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Impact of Na-selenite fertilization on the microbial biomass and enzymes of a soil under corn (*Zea mays* L.) cultivation

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Abstract

We tested the over time effect of different selenium doses [50 (D50) and 100 (D100) g ha^{-1} of Se as Na_2SeO_3] on a soil under corn (*Zea mays* L.) cultivation. The soil was sampled 18 (t1), 48 (t2) and 59 (t3) days after the addition of Se and analysed for total Se, organic carbon and nitrogen, water–extractable organic carbon, available P, microbial biomass–C (C_{mic}) contents, the cumulative basal respiration (ΣCO_2–C) and some enzymatic activities. Our findings showed Se fertilization increased the total soil Se content, although the differences between the treated and the untreated soils disappeared over time. Se fertilization had a negligible effect on the selected soil chemical and biochemical properties, with the exception of the ΣCO_2–C, and fluorescein diacetate hydrolysis and dehydrogenase activity. Indeed, these parameters showed lower values at t3 in the treated than in the untreated soils without significant decrease of the C_{mic}, suggesting a less energy demanded by the soil microorganisms for their own maintenance. This finding suggested a better adaptation of the microbial community to the modified conditions in the treated soils, where Se fertilization might have caused a shift in soil microbial community structure and/or promoted the survival of selected microorganisms. Overall, the obtained data highlighted that Se fertilization with Na–selenite, at the rate of 50 and 100 g ha^{-1}, had no negative impact on soil chemical and biochemical parameters, at least on a short term.

Keywords: enzyme activities; maize field; selenium; soil microbial C; soil Se fertigation.

Selenium (Se) is an essential micronutrient for animals and humans. Food is the main Se source for humans, but the concentration of Se in food depends on its content in the soil where the animals have been raised or plants have been grown. The application of Se–bearing fertilizers is an option to increase Se concentration in soils (De Feudis et al., 2019) and food crops (D'Amato et al., 2019). Sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) are the common Se forms used for agronomic biofortification in several countries. Both forms are water–soluble, but selenate is more mobile in the soil than selenite which is strongly adsorbed to soil particles with positively charged sites (Eich-Greatorex et al., 2007). Although Se–enriched fertilizers are widely used, in form of selenate or selenite, only a few studies have addressed the influence of Se on soil biochemical properties. In particular, they reported a reduction of both enzyme and microbial activities when large doses of Se were provided to soil (Espinosa-Ortiz et al., 2016; Nowak et al., 2002). Further, the previous studies did not investigate the soil biochemical properties through an over time field experiment, but they were conducted in laboratory conditions and/or measuring the biochemical parameters few days after Se addition.

In the present work, we tested the over time effect of 50 and 100 g ha^{-1} of Se as Na_2SeO_3 on some soil enzymatic activities, microbial biomass and basal respiration under corn cultivation. We tested the following hypotheses: 1) Se fertilization reduces soil microbial biomass and respiration, and enzymatic activities; 2) the negative effects of Se fertilization increase with the dose; 3) the influence of Se reduces over time.

The experiment was performed in 2015, at the Experimental Farm of the University of Perugia (Italy), located at 42° 96′ N, 12° 38′ E, with a total annual precipitation of 689 mm and a mean annul temperature of 15.3 °C. The soil was classified as fine, mixed, mesic, Typic Haplustept (Soil Survey Staff, 2014), and the Ap horizons (0-37 cm) had a silty clay texture, sub-alkaline reaction (pH_{H2O} 7.9), 5% carbonate content, and a cation exchange capacity of 33.13 $cmol_{(+)}$ kg^{-1} (for soil description see Table S1 of the Supplementary Materials).

On 12th April 2015, corn (*Zea mays* L. variety DKC 4316) was sowed with a density of 7.5 plants m^{-2}. At seeding, the field was fertilized with 150 kg N ha^{-1} and 75 kg P_2O_5 ha^{-1} in form of urea and triple superphosphate, respectively. Irrigation occurred on May 26th (20 mm), June 23th (100 mm), and July 6th (30 mm) by drip irrigation. To prevent weed occurrence, a pre-emergence treatment was performed with herbicides and hand hoeing. On June 10th, 36 plants were selected according to a completely randomized design within an area of 20x20 m. For each plant, a microplot was delimited by a PVC ring (Ø = 0.4 m; h = 0,35 m) which was placed around each plant stem and inserted vertically into the soil to 0.3 m depth. Specifically, 12 microplots were treated with 1 L of solution containing 1.423 mg of Na_2SeO_3, corresponding to 50 g Se ha^{-1} (D50), 12 microplots were treated with 1 L of solution containing 2.846 mg of Na_2SeO_3, corresponding to 100 g Se ha^{-1} (D100), and 12 microplots were treated with 1 L of distilled water and used as control (CTR).

The soil sampling was carried out on June 28th (t1), July 28th (t2) and September 8th (t3). At each sampling time the shoots of four plants per treatment were cut in correspondence of the neck and the root systems were sampled together with the soil volume delimited by the PVC ring until 0.3 m depth (Ap horizons). Once in the laboratory, the soil was isolated from the roots and sieved through a 2–mm mesh. An aliquot of each sample was stored at 4°C for the biochemical analyses, while the rest was allowed to air–dry.

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The fluorescein diacetate hydrolysis (FDA–H) rate was estimated using the method of Swisher and Carroll (1980) with some modifications. β–glucosidase, acid (acP) and alkaline (alkP) phosphatases, and arylsulphatase activities were determined according to Tabatabai (1994). Dehydrogenase activity (DHA) was evaluated according to von Mersi and Schinner (1991).

One–way ANOVA was performed to assess the effect of Se fertilization and sampling time on the selected soil chemical and biochemical parameters. Tukey's honest significant difference test was conducted for separation of the means at the 95% confidence level.

Our findings showed Se fertilization increased the total soil Se content, although the differences between the treated and the untreated soils disappeared over time (Table 1). The reduction of the Se content from the treated soils could be mainly due to volatilization processes performed by the soil heterotrophic microbial communities (e.g., Paul and Saha, 2019). This hypothesis is supported by a similar experiment performed in the same study site by De Feudis et al. (2019) which reported that the amount of Se taken–up by plants and loss by leaching can be considered negligible compared to Se added to the soil (on average, lesser than 1.5 and 3.5 %, respectively).

The generally similar values of TOC, WEOC and TN among the treatments and over time suggested an irrelevance of Se fertilization on soil organic matter mineralization, at least considering the corn growing season (Table 1). The higher amount of available P in treated than in untreated soils at t1 and t2 (Table 1) might be due to the competition of phosphate and selenite for the soil sorption sites (Dhillon and Dhillon, 2003). This effect disappeared at t3 when the Se content of the treated soils returned at the level of CTR.

The negligible effect of Se fertilization on Cmic and the decline of the total Se content in D50 and D100 over time can be attributed to the tolerance to Se of most soil microorganisms, which are able to transform this trace element from inorganic to organic and volatile species through methylation processes. Indeed, as reported by Paul and Saha (2019), the microorganisms play an important role in bioremediation of Se polluted soils through the methylation and reduction of Se. However, compared to CTR, the lower ΣCO_2–C, ΣCO_2–C–to–WEOC ratio and FDA-H of D50 and D100 at t3 alongside with the similar values of ΣCO_2–C–to–C_{mic} ratio (Table 1, Figures 1, 2 I) would indicate a less energy demanded by the soil microorganisms for their own maintenance.

This finding suggested a better adaptation of the microbial community (Massaccesi et al., 2015) to the modified conditions in the treated soils, where Se fertilization might have caused a shift in soil microbial community structure and/or promoted the survival of selected microorganisms.

Se addition did not influence the alkP and acP activities (Figure 2 III, IV). In all soils, the lower acid phosphatase activities observed at t2 and t3 compared to t1 were attributed to the decrease of P uptake by corn in its later growth stages (Bhadoria et al., 2004). The β–glucosidase activity did not generally show differences both among treatments and over time because of the absence of changes of TOC content (Figure 2 II). The higher arylsulphatase activity detected only at t1 in the Se treated soils than in the CTR (Figure 2 V) has been attributed to the chemical similarity of Se to sulphur (Golob et al., 2016). Thus, the decline of arylsulphatase activity from t2 for both D50 and D100 should be due to the reduction of the Se content in the treated soils. The chemical similarity of Se and sulphur might be involved also in the reduction of DHA in the treated soils (Figure 2 VI). Indeed, sulphur substitution by Se in the active centres of the enzyme produces a disruption of the enzyme-substrate complex reducing the speed of the enzymatic reactions (Nowak et al., 2002).

Our findings showed that Se addition in form of Na-selenite at the rates of 50 and 100 g ha^{-1} increased the soil Se concentration only on a short term. Indeed, after about three months from the addition, the total Se content in the treated soils reduced and reached similar values of CTR. Furthermore, the lack of differences between CTR and treated soils on TOC, TN, WEOC, and available P concentrations, β–glu, alkP and acP activities, and Cmic content revealed a negligible effect of Se fertilization on the organic carbon and phosphorus dynamics, and on the size of the microbial communities. Conversely, at the end of the experiment, the values of ΣCO_2–C, FDA-H and DHA were lower in the Se-treated soils than in CTR. This apparent reduction of activity together with an unaltered ΣCO_2–C–to–Cmic ratio would suggest a better adaptation of the microbial community in the treated than in the untreated soils. The obtained data highlighted that

Se fertilization with Na–selenite, at the rate of 50 and 100 g ha^{-1}, had no negative impact on some key indicators of the soil quality, at least on a short term.

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Highlights

- The effect over time of Se fertilization on some soil properties was evaluated
- Soil under corn cultivation was treated with Na–selenite at the rate of 50 and 100 g Se ha^{-1}
- Se addition did not affect the amounts of soil organic C, total N and available P
- Better adaptation of the microbial community in the Se–enriched soil
- On a short-term, Na–selenite fertigation had no negative impact on soil quality

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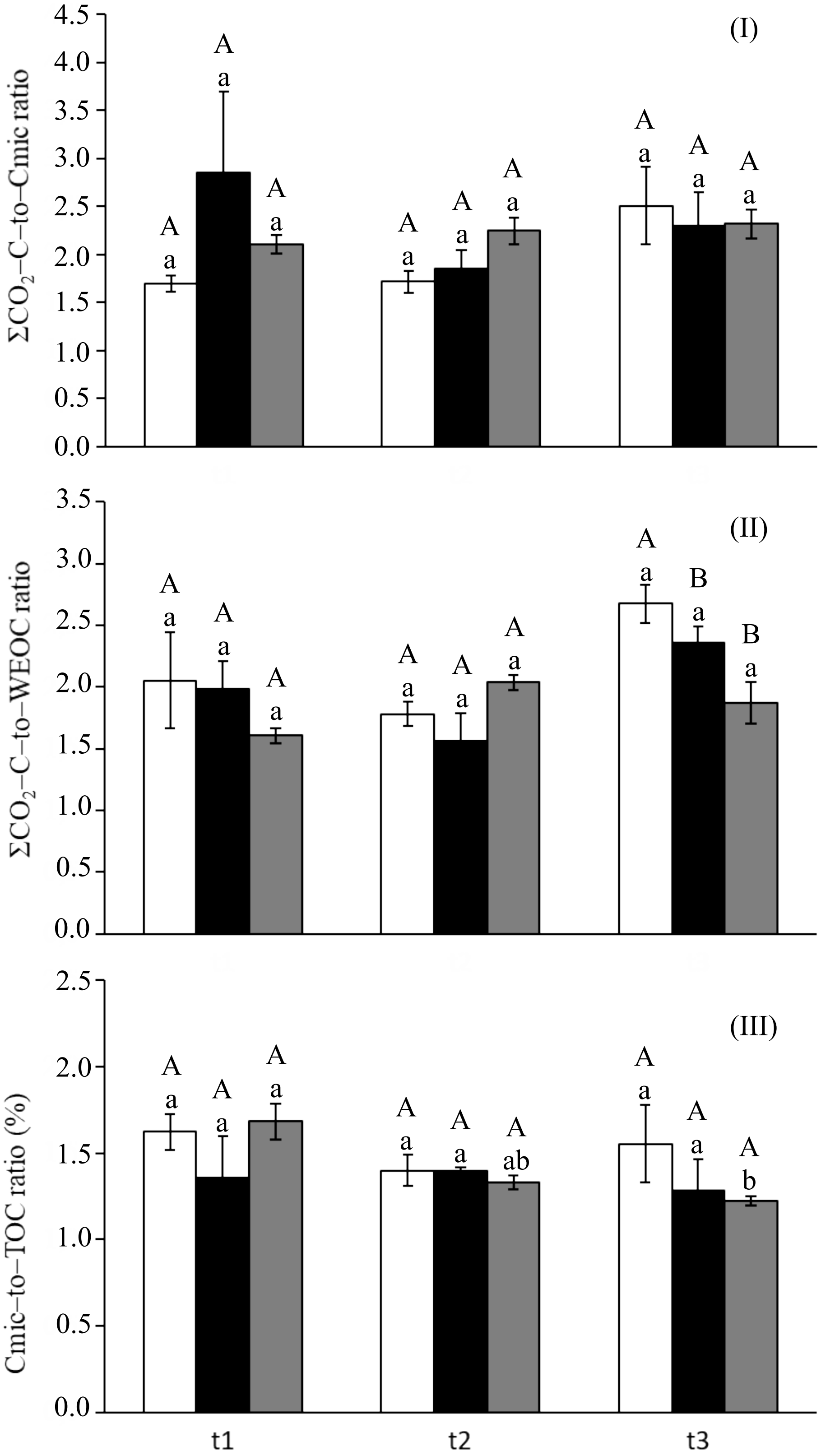
Figure captions

Figure 1. Mean values for soil ΣCO_2–C–to–C_{mic} ratio (I), ΣCO_2–C–to–WEOC ratio (II) and C_{mic}–to–TOC ratio (III) under unfertilized (white bars) and Se fertilized corn (*Zea mays* L.) plants at the rate of 50 (black bars) and 100 (grey bars) g Se ha^{-1} after 18, 48 and 59 days (t1, t2 and t3, respectively) soil Se fertilization as sodium selenite. Different capital letters indicate statistical differences among the treatments within each sampling date, different lower case letters indicate statistical differences among the sampling dates within each treatment (Tukey HSD test, $p < 0.05$). Error bars represent standard errors (n = 4). ΣCO_2–C = cumulative basal respiration; WEOC = water–extractable organic carbon; C_{mic} = microbial biomass–C; TOC = total organic carbon.

Figure 2. Mean values for soil fluorescein diacetate (FDA) hydrolysis (I), and activity of β-glucosidase (II), alkaline phosphatase (III), acid phosphatase (IV), arylsulphatase (V) and dehydrogenase (VI) under unfertilized (white bars) and Se fertilized corn (*Zea mays* L.) plants at the rate of 50 (black bars) and 100 (grey bars) g Se ha^{-1} after 18, 48 and 59 days (t1, t2 and t3, respectively) soil Se fertilization as sodium selenite. The results are expressed as as % of hydrolyzed FDA h^{-1} g^{-1} for FDA hydrolysis, μg *p*–nitrophenol g^{-1} h^{-1} for the activity of β-glucosidase, alkaline phosphatase, acid phosphatase and arylsulphatase, and *μ*g iodonitrotetrazolium formazan (INTF) g^{-1} $2h^{-1}$ for dehydrogenase activity. Different capital letters indicate statistical differences among the treatments within each sampling date, different lower case letters indicate statistical differences among the sampling dates within each treatment (Tukey HSD test, $p < 0.05$). Error bars represent standard errors (n = 4).

(I)
ΣCO2–C–to–Cmic ratio
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A a
A a
A a
A a
A a
A a
A a
A a
A a
(II)
ΣCO2–C–to–WEOC ratio
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A a
A a
A a
A a
A a
A a
A a
B a
B a
(III)
Cmic–to–TOC ratio (%)
2.5
2.0
1.5
1.0
0.5
0.0
A a
A a
A a
A a
A a
A ab
A a
A a
A b
t1
t2
t3

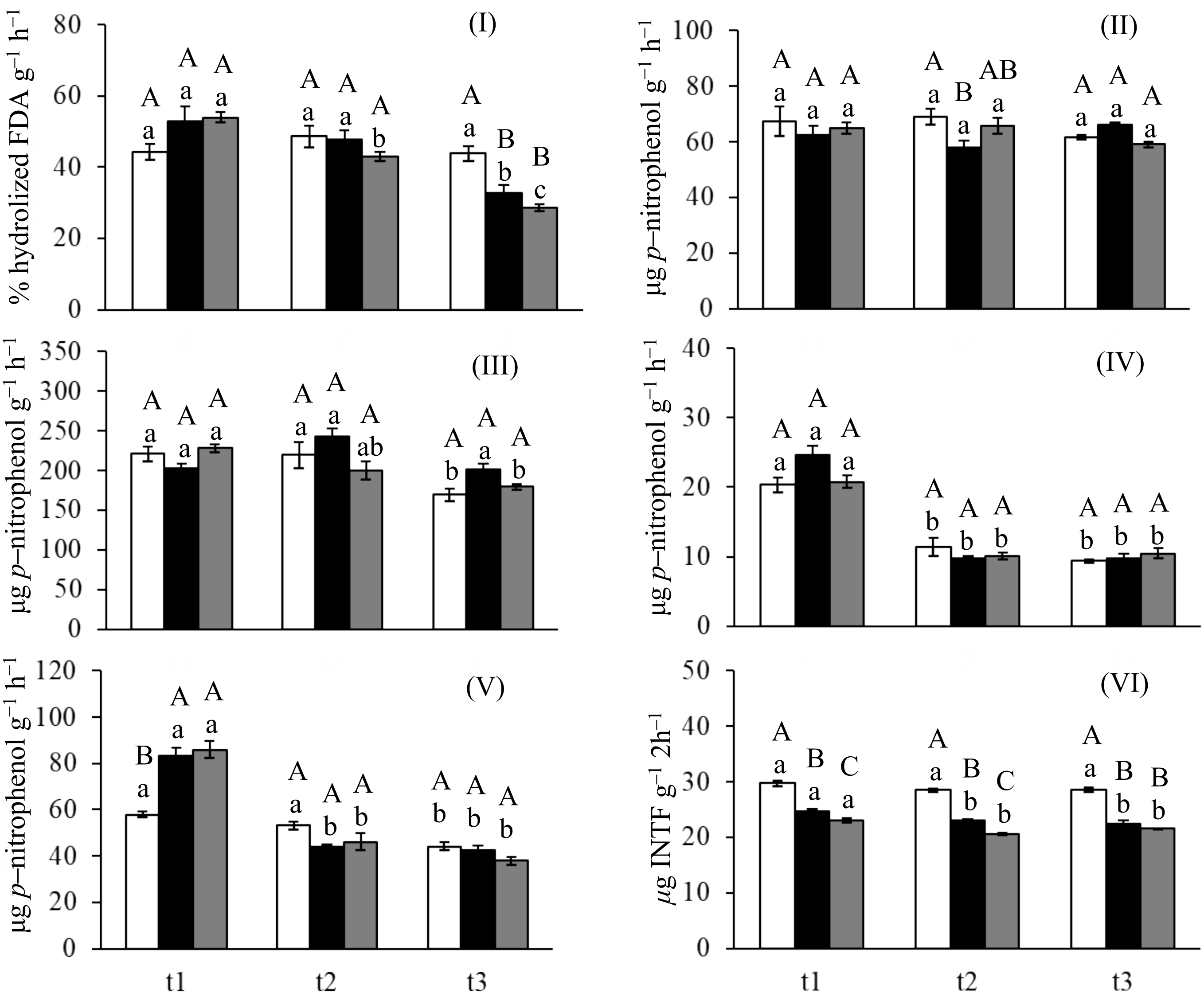

(I)
% hydrolized FDA g^{-1} h^{-1}
0
20
40
60
80
A a
A a
A a
A a
A a
A b
A a
B b
B c
(II)
µg *p*–nitrophenol g^{-1} h^{-1}
0
20
40
60
80
100
A a
A a
A a
A a
B a
AB a
A a
A a
A a
(III)
µg *p*–nitrophenol g^{-1} h^{-1}
0
50
100
150
200
250
300
350
A a
A a
A a
A a
A a
A ab
A b
A a
A b
(IV)
µg *p*–nitrophenol g^{-1} h^{-1}
0
10
20
30
40
A a
A a
A a
A b
A b
A b
A b
A b
A b
(V)
µg *p*–nitrophenol g^{-1} h^{-1}
0
20
40
60
80
100
120
B a
A a
A a
A a
A b
A b
A b
A b
A b
(VI)
µg INTF g^{-1} 2h^{-1}
0
10
20
30
40
50
A a
B a
C a
A a
B b
C b
A a
B b
B b
t1
t2
t3
t1
t2
t3

Table 1. Soil total Se (Se), total organic C (TOC), water–extractable organic C (WEOC), total N (TN), available P (AvP) and microbial C biomass (Cmic) contents, and cumulative soil basal respiration (ΣCO_2–C) under unfertilized (CTR) and Se fertilized corn (*Zea mays* L.) plants at the rate of 50 (D50) and 100 (D100) g Se ha^{-1} after 18, 48 and 59 days (t1, t2 and t3, respectively) soil Se fertilization as sodium selenite. Data presented are mean ± standard error (n= 4). Different capital letters indicate statistically significant differences among means within each sampling date, different lower case letters indicate statistical differences among means within each treatment (Tukey HSD test, $p < 0.05$).

	Time	CTR	D50	D100
Se	t1	241 ± 2 C a	277 ± 3 B a	846 ± 7 A a
μg kg^{-1}	t2	239 ± 1 B a	241 ± 6 B b	288 ± 4 A b
	t3	243 ± 2 A a	235 ± 5 A b	256 ± 7 A c
TOC	t1	15.2 ± 0.2 A a	14.8 ± 0.3 A a	12.9 ± 0.4 B b
g kg^{-1}	t2	16.3 ± 0.1 A a	17.0 ± 0.1 A a	16.0 ± 0.5 A a
	t3	16.6 ± 0.3 A a	16.9 ± 0.8 A a	17.8 ± 0.2 A a
WEOC	t1	0.217 ± 0.025 A a	0.250 ± 0.019 A ab	0.282 ± 0.011 A a
g kg^{-1}	t2	0.219 ± 0.006 B a	0.288 ± 0.020 A a	0.234 ± 0.005 B a
	t3	0.227 ± 0.009 AB a	0.198 ± 0.011 B b	0.274 ± 0.026 A a
TN	t1	1.13 ± 0.09 B b	1.30 ± 0.01 AB a	1.36 ± 0.02 A a
g kg^{-1}	t2	1.31 ± 0.01 A a	1.34 ± 0.01 A a	1.32 ± 0.01 A ab
	t3	1.33 ± 0.01 A a	1.59 ± 0.30 A a	1.26 ± 0.01 A b
AvP	t1	20.4 ± 0.6 B b	27.3 ± 2.1 A a	33.6 ± 2.1 A a
mg kg^{-1}	t2	18.8 ± 0.5 B b	28.6 ± 1.9 A a	19.9 ± 1.0 B b
	t3	27.3 ± 1.4 A a	27.3 ± 1.1 A a	22.3 ± 1.4 A b
Cmic	t1	247 ± 18 A a	202 ± 38 A a	216 ± 7 A a
mg kg^{-1}	t2	228 ± 15 A a	237 ± 5 A a	214 ± 12 A a
	t3	258 ± 37 A a	215 ± 30 A a	218 ± 4 A a
ΣCO_2–C	t1	421 ± 44 A b	486 ± 35 A a	453 ± 15 A a
mg kg^{-1}	t2	388 ± 14 A b	440 ± 51 A a	476 ± 11 A a
	t3	605 ± 26 A a	462 ± 3 B a	503 ± 26 B a

Declaration of interests

▯ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table S1. Morphological description of the soil of the Experimental Farm of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia, Papiano (Perugia, Italy). For symbols see legend.

Landform: plain; Altitude: 163 m a.s.l.; Parent material: fluvial and lacustrine sediments; Soil: fine, mixed, mesic Typic Haplustept (Soil Survey Staff, 2014).

Horizons	Depth cm	Colour[a]	Texture[b]	Structure[c]	Consistency and plasticity[d]	Roots[e]	Boundary[f]	Other observations
Ap1	0-8	2,5YR 4/2	sc	2fm sbk	mfi, wps, ws	0	cs	Skeleton (by volume): 5%; Ø < 0.5 cm
Ap2	8-23	2,5YR 4/3	sc	2m sbk	mfi, ws, wp	2 vf, f, m	cw	Skeleton (by volume): 2%; Ø < 0.5 cm
Ap3	23-37	2,5YR 4/3	sc	2m abk	mfi, ws, wp	2 vf, f	cs	Skeleton (by volume): 1- 2%; Ø < 1 cm
Bw	37-47	2,5YR 4/3	sc	1m-c abk	mfr, wvs, wvp	1 f	cs	Skeleton (by volume): 1- 2%; Ø < 1 cm
BC	47-76+	2,5YR 4/3	sc	1m-c abk	mfr, wvs, wvp	v_1 f	-	Skeleton (by volume): 5%; Ø < 1 cm

[a] moist and crushed, according to the Munsell Soil Color Charts.

[b] sc = silty clay

[c] 1 = weak, 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse; cr = crumb, abk = angular blocky, sbk = subangular blocky.

[d] m = moist, w = wet, fr = friable, fi = firm; s = sticky; vs = very sticky, ps = slightly plastic, p = plastic, vp = very plastic.

[e] 0 = absent, v_1 = very few, 1 = few, 2 = plentiful; vf = very fine, f = fine, m = medium, co = coarse.

[f] c = clear; w = wavy, s = smooth.