384	510	526	211	408	451	569
16	601	625	459	940	641	434	826	1140
17	913	1227	770	1323	843	1258	1169	1330
18	1115	1089	898	1504	956	994	1315	1360
19	371	392	208	449	411	483	393	560
20	642	802	713	1181	642	713	929	1116
21	696	770	523	897	597	812	768	1134
22	705	928	544	919	580	901	825	1051
23	740	823	467	917	757	882	705	896
24	618	1285	1014	1446	676	1169	1274	1435
Mean	580 c	726 b	670 bc	954 a	546 c	752 b	775 b	1036 a

Table A5. Concentrations of $Fe(II)_{HCl}$ and total Fe_{HCl} in Wetting−Drying Experiment

Soil	Reoxidized soil samples				Control soil samples			
	Steril	Native	Organic acids	Positive control	Steril	Native	Organic acids	Positive control
	$Fe(II)_{HCl}$ (mg kg^{-1})				**$Fe(II)_{HCl}$** (mg kg^{-1})			
1	248	257	375	466	299	304	489	400
2	241	292	393	655	306	344	707	490
3	47	88	136	367	91	136	365	288
4	85	161	202	376	117	201	374	308
5	82	110	155	257	92	123	176	221
6	168	208	297	472	196	315	644	388
7	118	143	203	319	149	243	310	324
8	107	145	207	409	167	238	420	299
9	107	198	412	605	98	336	868	518
10	194	281	388	659	176	400	907	479
11	332	379	437	635	294	464	680	591
12	292	326	355	479	257	389	491	437
13	135	188	306	438	98	278	594	374
14	123	176	280	468	94	278	525	437
15	71	110	188	271	56	154	325	349
16	228	256	356	458	186	314	699	477
17	509	596	721	750	441	650	986	701
18	303	266	463	586	223	334	994	540
19	188	213	278	309	121	246	356	296
20	291	352	520	658	243	436	801	656
21	298	276	395	508	174	340	689	489
22	454	480	487	599	334	540	664	589
23	363	375	443	536	215	443	649	599
24	199	240	339	573	93	338	854	802
Mean	216 d	255 c	347 b	494 a	188 d	327 c	607 a	460 b
	Total Fe_{HCl} (mg kg^{-1})				**Total Fe_{HCl}** (mg kg^{-1})			
1	408	404	546	520	391	378	482	501
2	498	518	850	904	442	475	725	906
3	127	212	392	465	110	172	379	496
4	163	382	425	429	143	274	380	472
5	133	206	278	351	119	137	185	298
6	414	521	706	656	379	527	653	642
7	278	292	429	382	243	279	323	334
8	333	436	621	510	290	458	476	450
9	182	573	948	928	146	594	906	1099
10	493	845	1030	1034	431	864	979	1074
11	410	664	755	778	389	642	684	934
12	376	533	564	575	358	531	525	595
13	185	416	690	632	153	395	623	641
14	187	436	633	715	168	441	549	772
15	97	208	330	331	97	187	340	367
16	434	458	809	660	383	459	723	696
17	684	1019	1211	942	668	932	1022	899
18	785	738	1358	950	724	724	1129	892
19	338	346	506	340	315	323	362	324
20	380	556	926	893	428	587	846	896
21	580	600	871	738	553	610	753	698
22	511	718	717	759	461	710	693	649
23	590	678	801	701	520	694	693	848
24	258	762	1018	953	231	794	1062	977
Mean	368 d	522 c	726 a	673 b	339 d	508 c	646 b	686 a

Table A6. SPAD values at 10, 17, 24 and 31 days after transplanting and dry matter weight at harvest, for the first crop (peanut). Mean ± standard error (n=3).

Treatment	Soil	SPAD10	SPAD17	SPAD24	SPAD31	Chlorophyll / area µg/cm^2	Dry matter g
flooded	1	26.9 ± 4.6	27.4 ± 1.1	31.7 ± 3.0	39.3 ± 2.1	17 ± 1.1	0.624 ± 0.063
control	1	30.4 ± 1.7	20.4 ± 2.0	21.7 ± 2.7	33.1 ± 1.8	14 ± 0.9	0.664 ± 0.052
flooded	2	35.4 ± 3.3	38.1 ± 4.6	36.5 ± 1.9	44.4 ± 2.6	20 ± 2.2	0.679 ± 0.114
control	2	32.6 ± 1.8	25.2 ± 1.3	26.1 ± 1.2	34.7 ± 0.8	15 ± 0.7	0.769 ± 0.031
flooded	3	34.3 ± 1.9	42.8 ± 2.4	38.8 ± 3.1	46.6 ± 3.8	22 ± 3.3	0.731 ± 0.107
control	3	28.3 ± 0.2	21.5 ± 1.8	11.1 ± 1.5	12.4 ± 1.9	4 ± 0.7	0.404 ± 0.064
flooded	4	29.7 ± 1.8	32.7 ± 6.8	30.0 ± 4.0	39.1 ± 4.0	17 ± 2.8	0.761 ± 0.184
control	4	16.2 ± 5.9	11.9 ± 2.0	11.0 ± 2.0	16.0 ± 4.3	6 ± 1.6	0.520 ± 0.086
flooded	5	31.8 ± 4.9	35.0 ± 2.9	32.7 ± 3.1	41.9 ± 2.6	19 ± 2.0	0.597 ± 0.070
control	5	28.2 ± 2.6	16.9 ± 2.3	11.9 ± 1.9	13.8 ± 2.4	4 ± 0.8	0.492 ± 0.033
flooded	6	31.1 ± 3.0	38.0 ± 4.1	35.9 ± 4.6	45.9 ± 4.6	24 ± 3.7	0.668 ± 0.046
control	6	28.6 ± 4.0	21.6 ± 2.7	21.8 ± 0.2	33.1 ± 1.0	13 ± 0.5	0.424 ± 0.060
flooded	7	28.5 ± 5.0	31.9 ± 3.4	28.8 ± 2.5	34.2 ± 2.5	15 ± 2.9	0.409 ± 0.044
control	7	31.0 ± 5.7	20.5 ± 3.3	17.5 ± 1.7	18.6 ± 2.4	6 ± 0.9	0.277 ± 0.043
flooded	8	30.6 ± 0.3	42.2 ± 1.1	37.6 ± 2.5	48.6 ± 2.2	26 ± 2.0	0.623 ± 0.059
control	8	30.9 ± 4.6	24.6 ± 3.0	19.3 ± 1.0	25.2 ± 0.2	9 ± 0.2	0.462 ± 0.034
flooded	9	35.5 ± 1.2	44.4 ± 0.8	40.5 ± 0.2	50.1 ± 0.6	28 ± 0.9	0.742 ± 0.010
control	9	32.4 ± 1.7	23.8 ± 1.0	17.7 ± 1.7	18.4 ± 2.0	6 ± 0.7	0.492 ± 0.096
flooded	10	32.7 ± 1.7	40.4 ± 2.1	32.0 ± 3.4	44.5 ± 1.9	24 ± 1.3	0.615 ± 0.101
control	10	28.8 ± 1.3	21.3 ± 1.1	25.6 ± 4.3	29.5 ± 5.2	12 ± 3.3	0.458 ± 0.017
flooded	11	29.3 ± 1.3	36.2 ± 2.7	32.5 ± 1.8	42.2 ± 2.0	20 ± 1.9	0.618 ± 0.036
control	11	30.6 ± 0.8	22.6 ± 1.9	13.3 ± 2.0	13.5 ± 1.8	5 ± 1.1	0.538 ± 0.029
flooded	12	32.6 ± 8.9	35.8 ± 10.4	33.5 ± 6.1	44.7 ± 4.6	23 ± 3.3	0.585 ± 0.092
control	12	27.9 ± 2.5	16.8 ± 1.5	13.2 ± 1.9	15.1 ± 3.5	5 ± 1.5	0.348 ± 0.045
flooded	13	31.5 ± 2.4	35.8 ± 4.7	38.1 ± 0.4	44.4 ± 3.0	22 ± 2.6	0.469 ± 0.115
control	13	48.7 ± 3.5	37.0 ± 1.5	22.0 ± 0.0	22.0 ± 0.0	6 ± 0.2	0.311 ± 0.015
flooded	14	33.0 ± 3.7	37.7 ± 6.5	35.7 ± 0.8	48.9 ± 0.4	26 ± 0.2	0.673 ± 0.025
control	14	28.9 ± 4.3	14.7 ± 2.6	5.5 ± 0.9	7.4 ± 1.0	2 ± 0.5	0.314 ± 0.026
flooded	15	37.3 ± 1.8	44.6 ± 0.5	38.5 ± 1.9	46.0 ± 0.9	21 ± 0.9	0.774 ± 0.052
control	15	35.7 ± 0.4	24.2 ± 1.8	13.9 ± 2.2	13.0 ± 2.4	4 ± 0.8	0.698 ± 0.052
flooded	16	33.7 ± 0.7	40.0 ± 3.6	36.6 ± 3.7	46.2 ± 2.4	25 ± 1.8	0.777 ± 0.131
control	16	32.8 ± 3.8	24.6 ± 1.7	29.0 ± 1.7	31.9 ± 1.2	12 ± 0.9	0.531 ± 0.073
flooded	17	34.0 ± 1.1	37.3 ± 4.6	30.5 ± 3.5	42.7 ± 1.7	21 ± 1.7	0.511 ± 0.054
control	17	33.0 ± 1.9	23.6 ± 4.2	24.7 ± 1.1	30.4 ± 3.3	12 ± 1.3	0.516 ± 0.159
flooded	18	34.0 ± 2.6	44.2 ± 0.6	41.0 ± 2.8	49.9 ± 1.4	27 ± 1.3	0.631 ± 0.048
control	18	36.9 ± 4.8	30.7 ± 1.8	32.1 ± 4.2	36.2 ± 1.3	19 ± 1.2	0.444 ± 0.095
flooded	19	30.8 ± 0.5	36.5 ± 0.6	33.4 ± 1.4	42.3 ± 1.8	20 ± 1.2	0.809 ± 0.133
control	19	25.0 ± 4.0	16.6 ± 3.6	16.8 ± 2.8	24.6 ± 3.6	9 ± 1.4	0.485 ± 0.025
flooded	20	31.4 ± 2.0	39.4 ± 2.4	38.5 ± 4.0	48.6 ± 2.3	23 ± 1.5	0.702 ± 0.072
control	20	34.2 ± 1.0	20.0 ± 0.7	16.6 ± 1.4	18.5 ± 2.2	6 ± 0.8	0.486 ± 0.071
flooded	21	31.7 ± 1.5	37.6 ± 2.9	32.1 ± 1.3	44.0 ± 0.3	22 ± 0.3	0.576 ± 0.031
control	21	23.9 ± 6.0	19.5 ± 1.8	21.9 ± 3.3	34.0 ± 2.4	15 ± 1.7	0.434 ± 0.045
flooded	22	28.3 ± 1.6	34.1 ± 1.9	39.9 ± 2.7	49.5 ± 2.1	28 ± 1.6	0.489 ± 0.057
control	22	33.6 ± 1.9	21.2 ± 3.2	15.4 ± 1.6	19.0 ± 3.3	7 ± 1.5	0.395 ± 0.018
flooded	23	33.7 ± 4.6	36.5 ± 3.4	33.0 ± 4.1	44.3 ± 0.9	22 ± 0.4	0.756 ± 0.040
control	23	24.7 ± 5.5	19.2 ± 2.0	21.8 ± 0.5	30.4 ± 1.3	12 ± 0.8	0.549 ± 0.047
flooded	24	36.8 ± 0.1	40.8 ± 1.5	38.4 ± 0.8	50.2 ± 0.7	27 ± 0.2	0.679 ± 0.044
control	24	35.0 ± 1.7	23.5 ± 1.9	14.5 ± 1.0	18.8 ± 1.4	6 ± 0.5	0.705 ± 0.149

Table A7. SPAD values at 10, 17 and 24 days after transplanting and dry matter weight at harvest, for the second crop (chickpea). Mean ± standard error (n=3).

Treatment	Soil	SPAD10	SPAD17	SPAD24	Chlorophyll / area	Dry matter
					µg/cm^2	g
flooded	1	33.0 ± 4.1	31.0 ± 0.9	24.5 ± 3.3	26 ± 3.6	0.119 ± 0.010
control	1	32.4 ± 0.91	33.77 ± 2.4	30.3 ± 1.7	39 ± 2.2	0.123 ± 0.003
flooded	2	38.3 ± 3.9	47.6 ± 3.4	46.4 ± 0.9	82 ± 3.3	0.123 ± 0.003
control	2	33.9 ± 1.7	42.37 ± 4.9	40.0 ± 3.4	58 ± 6.3	0.113 ± 0.024
flooded	3	30.9 ± 5.1	26.6 ± 3.2	25.6 ± 0.0	31 ± 0.3	0.106 ± 0.007
control	3	28.3 ± 2.36	27.35 ± 3.7	27.5 ± 0.8	32 ± 4.1	0.080 ± 0.011
flooded	4	38.0 ± 1.3	45.6 ± 1.6	42.3 ± 2.2	64 ± 1.6	0.117 ± 0.025
control	4	33.5 ± 1.39	26.37 ± 4.2	23.1 ± 2.7	25 ± 7.4	0.121 ± 0.001
flooded	5	31.7 ± 8.5	42.0 ± 7.8	37.1 ± 6.5	45 ± 13.1	0.094 ± 0.011
control	5	34.0 ± 4.35	21.73 ± 6.7	14.4 ± 3.7	16 ± 4.9	0.114 ± 0.012
flooded	6	41.1 ± 3.8	42.0 ± 4.4	43.0 ± 4.0	63 ± 7.4	0.118 ± 0.007
control	6	34.1 ± 1.59	44.37 ± 6.5	36.6 ± 6.3	47 ± 12.5	0.132 ± 0.019
flooded	7	43.0 ± 0.0	26.2 ± 0.0	17.6 ± 0.0	14 ± 0.0	0.121 ± 0.000
control	7	34.2 ± 2.7	25.3 ± 6.0	13.6 ± 1.3	16 ± 3.1	0.113 ± 0.004
flooded	8	36.2 ± 5.0	41.7 ± 4.0	39.9 ± 4.3	48 ± 7.7	0.126 ± 0.017
control	8	42.5 ± 4.23	36.7 ± 7.4	27.5 ± 5.4	35 ± 8.8	0.119 ± 0.004
flooded	9	36.4 ± 1.8	31.5 ± 4.1	39.5 ± 1.8	56 ± 3.4	0.095 ± 0.008
control	9	33.4 ± 2.08	32.0 ± 4.9	24.3 ± 8.0	36 ± 12.7	0.121 ± 0.006
flooded	10	43.8 ± 2.6	46.7 ± 0.4	46.8 ± 1.0	61 ± 0.5	0.129 ± 0.013
control	10	37.5 ± 4.41	32.7 ± 7.5	28.2 ± 4.1	28 ± 6.4	0.112 ± 0.007
flooded	11	38.3 ± 2.1	34.8 ± 1.2	25.3 ± 2.8	32 ± 4.1	0.145 ± 0.016
control	11	35.8 ± 4.27	19.3 ± 11.7	12.1 ± 6.5	13 ± 7.2	0.116 ± 0.011
flooded	12	41.7 ± 3.0	37.5 ± 0.4	31.6 ± 0.8	41 ± 1.3	0.104 ± 0.004
control	12	38.3 ± 3.07	24.23 ± 7.3	13.9 ± 3.9	15 ± 6.9	0.105 ± 0.006
flooded	13	36.2 ± 6.2	31.8 ± 3.4	23.0 ± 3.5	24 ± 4.2	0.122 ± 0.002
control	13	34.1 ± 3.91	31.93 ± 1.3	28.2 ± 4.3	35 ± 1.3	0.070 ± 0.006
flooded	14	42.1 ± 4.8	37.9 ± 3.8	39.5 ± 4.0	51 ± 9.1	0.120 ± 0.005
control	14	35.7 ± 3.53	26.63 ± 8.9	12.5 ± 2.1	15 ± 3.7	0.124 ± 0.015
flooded	15	42.0 ± 7.2	28.1 ± 1.6	20.4 ± 1.6	19 ± 2.3	0.137 ± 0.009
control	15	33.4 ± 5.89	20.23 ± 3.0	22.4 ± 5.4	21 ± 3.8	0.114 ± 0.014
flooded	16	39.8 ± 4.1	42.9 ± 6.9	34.5 ± 5.7	42 ± 12.3	0.109 ± 0.003
control	16	36.1 ± 4.08	33.1 ± 9.6	23.2 ± 8.7	31 ± 9.6	0.087 ± 0.016
flooded	17	43.1 ± 3.2	43.8 ± 4.0	38.1 ± 1.4	55 ± 2.6	0.121 ± 0.012
control	17	41.3 ± 1.27	28.2 ± 6.1	20.2 ± 4.7	21 ± 4.2	0.091 ± 0.005
flooded	18	42.0 ± 2.6	50.4 ± 0.5	46.8 ± 2.9	82 ± 5.8	0.113 ± 0.021
control	18	47.3 ± 4.81	41.6 ± 7.5	41.4 ± 2.6	69 ± 10.1	0.095 ± 0.012
flooded	19	31.9 ± 4.1	29.1 ± 7.8	20.6 ± 5.3	27 ± 8.4	0.124 ± 0.011
control	19	25.8 ± 8.07	15.57 ± 0.3	10.9 ± 1.6	11 ± 1.0	0.106 ± 0.014
flooded	20	29.8 ± 2.3	35.3 ± 3.7	31.0 ± 1.0	38 ± 3.2	0.108 ± 0.005
control	20	26.8 ± 4.71	14.27 ± 4.8	12.2 ± 3.3	11 ± 2.2	0.105 ± 0.010
flooded	21	39.5 ± 0.2	51.8 ± 1.0	42.1 ± 3.4	71 ± 15.0	0.140 ± 0.005
control	21	31.8 ± 1.6	28.27 ± 2.0	23.1 ± 6.1	30 ± 8.5	0.112 ± 0.012
flooded	22	39.6 ± 6.9	35.7 ± 6.2	28.1 ± 5.3	37 ± 12.3	0.090 ± 0.002
control	22	38.0 ± 1.99	37.0 ± 4.7	23.4 ± 1.5	23 ± 1.1	0.098 ± 0.006
flooded	23	34.5 ± 0.3	34.5 ± 6.2	36.9 ± 5.1	54 ± 13.5	0.118 ± 0.010
control	23	37.5 ± 1.54	42.8 ± 4.2	39.9 ± 1.4	50 ± 14.0	0.128 ± 0.004
flooded	24	38.8 ± 3.8	46.8 ± 5.0	40.7 ± 2.4	64 ± 5.6	0.122 ± 0.021
control	24	39.5 ± 4.38	22.17 ± 3.1	10.3 ± 2.4	14 ± 3.3	0.103 ± 0.008

Table A8. Mineral concentrations at harvest for the first crop (peanut). Mean ± standard error (n=3).

Treatment	Soil	P	K	Ca	Mg	Fe	Cu	Mn	Zn
		g kg^{-1}				mg kg^{-1}			
Flooded	1	3.9 ± 0.6	13.5 ± 0.6	25.0 ± 2.0	3.7 ± 0.2	71 ± 8	12 ± 2	68 ± 3	47 ± 2
Flooded	2	3.2 ± 0.4	12.1 ± 3.5	25.9 ± 2.9	3.3 ± 0.4	63 ± 11	21 ± 4	62 ± 8	46 ± 4
Flooded	3	3.7 ± 0.7	12.0 ± 3.5	25.5 ± 2.9	3.2 ± 0.3	61 ± 4	11 ± 3	61 ± 5	36 ± 5
Flooded	4	3.6 ± 0.3	11.8 ± 5.0	29.0 ± 3.5	3.3 ± 0.5	89 ± 12	13 ± 1	59 ± 9	31 ± 3
Flooded	5	4.0 ± 0.2	13.9 ± 1.2	26.3 ± 4.0	4.2 ± 0.4	132 ± 10	13 ± 2	68 ± 5	39 ± 1
Flooded	6	2.9 ± 0.5	13.3 ± 1.2	21.8 ± 1.6	3.2 ± 0.2	116 ± 12	13 ± 3	64 ± 4	38 ± 3
Flooded	7	4.1 ± 0.1	22.2 ± 1.1	37.5 ± 2.6	4.7 ± 0.2	140 ± 6	18 ± 2	66 ± 6	48 ± 3
Flooded	8	3.8 ± 0.3	14.5 ± 1.8	26.9 ± 0.7	3.3 ± 0.0	60 ± 3	12 ± 2	69 ± 3	30 ± 2
Flooded	9	3.1 ± 0.1	12.2 ± 0.5	20.7 ± 2.1	3.0 ± 0.0	53 ± 1	16 ± 2	66 ± 2	43 ± 2
Flooded	10	3.1 ± 0.2	18.0 ± 4.1	29.3 ± 2.7	4.1 ± 0.8	70 ± 20	11 ± 3	59 ± 4	43 ± 2
Flooded	11	3.4 ± 0.1	12.6 ± 2.0	30.5 ± 4.7	3.7 ± 0.2	122 ± 4	16 ± 1	67 ± 3	30 ± 2
Flooded	12	3.8 ± 0.4	15.8 ± 7.0	26.5 ± 5.5	3.5 ± 0.6	110 ± 9	13 ± 1	68 ± 7	43 ± 4
Flooded	13	4.2 ± 0.6	19.6 ± 7.3	23.9 ± 4.2	5.0 ± 1.6	134 ± 14	18 ± 4	72 ± 9	44 ± 5
Flooded	14	3.8 ± 0.6	14.5 ± 0.5	26.4 ± 2.6	3.2 ± 0.5	115 ± 4	15 ± 1	68 ± 0	38 ± 3
Flooded	15	2.9 ± 0.3	12.6 ± 0.5	23.9 ± 1.6	2.9 ± 0.1	53 ± 3	13 ± 2	57 ± 1	45 ± 1
Flooded	16	3.1 ± 0.3	13.2 ± 1.3	24.5 ± 2.7	3.0 ± 0.4	53 ± 5	19 ± 7	58 ± 6	47 ± 3
Flooded	17	3.8 ± 0.4	13.9 ± 0.8	29.2 ± 1.7	3.6 ± 0.8	113 ± 10	17 ± 1	74 ± 6	37 ± 2
Flooded	18	2.9 ± 0.1	11.8 ± 0.3	22.5 ± 1.2	3.5 ± 0.2	56 ± 9	14 ± 0	64 ± 4	47 ± 1
Flooded	19	3.1 ± 0.6	11.5 ± 1.2	25.2 ± 2.5	3.0 ± 0.3	47 ± 2	17 ± 7	61 ± 7	40 ± 0
Flooded	20	3.3 ± 0.5	11.4 ± 0.1	22.5 ± 2.9	3.0 ± 0.1	51 ± 0	13 ± 2	65 ± 3	32 ± 5
Flooded	21	3.8 ± 0.1	15.6 ± 0.9	21.6 ± 0.4	3.1 ± 0.1	67 ± 15	13 ± 1	72 ± 2	47 ± 2
Flooded	22	3.6 ± 0.1	14.1 ± 0.6	23.7 ± 1.1	3.5 ± 0.2	98 ± 3	18 ± 1	65 ± 4	49 ± 2
Flooded	23	3.3 ± 0.1	12.5 ± 0.7	25.8 ± 0.9	3.0 ± 0.1	67 ± 1	15 ± 1	60 ± 2	48 ± 2
Flooded	24	2.9 ± 0.1	12.1 ± 0.8	23.2 ± 3.6	3.2 ± 0.1	58 ± 1	20 ± 2	62 ± 1	48 ± 1
Control	1	2.9 ± 0.1	13.7 ± 0.7	19.3 ± 0.6	3.0 ± 0.1	59 ± 6	15 ± 2	68 ± 4	32 ± 1
Control	2	3.0 ± 0.2	12.1 ± 0.2	18.2 ± 0.6	2.8 ± 0.0	70 ± 1	17 ± 1	65 ± 1	33 ± 2
Control	3	3.5 ± 0.2	15.8 ± 2.2	22.5 ± 2.0	3.1 ± 0.4	61 ± 6	13 ± 1	96 ± 7	39 ± 1
Control	4	3.0 ± 0.2	12.6 ± 1.1	23.8 ± 1.3	2.6 ± 0.3	65 ± 2	13 ± 0	82 ± 6	35 ± 3
Control	5	3.1 ± 0.1	15.0 ± 1.7	21.8 ± 1.1	2.5 ± 0.2	60 ± 2	14 ± 0	73 ± 2	38 ± 1
Control	6	3.0 ± 0.0	12.9 ± 0.8	24.8 ± 0.8	2.9 ± 0.3	66 ± 4	15 ± 0	78 ± 6	39 ± 3
Control	7	2.9 ± 0.2	15.2 ± 1.9	24.1 ± 1.5	3.9 ± 0.5	74 ± 8	15 ± 2	67 ± 1	48 ± 4
Control	8	3.1 ± 0.1	13.3 ± 0.6	22.8 ± 2.1	2.9 ± 0.2	82 ± 11	14 ± 1	75 ± 1	34 ± 1
Control	9	3.1 ± 0.1	12.6 ± 0.8	22.4 ± 1.7	2.7 ± 0.4	78 ± 12	14 ± 1	74 ± 6	34 ± 3
Control	10	3.0 ± 0.0	11.7 ± 0.4	23.9 ± 0.3	2.6 ± 0.1	67 ± 7	13 ± 0	70 ± 4	35 ± 1
Control	11	3.2 ± 0.1	11.5 ± 0.2	24.3 ± 1.1	2.4 ± 0.1	56 ± 7	14 ± 0	69 ± 2	36 ± 1
Control	12	3.2 ± 0.0	12.1 ± 0.8	22.4 ± 1.3	3.1 ± 0.4	62 ± 4	13 ± 1	72 ± 9	38 ± 2
Control	13	3.1 ± 0.2	15.4 ± 1.0	23.2 ± 0.6	3.4 ± 0.1	67 ± 2	14 ± 0	79 ± 3	42 ± 1
Control	14	2.7 ± 0.2	12.3 ± 0.7	24.9 ± 0.9	3.5 ± 0.2	74 ± 7	14 ± 0	84 ± 2	40 ± 2
Control	15	3.0 ± 0.0	11.3 ± 0.2	19.4 ± 0.8	2.6 ± 0.1	66 ± 6	15 ± 1	61 ± 3	32 ± 0
Control	16	3.2 ± 0.2	12.2 ± 0.8	22.8 ± 1.9	2.5 ± 0.2	65 ± 6	17 ± 1	68 ± 7	38 ± 0
Control	17	3.4 ± 0.5	13.6 ± 0.6	19.4 ± 1.7	2.7 ± 0.4	78 ± 10	18 ± 0	72 ± 10	40 ± 2
Control	18	3.1 ± 0.3	12.1 ± 0.9	23.0 ± 2.6	2.9 ± 0.4	75 ± 8	20 ± 1	63 ± 1	39 ± 3
Control	19	3.1 ± 0.2	14.8 ± 0.4	23.9 ± 0.7	2.5 ± 0.0	65 ± 2	19 ± 1	69 ± 2	34 ± 1
Control	20	3.0 ± 0.1	13.0 ± 1.0	23.8 ± 1.2	2.7 ± 0.3	66 ± 1	19 ± 1	70 ± 2	40 ± 1
Control	21	3.1 ± 0.2	14.5 ± 1.3	23.6 ± 0.8	2.7 ± 0.2	65 ± 3	15 ± 2	78 ± 5	43 ± 2
Control	22	3.0 ± 0.4	13.3 ± 0.1	24.9 ± 0.1	2.9 ± 0.1	72 ± 2	19 ± 1	66 ± 1	43 ± 2
Control	23	3.1 ± 0.2	14.4 ± 0.5	18.7 ± 1.6	2.4 ± 0.1	68 ± 1	17 ± 1	65 ± 3	37 ± 1
Control	24	3.1 ± 0.1	13.4 ± 1.7	18.6 ± 1.7	2.3 ± 0.2	71 ± 4	16 ± 1	65 ± 6	35 ± 3

Table A9. Mineral concentrations at harvest for the second crop (chickpea). Mean ± standard error (n=3).

Treatment	Soil	P	K	Ca	Mg	Fe	Cu	Mn	Zn
		g kg^{-1}				mg kg^{-1}			
Flooded	1	2.7 ± 0.6	26.4 ± 7.1	28.2 ± 1.1	3.3 ± 0.1	40 ± 3	17 ± 1	43 ± 8	26 ± 6
Flooded	2	2.7 ± 0.5	25.3 ± 5.4	31.5 ± 1.3	3.7 ± 0.7	40 ± 1	16 ± 0	39 ± 8	26 ± 3
Flooded	3	2.7 ± 0.4	27.6 ± 4.1	38.6 ± 3.9	3.6 ± 0.1	39 ± 3	19 ± 1	43 ± 6	26 ± 3
Flooded	4	2.9 ± 0.5	31.2 ± 1.8	34.2 ± 4.8	3.8 ± 0.6	51 ± 3	19 ± 5	62 ± 11	39 ± 8
Flooded	5	3.8 ± 0.8	30.9 ± 2.2	27.0 ± 3.1	3.2 ± 0.5	44 ± 5	21 ± 2	39 ± 9	35 ± 6
Flooded	6	2.7 ± 0.4	24.3 ± 1.6	31.8 ± 0.4	3.5 ± 0.1	39 ± 3	17 ± 1	50 ± 10	25 ± 2
Flooded	7	4.6 ± 1.1	26.9 ± 0.5	34.6 ± 1.3	3.2 ± 0.6	50 ± 9	17 ± 3	66 ± 14	42 ± 8
Flooded	8	3.1 ± 0.6	24.8 ± 0.7	35.0 ± 1.1	2.8 ± 0.4	46 ± 2	16 ± 2	67 ± 9	39 ± 7
Flooded	9	4.2 ± 0.3	31.6 ± 3.4	37.5 ± 2.1	3.7 ± 0.6	44 ± 4	21 ± 2	50 ± 4	30 ± 3
Flooded	10	3.0 ± 0.9	26.5 ± 1.1	35.2 ± 2.0	3.6 ± 0.6	45 ± 4	15 ± 1	74 ± 17	38 ± 8
Flooded	11	2.7 ± 0.1	23.4 ± 2.0	28.8 ± 0.5	3.2 ± 0.2	38 ± 4	14 ± 1	94 ± 13	34 ± 11
Flooded	12	3.2 ± 0.4	31.4 ± 3.8	37.3 ± 3.1	3.7 ± 0.2	41 ± 1	19 ± 1	36 ± 4	25 ± 4
Flooded	13	2.9 ± 0.1	25.2 ± 0.7	34.7 ± 0.4	2.9 ± 0.1	48 ± 8	16 ± 0	52 ± 1	22 ± 1
Flooded	14	2.9 ± 0.4	20.0 ± 4.2	31.1 ± 0.8	3.0 ± 0.1	47 ± 1	16 ± 1	70 ± 10	23 ± 2
Flooded	15	2.4 ± 0.1	20.9 ± 6.8	28.3 ± 2.3	3.1 ± 0.1	51 ± 2	14 ± 1	83 ± 14	33 ± 10
Flooded	16	3.5 ± 0.1	24.4 ± 0.4	39.4 ± 1.9	3.9 ± 0.2	71 ± 16	18 ± 1	54 ± 6	26 ± 1
Flooded	17	3.1 ± 0.3	23.0 ± 3.3	33.2 ± 1.3	3.0 ± 0.0	83 ± 4	16 ± 2	73 ± 10	32 ± 7
Flooded	18	2.9 ± 0.4	21.6 ± 5.4	34.1 ± 1.0	3.4 ± 0.1	52 ± 8	18 ± 3	54 ± 22	29 ± 10
Flooded	19	3.4 ± 0.1	24.9 ± 3.3	32.4 ± 1.4	2.8 ± 0.1	59 ± 4	16 ± 2	69 ± 18	35 ± 6
Flooded	20	3.3 ± 0.2	22.5 ± 2.5	32.6 ± 2.3	3.1 ± 0.2	47 ± 3	18 ± 1	47 ± 7	26 ± 3
Flooded	21	3.7 ± 0.1	28.5 ± 1.4	32.5 ± 2.0	2.9 ± 0.1	64 ± 5	14 ± 1	80 ± 7	42 ± 2
Flooded	22	3.1 ± 0.1	28.0 ± 0.4	31.8 ± 1.9	3.8 ± 0.2	52 ± 3	22 ± 0	30 ± 1	24 ± 1
Flooded	23	3.4 ± 0.2	27.2 ± 0.8	32.0 ± 1.7	3.1 ± 0.0	55 ± 3	17 ± 1	56 ± 15	33 ± 5
Flooded	24	2.9 ± 0.3	28.2 ± 5.2	29.3 ± 3.6	3.4 ± 0.3	57 ± 5	17 ± 2	64 ± 17	35 ± 10
Control	1	2.5 ± 0.0	30.1 ± 1.1	34.2 ± 0.9	3.1 ± 0.2	50 ± 3	16 ± 0	60 ± 4	29 ± 0
Control	2	2.6 ± 0.0	33.0 ± 0.5	30.0 ± 3.9	3.0 ± 0.3	50 ± 4	20 ± 5	87 ± 14	34 ± 2
Control	3	2.8 ± 0.1	32.2 ± 0.4	29.1 ± 1.5	3.0 ± 0.1	49 ± 2	25 ± 2	54 ± 0	26 ± 1
Control	4	2.6 ± 0.0	28.5 ± 1.0	35.9 ± 0.7	2.8 ± 0.0	53 ± 1	16 ± 0	68 ± 2	24 ± 1
Control	5	2.5 ± 0.1	29.9 ± 1.0	33.7 ± 2.8	2.9 ± 0.0	53 ± 2	18 ± 2	69 ± 9	24 ± 2
Control	6	2.5 ± 0.2	28.6 ± 0.4	34.3 ± 0.4	2.9 ± 0.1	57 ± 3	16 ± 2	99 ± 25	36 ± 6
Control	7	2.4 ± 0.3	27.2 ± 1.3	35.6 ± 1.1	2.9 ± 0.0	53 ± 2	17 ± 1	70 ± 8	24 ± 2
Control	8	2.8 ± 0.1	26.1 ± 1.3	35.9 ± 1.0	2.9 ± 0.1	54 ± 2	17 ± 1	78 ± 7	25 ± 2
Control	9	2.7 ± 0.0	26.4 ± 1.3	36.0 ± 0.7	2.9 ± 0.0	54 ± 1	16 ± 1	78 ± 16	24 ± 1
Control	10	2.8 ± 0.1	21.6 ± 0.8	36.3 ± 1.1	3.0 ± 0.1	51 ± 2	18 ± 1	56 ± 5	23 ± 1
Control	11	2.7 ± 0.1	22.2 ± 3.0	35.2 ± 0.5	2.9 ± 0.1	55 ± 3	17 ± 2	75 ± 18	25 ± 2
Control	12	2.6 ± 0.1	20.1 ± 0.3	32.3 ± 1.2	2.8 ± 0.0	49 ± 0	19 ± 1	56 ± 7	24 ± 1
Control	13	2.8 ± 0.1	22.3 ± 0.3	33.8 ± 2.6	3.2 ± 0.1	53 ± 3	28 ± 1	55 ± 3	33 ± 1
Control	14	2.8 ± 0.1	29.1 ± 4.3	34.1 ± 1.1	2.9 ± 0.1	51 ± 2	16 ± 2	114 ± 28	34 ± 6
Control	15	2.8 ± 0.1	26.1 ± 4.3	35.2 ± 0.3	2.9 ± 0.0	53 ± 2	18 ± 2	86 ± 34	30 ± 7
Control	16	2.7 ± 0.2	21.5 ± 2.9	35.1 ± 2.5	3.2 ± 0.2	53 ± 2	24 ± 4	66 ± 13	27 ± 3
Control	17	2.7 ± 0.1	20.2 ± 0.5	34.8 ± 1.5	2.9 ± 0.1	45 ± 2	22 ± 1	52 ± 2	25 ± 1
Control	18	2.7 ± 0.1	22.2 ± 0.7	37.9 ± 0.7	3.0 ± 0.1	47 ± 1	21 ± 2	57 ± 3	28 ± 5
Control	19	2.9 ± 0.2	20.5 ± 1.2	36.5 ± 0.4	2.9 ± 0.0	50 ± 2	19 ± 2	73 ± 17	24 ± 6
Control	20	3.2 ± 0.2	20.2 ± 0.9	32.8 ± 3.5	3.0 ± 0.0	50 ± 3	19 ± 2	76 ± 9	22 ± 3
Control	21	3.1 ± 0.2	19.6 ± 1.3	32.9 ± 2.9	2.9 ± 0.0	52 ± 3	18 ± 2	93 ± 27	24 ± 3
Control	22	3.3 ± 0.4	21.8 ± 0.6	36.9 ± 0.4	3.1 ± 0.1	49 ± 1	20 ± 1	62 ± 6	21 ± 0
Control	23	3.0 ± 0.1	21.7 ± 2.9	36.6 ± 0.7	2.9 ± 0.0	53 ± 2	15 ± 0	138 ± 6	29 ± 3
Control	24	3.6 ± 0.2	19.1 ± 0.3	30.9 ± 2.2	3.0 ± 0.1	54 ± 3	19 ± 1	65 ± 11	24 ± 1

ANNEX 3

Chapter 4

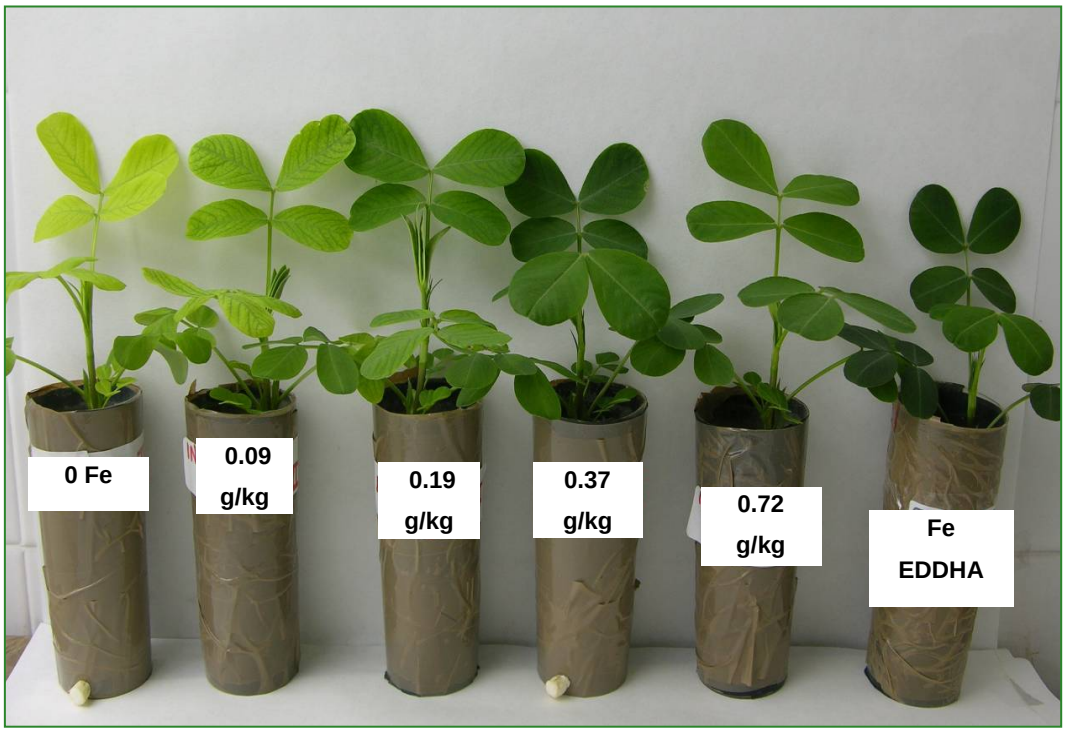


Figure A4. Peanut grown in soil inducing iron chlorosis fertilized with different rates of siderite (0.09 , 0.19, 0.37,and 0.72 g kg^{-1}) and FeEDDHA.

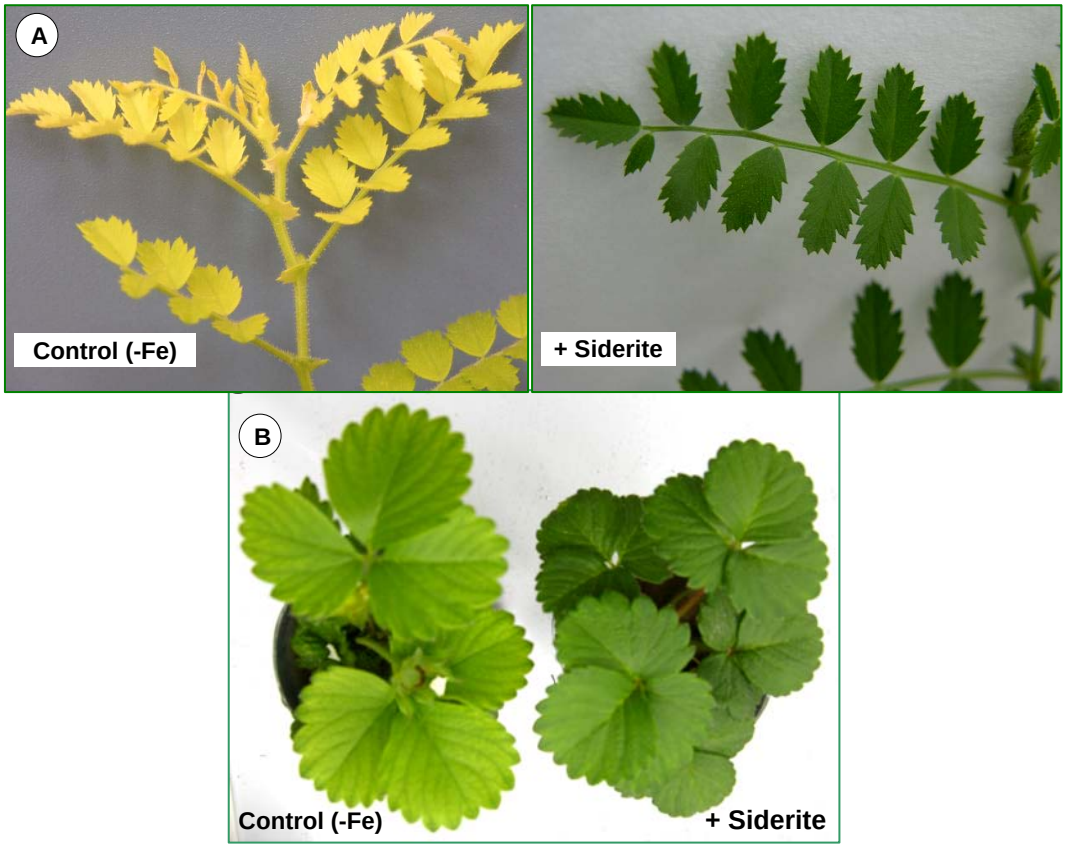


Figure A5. Chickpea (A) and strawberry (B) plants grown in a soil inducing iron chlorosis (left) and in the same soil fertilized with siderite (right).

Table A10. Mineral concentrations at harvest for the first crop (chickpea) of the 3th Experiment (effect of siderite rate)

Treatment	Siderite rate	Repetition	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	g kg^{-1} soil		g kg^{-1}				mg kg^{-1}			
SID3	0.24	1	2.3	49	46	4.0	40	21	41	28
SID3	0.24	2	2.3	44	47	4.0	38	20	51	27
SID3	0.24	3	2.1	40	49	3.8	31	16	45	27
SID3	0.24	4	2.1	56	55	4.8	43	22	53	38
SID3	0.46	1	2.9	55	36	3.8	35	19	49	33
SID3	0.46	2	2.2	53	45	4.4	54	29	44	32
SID3	0.46	3	2.3	54	39	3.9	37	19	43	28
SID3	0.46	4	2.2	45	40	4.0	41	22	39	24
SID3	0.93	1	2.5	66	40	4.2	35	18	39	24
SID3	0.93	2	2.0	50	41	4.2	47	25	38	30
SID3	0.93	3	2.2	66	40	4.4	31	17	41	31
SID3	0.93	4	2.0	59	44	4.2	45	23	42	24
SID3	1.4	1	2.4	46	29	3.4	76	22	42	28
SID3	1.4	2	2.6	65	29	3.8	46	24	44	35
SID3	1.4	3	2.5	47	23	3.6	48	25	46	32
SID3	1.4	4	2.6	58	27	3.6	48	25	46	30
SIDP3	0.24	1	2.0	39	51	4.2	32	17	51	28
SIDP3	0.24	2	1.8	32	42	3.8	58	31	43	38
SIDP3	0.24	3	1.7	38	54	4.5	48	25	49	38
SIDP3	0.24	4	2.0	43	48	4.1	38	20	42	33
SIDP3	0.46	1	1.9	36	38	3.6	43	23	38	23
SIDP3	0.46	2	2.2	53	42	4.2	32	17	39	26
SIDP3	0.46	3	1.9	39	42	3.6	39	21	46	34
SIDP3	0.46	4	1.8	39	41	3.8	50	26	40	40
SIDP3	0.93	1	3.2	57	29	3.6	29	15	34	30
SIDP3	0.93	2	1.9	47	31	3.4	28	15	33	28
SIDP3	0.93	3	2.0	41	28	3.4	53	28	31	34
SIDP3	0.93	4	2.0	48	32	3.4	33	18	32	28
SIDP3	1.4	1	1.8	48	27	3.2	46	24	37	35
SIDP3	1.4	2	2.2	58	30	3.7	42	22	37	33
SIDP3	1.4	3	2.6	53	26	3.4	37	20	36	32
SIDP3	1.4	4	3.0	52	28	3.4	47	17	31	25
FeEDDHA	0	1	3.4	30	24	3.0	51	27	22	34
FeEDDHA	0	2	2.4	31	21	2.5	54	28	20	36
FeEDDHA	0	3	2.8	27	24	2.6	73	22	19	31
FeEDDHA	0	4	2.7	35	21	2.3	98	52	14	49
CONTROL	0	1	1.7	33	48	3.8	61	32	54	30
CONTROL	0	2	2.4	36	57	4.5	36	19	68	26
CONTROL	0	3	2.4	33	53	4.2	38	20	64	30
CONTROL	0	4	2.4	—	46	3.7	141	24	53	34

Table A11. Mineral concentrations at harvest for the second crop (chickpea) of the 3th Experiment (effect of siderite rate)

Treatment	Siderite rate	Repetition	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	g kg^{-1} soil		g kg^{-1}				mg kg^{-1}			
SID3	0.24	1	5.5	39	68	5.7	68	27	35	30
SID3	0.24	2	5.4	25	57	4.9	44	21	45	31
SID3	0.24	3	7.2	37	48	4.6	55	23	41	34
SID3	0.24	4	5.4	33	44	4.3	50	16	35	29
SID3	0.46	1	5.3	32	36	4.2	52	14	29	22
SID3	0.46	2	5.9	39	51	5.2	53	8	37	26
SID3	0.46	3	5.0	24	32	3.5	50	12	29	35
SID3	0.46	4	5.5	42	51	4.9	52	17	32	31
SID3	0.93	1	6.6	38	30	3.4	59	17	33	28
SID3	0.93	2	4.9	28	29	3.6	64	13	23	24
SID3	0.93	3	3.4	32	29	3.2	71	21	27	22
SID3	0.93	4	5.1	31	28	3.3	56	15	26	29
SID3	1.4	1	4.1	32	50	4.1	493	16	35	29
SID3	1.4	2	4.8	18	31	4.4	59	19	29	21
SID3	1.4	3	4.4	34	26	3.5	62	14	22	24
SID3	1.4	4	4.0	29	26	3.5	46	19	23	21
SIDP3	0.24	1	—	—	—	—	—	—	—	—
SIDP3	0.24	2	5.5	20	45	4.4	54	11	35	23
SIDP3	0.24	3	5.4	22	42	4.1	89	10	34	31
SIDP3	0.24	4	5.5	24	61	4.7	60	10	34	28
SIDP3	0.46	1	5.6	30	31	3.4	53	15	29	29
SIDP3	0.46	2	4.9	33	37	3.9	65	16	31	17
SIDP3	0.46	3	4.5	30	34	3.6	47	19	31	37
SIDP3	0.46	4	5.4	24	34	3.7	87	12	30	17
SIDP3	0.93	1	4.5	22	27	3.4	48	14	28	51
SIDP3	0.93	2	4.3	39	38	4.5	81	13	29	24
SIDP3	0.93	3	4.8	24	35	4.0	59	16	30	26
SIDP3	0.93	4	4.8	23	28	3.9	58	13	27	23
SIDP3	1.4	1	5.4	34	27	3.4	52	17	25	27
SIDP3	1.4	2	—	—	—	—	—	—	—	—
SIDP3	1.4	3	4.7	35	25	3.1	41	8	22	21
SIDP3	1.4	4	3.4	38	30	4.1	27	12	24	22
CONTROL	0	1	7.0	26	85	5.8	50	29	43	29
CONTROL	0	2	4.3	24	48	3.9	32	16	36	25
CONTROL	0	3	5.7	29	64	5.3	65	16	40	32
CONTROL	0	4	4.5	24	60	4.5	44	13	37	23
FeEDDHA (1st crop)	0	1	4.6	35	26	2.5	82	17	13	37
FeEDDHA (1st crop)	0	2	8.5	32	27	2.8	126	19	15	39
FeEDDHA (1st crop)	0	3	6.1	32	29	2.7	170	17	16	26
FeEDDHA (1st crop)	0	4	4.7	35	25	2.5	58	19	13	34
FeEDDHA	0	1	8.0	32	22	2.7	79	17	12	33
FeEDDHA	0	2	6.2	34	23	2.9	96	18	11	38
FeEDDHA	0	3	6.8	34	21	2.5	78	—	9	41
FeEDDHA	0	4	9.3	34	24	2.6	74	—	14	37

Table A12. Mineral concentrations at harvest for the third crop (peanut) of the 3th Experiment (effect of siderite rate)

Treatment	Siderite rate	Repetition	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	g kg^{-1} soil		g kg^{-1}				mg kg^{-1}			
SID3	0.24	1	3.9	29	39	3.3	28	7.0	37	21
SID3	0.24	2	4.3	26	45	3.1	149	8.3	42	25
SID3	0.24	3	4.1	12	33	2.9	68	7.6	42	19
SID3	0.24	4	3.6	13	36	2.8	62	8.2	41	22
SID3	0.46	1	4.8	11	28	2.4	43	8.1	33	26
SID3	0.46	2	4.5	9	30	2.6	41	7.3	30	23
SID3	0.46	3	4.8	22	34	3.0	28	4.6	33	19
SID3	0.46	4	4.2	24	36	2.4	38	7.2	32	19
SID3	0.93	1	4.2	15	22	2.2	42	6.4	25	19
SID3	0.93	2	3.6	27	31	2.5	27	5.8	22	18
SID3	0.93	3	3.6	3	27	2.3	44	6.0	19	17
SID3	0.93	4	4.5	3	24	2.1	31	5.5	15	21
SID3	1.4	1	5.7	20	28	2.6	38	10.2	4	24
SID3	1.4	2	5.2	15	24	2.3	56	9.0	4	23
SID3	1.4	3	2.5	16	21	2.2	38	7.1	5	17
SID3	1.4	4	4.3	7	23	1.9	36	6.4	17	21
SIDP3	0.24	1	3.7	20	33	2.8	23	5.9	40	18
SIDP3	0.24	2	4.0	25	34	2.8	33	7.7	39	22
SIDP3	0.24	3	4.3	13	36	3.0	33	7.2	44	23
SIDP3	0.24	4	3.6	12	27	2.5	34	7.2	47	18
SIDP3	0.46	1	3.5	24	32	2.5	31	6.5	32	20
SIDP3	0.46	2	4.4	25	31	2.4	33	7.1	31	20
SIDP3	0.46	3	6.0	20	36	3.0	43	8.2	36	22
SIDP3	0.46	4	5.3	14	30	2.7	33	7.9	35	20
SIDP3	0.93	1	3.6	17	25	2.4	26	5.7	22	15
SIDP3	0.93	2	4.5	25	31	2.3	26	6.8	21	21
SIDP3	0.93	3	3.2	24	27	2.2	26	5.1	17	16
SIDP3	0.93	4	3.8	9	22	2.1	27	6.9	20	16
SIDP3	1.4	1	3.7	16	24	2.2	38	8.1	18	22
SIDP3	1.4	2	4.7	18	25	2.5	29	6.4	18	21
SIDP3	1.4	3	4.6	15	24	2.5	37	7.3	18	21
SIDP3	1.4	4	4.2	13	23	2.4	28	6.5	17	23
FeEDDHA (1st crop)	0	1	—	—	—	—	—	—	—	—
FeEDDHA (1st crop)	0	2	4.3	21	36	3.2	74	6.8	19	20
FeEDDHA (1st crop)	0	3	3.6	3	26	2.1	31	5.0	14	19
FeEDDHA (1st crop)	0	4	3.6	5	30	2.1	33	6.3	12	23
FeEDDHA	0	1	3.9	2	23	2.2	57	5.3	9	20
FeEDDHA	0	2	3.6	4	21	2.2	49	6.5	10	25
FeEDDHA	0	3	4.4	3	23	2.2	41	6.6	10	26
FeEDDHA	0	4	4.1	3	24	2.4	46	6.2	10	21
CONTROL	0	1	4.3	3	38	3.5	38	6.8	40	23
CONTROL	0	2	4.7	4	38	3.4	28	8.0	38	25
CONTROL	0	3	4.8	13	35	3.1	38	8.6	55	25
CONTROL	0	4	5.3	19	42	4.2	29	8.9	79	27

Table A13. Mineral concentrations at harvest for the fourth crop (peanut) of the 3th Experiment (effect of siderite rate)

Treatment	Siderite rate	Repetition	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	g kg^{-1} soil		g kg^{-1}				mg kg^{-1}			
SID3	0.24	1	3.5	14	23	2.3	32	8.2	23	22
SID3	0.24	2	5.5	11	28	3.1	26	7.7	27	17
SID3	0.24	3	4.5	17	26	2.7	37	8.4	32	14
SID3	0.24	4	4.1	17	26	2.7	33	7.3	27	18
SID3	0.46	1	5.8	13	24	2.8	39	8.9	27	27
SID3	0.46	2	3.7	9	25	3.0	36	8.9	22	20
SID3	0.46	3	6.7	11	29	3.1	26	8.3	23	20
SID3	0.46	4	5.0	15	27	3.2	32	6.7	24	16
SID3	0.93	1	4.8	4	15	2.1	52	5.2	15	16
SID3	0.93	2	3.1	14	20	2.5	58	7.6	21	21
SID3	0.93	3	3.2	13	17	2.2	40	7.1	19	20
SID3	0.93	4	3.0	6	18	2.2	31	5.3	15	9
SID3	1.4	1	3.2	10	16	2.2	41	6.7	16	18
SID3	1.4	2	3.2	10	18	2.2	38	6.8	16	11
SID3	1.4	3	3.3	28	18	2.5	50	6.2	16	13
SID3	1.4	4	3.9	8	19	2.6	52	6.7	19	18
SIDP3	0.24	1	2.5	10	21	2.2	38	9.4	31	25
SIDP3	0.24	2	3.0	4	21	2.4	31	8.2	20	24
SIDP3	0.24	3	4.0	9	28	2.8	32	10.6	28	33
SIDP3	0.24	4	3.3	13	30	3.2	38	10.0	33	33
SIDP3	0.46	1	3.7	16	19	2.0	37	11.5	28	35
SIDP3	0.46	2	3.8	10	21	2.2	38	10.4	26	35
SIDP3	0.46	3	3.2	12	22	2.6	37	10.0	24	29
SIDP3	0.46	4	3.5	20	26	3.0	38	11.2	34	31
SIDP3	0.93	1	3.5	12	19	2.6	48	10.6	23	28
SIDP3	0.93	2	3.4	11	18	2.6	56	8.5	25	27
SIDP3	0.93	3	2.6	14	20	2.1	47	8.6	27	21
SIDP3	0.93	4	2.3	14	19	2.3	44	8.0	27	16
SIDP3	1.4	1	2.9	9	15	2.2	55	8.8	21	24
SIDP3	1.4	2	3.1	10	15	2.0	54	9.9	27	25
SIDP3	1.4	3	3.3	10	16	2.2	50	7.7	20	24
SIDP3	1.4	4	3.2	0	16	2.4	89	10.8	23	24
FeEDDHA (1st crop)	0	1	3.6	9	23	2.5	22	6.4	16	25
FeEDDHA (1st crop)	0	2	2.9	17	26	2.8	15	4.0	13	16
FeEDDHA (1st crop)	0	3	3.6	15	28	2.8	17	5.3	13	19
FeEDDHA (1st crop)	0	4	3.3	8	23	2.3	25	5.5	12	27
FeEDDHA	0	1	2.7	9	21	2.2	30	8.3	17	13
FeEDDHA	0	2	2.6	6	21	2.2	27	8.2	17	13
FeEDDHA	0	3	3.3	18	19	2.1	24	6.8	14	12
FeEDDHA	0	4	4.2	12	24	2.4	27	7.6	22	14
CONTROL	0	1	3.4	14	26	2.3	34	9.9	23	31
CONTROL	0	2	3.1	8	21	2.6	27	9.3	21	34
CONTROL	0	3	3.1	4	22	2.6	33	10.0	23	37
CONTROL	0	4	3.8	11	24	2.4	25	10.7	28	43

Table A14. Mineral concentrations at harvest for the fifth crop (strawberry) of the 3th Experiment (effect of siderite rate)

Treatment	Siderite rate	Repetition	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	g kg^{-1} soil		g kg^{-1}				mg kg^{-1}			
SID3	0.24	1	2.0	26	18	3.9	138	2.1	106	22
SID3	0.24	2	2.1	29	22	5.1	125	2.3	102	24
SID3	0.24	3	0.9	25	12	3.2	122	2.2	124	16
SID3	0.24	4	1.3	20	21	3.5	551	3.1	132	19
SID3	0.46	1	1.2	23	14	2.5	131	2.0	61	22
SID3	0.46	2	1.8	30	15	3.6	94	1.9	79	13
SID3	0.46	3	1.0	28	14	3.5	112	4.3	72	16
SID3	0.46	4	1.4	30	15	3.2	102	1.8	83	14
SID3	0.93	1	2.4	34	18	2.8	261	2.0	79	19
SID3	0.93	2	1.7	28	12	2.4	117	2.2	77	10
SID3	0.93	3	2.5	30	14	2.8	145	1.8	86	16
SID3	0.93	4	1.9	23	14	3.1	127	2.2	73	14
SID3	1.4	1	1.5	27	14	2.8	187	3.0	55	21
SID3	1.4	2	1.6	29	12	2.4	141	2.3	60	15
SID3	1.4	3	2.1	29	11	2.6	90	2.2	61	23
SID3	1.4	4	1.6	29	13	2.4	167	1.5	62	11
SIDP3	0.24	1	1.4	23	14	3.2	123	2.2	89	19
SIDP3	0.24	2	1.6	25	16	3.9	198	2.1	98	10
SIDP3	0.24	3	1.7	23	16	3.5	98	2.2	73	15
SIDP3	0.24	4	1.5	26	13	3.2	61	1.8	90	15
SIDP3	0.46	1	1.9	26	17	3.7	109	3.3	128	19
SIDP3	0.46	2	1.4	20	15	3.1	68	2.4	117	18
SIDP3	0.46	3	1.4	21	19	4.5	135	5.7	121	20
SIDP3	0.46	4	1.1	19	16	3.6	163	2.8	124	16
SIDP3	0.93	1	1.4	28	17	3.4	133	2.0	74	14
SIDP3	0.93	2	1.4	28	14	3.2	69	4.3	73	21
SIDP3	0.93	3	1.1	26	12	2.5	125	1.9	66	17
SIDP3	0.93	4	2.0	31	14	3.0	134	2.0	66	17
SIDP3	1.4	1	1.5	29	16	3.9	170	2.8	94	17
SIDP3	1.4	2	1.2	28	17	3.1	186	2.8	62	17
SIDP3	1.4	3	1.9	26	11	3.0	184	2.1	72	21
SIDP3	1.4	4	1.2	20	15	3.5	105	2.0	80	21
FeEDDHA (1st crop)	0	1	1.8	29	16	3.8	288	4.2	136	21
FeEDDHA (1st crop)	0	2	1.7	29	17	3.8	285	3.4	112	29
FeEDDHA (1st crop)	0	3	2.0	28	17	3.8	182	2.9	158	14
FeEDDHA (1st crop)	0	4	1.4	20	13	3.8	187	3.3	93	29
FeEDDHA	0	1	1.6	20	16	3.2	95	2.5	50	14
FeEDDHA	0	2	1.6	19	18	3.8	143	2.0	67	15
FeEDDHA	0	3	2.0	19	15	3.4	129	2.4	32	15
FeEDDHA	0	4	1.9	20	15	3.8	155	2.3	57	29
CONTROL	0	1	1.6	25	22	4.9	193	3.0	165	26
CONTROL	0	2	2.1	26	23	5.0	146	2.9	173	18
CONTROL	0	3	1.8	19	17	4.1	98	2.4	138	23
CONTROL	0	4	1.6	20	19	4.8	110	4.7	128	18

ANNEX 4

Chapter 5

Figure A6. Andalusia map indicating the location of the olive trees experimental orchards.

Figure A7. Overview of the experimental orchard of Córdoba (Baena). The different colors that are painted the trunks of the trees correspond to the different fertilization treatments applied.

Table A15. SPAD value in 2008, 2009 and 2010 in Jaén. Values represent the mean SPAD of the experimental plot (4 trees and 30 leaves/tree) ± standard error.

		SPAD														
		2008				2009			2010		
Bl[a]	Treat[b]	May	June	July	October	June	July	November	June	July	October
R	0	47.63 ± 0.56	49.59 ± 0.51	51.32 ± 0.64	57.67 ± 0.79	45.75 ± 3.33	50.62 ± 0.65	60.76 ± 0.65	47.42 ± 0.60	45.41 ± 0.53	55.24 ± 0.90
S	0	46.26 ± 0.71	53.39 ± 0.57	52.38 ± 0.66	58.21 ± 0.64	50.38 ± 0.63	57.12 ± 0.61	63.20 ± 0.51	50.09 ± 0.53	49.17 ± 0.62	58.47 ± 0.59
T	0	47.04 ± 0.51	53.72 ± 0.47	51.35 ± 0.60	58.54 ± 0.55	49.25 ± 0.59	55.67 ± 0.59	65.56 ± 0.50	47.05 ± 0.56	45.30 ± 0.49	54.80 ± 0.72
R	3	46.89 ± 0.58	55.23 ± 0.48	58.57 ± 0.63	61.35 ± 0.85	54.43 ± 0.50	61.60 ± 0.52	67.37 ± 0.42	54.43 ± 0.41	59.69 ± 0.63	65.27 ± 0.46
S	3	50.38 ± 0.58	56.93 ± 0.47	57.08 ± 0.63	61.53 ± 0.55	53.97 ± 0.55	60.97 ± 0.62	67.77 ± 0.46	52.03 ± 0.38	57.50 ± 0.70	62.84 ± 0.72
T	3	46.19 ± 0.63	56.08 ± 0.46	58.97 ± 0.70	58.46 ± 0.56	56.57 ± 0.69	61.65 ± 0.53	66.44 ± 0.55	54.16 ± 0.53	60.24 ± 0.61	66.35 ± 0.53
R	4	42.90 ± 0.53	56.22 ± 0.59	57.69 ± 0.65	61.00 ± 0.75	60.02 ± 3.67	61.73 ± 0.57	68.47 ± 0.51	54.86 ± 0.46	60.10 ± 0.51	66.23 ± 0.45
S	4	44.52 ± 0.66	56.89 ± 0.49	56.30 ± 0.64	58.57 ± 0.49	53.57 ± 0.52	59.40 ± 0.50	66.38 ± 0.42	53.51 ± 0.54	58.02 ± 0.63	65.99 ± 0.70
T	4	48.93 ± 0.60	55.65 ± 0.41	56.70 ± 0.57	60.84 ± 0.81	55.91 ± 0.57	60.68 ± 0.57	65.50 ± 0.52	54.31 ± 0.40	58.48 ± 0.68	65.17 ± 0.65

[a] Block; [b] Treatment: 0: Control (-Fe); 3: Siderite; 4: Siderite+P

Table A16. SPAD value in 2008, 2009 and 2010 in Córdoba. Values represent the mean SPAD of 20 leaves ± standard error.

		SPAD															
		2008				2009			2010		
Bl[a]	Treat[b]	April	June	July	October	June	July	November	June	July	October
R	0	32.89 ± 1.66	29.30 ± 0.54	34.11 ± 1.07	36.27 ± 1.35	34.14 ± 1.64	37.77 ± 2.27	45.28 ± 2.24	30.41 ± 0.99	36.50 ± 1.37	30.46 ± 1.95
S	0	44.07 ± 1.38	33.95 ± 0.69	40.25 ± 1.16	43.12 ± 1.11	42.62 ± 1.65	49.49 ± 1.80	62.18 ± 2.02	37.79 ± 0.85	41.42 ± 0.88	51.12 ± 1.28
T	0	35.25 ± 2.50	34.29 ± 0.71	38.83 ± 1.09	42.89 ± 1.55	46.53 ± 1.96	57.67 ± 2.29	69.70 ± 1.35	39.45 ± 0.79	40.44 ± 1.07	48.90 ± 1.75
U	0	41.68 ± 2.78	32.59 ± 0.76	37.92 ± 0.86	53.76 ± 1.54	41.36 ± 1.54	61.07 ± 1.99	64.83 ± 2.36	38.97 ± 0.90	44.73 ± 1.16	55.74 ± 1.30
W	0	38.86 ± 1.57	30.53 ± 0.80	35.59 ± 0.92	38.72 ± 0.71	37.82 ± 1.36	49.23 ± 2.04	62.07 ± 2.74	41.78 ± 1.33	36.86 ± 1.21	46.95 ± 1.82
X	0	44.19 ± 1.24	32.68 ± 0.73	39.91 ± 1.06	40.22 ± 1.31	42.61 ± 1.20	55.87 ± 2.09	64.88 ± 2.11	36.44 ± 1.11	37.44 ± 0.97	44.19 ± 2.11
R	1	31.75 ± 2.02	34.88 ± 0.70	45.47 ± 1.42	55.00 ± 2.27	46.53 ± 1.58	42.52 ± 1.62	51.50 ± 1.80	46.72 ± 0.69	52.76 ± 1.63	54.29 ± 1.81
S	1	32.50 ± 1.70	33.02 ± 0.85	43.65 ± 1.74	46.82 ± 1.93	52.42 ± 1.77	48.68 ± 1.50	50.89 ± 2.63	47.29 ± 1.38	51.87 ± 1.88	53.88 ± 1.97
T	1	44.58 ± 1.81	37.31 ± 1.19	53.06 ± 1.50	60.97 ± 1.49	52.61 ± 1.08	56.95 ± 1.49	64.28 ± 1.83	46.38 ± 1.05	51.72 ± 1.38	54.17 ± 1.10
U	1	47.33 ± 1.43	38.76 ± 1.36	47.14 ± 1.48	53.43 ± 1.01	48.08 ± 1.07	55.46 ± 2.08	62.99 ± 2.04	45.73 ± 1.02	50.89 ± 1.10	58.95 ± 1.65
W	1	43.97 ± 1.17	42.20 ± 1.40	54.04 ± 1.25	55.42 ± 1.22	49.09 ± 0.92	67.62 ± 1.37	76.26 ± 1.17	43.75 ± 0.99	51.03 ± 1.64	51.69 ± 2.13
X	1	46.88 ± 1.56	36.51 ± 0.70	56.96 ± 1.34	59.54 ± 0.91	48.62 ± 0.96	53.79 ± 2.16	61.93 ± 2.38	45.42 ± 0.63	54.09 ± 1.23	63.73 ± 3.47
R	2	39.07 ± 1.32	36.60 ± 1.10	54.22 ± 1.81	59.76 ± 1.15	51.06 ± 1.48	43.00 ± 1.45	51.77 ± 1.91	46.75 ± 1.41	52.61 ± 1.89	52.53 ± 2.54
S	2	44.06 ± 1.12	37.23 ± 1.16	52.22 ± 1.71	62.12 ± 1.09	49.75 ± 0.96	62.61 ± 2.03	65.39 ± 1.15	45.57 ± 0.89	51.91 ± 1.45	60.36 ± 0.99
T	2	33.12 ± 1.73	31.98 ± 0.75	44.38 ± 1.13	49.46 ± 1.63	50.59 ± 1.53	51.41 ± 2.06	65.48 ± 2.34	44.12 ± 1.32	47.46 ± 2.03	53.78 ± 1.91
U	2	38.07 ± 2.01	35.96 ± 0.91	50.18 ± 1.10	52.85 ± 1.97	51.32 ± 0.90	51.96 ± 1.52	58.42 ± 2.34	48.51 ± 0.72	52.27 ± 1.06	55.56 ± 1.89
W	2	37.80 ± 1.52	31.49 ± 0.69	47.80 ± 1.77	49.29 ± 1.89	47.96 ± 0.75	54.20 ± 2.18	61.40 ± 2.17	47.81 ± 0.78	49.34 ± 1.15	51.46 ± 2.04
X	2	53.25 ± 1.28	45.28 ± 0.84	54.37 ± 1.29	57.14 ± 0.99	46.97 ± 0.66	55.42 ± 2.40	60.98 ± 2.81	47.05 ± 0.65	52.81 ± 1.11	59.41 ± 1.98
R	3	40.74 ± 1.13	37.21 ± 0.69	50.23 ± 2.12	47.94 ± 2.15	48.47 ± 1.28	41.74 ± 1.31	42.37 ± 2.31	44.48 ± 1.04	52.05 ± 1.76	55.04 ± 1.96
S	3	39.31 ± 1.35	37.15 ± 1.10	48.43 ± 1.79	51.75 ± 2.20	45.66 ± 0.95	57.81 ± 1.80	64.15 ± 1.98	41.49 ± 0.98	49.21 ± 1.79	57.39 ± 1.63
T	3	42.59 ± 1.95	34.54 ± 0.70	43.49 ± 2.25	45.75 ± 1.70	50.28 ± 1.26	51.19 ± 1.74	60.02 ± 2.71	46.98 ± 1.01	49.63 ± 1.75	58.57 ± 1.09
U	3	27.75 ± 1.71	44.22 ± 15.17	34.36 ± 0.96	33.64 ± 1.28	41.52 ± 1.31	47.52 ± 1.96	56.14 ± 1.53	33.16 ± 0.98	42.42 ± 1.63	51.94 ± 1.29
W	3	43.66 ± 2.21	38.02 ± 0.84	46.63 ± 1.06	52.17 ± 1.75	46.23 ± 0.96	62.64 ± 1.54	62.84 ± 1.12	46.05 ± 0.72	49.39 ± 1.52	57.11 ± 1.50
X	3	46.47 ± 1.56	43.94 ± 1.66	51.50 ± 1.05	60.01 ± 1.32	52.30 ± 1.07	61.08 ± 1.63	62.37 ± 2.09	45.41 ± 1.15	48.26 ± 1.26	58.97 ± 2.06
R	4	45.40 ± 1.90	41.22 ± 0.96	54.02 ± 1.12	55.37 ± 2.28	49.90 ± 1.78	40.19 ± 1.38	46.20 ± 2.17	47.68 ± 1.13	52.91 ± 1.66	55.94 ± 1.84
S	4	51.09 ± 2.29	48.82 ± 1.19	59.60 ± 1.33	64.69 ± 1.22	53.48 ± 0.88	55.79 ± 2.06	65.78 ± 1.85	55.68 ± 0.53	59.48 ± 1.42	64.33 ± 0.99
T	4	44.47 ± 1.97	35.25 ± 1.15	53.49 ± 1.51	56.94 ± 1.18	47.46 ± 1.27	57.72 ± 1.35	63.15 ± 1.30	42.11 ± 1.02	45.34 ± 1.76	56.53 ± 2.42
U	4	44.48 ± 1.35	37.34 ± 0.77	54.11 ± 1.12	42.42 ± 2.17	50.50 ± 0.91	50.94 ± 1.78	58.41 ± 1.01	45.31 ± 1.04	52.96 ± 0.75	60.95 ± 0.78
W	4	38.38 ± 1.73	32.97 ± 0.85	47.01 ± 2.50	51.15 ± 2.08	49.11 ± 1.62	55.14 ± 2.15	66.55 ± 1.64	41.71 ± 0.69	48.62 ± 0.96	57.09 ± 3.27
X	4	39.45 ± 1.27	33.38 ± 0.76	47.49 ± 1.48	55.38 ± 0.86	52.03 ± 1.18	58.92 ± 1.81	61.62 ± 1.28	44.72 ± 1.04	48.99 ± 1.60	55.57 ± 1.72
R	5	52.13 ± 1.84	46.30 ± 0.77	54.40 ± 1.16	60.10 ± 0.91	49.75 ± 1.46	39.44 ± 0.99	45.26 ± 1.36	39.57 ± 1.15	47.22 ± 1.49	50.16 ± 1.38
S	5	46.17 ± 2.04	41.62 ± 1.26	55.07 ± 1.95	58.04 ± 2.13	46.26 ± 0.84	60.01 ± 1.16	63.94 ± 1.52	39.51 ± 1.05	41.87 ± 1.85	49.35 ± 2.28
T	5	34.60 ± 1.87	39.13 ± 1.11	53.49 ± 1.32	55.67 ± 1.49	51.68 ± 1.10	56.63 ± 1.51	68.95 ± 1.37	37.34 ± 0.80	43.06 ± 1.21	48.08 ± 1.77
U	5	41.28 ± 1.79	40.34 ± 0.83	53.56 ± 1.22	62.63 ± 1.01	52.70 ± 1.25	53.25 ± 1.89	68.00 ± 0.96	39.50 ± 1.46	45.48 ± 1.54	54.70 ± 1.24
W	5	42.39 ± 2.03	43.19 ± 1.04	53.35 ± 1.80	55.90 ± 1.35	50.44 ± 0.85	58.23 ± 1.39	65.20 ± 1.09	38.77 ± 1.22	41.11 ± 0.99	46.84 ± 2.17
X	5	39.77 ± 1.42	45.03 ± 1.06	56.22 ± 1.56	60.85 ± 1.22	41.23 ± 1.77	54.66 ± 1.30	57.97 ± 1.31	36.91 ± 1.26	38.56 ± 1.34	40.07 ± 2.03

[a] Block; [b] Treatment: 0: Control (-Fe); 1: Vivianite; 2: Vivianite + humic acids; 3: Siderite; 4: Siderite+P; 5: FeEDDHA.

Table A17. SPAD value in 2008, 2009 and 2010 in Seville. Values represent the mean SPAD of 30 leaves ± standard error.

		SPAD															
		2008				2009			2010		
Bl[a]	Treat[b]	April	June	July	October	June	July	November	May	July	October
R	0	53.52 ± 1.60	44.03 ± 1.55	51.71 ± 0.73	53.88 ± 0.94	47.91 ± 0.86	55.22 ± 1.20	62.56 ± 1.13	46.59 ± 1.21	48.96 ± 1.20	56.48 ± 1.22
R	1	54.11 ± 2.48	46.76 ± 0.86	51.83 ± 0.82	53.04 ± 0.80	48.55 ± 0.70	61.69 ± 0.56	65.53 ± 0.80	46.05 ± 1.04	52.38 ± 0.99	61.70 ± 0.63
R	2	52.30 ± 1.92	50.12 ± 0.61	53.94 ± 0.95	54.14 ± 0.57	47.25 ± 0.84	58.60 ± 0.81	59.52 ± 0.57	48.00 ± 0.95	49.40 ± 0.80	59.11 ± 0.54
R	3	53.75 ± 2.39	46.17 ± 0.92	53.74 ± 0.73	52.55 ± 0.88	47.82 ± 0.93	55.16 ± 0.71	59.45 ± 0.92	45.96 ± 0.84	48.30 ± 0.79	57.25 ± 0.66
R	4	55.55 ± 1.86	46.35 ± 0.79	55.40 ± 0.60	51.10 ± 0.58	51.11 ± 0.86	60.56 ± 1.03	68.34 ± 0.59	43.98 ± 1.09	49.54 ± 0.90	61.84 ± 0.96
R	5	52.11 ± 2.01	45.44 ± 0.72	52.17 ± 1.22	56.21 ± 0.72	48.47 ± 0.94	55.91 ± 0.80	57.97 ± 0.78	41.92 ± 0.87	47.59 ± 1.15	55.61 ± 0.73
S	0	49.14 ± 1.32	52.38 ± 0.85	54.47 ± 0.61	56.47 ± 0.63	43.90 ± 0.69	52.25 ± 0.87	56.69 ± 0.84	44.73 ± 1.36	43.64 ± 1.10	52.23 ± 1.75
S	1	48.53 ± 1.15	47.79 ± 0.96	52.36 ± 0.91	54.49 ± 0.88	49.54 ± 0.74	57.57 ± 1.24	61.85 ± 1.04	50.08 ± 1.11	50.67 ± 1.17	61.34 ± 0.73
S	2	45.56 ± 0.62	49.92 ± 0.57	55.89 ± 0.61	53.91 ± 0.64	50.22 ± 1.12	53.82 ± 0.99	57.73 ± 1.27	48.17 ± 1.08	51.32 ± 1.19	54.68 ± 1.34
S	3	44.69 ± 1.55	49.83 ± 0.62	56.44 ± 0.70	52.16 ± 0.49	46.66 ± 0.83	57.31 ± 0.80	57.56 ± 0.78	45.21 ± 1.01	48.19 ± 0.89	59.21 ± 0.81
S	4	53.37 ± 1.50	48.72 ± 0.57	55.03 ± 0.69	53.02 ± 0.79	53.20 ± 0.76	61.38 ± 0.71	61.87 ± 0.74	46.32 ± 0.94	52.97 ± 1.08	60.00 ± 0.72
S	5	51.53 ± 1.66	50.89 ± 0.90	55.64 ± 0.68	53.21 ± 0.71	49.51 ± 0.84	59.22 ± 0.63	60.32 ± 0.79	46.74 ± 1.32	47.50 ± 0.90	63.79 ± 0.70
T	0	49.93 ± 0.75	49.54 ± 0.72	53.75 ± 0.71	54.53 ± 0.62	48.32 ± 0.69	59.61 ± 0.62	61.09 ± 0.70	44.46 ± 0.83	56.47 ± 0.94	61.73 ± 0.65
T	1	50.09 ± 0.81	51.42 ± 0.84	49.33 ± 1.05	54.41 ± 1.15	51.95 ± 0.91	61.41 ± 0.70	62.66 ± 0.67	47.35 ± 1.02	53.21 ± 1.01	62.24 ± 1.12
T	2	48.27 ± 1.14	47.81 ± 0.88	56.15 ± 0.80	57.45 ± 0.82	50.30 ± 1.09	57.46 ± 1.18	65.01 ± 0.72	49.05 ± 0.98	57.50 ± 1.23	64.93 ± 0.76
T	3	48.11 ± 0.91	49.78 ± 0.93	56.57 ± 0.84	53.88 ± 0.67	51.05 ± 1.02	59.42 ± 0.95	65.84 ± 0.88	50.05 ± 0.99	50.44 ± 1.07	62.88 ± 1.12
T	4	51.74 ± 2.03	52.33 ± 0.81	54.01 ± 0.74	52.68 ± 0.68	50.61 ± 0.85	56.50 ± 0.90	60.93 ± 0.68	49.80 ± 0.81	52.35 ± 1.05	61.22 ± 0.56
T	5	47.41 ± 2.02	49.17 ± 0.74	56.71 ± 0.65	57.35 ± 0.96	54.99 ± 0.97	61.80 ± 1.08	59.77 ± 0.86	49.87 ± 0.80	57.52 ± 0.90	65.02 ± 0.81
U	0	49.03 ± 1.23	49.53 ± 0.72	53.34 ± 0.63	54.22 ± 0.69	45.20 ± 0.71	58.87 ± 0.70	61.76 ± 0.55	47.54 ± 0.87	49.71 ± 1.02	58.67 ± 1.04
U	1	49.50 ± 1.34	51.51 ± 0.81	53.44 ± 0.59	53.66 ± 0.47	50.33 ± 0.87	62.81 ± 0.74	65.78 ± 0.68	48.10 ± 0.81	55.97 ± 1.18	64.58 ± 0.79
U	2	49.27 ± 1.03	47.71 ± 0.82	57.24 ± 0.89	57.52 ± 0.99	53.16 ± 0.65	62.27 ± 1.00	68.33 ± 0.70	48.93 ± 1.07	58.43 ± 1.49	66.95 ± 0.63
U	3	51.90 ± 1.76	48.70 ± 0.80	53.35 ± 0.87	— ± —	49.44 ± 0.83	57.23 ± 0.61	57.62 ± 0.77	49.77 ± 0.92	55.16 ± 0.91	56.22 ± 0.55
U	4	48.87 ± 0.96	54.84 ± 0.83	55.95 ± 0.84	54.58 ± 0.67	48.20 ± 0.71	59.03 ± 0.69	61.88 ± 0.81	45.53 ± 1.16	52.20 ± 0.96	64.29 ± 0.65
U	5	48.51 ± 0.84	48.75 ± 0.84	55.71 ± 0.64	55.45 ± 0.92	49.03 ± 0.82	59.49 ± 0.93	61.99 ± 0.71	43.89 ± 1.06	54.33 ± 1.33	62.18 ± 0.67
W	0	47.99 ± 0.78	50.85 ± 1.02	56.37 ± 0.90	56.81 ± 0.60	47.74 ± 0.96	53.41 ± 1.24	56.24 ± 0.67	44.28 ± 0.80	49.94 ± 1.21	61.40 ± 0.89
W	1	51.45 ± 1.00	54.70 ± 0.88	54.43 ± 0.54	56.71 ± 0.50	48.37 ± 0.73	59.76 ± 0.83	61.12 ± 0.96	47.90 ± 1.16	57.39 ± 0.96	64.50 ± 0.61
W	2	50.32 ± 1.15	47.64 ± 0.87	54.98 ± 0.73	53.83 ± 0.53	49.10 ± 1.05	59.89 ± 0.65	60.53 ± 0.69	50.54 ± 0.77	54.51 ± 0.84	58.25 ± 0.68
W	3	54.16 ± 1.55	49.67 ± 0.74	54.66 ± 0.73	54.28 ± 0.62	52.41 ± 0.90	60.54 ± 0.70	62.96 ± 0.84	51.62 ± 1.19	56.17 ± 1.24	67.61 ± 0.70
W	4	51.34 ± 2.14	48.08 ± 0.88	53.13 ± 0.96	64.97 ± 13.67	46.56 ± 0.82	57.29 ± 0.94	59.60 ± 0.70	45.21 ± 0.80	52.24 ± 1.27	64.24 ± 0.67
W	5	49.00 ± 0.91	51.75 ± 0.70	55.37 ± 0.86	55.61 ± 0.96	49.52 ± 0.81	64.99 ± 0.73	70.55 ± 1.05	44.55 ± 1.16	54.67 ± 0.99	65.75 ± 0.70
X	0	46.39 ± 0.99	50.47 ± 1.12	55.06 ± 0.83	53.59 ± 0.61	44.36 ± 0.77	55.94 ± 1.12	62.10 ± 1.23	45.39 ± 0.92	49.43 ± 1.02	65.19 ± 0.63
X	1	50.17 ± 0.83	51.57 ± 0.85	54.81 ± 0.68	55.20 ± 0.66	50.57 ± 1.07	60.66 ± 0.73	62.54 ± 0.58	48.56 ± 0.86	55.72 ± 1.10	64.18 ± 0.67
X	2	49.68 ± 1.11	56.41 ± 0.79	57.08 ± 0.80	57.86 ± 0.70	49.49 ± 0.88	61.26 ± 0.85	64.58 ± 1.01	50.63 ± 1.25	56.80 ± 1.29	67.00 ± 0.63
X	3	48.34 ± 0.93	49.16 ± 1.69	57.60 ± 0.91	53.43 ± 0.56	47.10 ± 0.95	59.59 ± 0.63	66.65 ± 1.10	47.09 ± 0.88	55.72 ± 0.90	64.99 ± 0.68
X	4	47.97 ± 1.56	48.68 ± 0.56	56.98 ± 0.89	59.16 ± 0.71	48.66 ± 1.09	57.91 ± 0.90	60.35 ± 0.88	48.17 ± 0.73	54.43 ± 1.03	62.37 ± 0.65
X	5	46.85 ± 1.11	49.57 ± 0.69	59.12 ± 0.93	61.42 ± 1.09	47.71 ± 0.75	58.88 ± 0.76	63.80 ± 0.91	47.29 ± 0.95	56.05 ± 1.17	72.31 ± 0.75

[a] Block; [b] Treatment: 0: Control (-Fe); 1: Vivianite; 2: Vivianite + humic acids; 3: Siderite; 4: Siderite+P; 5: FeEDDHA.

Table A18. Diameter of the olive trees trunks in Cordoba and Seville orchards at the beginning of the experiment (2008) and end (2011).

Block	Treatment	Trunk diameter (cm)			
		Córdoba (Baena)		Seville (Estepa)	
		2008	2011	2008	2011
R	Control	11.78	14.32	14.48	15.12
R	Vivianite	12.41	14.96	15.76	16.87
R	Vivianite + humic acids	12.41	14.01	14.48	16.39
R	Siderite	12.73	14.01	15.12	16.23
R	P-Siderite	11.14	13.05	11.94	13.37
R	FeEDDHA	12.41	13.69	12.57	13.37
S	Control	10.82	12.41	24.19	29.92
S	Vivianite	11.14	13.05	14.16	16.39
S	Vivianite + humic acids	11.14	12.41	10.66	12.89
S	Siderite	11.14	12.41	14.64	15.28
S	P-Siderite	8.44	9.55	13.05	15.28
S	FeEDDHA	13.05	14.96	14.32	17.83
T	Control	12.10	13.69	12.10	14.64
T	Vivianite	12.73	14.32	15.60	19.10
T	Vivianite + humic acids	12.10	13.69	15.60	16.55
T	Siderite	13.69	15.28	13.69	15.92
T	P-Siderite	12.41	14.01	15.44	17.03
T	FeEDDHA	13.05	15.28	13.37	15.60
U	Control	11.78	13.05	15.60	17.19
U	Vivianite	12.73	14.32	12.10	13.69
U	Vivianite + humic acids	12.73	14.64	12.89	15.28
U	Siderite	12.73	14.01	15.44	15.44
U	P-Siderite	12.73	14.01	11.62	13.69
U	FeEDDHA	12.73	14.01	14.16	16.23
W	Control	13.37	14.32	13.05	15.12
W	Vivianite	10.82	12.10	13.37	15.44
W	Vivianite + humic acids	13.37	14.64	14.01	10.50
W	Siderite	13.69	14.96	9.39	11.78
W	P-Siderite	12.73	14.01	16.71	17.03
W	FeEDDHA	13.37	14.64	13.37	15.76
X	Control	12.26	13.05	12.73	15.12
X	Vivianite	6.84	9.55	10.19	12.73
X	Vivianite + humic acids	11.94	14.01	13.37	14.64
X	Siderite	10.82	12.41	11.46	14.32
X	P-Siderite	13.37	14.64	14.80	16.23
X	FeEDDHA	13.05	14.32	17.51	21.17

Table A19. Diameter of the olive trees trunks in Jaén orchard at the beginning of the experiment (2008) and end (2011). Four trees per plot.

Block	Treatment	Trunk diameter (cm) Jaén (Mancha Real)	
		2008	2011
R	Control	16.09	17.42
R	Siderite	15.97	17.92
R	P-Siderite	17.03	19.54
S	Control	16.79	18.33
S	Siderite	16.08	17.37
S	P-Siderite	16.13	18.32
T	Control	15.68	17.83
T	Siderite	16.03	18.31
T	P-Siderite	16.01	17.84

Table A20. Production data, oil yield and content of polyphenols in Jaén.

Orchard	Treatment	Block	Tree	2008			2009			2010			Cumulative (3 years)	
				Olive	Oil yield	Total Polyphenols	Olive	Oil yield	Total Polyphenols	Olive	Oil yield	Total Polyphenols	Olive	Oil yield
				kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	
Jaén	Control	R	1	38.5	24.5	1071	54.3	26.7	654	29.9	28.2	551	122.7	32.4
Jaén	Control	R	2	40.2	25.5	1056	76.2	25.2	—	44.7	26.3	482	161.0	41.2
Jaén	Control	R	3	52	24.7	949	70.0	27.1	605	35.5	19.5	188	157.5	38.7
Jaén	Control	R	4	31.7	27.7	1103	64.9	25.9	756	55.2	24.3	473	151.7	39.0
Jaén	Siderite	R	1	34	23.3	883	68.9	25	681	45.0	23.2	427	147.8	35.6
Jaén	Siderite	R	2	56.9	23.5	956	78.4	20	699	51.7	24.6	453	187.0	41.8
Jaén	Siderite	R	3	69.5	21	1066	105.4	18.3	845	93.8	22.1	493	268.7	54.6
Jaén	Siderite	R	4	47.5	22	1076	77.7	21.3	751	61.2	23.7	602	186.4	41.5
Jaén	P-Siderite	R	1	55.6	22.3	778	75.7	20.3	791	59.7	23.9	389	191.0	42.0
Jaén	P-Siderite	R	2	58.6	23.8	937	76.7	20	689	66.5	21.9	461	201.7	43.8
Jaén	P-Siderite	R	3	29.3	23	813	72.8	21.4	628	57.1	23.9	496	159.3	36.0
Jaén	P-Siderite	R	4	48.9	21.3	845	85.4	20.8	708	59.4	23.7	428	193.6	42.2
Jaén	Control	S	1	44.6	22.4	842	74.5	20.5	606	38.6	23.5	317	157.7	34.3
Jaén	Control	S	2	44.1	24.2	888	76.0	22.1	807	25.0	18.9	240	145.1	32.2
Jaén	Control	S	3	28	24.4	891	67.9	20.2	—	62.0	26.4	543	157.9	36.9
Jaén	Control	S	4	55.3	21.9	873	94.8	20.8	722	57.3	21.9	444	207.5	44.4
Jaén	Siderite	S	1	41.8	23.9	902	71.2	26.2	635	20.9	20.5	178	133.9	32.9
Jaén	Siderite	S	2	46.5	26	1034	86.1	23.1	765	67.2	25.7	574	199.8	49.3
Jaén	Siderite	S	3	43.9	24.6	1105	75.1	23.4	647	49.4	25.9	606	168.3	41.2
Jaén	Siderite	S	4	34.7	27.5	1176	60.1	27	650	42.4	25.6	445	137.2	36.6
Jaén	P-Siderite	S	1	50.6	21.9	1041	73.9	22.8	642	66.9	24.8	642	191.3	44.5
Jaén	P-Siderite	S	2	44.2	23.3	903	82.4	21.6	751	65.2	22	515	191.8	42.4
Jaén	P-Siderite	S	3	43	23	961	85.8	21.5	721	69.9	23.8	609	198.7	45.0
Jaén	P-Siderite	S	4	39.1	24.2	934	79.2	20.7	—	25.3	21.4	194	143.6	31.3
Jaén	Control	T	1	39.3	26.5	1110	69.6	23.3	769	29.3	24.6	508	138.2	33.8
Jaén	Control	T	2	42	25.6	968	64.7	22.2	706	25.7	25.3	332	132.5	31.6
Jaén	Control	T	3	53.6	25.2	797	76.0	24.2	667	21.8	22.6	360	151.5	36.8
Jaén	Control	T	4	44.4	25.5	1025	72.8	26.3	806	29.1	25.3	401	146.3	37.8
Jaén	Siderite	T	1	58.6	19.8	1056	98.5	18.5	650	83.5	24.2	577	240.6	50.0
Jaén	Siderite	T	2	51.7	22.2	936	105.6	19.5	692	80.2	23.6	593	237.4	51.0
Jaén	Siderite	T	3	47.1	21.3	1148	57.8	23.9	683	42.7	25.9	647	147.6	34.9
Jaén	Siderite	T	4	58.7	23.9	947	79.8	21.7	—	56.1	24	545	194.5	44.8
Jaén	P-Siderite	T	1	42.5	24.1	1255	70.9	23.4	645	46.0	26.5	690	159.3	39.0
Jaén	P-Siderite	T	2	30	26	1182	74.7	23.2	626	42.0	27.5	490	146.7	36.7
Jaén	P-Siderite	T	3	40.4	25.6	1097	61.8	23.2	—	36.2	25.3	460	138.5	33.9
Jaén	P-Siderite	T	4	32.3	25.8	1129	72.2	21.7	697	61.6	26.5	727	166.1	40.3

Table A21. Production data, oil yield and content of polyphenols in Córdoba.

Orchard	Treatment	Block	2008			2009			2010	Cumulative (3 years)	
			Olive	Oil yield	Total Polyphenols	Olive	Oil yield	Total Polyphenols	Olive	Olive	Oil yield
			kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	kg tree^{-1}	
Córdoba	Control	R	0	–	–	23.2	24.8	851	3.3	26.5	5.8
Córdoba	Vivianite	R	1.7	23.4	625	24.5	24.8	874	8.1	34.3	6.5
Córdoba	Vivianite + humic acids	R	2.5	23.9	694	23.1	24.4	879	4.0	29.6	6.2
Córdoba	Siderite	R	3.4	24.2	788	23.8	25.0	791	19.9	47.1	6.8
Córdoba	P-Siderite	R	1.5	24.4	685	21.6	23.6	796	3.2	26.3	5.5
Córdoba	FeEDDHA	R	0.1	23.9	579	22.7	24.4	891	1.8	24.5	5.6
Córdoba	Control	S	0	–	–	16.3	27.8	879	7.3	23.7	4.5
Córdoba	Vivianite	S	0.4	25.8	579	9.3	25.8	604	21.3	30.9	2.5
Córdoba	Vivianite + humic acids	S	0	25.5	601	15.8	25.7	954	1.4	17.2	4.1
Córdoba	Siderite	S	5.1	26.2	714	15.2	26.9	842	6.7	27.0	5.4
Córdoba	P-Siderite	S	2.7	22.9	867	12.6	23.0	648	11.0	26.3	3.5
Córdoba	FeEDDHA	S	0	23.9	579	26.0	25.0	670	8.8	34.8	6.5
Córdoba	Control	T	3.2	24.2	623	18.3	25.1	774	11.3	32.8	5.4
Córdoba	Vivianite	T	0.3	25.8	579	29.3	22.3	924	0.5	30.1	6.6
Córdoba	Vivianite + humic acids	T	0.6	25.5	601	20.5	25.8	781	12.6	33.7	5.4
Córdoba	Siderite	T	0	–	–	21.9	26.5	719	10.5	32.4	5.8
Córdoba	P-Siderite	T	0	–	–	20.5	28.4	722	7.8	28.3	5.8
Córdoba	FeEDDHA	T	1.5	23.9	579	28.7	24.5	843	6.2	36.4	7.4
Córdoba	Control	U	3.4	26.4	918	10.6	28.3	595	17.9	31.9	3.9
Córdoba	Vivianite	U	0.1	–	–	20.2	25.0	744	1.4	21.6	5.1
Córdoba	Vivianite + humic acids	U	0.2	–	–	20.7	23.9	837	1.5	22.4	4.9
Córdoba	Siderite	U	0	–	–	16.3	21.2	869	1.6	17.9	3.5
Córdoba	P-Siderite	U	0.1	–	–	26.1	21.6	977	0.4	26.6	5.6
Córdoba	FeEDDHA	U	0.3	–	–	23.3	21.5	913	2.6	26.3	5.0
Córdoba	Control	W	5.9	23.8	746	13.7	25.3	701	6.9	26.5	4.9
Córdoba	Vivianite	W	6.6	25.7	704	21	23.7	977	1.9	29.5	6.7
Córdoba	Vivianite + humic acids	W	0.7	24.8	552	17.4	23.8	771	4.2	22.3	4.3
Córdoba	Siderite	W	11.7	26.2	836	17.6	27.2	652	20.6	49.9	7.9
Córdoba	P-Siderite	W	0.5	24.3	612	16.7	23.4	831	4.5	21.8	4.0
Córdoba	FeEDDHA	W	4.5	25.7	701	19.5	25.4	777	0.9	24.9	6.1
Córdoba	Control	X	2.3	25.8	599	12.9	26.5	655	6.0	21.2	4.0
Córdoba	Vivianite	X	0	–	–	10.1	22.5	764	0.6	10.7	2.3
Córdoba	Vivianite + humic acids	X	3.6	25.5	655	22.4	25	845	2.9	28.9	6.5
Córdoba	Siderite	X	2.6	25.9	818	19.5	22.6	813	4.5	26.5	5.1
Córdoba	P-Siderite	X	1.7	25.1	729	23.2	23	796	5.6	30.4	5.8
Córdoba	FeEDDHA	X	16.2	23.7	1056	16.9	27.2	682	12.2	45.3	8.4

Table 22. Production data, oil yield and content of polyphenols in Seville.

Orchard	Treatment	Block	2008			2010			Cumulative (2 years)	
			Olive	Oil yield	Total Polyphenols	Olive	Oil yield	Total Polyphenols	Olive	Oil yield
			kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	% P/P	mg kg^{-1} oil	kg tree^{-1}	
Seville	Control	R	0.8	0	0	35.7	25	678	36.5	8.9
Seville	Vivianite	R	0	0	0	17.9	26.1	546	17.9	4.7
Seville	Vivianite + humic acids	R	0	0	0	12.3	25.4	492	12.3	3.1
Seville	Siderite	R	0	0	0	13.9	25.9	469	13.9	3.6
Seville	P-Siderite	R	0	0	0	12.2	26.6	484	12.2	3.2
Seville	FeEDDHA	R	0	0	0	5.8	–	–	5.8	0.0
Seville	Control	S	0	0	0	7.0	–	–	7.0	0.0
Seville	Vivianite	S	0	0	0	20.1	24.9	602	20.1	5.0
Seville	Vivianite + humic acids	S	0	0	0	27.7	16.6	831	27.7	4.6
Seville	Siderite	S	0	0	0	8.8	–	–	8.8	0.0
Seville	P-Siderite	S	0	0	0	15.1	24	510	15.1	3.6
Seville	FeEDDHA	S	0	0	0	9.4	27.4	544	9.4	2.6
Seville	Control	T	0	0	0	9.2	12.8	426	9.2	1.2
Seville	Vivianite	T	0	0	0	11.2	26.6	456	11.2	3.0
Seville	Vivianite + humic acids	T	24.2	0	0	47.0	23.1	801	71.3	10.9
Seville	Siderite	T	0.7	0	0	19.5	23.7	501	20.3	4.6
Seville	P-Siderite	T	0.7	0	0	14.5	25.4	357	15.2	3.7
Seville	FeEDDHA	T	1.7	0	0	27.3	19.3	702	29.0	5.3
Seville	Control	U	0.6	0	0	19.6	24.1	352	20.3	4.7
Seville	Vivianite	U	0	0	0	19.7	24.6	626	19.7	4.8
Seville	Vivianite + humic acids	U	0	0	0	30.6	25.7	669	30.6	7.9
Seville	Siderite	U	0	0	0	6.4	19.3	554	6.4	1.2
Seville	P-Siderite	U	0	0	0	26.6	24.3	538	26.6	6.5
Seville	FeEDDHA	U	0	0	0	13.6	21.7	417	13.6	2.9
Seville	Control	W	0.8	0	0	26.1	23.4	525	26.8	6.1
Seville	Vivianite	W	0.7	0	0	25.0	23.8	669	25.7	5.9
Seville	Vivianite + humic acids	W	0	0	0	5.1	24.7	421	5.1	1.3
Seville	Siderite	W	0	0	0	12.8	25.7	557	12.8	3.3
Seville	P-Siderite	W	1.3	0	0	39.5	25	712	40.7	9.9
Seville	FeEDDHA	W	0	0	0	13.6	20.1	339	13.6	2.7
Seville	Control	X	2.2	0	0	22.7	21.3	317	24.9	4.8
Seville	Vivianite	X	0.2	0	0	14.0	22.1	453	14.2	3.1
Seville	Vivianite + humic acids	X	0	0	0	2.2	22.8	431	2.2	0.5
Seville	Siderite	X	1.4	0	0	22.0	24.3	505	23.4	5.3
Seville	P-Siderite	X	0.3	0	0	13.9	23.3	399	14.2	3.2
Seville	FeEDDHA	X	11.4	0	0	67.0	23.4	738	78.4	15.7

Curriculum Vitae

Inmaculada Sánchez Alcalá was born in Córdoba (Spain), on September 15th, 1979. In 1997 she obtained her high school qualification at the I.E.S. “Séneca” in Córdoba. In the same year, she started her Agronomy studies at University of Córdoba. In 2004-2005 she was student collaborator at the Plant Disease Group of that University. After she obtained her Degree in agricultural engineering in 2005, she cursed the Official Master’s Programme “Plant Breeding, Protection and Improvement” at the University of Córdoba (2006-2007). She obtained a FPI grant from the Ministry of Education and Science for the realization of this thesis with the Soil Science Group of the University of Córdoba. Between 2008 and 2009 she made two short stays (5 months in 2008 and 1.5 months in 2009) at the University of Vienna, to carry out some experiments on microbial reduction of Fe. The results of the work of these years appear in the current book.

List of publications

Sánchez-Alcalá, I., M.C. del Campillo, J. Torrent, K.L. Straub and S.M. Kraemer. 2011. Iron(III) reduction in anaerobically incubated suspensions of highly calcareous agricultural soils. Soil Sci. Soc. Am. J.

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