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RESEARCH ARTICLE

Intercropping with grasses helps to reduce iron chlorosis in olive

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Abstract

Grasses are more efficient than dicots in acquiring Fe from calcareous soils. We studied whether intercropping with grasses alleviates Fe chlorosis in olive and whether the effect persists in succeeding dicot crops. Three different pot experiments were conducted. In the first, olive plants were intercropped with 6 different grass species (purple false brome, annual ryegrass, compact brome, goatgrass, barley and red fescue); in the second, the two species best performing in the previous experiment were studied in various calcareous soils and; in the third, chickpea and peanut were grown in pots previously used to cultivate the two grasses. Intercropping with purple false brome and barley increased leaf chlorophyll concentrations and/or boosted growth of olive trees on three different calcareous soils. Olive growth was adversely affected by intercropping in one soil as a result of competition for water. Intercropping increased Fe, Mn, Cu and Zn leaf contents in olive. Also, grass cropping generally raised available levels of soil Fe, Mn, Cu and Zn; this effect, however, resulted in no substantial alleviation of Fe chlorosis in succeeding chickpea or peanut crops. Intercropping with purple false brome and barley appears to be a promising remedy for Fe chlorosis in olive orchards affected by Fe chlorosis.

Keywords: Calcareous soils, gramineae, iron chlorosis, iron deficiency, intercropping, phytosiderophores

1. Introduction

Lime-induced iron (Fe) chlorosis is commonplace in crops grown on calcareous soils (Rombolà and Tagliavini 2006). This deficiency is characterized by interveinal yellowing of the youngest leaves by effect of poor Fe redistribution in chlorotic plants (Korcak 1987). Olive (*Olea europaea* L.) additionally exhibits shorter internodes and shoots with few, short leaves in cases of severe Fe chlorosis. Alcántara *et al*. (2003) recommend using tolerant cultivars in order to avoid the problems deriving from Fe deficiency in olive.

A number of Fe fertilizers including synthetic chelates (Lucena, 2006) applied either to soil or leaves efficiently alleviate Fe chlorosis; however, their cost and time-limited effectiveness often make them uneconomical. This shortcoming has promoted extensive research into the mechanisms whereby higher plants acquire Fe from soil and ways to exploit them. Strategy I plants, which include dicots and nongraminaceous monocots, respond to Fe deficiency by releasing protons and reductive substances (e.g., phenols, organic acids) from their roots, and also by increasing ferric reduction activity at the root plasma membrane (Chaney *et al*. 1972). Root exudation of organic acid anions such as citrate, malate or oxalate is thought to provide one of the main strategies for plants growing on calcareous soils to boost nutrient mobilization and acquisition under micronutrient limiting conditions (Jones, 1998).

Solubilized Fe must be reduced from Fe^{3+} to Fe^{2+} at the plasma membrane before Fe^{2+} is mobilized in root cells by a specific Fe^{2+} carrier. By contrast, Strategy II plants such as graminaceae respond to low Fe availability by releasing chelating agents called "phytosiderophores" (PS), which possess a high affinity for Fe^{3+}, into the rhizosphere (Takagi 1976), the Fe^{3+}–PS complexes thus formed being acquired by the plants (Römheld and Marschner 1986). By virtue of their mechanisms of response to Fe deficiency being less sensitive to a high pH and increased bicarbonate levels, grasses are generally more efficient than dicots in acquiring Fe from calcareous soils (Römheld 1991). In addition, grasses can use this chelation strategy to obtain Zn from soil (Marschner *el al*. 1989).

Several studies have shown that intercropping with grasses provides a sustainable strategy for preventing Fe deficiency chlorosis in tree crops (Kamal *et al*. 2000; Tagliavini *et al*. 2000; Zuo *el al*. 2000; Rombolà *et al*. 2003; Cesco *et al*. 2006; Bavaresco *et al*. 2010). This is a result of Fe^{3+}–PS complexes efficiently supplying Fe to dicots after reduction at the plasma membrane (Römheld and Marschner 1986), which may account for some beneficial effects of intercropping dicots with grasses (e.g., re-greening).

Grass covers are widely used in olive orchards to improve soil fertility and prevent erosion. Whether they can alleviate Fe chlorosis in olive is unknown, however. This work was undertaken to assess the effects of intercropping olive tree (*Olea europaea L.)* with grass species on the status of Fe in the olive plant. For this purpose two pot experiments with Fe chlorosis-inducing calcareous soils were carried out where olive (*Olea europaea* L.) plants were intercropped with different grass species. In a third pot experiment, the residual anti-Fe chlorosis effect of grasses was assessed by cultivating two dicots (chickpea and peanut) in soils previously cropped with grasses. The specific grass species studied were *Brachypodium distachyum* L., *Lolium rigidum* L., *Bromus madritensis* L., *Aegilops ventricosa* Tausch and *Hordeum vulgare* L., which had previously proved effective for erosion control in intercropped olive (Junta de Andalucía, 2007), and *Festuca rubra* L., the beneficial effect of which was apparent from its leaf re-greening of intercropped citrumelo plants (Cesco *et al*., 2006).

2. Materials and Methods

2.1. Soil selection and analysis

Soil samples were obtained from the surface horizon (0–30 cm) of three soils from an area in southern Spain where olive trees and vines exhibit the typical symptoms of Fe chlorosis. The samples were air-dried, ground to <2 mm and analysed in duplicate in the laboratory for their content in clay-sized particles (pipette method following dispersion with sodium hexametaphosphate), pH (potentiometric measurement in a 1:2.5 soil mass:water volume suspension), cation exchange capacity (CEC) (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), "active lime" or active calcium carbonate equivalent (ACCE) (Drouineau 1942), electrical conductivity (EC) in a 1:5 soil:water suspension (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)]. Citrate/bicarbonate/dithionite-extractable Fe (Fe_d) was determined according to Mehra and Jackson (1960) except that extraction was performed 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 substantial pH changes by effect of dissolution of soil carbonates during extraction (a modification proposed by Benítez *et al*., 2002). Finally, citrate/ascorbate-extractable Fe (Fe_{ca}) was determined according to Reyes and Torrent (1997), and diethylenetriaminepentaacetic acid-extractable Fe, Mn, Zn, and Cu (Fe_{DTPA}, Mn_{DTPA}, Zn_{DTPA} and Cu_{DTPA}, respectively) according to Lindsay and Norvell (1978).

2.2. Pot experiment 1: selection of grasses for intercropping with olive

Five month old olives [*Olea europaea* L, 'Arbequina', which was deemed one of the most sensitive Fe chlorosis sensitive species by Alcántara *et al*. (2003)] were transplanted into plastic pots filled with ~1.3 kg of soil 1 (Table 1) and placed in a growth chamber with a photoperiod of 14 h (270 µmol photons m^{-2} s^{-1} at leaf level) and 10 h of darkness at a temperature of 24 ºC and a mean relatively humidity of ~70%. Pots were randomly arranged in complete blocks, each block containing plants of similar height. One month later, when olive plants showed symptoms of Fe chlorosis in the youngest leaves, 10 seeds of the selected grass species were sown in each pot (1400 seeds m^{-2}). The experiment comprised the following seven treatments: (1) olive cultivated on bare soil (Control); and olive intercropped with (2) *Brachypodium distachyum* L. (purple false brome), (3) *Lolium rigidum* L. (annual ryegrass), (4) *Bromus madritensis* L. (compact brome), (5) *Aegilops ventricosa* Tausch (goatgrass), (6) *Hordeum vulgare* L. (barley) and (7) *Festuca rubra* L. (red fescue). To the authors' knowledge, none of the grass species studied has an allelopathic effect on olive. Each treatment was replicated 5 times. The soil was watered to the required moisture content by weighing the pots on a daily basis. For each control pot, a total of 250 mL of a Fe-free modified Hoagland nutrient solution [2.5 mM $Ca(NO_3)_2$, 2.5 m*M* KNO_3, 1 m*M* MgSO4, 1 mM KH_2PO_4, 0.1 mM KCl, 50 µM H_3BO_3, 4 µ*M* $MnSO_4$, 4 µM $ZnSO_4$, 1 µM $CuSO_4$ and 0.1 µ*M* $(NH_4)_6Mo_7O_{24}$] was distributed in ten applications over the growth period. Pots containing intercropped olive plants received an additional amount of nutrients (20 % more than the control) in order to meet the nutritional requirements of grasses.

Olive plant height and chlorophyll content were measured on a weekly basis. The chlorophyll content of the youngest olive leaves was estimated from SPAD value as measured with an SPAD 502 portable chlorophyll meter (Minolta Camera Co., Osaka, Japan). Three SPAD measurements per leaf in the six youngest leaves of each olive plant were made, also on a weekly basis. The SPAD × plant height product was additionally calculated weekly as an indicator of plant performance. After the last SPAD reading was obtained at the end of the experiment, the youngest fully expanded leaves were cut off and a piece from the middle about 0.5 cm^2 in size of each was weighed and placed in a tube containing 10 cm^3 of methanol at 25 ºC in the dark for 24 h. The absorbance of the resulting extract was measured at 665 nm (chlorophyll a) and 650 nm (chlorophyll b), and the chlorophyll content per unit leaf weight calculated according to Holden (1976). Leaf chlorophyll concentration per unit weight and SPAD values were highly correlated ($r = 0.92$ ***). Total water consumption, fresh leaf weight and fresh weight of the aerial part were determined at the end of the experiment. A solution obtained by digesting about ten olive leaves previously dried at 65 ºC for at least 72 h with nitric–perchloric acid (Zazoski and Burau 1977) was analysed for Ca, Mg, Fe, Mn, Cu and Zn by atomic absorption spectrophotometry, K by flame emission spectrophotometry and P with the Molybdenum Blue method of Murphy and Riley (1962).

2.3. Pot experiment 2: intercropping of olive with purple false brome and barley

Experiment 2 was conducted in order to confirm the results of Experiment 1, using the same conditions; by exception the soils used were no. 2 and 3 in Table 1, and the pots were smaller (~0.9 kg soil) and used to crop only the grasses best performing in terms of leaf chlorophyll concentration in Experiment 1. One month after transplantation of olive plants to soils 2 and 3, the youngest leaves showed symptoms of Fe chlorosis; at that time, 10 seeds (1400 seeds m^{-2}) of the selected grass species were sown in each pot. The treatments used included (1) cultivation on bare soil (Control); and intercropping of olive plants with (2) *Brachypodium distachyum* L. (purple false brome) and (3) *Hordeum vulgare* L. (barley). The measured parameters were the same as in Experiment 1. Also, after harvesting, soil was removed from the pots, air-dried, ground and analysed for Fe_{ox}, Fe_{DTPA}, Mn_{DTPA}, Zn_{DTPA} and Cu_{DTPA} as described above.

Table 1. Selected properties of the soils

OC, organic carbon; CCE, calcium carbonate equivalent; ACCE, active calcium carbonate equivalent (active lime); EC, electrical conductivity; CEC, cation exchange capacity; Fed, dithionite-extractable Fe; Fe_{ox}, oxalate-extractable Fe; Fe_{ca}, citrate/ascorbate-extractable Fe. All data are means of four replications.

2.4. Pot experiment 3: effect of grass cropping on succeeding crops

Persistence of the anti-Fe chlorosis effect of grass was assessed by sowing about forty seeds (680 seeds m^{-2}) of purple false brome or barley in 28×21×11 cm pots containing 4 kg of soil 2 or 3. A pot containing bare soil was used as control. After 12 weeks of cultivation, the grasses were dry and the soil was removed from the pots and air-dried. Pots filled with an amount of 0.25 kg of these soils were planted with two seedlings of chickpea (*Cicer arietinum* L., cv ICC 11224) or peanut (*Arachis hypogaea* L.) from seeds that were previously germinated on a moistened paper tower for 2–4 days. Only one plant per pot was left one week after transplanting. Pots were randomly arranged in the growth chamber as in Experiment 1, and supplied with 40 ml of Fe-free modified Hoagland nutrient solution distributed in one application each week. SPAD and plant height were measured on a weekly basis. After 3 weeks, plants were harvested and analysed as in the previous experiments, and soil was air-dried and homogenized before analysis for Fe forms.

2.5. Statistical analysis

An analysis of variance (ANOVA) was performed with Statistix 9.0 from Analytical Software (Tallahassee, FL, USA). Means were separated via the LSD test. Pearson correlation was used to relate measured variables. The term "significant" is used here to indicate significance at the $p < 0.05$ level and one, two and three asterisks represent a 0.05, 0.01 and 0.001 probability level, respectively.

3. Results and Discussion

3.1. Soil properties

Table 1 illustrates selected properties of the three soils. As can be seen, all soil samples had high CCE (553–699 g kg^{-1}) and ACCE values (139–295 g kg^{-1}), which is consistent with their alkaline nature (pH 8.3–8.6), but low OC values (7.8–12.4 g kg^{-1}). Soil 1 was loam, and soils 2 and 3 were clayey. CEC ranged from 14.8 $cmol_c$ kg^{-1} (soil 1) to 20 $cmol_c$ kg^{-1} (soil 2). The content in soluble salts of the soils was low (EC < 0.17 dS m^{-1}). The three soils exhibited available P and K contents above critical levels. The concentration of Fe present in the form of Fe oxides as estimated from Fe_d was also low (1.0–3.7 g kg^{-1}) in all soils. Soils 1 and 3 exhibited Fe_{ox} values (i.e., Fe in the form of poorly crystalline Fe oxides) below the critical level proposed by Benítez *el al.* (2002) for Fe chlorosis in olive: 0.35 g kg^{-1}. Also, FeD_{TPA} in all soils was below the critical level proposed by Lindsay and Norvell (1978) for Fe chlorosis, and soil 2 had a Fe_{ox} content above the critical value.

Table 2. Experiment 1. Water consumption, fresh leaf and plant weight, plant height, chlorophyll concentration, SPAD and SPAD × plant height (mean ± standard error) at the end of experiment in olive intercropped with various grasses on soil 1.

Treatment	Water consumption ml	Fresh leaf weight g	Fresh plant weight g	Plant height cm	Chlorophyll mg g^{-1}	SPAD	SPAD × plant height SPAD × cm
Control	1160 ± 70 d	0.14 ± 0.02	11.9 ± 1.2	33.9 ± 3.3	0.89 ± 0.18 b	28.2 ± 3.3 b	960 ± 160
Barley	2420 ± 60 a	0.12 ± 0.01	8.3 ± 1.1	30.3 ± 2.6	1.67 ± 0.24 a	42.6 ± 4.4 a	1330 ± 220
Purple false brome	1480 ± 40 b	0.15 ± 0.02	11.0 ± 0.9	34.9 ± 3.3	1.55 ± 0.21 a	36.7 ± 3.9 ab	1320 ± 220
Red fescue	1210 ± 110 cd	0.11 ± 0.02	11.3 ± 1.7	32.9 ± 3.0	1.01 ± 0.13 b	31.7 ± 2.6 b	1040 ± 112
Annual ryegrass	1110 ± 80 d	0.10 ± 0.02	8.7 ± 1.7	32.4 ± 4.0	0.99 ± 0.23 b	26.5 ± 4.6 b	900 ± 220
Compact brome	1340 ± 50 bc	0.11 ± 0.00	11.4 ± 1.2	31.8 ± 2.6	0.96 ± 0.09 b	31.3 ± 2.9 b	1000 ± 150
Goatgrass	2310 ± 50 a	0.12 ± 0.01	8.4 ± 1.7	30.9 ± 3.5	0.90 ± 0.14 b	26.7 ± 3.4 b	850 ± 160
P-ANOVA	0.000	0.326	0.085	0.227	0.013	0.033	0.140

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

3.2. Effect of intercropping with grasses on olive performance

Table 2 shows the results obtained by intercropping olive plants with six different grasses on soil 1. As can be seen, the highest leaf chlorophyll concentrations and SPAD values were obtained with purple false brome and barley. There were significant differences in leaf chlorophyll concentration between control olive plants and both purple false brome- and barley-intercropped plants. By contrast, SPAD only differed significantly between purple false brome-intercropped and control olive plants. There were no significant differences in fresh leaf weight, plant weight or height between treatments. However, intercropping resulted in significantly increased water consumption with four of the six grasses —but especially barley— relative to the control.

Figure 1 shows the weekly variation of SPAD value, plant height and SPAD × plant height. Based on the SPAD × plant height results, purple false brome and barley were tendentiously more efficient than the other grasses. Leaf mineral element concentrations in grass-intercropped olive plants (results not shown) invariably fell above the critical levels compiled by Fernández-Escobar (2008).

3.3. Performance of olive intercropped with purple false brome and barley on two soils

This experiment was used to confirm the results of Experiment 1 on other soils (viz., no. 2 and 3). These soils had increased clay contents and differed in Fe content, which was high in soil 2 and low in soil 3 (see Table 1). Figure 2 shows the temporal variation of SPAD, plant height and SPAD × plant height for olive plants intercropped with purple false brome and barley on soils 2 and 3. Symptoms of Fe chlorosis were stronger in the plants grown on soil 3, where SPAD values was thus lower than in soil 2. This is consistent with the low Feox content of soil 3 relative to soil 2. At the end of the experiment, intercropping had a positive effect on SPAD that was significant for both grasses in soil 2. On the other hand, plant height was negatively affected in a significant manner by intercropping in soil 2, probably by effect of competition for water between olive plants and grasses as suggested by the results of Experiment 1. Although the pots were watered daily, the grasses demanded so much water that olive growth was adversely affected from the fourth week of the experiment owing to the high density of roots in the pots. The SPAD × plant height product, which was used as a measure of plant performance, was considerably affected by intercropping after only one week in soil 2.

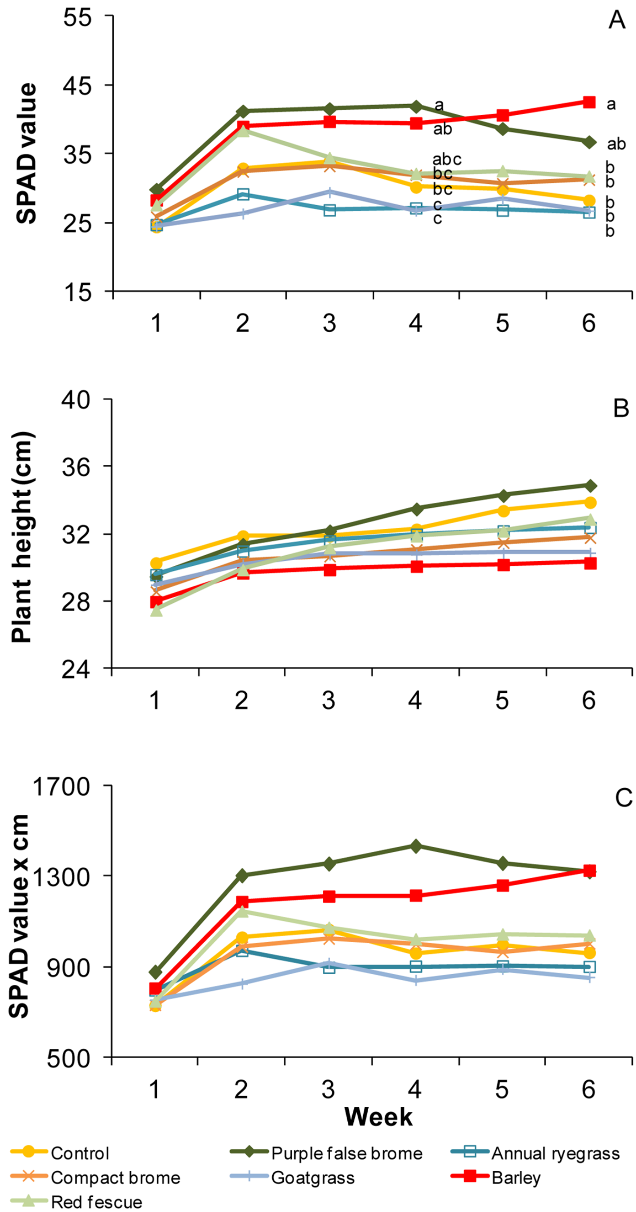

Figure 1. Experiment 1. Variation of SPAD (A), plant height (B) and SPAD × plant height (C) in olive intercropped with six different grasses on soil 1. Different letters for the same week indicate significant differences ($p < 0.05$, LSD test) between treatments.

In this way, the increased SPAD values of the intercropped olive plants were somewhat countered by their decreased growth. As in the previous experiment, no mineral element deficiency was detected (results not shown).

The levels of available Fe, Mn, Zn and Cu as measured with the DTPA test of Lindsay and Norwell (1978) at the end of the experiment were higher in nearly all cases. The effect was significant for Mn_{DTPA}, Zn_{DTPA} and Cu_{DTPA} in soil 3, and for Fe_{DTPA} in both soils; also, Fe_{ox} was slightly but significantly higher in soil 2 under olive-barley intercropping than in its olive-only counterpart (Table 3).

These results can be ascribed to organic acids or phytosiderophores released by grasses (especially barley), which can mobilize cationic micronutrients and make them more readily available to olive, the effect being particularly marked for Fe.

Table 3. Experiment 2. Oxalate-extractable Fe (Fe_{ox}) and diethylenetriaminepentaacetic acid-extractable Fe, Mn, Zn and Cu (Fe_{DTPA}, Mn_{DTPA}, Zn_{DTPA} and Cu_{DTPA}) in soils 2 and 3 after harvesting of olive intercropped with purple false brome and barley (mean ± standard error)

Soil	Treatment	Fe_{ox}	Fe_{DTPA}	Mn_{DTPA}	Zn_{DTPA}	Cu_{DTPA}
		g kg^{-1}	mg kg^{-1}			
2	Control	0.39 ± 0.01 b	2.94 ± 0.34 b	2.33 ± 0.28	0.33 ± 0.01	5.75 ± 1.14
	Purple false brome	0.41 ± 0.00 ab	4.78 ± 0.37 a	3.62 ± 0.84	0.35 ± 0.01	4.85 ± 0.04
	Barley	0.42 ± 0.01 a	4.97 ± 0.37 a	2.50 ± 0.13	0.35 ± 0.01	4.84 ± 0.04
	P- ANOVA	0.018	0.001	0.129	0.410	0.549
3	Control	0.22 ± 0.00	0.96 ± 0.11 c	1.68 ± 0.44 b	0.56 ± 0.02 b	5.46 ± 0.18 b
	Purple false brome	0.23 ± 0.01	2.00 ± 0.08 b	3.84 ± 0.13 a	0.64 ± 0.01 a	5.95 ± 0.11 a
	Barley	0.23 ± 0.01	2.49 ± 0.16 a	4.87 ± 0.41 a	0.57 ± 0.01 b	6.25 ± 0.10 a
	P- ANOVA	0.439	0.000	0.001	0.007	0.005

Different letters in the same column for each soil indicate significant differences ($p < 0.05$, LSD test) between treatments

3.4. Effects of grass cropping on succeeding chickpea and peanut crops

All chickpea and peanut plants showed symptoms of Fe chlorosis throughout the experiments, but especially in the plants grown on soil 3. As can be seen from Table 4, chickpea grown after a grass crop (i.e., not in intercropping) on soils 2 and 3 exhibited —not significantly— higher SPAD values than the control during the first week. The differences disappeared in the second sampling and were reversed in the third week (harvest), the control plants then exhibiting increased SPAD values in both soils. No plant height differences were found, however. Peanut grown after grass exhibited increased SPAD values in the first week (only on soil 3) and also increased stem length at the end of the experiment. This lack of response contradicts the highly significant increases in the more labile form of Fe (as measured by Fe_{ox} and Fe_{DTPA}) brought about by cropping of purple false brome or barley on soil 2 (Table 4). Thus, mobilization of Fe by these grasses had, at most, a short-lived effect on Fe acquisition by succeeding dicots. The reduced efficiency observed may have resulted from degradation of organic acids or phytosiderophores or from exchange of complexed Fe with other cations.

Table 5 shows the contents in mineral elements of the aerial part of chickpea and peanut plants. Previous cropping of purple false brome or barley resulted in significantly increased concentrations of P, Cu and Zn (soil 2), and Mg, Mn, Cu and Zn (soil 3) in chickpea; and also in P, Cu and Zn (soil 2), and P and Zn (soil 3) in peanut.

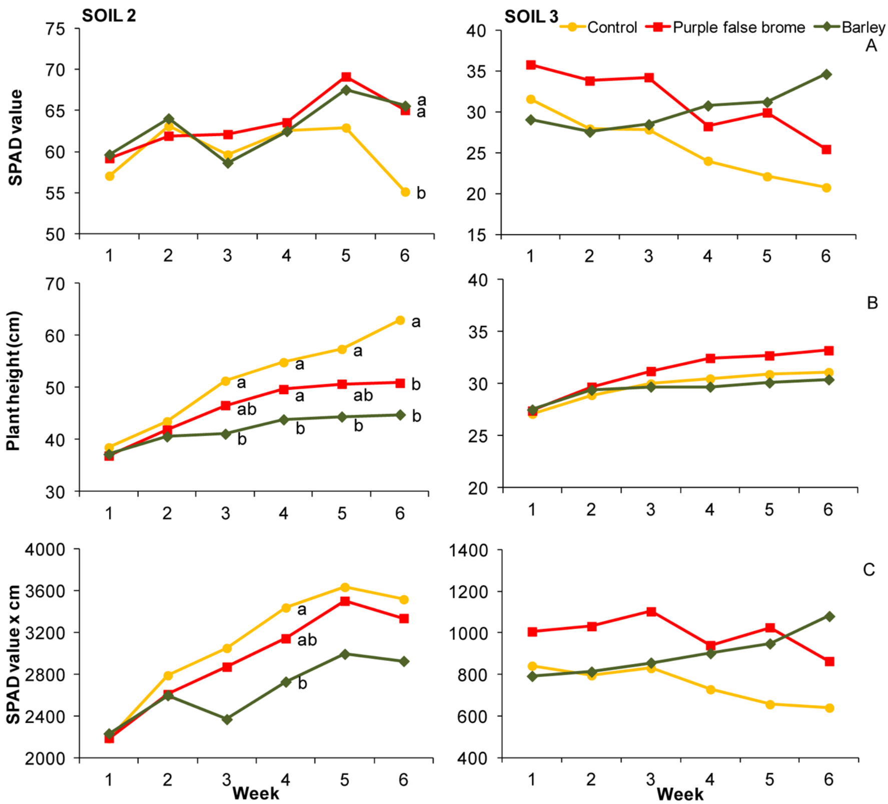

Figure 2. Experiment 2. Variation of SPAD (A), plant height (B) and SPAD × plant height (C) in olive intercropped with purple false brome and barley on soils 2 and 3. Different letters for the same week indicate significant differences ($p < 0.05$, LSD test) between treatments.

Overall, these results are consistent with the increase in available forms of Mn, Zn and Cu (Mn_{DTPA}, Zn_{DTPA} and Cu_{DTPA} , respectively) in Experiment 2 (Table 3) and are similar to those previously obtained by Zuo and Zhang (2008), who found Zn and Cu contents in peanut intercropped with grass to be significantly increased relative to monocropping. Similarly, Inal *et al*. (2007) found peanut in association with maize to result in improved nutrition of plants with Zn, P and K, most probably by effect of biological and chemical processes in the rhizosphere. To what extent chelation of Mn, Cu and Zn contributes to increasing uptake of these nutrients is difficult to assess, however.

Enhanced P uptake by chickpea and peanut (soil 2), and peanut (soil 3), after grass cropping (Table 5) can also be ascribed to changes in P availability induced by root-excreted organic compounds or to hydrolysis of the most labile organic P forms present in grass roots.

Table 4. Experiment 3. Variation of SPAD, plant height and SPAD × plant height in chickpea and peanut at the end of experiment, and of Fe_{ox} (oxalate-extractable Fe) and Fe_{DTPA} (diethylenetriaminepentaacetic acid-extractable Fe) after cropping purple false brome and barley on soils 2 and 3 (mean ± standard error)

Soil	Crop	Treatment	SPAD Week 1	SPAD Week 2	SPAD Week 3	Plant height cm	SPAD × plant height SPAD × cm	Fe_{ox} g kg^{-1}	Fe_{DTPA} mg kg^{-1}
2	Chickpea	Control	25.3 ± 2.9	27.0 ± 1.8	36.0 ± 2.7 a	12.8 ± 0.3	463 ± 43	0.35 ± 0.01 b	2.6 ± 0.1 b
		Purple false brome	32.5 ± 1.5	25.6 ± 1.6	25.1 ± 1.9 b	13.3 ± 0.8	341 ± 45	0.37 ± 0.01 a	3.7 ± 0.3 a
		Barley	31.8 ± 2.5	24.7 ± 2.3	22.9 ± 2.5 b	13.8 ± 0.5	320 ± 42	0.38 ± 0.00 a	3.6 ± 0.1 a
		P- ANOVA	0.096	0.715	0.004	0.455	0.078	0.010	0.001
	Peanut	Control	35.9 ± 4.2	34.2 ± 2.0	37.0 ± 0.9	14.5 ± 0.7	537 ± 25	0.30 ± 0.00 c	5.0 ± 0.1 b
		Purple false brome	34.9 ± 1.8	36.1 ± 0.8	38.6 ± 1.7	15.4 ± 0.5	596 ± 39	0.38 ± 0.02 b	7.6 ± 0.1 a
		Barley	33.9 ± 0.9	37.5 ± 1.1	37.8 ± 1.5	15.2 ± 0.2	575 ± 27	0.41 ± 0.01 a	7.2 ± 0.3 a
		P- ANOVA	0.872	0.285	0.736	0.522	0.423	0.000	0.000
3	Chickpea	Control	21.4 ± 1.3	15.2 ± 1.5	23.6 ± 1.4	11.5 ± 0.2	272 ± 17	0.18 ± 0.01	1.1 ± 0.2
		Purple false brome	27.4 ± 0.9	18.9 ± 2.2	17.1 ± 2.2	11.0 ± 0.4	186 ± 19	0.18 ± 0.01	1.8 ± 0.3
		Barley	27.0 ± 3.3	18.5 ± 2.0	20.2 ± 2.9	10.8 ± 0.3	218 ± 29	0.18 ± 0.01	1.8 ± 0.2
		P- ANOVA	0.112	0.346	0.169	0.233	0.051	0.823	0.103
	Peanut	Control	28.9 ± 2.4	25.6 ± 2.0	27.4 ± 1.0	12.5 ± 0.4 b	343 ± 19	0.23 ± 0.04	2.8 ± 0.1
		Purple false brome	29.9 ± 3.4	25.7 ± 3.3	25.5 ± 2.9	13.9 ± 0.5 a	356 ± 49	0.21 ± 0.00	3.2 ± 0.3
		Barley	37.6 ± 2.3	32.2 ± 3.0	27.8 ± 2.4	14.2 ± 0.2 a	380 ± 36	0.21 ± 0.01	3.1 ± 0.3
		P- ANOVA	0.087	0.204	0.839	0.020	0.776	0.900	0.409

Different letters in the same column for each soil and crop indicate significant differences ($p< 0.05$, LSD test) between treatments

Table 5. Experiment 3. Mineral element concentrations (mean ± standard error) in the aerial part of chickpea and peanut grown after purple false brome and barley on soils 2 and 3

Soil	Crop	Treatment	Ca	Mg	K	P	Fe	Mn	Cu	Zn
			g kg^{-1}				mg g^{-1}			
2	Chickpea	Control	72.5 ± 6.0	4.2 ± 0.3	27.6 ± 4.2	1.2 ± 0.1 b	136.1 ± 28.3	38.7 ± 2.4	8.2 ± 0.4 b	14.8 ± 0.6 b
		Purple false brome	61.8 ± 6.3	5.0 ± 0.5	29.0 ± 4.7	4.3 ± 0.4 a	105.0 ± 10.7	31.1 ± 3.1	11.5 ± 0.8 a	32.7 ± 3.1 a
		Barley	53.3 ± 6.6	4.0 ± 0.5	20.3 ± 4.1	4.7 ± 0.6 a	92.2 ± 25.2	34.9 ± 2.4	10.9 ± 1.0 a	26.5 ± 2.9 a
		P- ANOVA	0.138	0.317	0.353	0.000	0.402	0.171	0.025	0.001
	Peanut	Control	59.7 ± 1.1	4.8 ± 0.2	27.0 ± 5.2	2.2 ± 0.1 b	62.2 ± 5.4	73.0 ± 4.9 a	9.9 ± 0.4 b	21.8 ± 1.3 b
		Purple false brome	55.8 ± 1.2	4.8 ± 0.1	27.1 ± 3.1	3.5 ± 0.3 a	68.7 ± 9.1	49.3 ± 4.1 b	11.8 ± 0.6 a	29.0 ± 1.3 a
		Barley	62.1 ± 2.2	5.6 ± 0.4	24.8 ± 3.2	3.2 ± 0.2 a	53.5 ± 2.9	69.9 ± 4.3 a	13.1 ± 0.4 a	27.3 ± 1.4 a
		P- ANOVA	0.050	0.053	0.900	0.003	0.274	0.005	0.003	0.005
3	Chickpea	Control	86.7 ± 2.2	4.9 ± 0.1 b	19.7 ± 4.4	6.0 ± 0.6	78.4 ± 7.0 ab	34.3 ± 1.1 b	7.7 ± 0.2 b	17.3 ± 0.5 b
		Purple false brome	90.3 ± 5.0	6.0 ± 0.3 a	23.3 ± 2.6	5.1 ± 0.1	89.1 ± 14.5 a	41.0 ± 2.5 ab	13.8 ± 0.4 a	42.8 ± 1.0 a
		Barley	90.5 ± 2.6	6.0 ± 0.2 a	27.5 ± 4.6	4.9 ± 0.2	49.4 ± 3.1 b	44.7 ± 3.7 a	14.1 ± 0.3 a	43.1 ± 2.1 a
		P- ANOVA	0.695	0.004	0.407	0.117	0.031	0.048	0.000	0.000
	Peanut	Control	73.0 ± 0.5	6.2 ± 0.2	29.9 ± 3.9	2.2 ± 0.1 b	45.6 ± 3.4	68.7 ± 4.1	10.6 ± 0.4	23.5 ± 0.7 b
		Purple false brome	66.7 ± 4.5	5.4 ± 0.5	26.2 ± 2.6	3.5 ± 0.3 a	48.9 ± 3.2	60.2 ± 4.7	12.0 ± 0.7	30.6 ± 0.8 a
		Barley	69.9 ± 3.0	5.3 ± 0.6	22.9 ± 3.3	3.2 ± 0.2 a	55.2 ± 4.9	65.7 ± 7.5	13.0 ± 0.7	29.1 ± 1.1 a
		P- ANOVA	0.393	0.388	0.357	0.003	0.252	0.574	0.054	0.000

Different letters in the same column for each soil and crop indicate significant differences ($p < 0.05$, LSD test) between treatments

4. Conclusions

Only purple false brome and barley among the six grass species studied succeeded in alleviating Fe deficiency symptoms in olive intercropped with them. Our results also signal the need for appropriate management of the grass cover in order to avoid competition with water. Overall, grass cropping significantly increased the content in available Fe of soil as measured by oxalate- or DTPA-extractable Fe, and also the contents of other micronutrients such as Mn, Cu and Zn. The residual anti-Fe chlorosis effect that grasses had on the succeeding dicot crops (chickpea and peanut) was, however, short-lived or insignificant —which suggests fast degradation of organic acids or Fe-phytosiderophores excreted by grasses. By contrast, intercropping improved plant uptake of Mn, Cu and Zn.

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