Soil nutrients effects on the performance of durum wheat inoculated with entomopathogenic fungi

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SUPPLEMENTARY MATERIAL

Table S1: Additional soil properties‡ of the soils described in Sacristán et al. (2019)

Soil	Soil mass per pot	HMF	Fe_d	Exchangeable cations				Clay fraction composition				
				Ca	Mg	Na	K	S	V	C	I	K
	g		g kg^{-1}	cmol$_c$ kg^{-1}				%				
Non-calcareous soils												
2	300	0.979	19.2	8.4	2.00	0.25	0.38	0	0	0	72	28
4	465	0.992	13.5	2.4	0.34	0.08	0.2	0	29	0	26	45
8	260	0.943	35.9	39.5	3.20	0.35	0.75	0	14	0	39	47
Calcareous soils												
19	255	0.973	4.8		1.50	0.13	0.98	0	37	0	54	9
21	335	0.981	7.5		1.00	0.14	0.26	63	0	0	36	1
22	340	0.982	7.2		0.80	0.14	0.22	64	0	0	35	1
26	265	0.959	5.6		7.00	0.27	1.5	77	0	0	18	5
27	265	0.964	5.8		7.10	0.33	1.7	67	0	0	28	5
28	245	0.972	2.0		1.40	0.16	0.65	75	0	4	19	2
31	295	0.973	19.1		1.60	0.19	1.4	0	0	1	95	4
37	275	0.980	9.6		1.00	0.15	0.42	7	0	0	80	14
48	315	0.972	8.4		3.00	0.14	1.5	42	0	0	50	8

‡ HMF: Hygroscopic moisture factor; Fe_d: citrate/bicarbonate/dithionite-extractable Fe. S, Smectite; V, Vermiculite; C, Chlorite; I, Illite; K, Kaolinite.

Organic C (OC) was determined by rapid dichromate oxidation; particle size distribution by Gee and Bauder, (1986); pH by potentiometric measurement in a 1:2.5 w/v soil:water suspension; total calcium carbonate ($CaCO_3$) equivalent by van Wesemael, (1955); electrical conductivity (EC) in a 1:5 soil:water suspension with a conductivity meter; and cation exchange capacity (CEC) by extraction with 1 M NH_4OAc buffered at pH 7. The different forms of iron (Fe) were extracted by *Mehra* and *Jackson*, (1960) and *Schwertmann*, (1964) and measured with the *o*-phenanthroline colorimetric method [6]. Micronutrients (Fe, Cu, Mn and Zn) were extracted with diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978) for measurement by atomic absorption spectrophotometry. Available soil P by Olsen et al. (1954) and phosphorus in the 0.01 M $CaCl_2$ was used as a proxy for P in the soil solution determined in a 1:10 w:v soil:solution suspension stirring it 30 min. and measured both with the method of Murphy and Riley (1962). Clay fraction composition was performed on oriented mounts (Mg saturation, Mg saturation/ ethylene glycol solvation, and K saturation treatments) examined by X-ray diffraction using a Bruker D8 ADVANCE instrument with monochromatic Cu Kα radiation. Semiquantitative estimates of the proportions of different minerals were obtained by using the method of [10].

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Table S2: One-way ANOVA or Kruskal–Wallis test and comparison of means ‡ for wheat biomass, nutrient uptake and grain nutrient concentration (mean value, n = 18) on the different soils at harvest.

Soil code	Biomass						Nutrient uptake										Grain nutrient concentration										Grain P/Zn ratio	
	Grain yield		ADM§		Harvest index		P		K		Fe		Mn		Zn		P		K		Fe		Mn		Zn			
	g						mg plant^{-1}				µg plant^{-1}						mg g^{-1}				µg g^{-1}							
2	0.82	abcd	2.54	a	0.32	e	5.0	c	57	b	143	c	57	c	47	bcde	3.8	abc	5.5	b	46	bc	27	c	33	bcde	124	ab
4	0.50	cde	1.32	c	0.40	bcd	1.4	h	29	e	71	g	102	a	48	cde	2.5	cd	4.8	ef	57	bc	49	a	67	ab	40	ef
8	0.54	bcde	1.56	bc	0.34	de	1.9	fg	33	de	88	f	43	d	48	bcde	3.2	abc	5.2	bcd	48	bc	30	abc	61	ab	53	bcdef
19	0.82	ab	1.61	bc	0.51	a	2.1	ef	36	d	112	e	36	e	66	ab	2.3	cd	5.2	bcde	41	c	28	bc	53	ab	44	def
21	0.61	bcde	2.55	a	0.24	f	5.6	b	59	b	201	a	85	b	75	a	4.9	ab	5.5	b	60	ab	44	ab	65	a	77	abc
22	0.82	abc	1.92	ab	0.43	bc	2.3	ef	46	c	114	e	32	f	50	abcd	2.5	cd	4.9	cdef	44	bc	23	c	43	abcd	60	bcde
26	0.80	abcd	2.21	ab	0.35	de	2.8	d	58	b	159	b	25	g	60	abc	2.9	bc	4.9	cdef	50	bc	20	c	53	abc	61	bcde
27	0.53	de	1.16	c	0.45	ab	1.6	gh	33	de	87	f	16	h	26	ef	2.3	cd	7.7	a	51	abc	22	c	31	cde	82	abcd
28	0.48	e	1.27	c	0.38	cde	1.0	i	28	e	91	f	26	g	41	def	1.8	d	4.5	f	42	c	27	bc	55	a	32	f
31	0.37	e	2.00	ab	0.19	f	2.5	de	55	b	120	de	79	b	40	def	2.4	cd	5.4	bc	71	a	44	ab	54	abc	51	cdef
37	0.66	abcde	1.58	bc	0.40	bcd	1.9	fg	36	d	70	g	37	e	21	f	2.5	cd	4.8	def	42	c	30	abc	20	e	122	ab
48	1.05	a	2.73	a	0.38	cde	6.3	a	66	a	127	d	39	de	44	def	5.0	a	4.6	f	43	c	21	c	25	de	206	a

‡When differences were significant, the LSD (ANOVA) or Dunn's (Kruskal–Wallis) post-hoc test was performed, different letters denoting significant differences between soils. The Kruskal–Wallis test was applied to biomass, grain nutrient concentration and P/Zn ratio in grain because the variance homoscedasticity condition was not fulfilled .
§Aerial dry matter

Table S3: Factorial ANOVA (soil × fungal treatment) and Kruskall–Wallis test (†, when data failed to fulfill the criteria for parametric analysis) for grain yield, straw and nutrient uptake of durum wheat plants grown on GSGYI < 15.§ Significant differences (P < 0.05) are in boldface.

Treatment	Biomass		Plant nutrient uptake							
	Grain yield	Straw	P	K	Mg	Ca	Na	Fe	Mn	Zn
	g		mg plant^{-1}				µg plant^{-1}			
Control	0.64 ± 0.04	0.88 ± 0.05	1.83 ± 0.11	36.3 ± 1.9	3.11 ± 0.18	3.34 ± 0.24	475 ± 41	99 ± 2	32.1 ± 3.3	43.9 ± 2.9
B. bassiana	0.63 ± 0.04	0.92 ± 0.06	1.75 ± 0.13	36.1 ± 1.9	3.26 ± 0.19	3.27 ± 0.21	486 ± 43	102 ± 3	32.2 ± 3.3	44.9 ± 2.8
P_{FT}‡	0.888	0.916	0.415	0.778	0.158	0.706	0.057	0.250	0.766	0.546
$P_{interaction}$	†	†	0.867	**0.002**	0.818	†	**0.002**	0.517	0.255	0.653
Control	0.61 ± 0.03	1.08 ± 0.08	2.59 ± 0.28	39.9 ± 2.1	3.62 ± 0.23	4.34 ± 0.39	506 ± 36	114 ± 7	43.0 ± 4.5	46.9 ± 3.3
M. brunneum	0.59 ± 0.03	1.08 ± 0.09	2.27 ± 0.23	43.6 ± 3.1	3.63 ± 0.25	3.94 ± 0.30	515 ± 42	111 ± 7	42.9± 4.6	46.8 ± 3.7
P_{FT}	0.688	0.605	0.474	0.118	0.453	0.500	0.112	0.418	0.664	0.355
$P_{interaction}$	†	†	0.310	**0.048**	0.556	0.281	0.079	0.256	0.072	0.279

†Absence of *P* for the interaction means that the variance fulfilled neither the homoscedasticity nor the normality condition, so a Kruskal–Wallis test was used instead.
§Group of soils in which the increase in grain yield was lower than 15% (Soils 19, 22, 27, 28 and 31, $n = 30$, with *B. bassiana*; and Soils 19, 21, 22, 27, 28, 31 and 37, $n = 42$, with *M. brunneum*).
‡Only the probability values (*P*) for the fungal treatment (P_{FT}) and the soil × fungal treatment interaction ($P_{interaction}$) are shown because those for the factor soil (P_{soil}) were all significant ($P < 0.05$).

Table S4: Factorial ANOVAs for nutrient uptake and grain nutrient concentration (mean ± standard error) with soil and fungal treatment as factors for the GSGYI > 15. Significant differences ($P < 0.05$) are in boldface.

	Plant nutrient uptake				Grain nutrient concentration			
Treatment	Mg	Ca	Fe	Mn	Mg	Ca	Fe	Mn
	mg plant^{-1}		μg plant^{-1}		g kg^{-1}	mg kg^{-1}		
Control	5.10 ± 0.31	4.67 ± 0.37	128 ± 8	57.9 ± 5.5	2.34 ± 0.07	210 ± 7	51.8 ± 2.3	33.6 ± 2.3
B. bassiana	4.95 ± 0.31	4.10 ± 0.25	123 ± 7	53.4 ± 4.4	2.09 ± 0.06	214 ± 6	47.6 ± 2.0	29.4 ± 2.1
P_{FT}‡	0.452	0.107	0.914	0.212	**0.008**	0.661	0.146	0.051
$P_{interaction}$	0.604	0.169	0.076	0.344	†	0.188	0.828	0.944
Control	5.18 ± 0.38	3.71 ± 0.19	117 ± 6	52.3 ± 6.6	2.32 ± 0.08	201 ± 7	51.1 ± 2.8	29.9 ± 2.7
M. brunneum	4.98 ± 0.39	3.66 ± 0.23	121 ± 8	54.8 ± 7.1	2.19 ± 0.08	189 ± 4	46.2 ± 3.0	30.4 ± 4.0
P_{FT}§	0.146	0.88	0.092	0.711	0.491	**0.037**	0.193	0.973
$P_{interaction}$	0.755	0.299	0.108	0.996	0.678	**0.017**	0.976	0.889

†Absence of P for the interaction means that the variance fulfilled neither the homoscedasticity nor the normality condition, so a Kruskal–Wallis test was used instead.

§ Group of soils in which grain yield increased by more than 15% (Soils 2, 4, 8, 21, 26, 37 and 48, $n = 42$, with *B. bassiana*; and Soils 2, 4, 8, 26 and 48, $n = 30$, with *M. brunneum*).

‡Only the probability values (P) for the fungal treatment (P_{FT}) and the soil × fungal treatment interaction ($P_{interaction}$) are shown because those for the factor soil (P_{soil}) were all significant ($P < 0.05$).

Table S5: Factorial ANOVA (soil × fungus) and Kruskall–Wallis test (†, when data failed to fulfill the ANOVA criteria) for grain nutrient concentration in the GSGYI < 15.§ Significant differences ($P < 0.05$) are in boldface**.**

Treatment	P	K	Mg	Ca	Na	Fe	Mn	Zn	Grain P/Zn ratio
	mg kg^{-1}			μg kg^{-1}					
Control	2.40 ± 0.17	5.55 ± 0.29	1.96 ± 0.08	212 ± 6	46.9 ± 3.3	45.7 ± 2.4	27.5 ± 1.7	44.2 ± 2.9	62.3 ± 7.6
B. bassiana	2.07 ± 0.11	5.52 ± 0.30	2.03 ± 0.09	217 ± 6	44.8 ± 2.8	48.7 ± 1.9	26.3 ± 1.3	46.9 ± 2.2	48.3 ± 4.1
P_{FT}‡	0.333	0.626	0.286	0.806	0.185	0.328	0.334	0.146	0.615
$P_{interaction}$	†	0.141	0.683	†	†	0.799	0.711	0.361	†
Control	2.97 ± 0.23	5.38 ± 0.23	2.07 ± 0.07	223 ± 6	45.6 ± 2.4	47.9 ± 2.1	31.7 ± 1.8	46.6 ± 3.2	73.3 ± 7.0
M. brunneum	2.59 ± 0.17	5.44 ± 0.16	2.04 ± 0.09	216 ± 6	45.9 ± 2.7	49.7 ± 2.7	29.6 ± 1.5	47.9 ± 2.9	61.9 ± 6.3
P_{FT}	0.24	0.098	0.947	0.551	0.99	0.334	0.077	0.812	0.157
$P_{interaction}$	0.685	**0.029**	0.424	†	†	0.454	0.321	†	†

†Absence of *P* for the interaction means that the variance fulfilled neither the homoscedasticity nor the normality condition was fulfilled, so a Kruskal–Wallis test was used instead.

§Group of soils in which grain yield increased by less than 15% (Soils 19, 22, 27, 28 and 31, $n = 30$, with *B. bassiana*; and Soils 19, 21, 22, 27, 28, 31 and 37, $n = 42$, with *M. brunneum*).

‡Only the probability values (*P*) for the fungal treatment (P_{FT}) and the soil × fungal treatment interaction ($P_{interaction}$) are shown because those for the factor soil (P_{soil}) were all significant ($P < 0.05$).

Table S6: Paired *t*-test for nutrient uptake (mean ± SE) of fungus-treated plants against non-inoculated plants ($n = 72$) at harvest (102 DAS). Significant P values ($P < 0.05$) are in boldface.

Treatment	P	K	Mg	Ca	Na	Fe	Mn	Zn
	mg plant^{-1}				µg plant^{-1}			
Control	2.99 ± 0.25	41.9 ± 1.7	4.24 ± 0.23	4.08 ± 0.25	735 ± 89	115 ± 5	46.7 ± 3.8	48.6 ± 2.1
Bb	2.69 ± 0.22	44.2 ± 1.9	4.22 ± 0.22	3.73 ± 0.18	832 ± 10	114 ± 5	44.2 ± 3.2	47.5 ± 1.8
P	0.053	0.205	0.969	0.286	0.136	0.677	0.221	0.337
Mb	2.83 ± 0.24	46.9 ± 2.5	4.19 ± 0.23	3.83 ± 0.21	801 ± 110	116 ± 6	47.8 ± 4.0	47.8 ± 2.2
P	0.572	0.081	0.757	0.574	0.106	0.214	0.874	0.870

Table S7: Paired *t*-test for grain nutrient concentration (mean ± SE) of fungus-treated plants against non-inoculated plants ($n = 72$) at harvest (102 DAS). Significant P values ($P < 0.05$) are in boldface.

Treatment	P	K	Ca	Mg	Na	Fe	Mn	Zn	Grain P / Zn ratio
	mg kg^{-1}				µg kg^{-1}				
Control	3.29 ± 0.18	5.08 ± 0.15	212 ± 5	2.17 ± 0.06	49 ± 2	49.2 ± 1.7	31.0 ± 1.5	48.4 ± 2.8	83.9
Bb	2.70 ± 0.15	5.26 ± 0.16	216 ± 4	2.06 ± 0.06	51.3 ± 2.85	48.1 ± 1.4	28.1 ± 1.3	44.4 ± 2.2	72.4
P	**0.002**	0.175	0.417	0.105	0.689	0.265	**0.004**	0.126	0.066
Mb	2.98 ± 0.16	5.26 ± 0.11	205 ± 4	2.11 ± 63.1	49.5 ± 2.4	48.2 ± 2.0	29.9 ± 1.9	47.3 ± 2.7	78.2
P	**0.012**	0.115	0.369	0.249	0.888	0.322	0.19	0.715	0.205

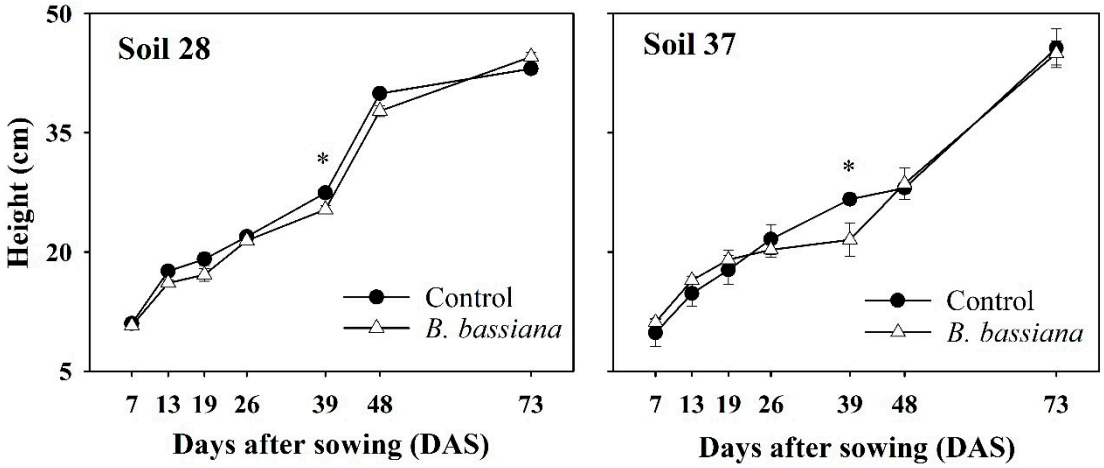

Figure S1: Time course of plant height (mean ± standard error, $n = 6$) in plants inoculated with *B. bassiana* and non-inoculated (Control) plants grown on Soils 28 and 37. Significant ($P < 0.05$) differences are marked with an asterisk

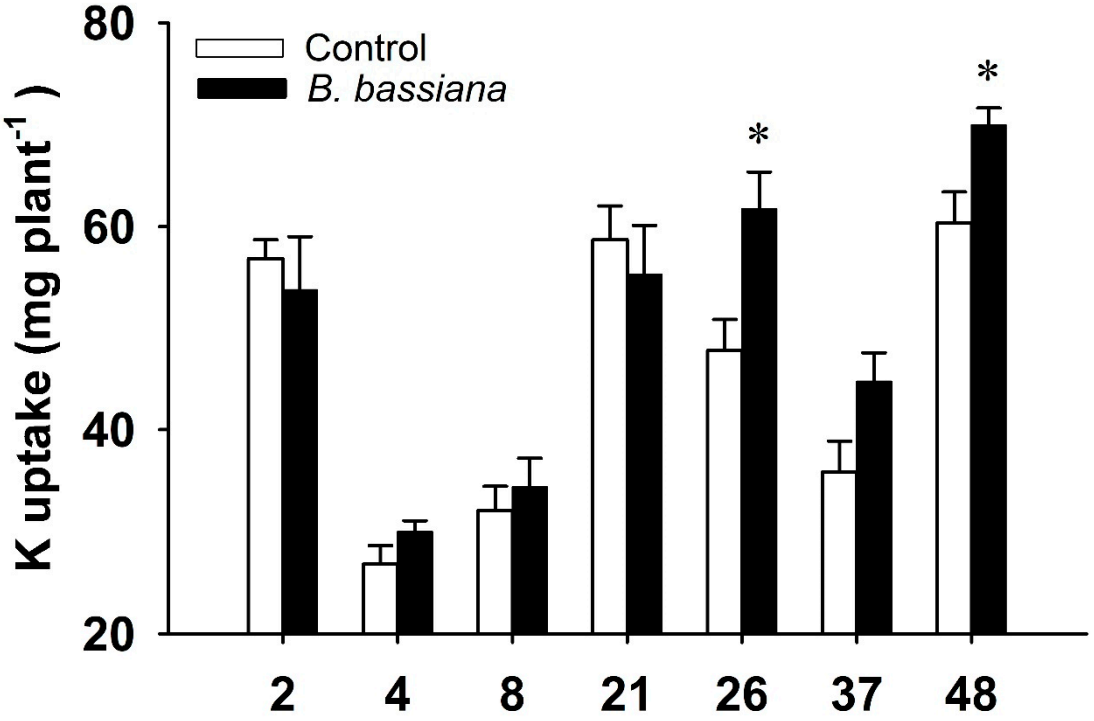

Figure S2: Nature of soil × fungal treatment interaction in K uptake by plants on soils pertaining to the GSGYI > 15 (those where grain yield was increased by more than 15%) inoculated with *B. bassiana*. Significant ($P < 0.05$) differences are marked with an asterisk

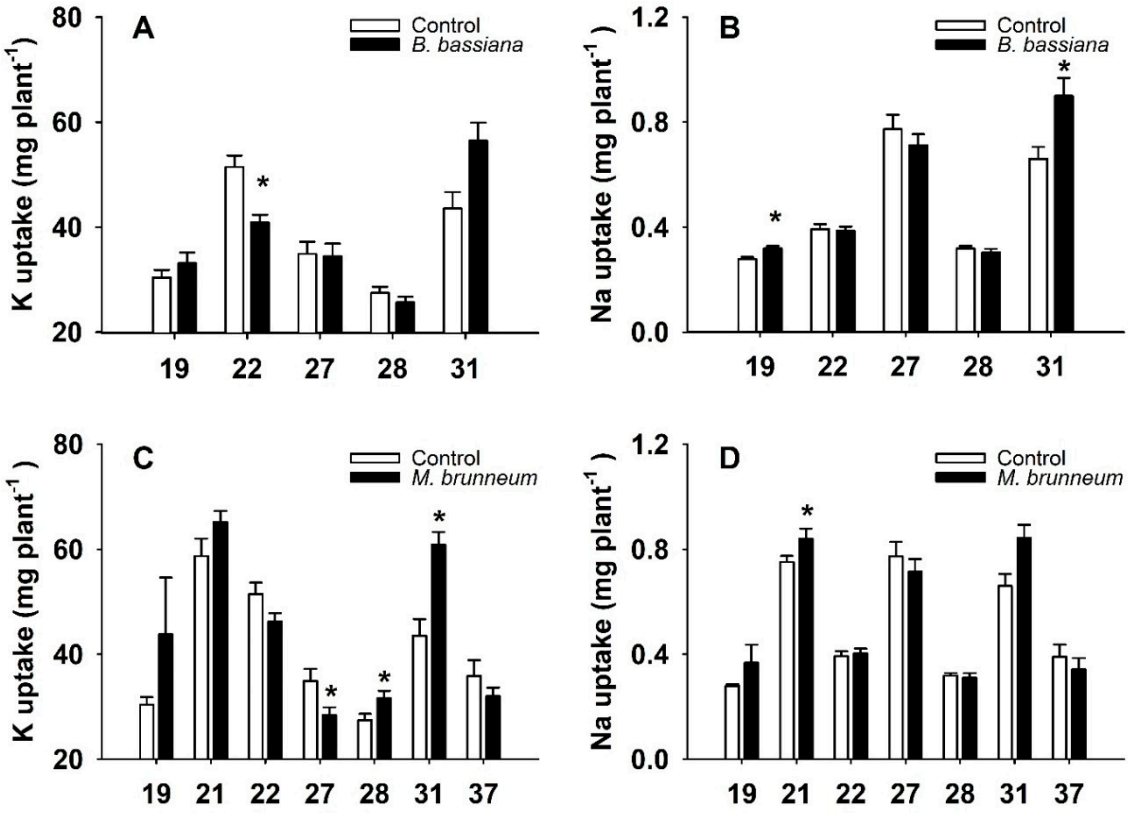

Figure S3: Nature of soil × fungal treatment interaction in K and Na uptake by plants on soils pertaining to the GSGYI < 15 (those where grain yield was increased by less than 15%) inoculated with *B. bassiana* (A and B) or *M. brunneum* (C and D). Significant (P <0.05) differences are marked with an asterisk

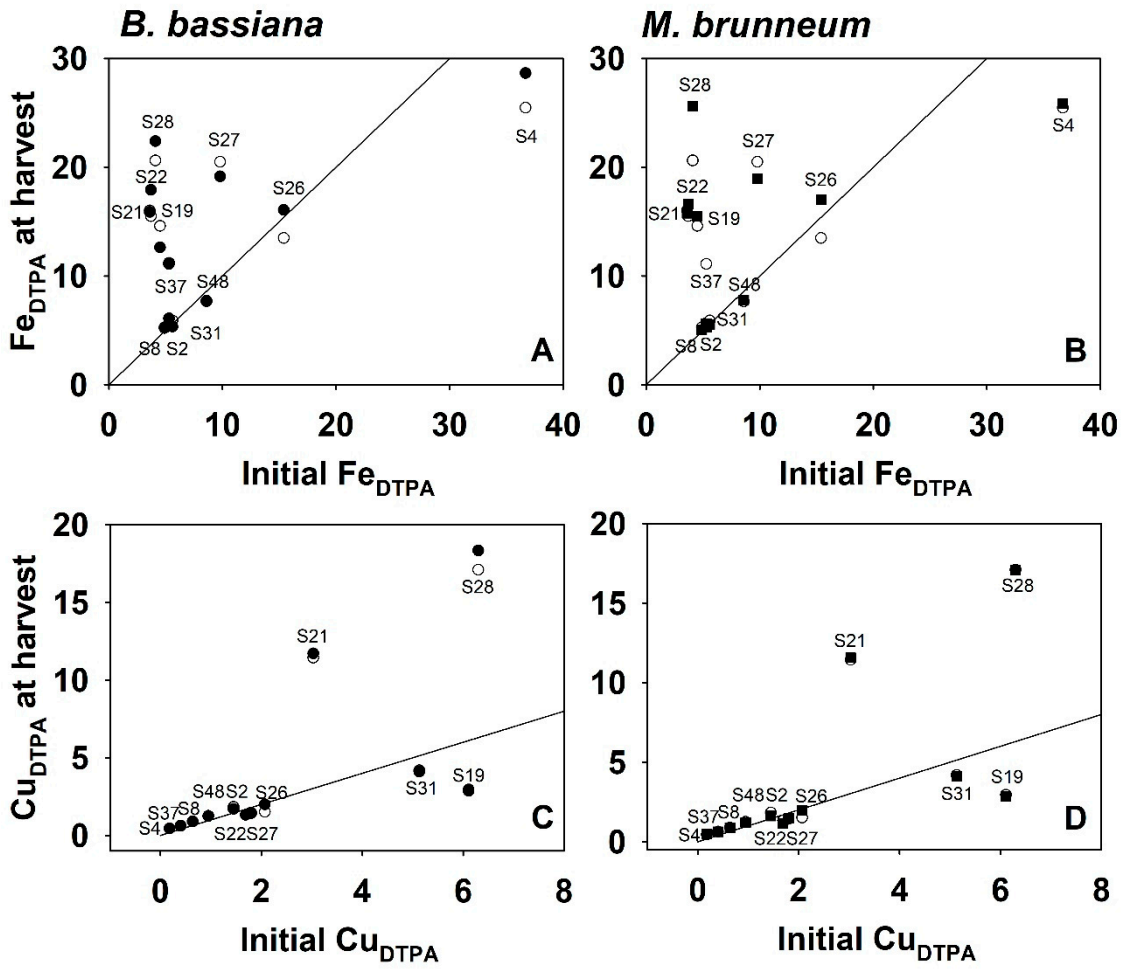

Figure S4: Nutrient availability (Fe and Cu) in soils before (*x* axis) and after (*y* axis) crop, showing the different values for Control (white circles), *B. bassiana* (black circles) and *M. brunneum* (black squares).