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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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## Manuscript Details

<b>Manuscript number</b>	GEODER_2019_2650_R2
<b>Title</b>	Impact of Na-selenite fertilization on the microbial biomass and enzymes of a soil under corn ( <i>Zea mays</i> L.) cultivation
<b>Article type</b>	Short Communication

### Abstract

We tested the over time effect of different selenium doses [50 (D50) and 100 (D100) g ha<sup>-1</sup> of Se as Na<sub>2</sub>SeO<sub>3</sub>] 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<sub>mic</sub>) contents, the cumulative basal respiration ( $\Sigma$ CO<sub>2</sub>-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  $\Sigma$ CO<sub>2</sub>-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<sub>mic</sub>, 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<sup>-1</sup>, had no negative impact on soil chemical and biochemical parameters, at least on a short term.

<b>Keywords</b>	enzyme activities; maize field; selenium; soil microbial C; soil Se fertigation
<b>Taxonomy</b>	Soil Quality, Agricultural Soil, Soil Biochemistry, Fertilizer, Soil Enzyme Activity
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Bologna, April 28<sup>th</sup> 2020

The Editor in Chief of Geoderma

Dear Editor,

I resubmit the revised short communication “Impact of Na-selenite fertilization on the microbial biomass and enzymes of a soil under corn (*Zea mays* L.) cultivation” by De Feudis, M.\*, Massaccesi, L., Roberto D’Amato, R., Businelli, B., Casucci, C., Agnelli, A. (GEODER\_2019\_2650\_R1), for publication in Geoderma. The manuscript was revised according to the suggestions of the Editor and Reviewer.

Sincerely yours

Mauro De Feudis

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## Editor

*Line 30: The soil was sampled 18... (t3) days after the addition of Se and...*

We changed as suggested. Thank you. (lines 30-31 of the new version of the manuscript)

*Line 34: ...due to volatilization and...*

According to Reviewer 2 we changed this part as follow: "Our findings showed Se fertilization increased the total soil Se content, although the differences between the treated and the untreated soils disappeared over time" (lines 33-35 of the new version of the manuscript)

*Line 39: The reduction in respiration without a reduction in biomass, suggests a change in carbon use efficiency. I think that this should be developped in the discussion, as the reviewer suggest.*

We changed this part in the abstract (lines 37-42) and we developed the concept in the discussion by explaining better the possible causes that produced a reduced respiration without a reduction in biomass (lines 128-133 of the new version of the manuscript), as suggested by the Reviewer.

*Line 41: I don't think that you can really state this with your data. Soil quality has to be defined and there does appear to be a change is microbial functioning...*

We replaced "soil quality" with "soil chemical and biochemical parameters". Thank you

*Line 53: only a few studies have addressed*

We changed accordingly. Thank you (line 56 of the new version of the manuscript)

*Line 55: So why is this of interest, important? I think that it is here that you need to develop the introduction to show the novelty.*

At lines 58-61 of the new version we better clarified the novelty of the present research

*Line 58: tested*

Done. Thank you (line 63 of the new version)

*Line 100: You should use repeated measures ANOVA for the sampling time.*

Thanks for pointing out this issue. We decided to do not perform the repeated measures ANOVA because in our case the investigated parameters were not measured more than once on the same subjects. In fact, our experiment can be considered as a pot experiment where for each treatment we prepared 12 pots, and for each sampling time we sampled 4 pots in destructive way. Therefore, the soil samples are not related to each other and, as a consequence, to perform a repeated measures ANOVA should be not appropriate.

## Reviewer 2

*The manuscript entitled "Impact of Na-selenite fertilization on the microbial biomass and enzymes of a soil under corn (Zea mays L.) cultivation" was modified and improved according Editor and Reviewers suggestions. However, it still needs a moderate revision before publication in Geoderma.*

*General comments:*

*The manuscript novelty was not clearly described in the introduction. Authors should highlight it better*

At lines 58-61 of the new version we better clarified the novelty of the present research. In particular, we believe that the novelty of our study is the combination of an open-field experiment with an over time monitoring of the effect of Se addition on the chemical and biochemical soil properties.

*Line 34: delete "due to the volatilization processes". Isn't a manuscript result, it was inferred from other works*

We changed this part as follow: "Our findings showed Se fertilization increased the total soil Se content, although the differences between the treated and the untreated soils disappeared over time" (lines 33-35 of the new version of the manuscript)

*Some new discussion is still needed (see comments below)*

*Lines 116-118: a better explanation/discussion is needed*

*Lines 120-122: a better explanation/discussion is needed*

This part of the Discussion section was improved to better explain both biomethylation processes and microbial adaptation as follow "The negligible effect of Se fertilization on  $C_{mic}$  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  $\Sigma CO_2-C$ ,  $\Sigma CO_2-C$ -to-WEOC ratio and FDA-H of D50 and D100 at t3 alongside with the similar values of  $\Sigma CO_2-C$ -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." (lines 123-133 of the new version of the manuscript).

*Line 132: Figure 2 instead 6*

We corrected it in the revised manuscript, thank you (line 143 of the new version).

*Figure 1: explain abbreviations. Some symbols are missing (squares instead the symbols)*

*Lines 119-120: some symbols are missing (squares instead the symbols)*

*Lines 141 and 143: some symbols are missing (squares instead the symbols)*

*Table 1: line 7: treatment instead column. Some symbols are missing (squares instead the symbols)*

The abbreviations in the caption of Figure 1 were explained.

During the conversion of word file to PDF file through the submission process, the symbols " $\Sigma$ " were converted to squares. We are apologies for the carelessness during the check of PDF file, however in the new version the symbols " $\Sigma$ " are present (lines 128-129 of the new version and Table 1). Further, we changed column with treatment. Thank you.

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

3

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## Abstract

We tested the over time effect of different selenium doses [50 (D50) and 100 (D100) g ha<sup>-1</sup> of Se as Na<sub>2</sub>SeO<sub>3</sub>] 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<sub>mic</sub>) contents, the cumulative basal respiration (ΣCO<sub>2</sub>-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<sub>2</sub>-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<sub>mic</sub>, 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<sup>-1</sup>, 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<sub>2</sub>SeO<sub>4</sub>) and sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) 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<sup>-1</sup> of Se as Na<sub>2</sub>SeO<sub>3</sub> 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 annual 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<sub>H2O</sub> 7.9), 5% carbonate content, and a cation exchange capacity of 33.13 cmol<sub>(+)</sub> kg<sup>-1</sup> (for soil description see Table S1 of the Supplementary Materials).

On 12<sup>th</sup> April 2015, corn (*Zea mays* L. variety DKC 4316) was sowed with a density of 7.5 plants m<sup>-2</sup>. At seeding, the field was fertilized with 150 kg N ha<sup>-1</sup> and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in form of urea and triple superphosphate, respectively. Irrigation occurred on May 26<sup>th</sup> (20 mm), June 23<sup>th</sup> (100 mm), and July 6<sup>th</sup> (30 mm) by drip irrigation. To prevent weed occurrence, a pre-emergence treatment was performed with herbicides and hand hoeing. On June 10<sup>th</sup>, 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



80 plant stem and inserted vertically into the soil to 0.3 m depth. Specifically, 12 microplots were  
81 treated with 1 L of solution containing 1.423 mg of  $\text{Na}_2\text{SeO}_3$ , corresponding to 50 g Se  $\text{ha}^{-1}$  (D50),  
82 12 microplots were treated with 1 L of solution containing 2.846 mg of  $\text{Na}_2\text{SeO}_3$ , corresponding  
83 to 100 g Se  $\text{ha}^{-1}$  (D100), and 12 microplots were treated with 1 L of distilled water and used as  
84 control (CTR).

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

91 The total Se content was measured according to De Feudis et al. (2019), the total organic C (TOC)  
92 content was estimated by K-dichromate digestion, heating the suspension at 180 °C for 30 min.  
93 Water-extractable organic matter (WEOM) was obtained according to Agnelli et al. (2016) and  
94 its organic C content (WEOC) was determined by a TOC-500A analyser (Shimatzu, Kyoto, Japan)  
95 after the addition of few drops of concentrated  $\text{H}_3\text{PO}_4$  to remove carbonates. The total N (TN)  
96 content was determined by the Kjeldahl method, while available P was estimated according to  
97 Olsen et al. (1954). The soil microbial biomass-C ( $\text{C}_{\text{mic}}$ ) was determined by the fumigation-  
98 extraction protocol, after 51 days of incubation at 25 °C and at 50% of soil water holding capacity.  
99 During the incubation, basal respiration was periodically measured by alkali (1 M NaOH solution)  
100 absorption of the developed  $\text{CO}_2$  and back-titration of the residual  $\text{OH}^-$  with a standardized HCl  
101 solution. The total amount of  $\text{CO}_2$  evolved during the 51 days of incubation was expressed as the  
102 cumulative amount of  $\text{CO}_2\text{-C}$  evolved during the experiment ( $\Sigma\text{CO}_2\text{-C}$ ).

103 The fluorescein diacetate hydrolysis (FDA-H) rate was estimated using the method of Swisher and  
104 Carroll (1980) with some modifications.  $\beta$ -glucosidase, acid (acP) and alkaline (alkP) phosphatases,

105 and arylsulphatase activities were determined according to Tabatabai (1994). Dehydrogenase activity  
 106 (DHA) was evaluated according to von Mersi and Schinner (1991).

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

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

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

123 The negligible effect of Se fertilization on  $C_{mic}$  and the decline of the total Se content in D50 and  
 124 D100 over time can be attributed to the tolerance to Se of most soil microorganisms, which are  
 125 able to transform this trace element from inorganic to organic and volatile species through  
 126 methylation processes. Indeed, as reported by Paul and Saha (2019), the microorganisms play an  
 127 important role in bioremediation of Se polluted soils through the methylation and reduction of Se.  
 128 However, compared to CTR, the lower  $\Sigma CO_2-C$ ,  $\Sigma CO_2-C$ -to-WEOC ratio and FDA-H of D50 and  
 129 D100 at t3 alongside with the similar values of  $\Sigma CO_2-C$ -to- $C_{mic}$  ratio (Table 1, Figures 1, 2 I)  
 130 would indicate a less energy demanded by the soil microorganisms for their own maintenance.

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

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

146 Our findings showed that Se addition in form of Na-selenite at the rates of 50 and 100 g ha<sup>-1</sup>  
147 increased the soil Se concentration only on a short term. Indeed, after about three months from the  
148 addition, the total Se content in the treated soils reduced and reached similar values of CTR.  
149 Furthermore, the lack of differences between CTR and treated soils on TOC, TN, WEOC, and  
150 available P concentrations,  $\beta$ -glu, alkP and acP activities, and Cmic content revealed a negligible  
151 effect of Se fertilization on the organic carbon and phosphorus dynamics, and on the size of the  
152 microbial communities. Conversely, at the end of the experiment, the values of  $\Sigma\text{CO}_2\text{-C}$ , FDA-H  
153 and DHA were lower in the Se-treated soils than in CTR. This apparent reduction of activity  
154 together with an unaltered  $\Sigma\text{CO}_2\text{-C}$ -to-Cmic ratio would suggest a better adaptation of the  
155 microbial community in the treated than in the untreated soils. The obtained data highlighted that

156 Se fertilization with Na-selenite, at the rate of 50 and 100 g ha<sup>-1</sup>, had no negative impact on some  
157 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<sup>-1</sup>
- 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

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

3

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## Abstract

We tested the over time effect of different selenium doses [50 (D50) and 100 (D100) g ha<sup>-1</sup> of Se as Na<sub>2</sub>SeO<sub>3</sub>] 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<sub>mic</sub>) contents, the cumulative basal respiration (ΣCO<sub>2</sub>-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<sub>2</sub>-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<sub>mic</sub>, 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<sup>-1</sup>, 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<sub>2</sub>SeO<sub>4</sub>) and sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) 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<sup>-1</sup> of Se as Na<sub>2</sub>SeO<sub>3</sub> 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 annual 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<sub>H2O</sub> 7.9), 5% carbonate content, and a cation exchange capacity of 33.13 cmol<sub>(+)</sub> kg<sup>-1</sup> (for soil description see Table S1 of the Supplementary Materials).

On 12<sup>th</sup> April 2015, corn (*Zea mays* L. variety DKC 4316) was sowed with a density of 7.5 plants m<sup>-2</sup>. At seeding, the field was fertilized with 150 kg N ha<sup>-1</sup> and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in form of urea and triple superphosphate, respectively. Irrigation occurred on May 26<sup>th</sup> (20 mm), June 23<sup>th</sup> (100 mm), and July 6<sup>th</sup> (30 mm) by drip irrigation. To prevent weed occurrence, a pre-emergence treatment was performed with herbicides and hand hoeing. On June 10<sup>th</sup>, 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

80 plant stem and inserted vertically into the soil to 0.3 m depth. Specifically, 12 microplots were  
 81 treated with 1 L of solution containing 1.423 mg of  $\text{Na}_2\text{SeO}_3$ , corresponding to 50 g Se  $\text{ha}^{-1}$  (D50),  
 82 12 microplots were treated with 1 L of solution containing 2.846 mg of  $\text{Na}_2\text{SeO}_3$ , corresponding  
 83 to 100 g Se  $\text{ha}^{-1}$  (D100), and 12 microplots were treated with 1 L of distilled water and used as  
 84 control (CTR).

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

91 The total Se content was measured according to De Feudis et al. (2019), the total organic C (TOC)  
 92 content was estimated by K-dichromate digestion, heating the suspension at 180 °C for 30 min.  
 93 Water-extractable organic matter (WEOM) was obtained according to Agnelli et al. (2016) and  
 94 its organic C content (WEOC) was determined by a TOC-500A analyser (Shimatzu, Kyoto, Japan)  
 95 after the addition of few drops of concentrated  $\text{H}_3\text{PO}_4$  to remove carbonates. The total N (TN)  
 96 content was determined by the Kjeldahl method, while available P was estimated according to  
 97 Olsen et al. (1954). The soil microbial biomass-C ( $\text{C}_{\text{mic}}$ ) was determined by the fumigation-  
 98 extraction protocol, after 51 days of incubation at 25 °C and at 50% of soil water holding capacity.  
 99 During the incubation, basal respiration was periodically measured by alkali (1 M NaOH solution)  
 100 absorption of the developed  $\text{CO}_2$  and back-titration of the residual  $\text{OH}^-$  with a standardized HCl  
 101 solution. The total amount of  $\text{CO}_2$  evolved during the 51 days of incubation was expressed as the  
 102 cumulative amount of  $\text{CO}_2$ -C evolved during the experiment ( $\Sigma\text{CO}_2\text{-C}$ ).

103 The fluorescein diacetate hydrolysis (FDA-H) rate was estimated using the method of Swisher and  
 104 Carroll (1980) with some modifications.  $\beta$ -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  $C_{mic}$  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  $\Sigma CO_2-C$ ,  $\Sigma CO_2-C$ -to-WEOC ratio and FDA-H of D50 and D100 at t3 alongside with the similar values of  $\Sigma CO_2-C$ -to- $C_{mic}$  ratio (Table 1, Figures 1, 2 I) would indicate a less energy demanded by the soil microorganisms for their own maintenance.

131 This finding suggested a better adaptation of the microbial community (Massaccesi et al., 2015)  
132 to the modified conditions in the treated soils, where Se fertilization might have caused a shift in  
133 soil microbial community structure and/or promoted the survival of selected microorganisms.  
134 Se addition did not influence the alkP and acP activities (Figure 2 III, IV). In all soils, the lower  
135 acid phosphatase activities observed at t2 and t3 compared to t1 were attributed to the decrease of  
136 P uptake by corn in its later growth stages (Bhadoria et al., 2004). The  $\beta$ -glucosidase activity did  
137 not generally show differences both among treatments and over time because of the absence of  
138 changes of TOC content (Figure 2 II). The higher arylsulphatase activity detected only at t1 in the  
139 Se treated soils than in the CTR (Figure 2 V) has been attributed to the chemical similarity of Se  
140 to sulphur (Golob et al., 2016). Thus, the decline of arylsulphatase activity from t2 for both D50  
141 and D100 should be due to the reduction of the Se content in the treated soils. The chemical  
142 similarity of Se and sulphur might be involved also in the reduction of DHA in the treated soils  
143 (Figure 2 VI). Indeed, sulphur substitution by Se in the active centres of the enzyme produces a  
144 disruption of the enzyme-substrate complex reducing the speed of the enzymatic reactions (Nowak  
145 et al., 2002).

146 Our findings showed that Se addition in form of Na-selenite at the rates of 50 and 100 g ha<sup>-1</sup>  
147 increased the soil Se concentration only on a short term. Indeed, after about three months from the  
148 addition, the total Se content in the treated soils reduced and reached similar values of CTR.  
149 Furthermore, the lack of differences between CTR and treated soils on TOC, TN, WEOC, and  
150 available P concentrations,  $\beta$ -glu, alkP and acP activities, and Cmic content revealed a negligible  
151 effect of Se fertilization on the organic carbon and phosphorus dynamics, and on the size of the  
152 microbial communities. Conversely, at the end of the experiment, the values of  $\Sigma\text{CO}_2\text{-C}$ , FDA-H  
153 and DHA were lower in the Se-treated soils than in CTR. This apparent reduction of activity  
154 together with an unaltered  $\Sigma\text{CO}_2\text{-C}$ -to-Cmic ratio would suggest a better adaptation of the  
155 microbial community in the treated than in the untreated soils. The obtained data highlighted that

156 Se fertilization with Na-selenite, at the rate of 50 and 100 g ha<sup>-1</sup>, had no negative impact on some  
157 key indicators of the soil quality, at least on a short term.

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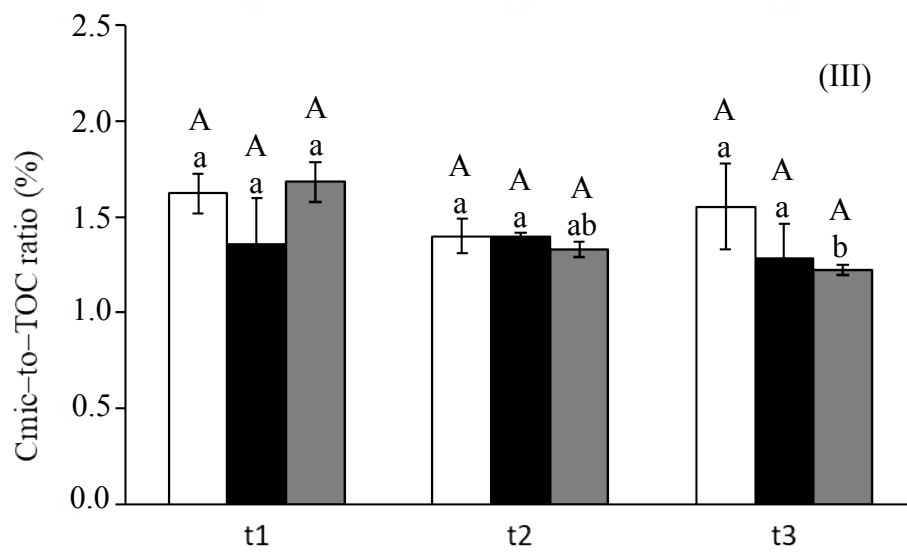
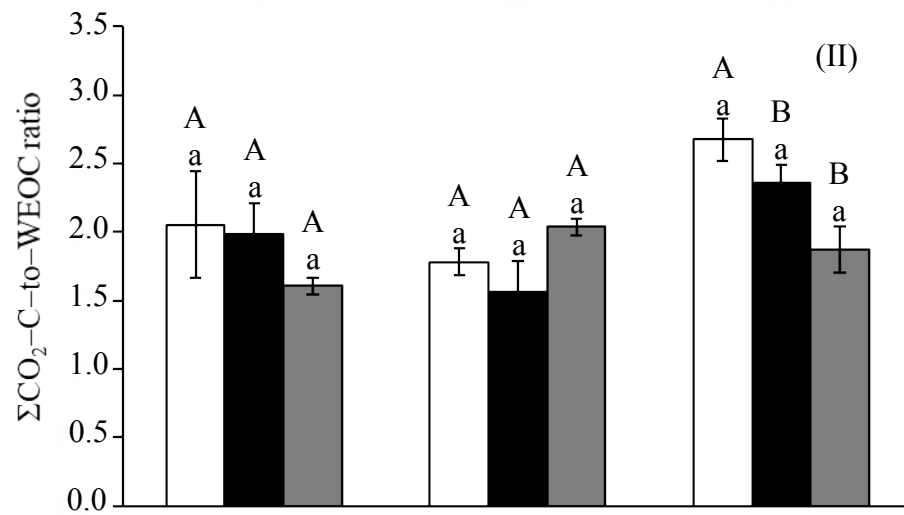
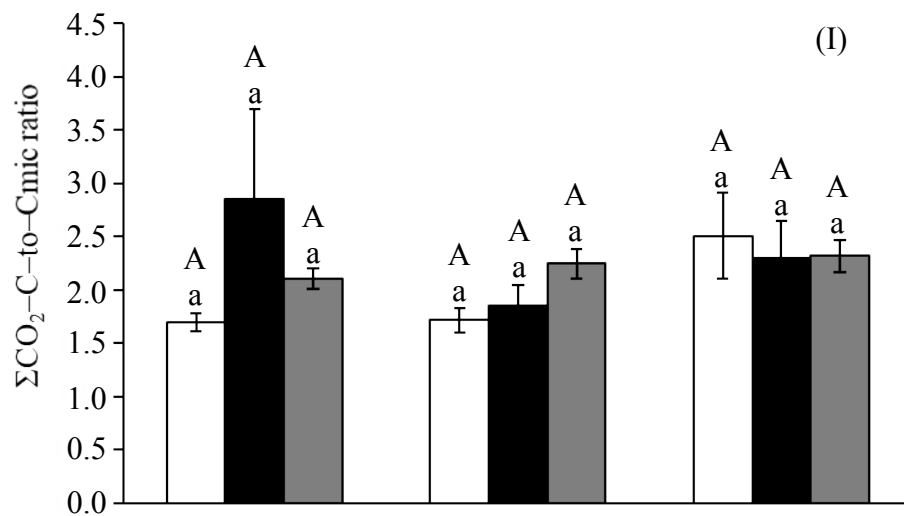
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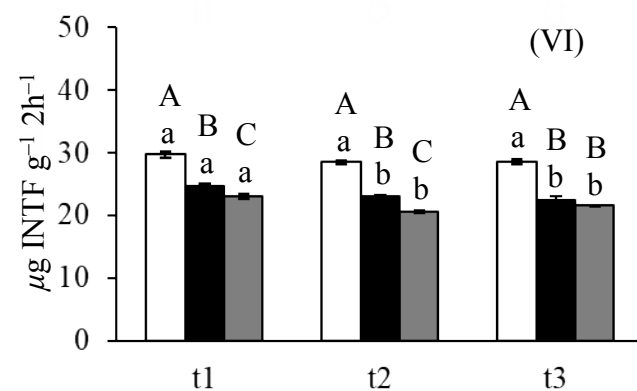
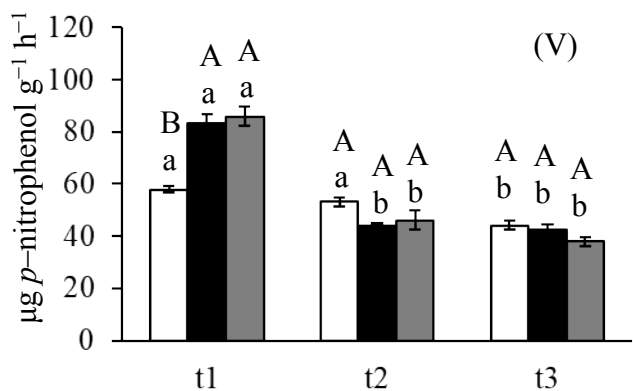
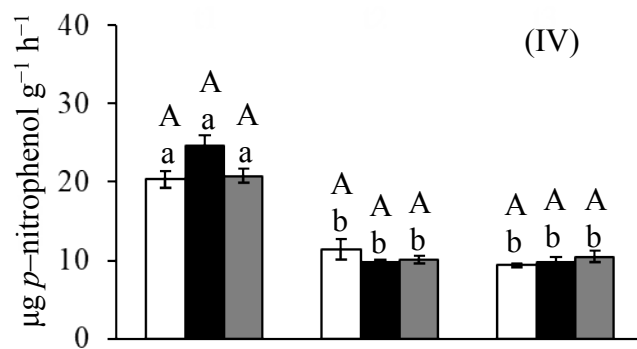
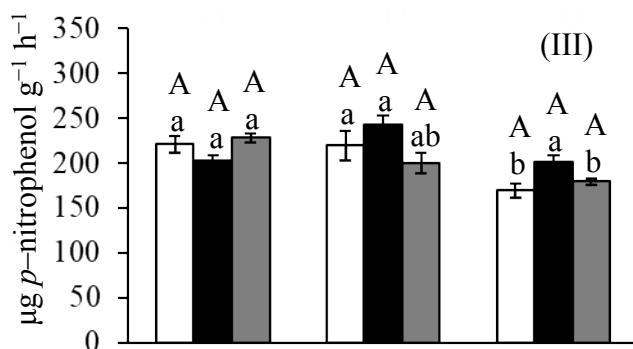
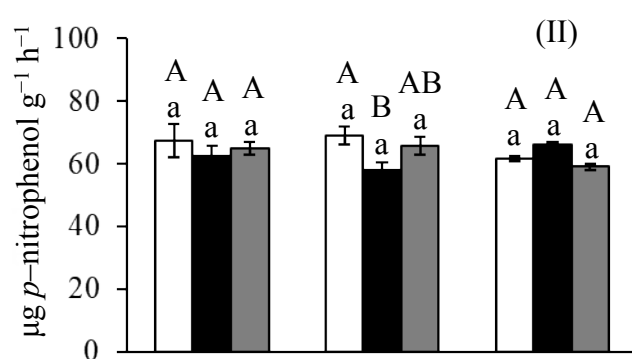
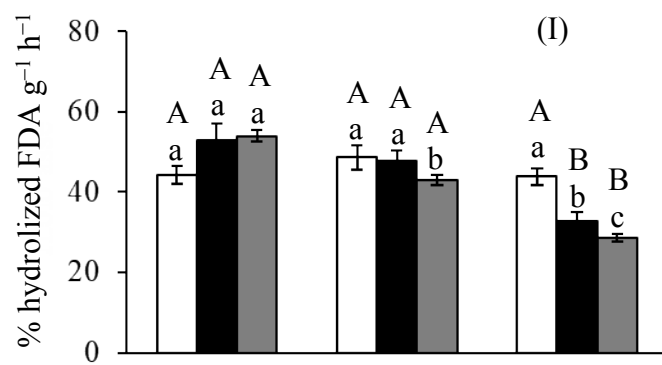
## Figure captions

**Figure 1.** Mean values for soil  $\Sigma\text{CO}_2\text{-C-to-C}_{\text{mic}}$  ratio (I),  $\Sigma\text{CO}_2\text{-C-to-WEOC}$  ratio (II) and  $\text{C}_{\text{mic-to-TOC}}$  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<sup>-1</sup> 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 ( $n = 4$ ).  $\Sigma\text{CO}_2\text{-C}$  = cumulative basal respiration; WEOC = water-extractable organic carbon;  $\text{C}_{\text{mic}}$  = microbial biomass-C; TOC = total organic carbon.

**Figure 2.** Mean values for soil fluorescein diacetate (FDA) hydrolysis (I), and activity of  $\beta$ -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<sup>-1</sup> after 18, 48 and 59 days (t1, t2 and t3, respectively) soil Se fertilization as sodium selenite. The results are expressed as % of hydrolyzed FDA h<sup>-1</sup> g<sup>-1</sup> for FDA hydrolysis,  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  for the activity of  $\beta$ -glucosidase, alkaline phosphatase, acid phosphatase and arylsulphatase, and  $\mu\text{g iodonitrotetrazolium formazan (INTF) g}^{-1} \text{ 2h}^{-1}$  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 ( $n = 4$ ).







**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 ( $\Sigma\text{CO}_2\text{-C}$ ) under unfertilized (CTR) and Se fertilized corn (*Zea mays* L.) plants at the rate of 50 (D50) and 100 (D100) g Se ha<sup>-1</sup> after 18, 48 and 59 days (t1, t2 and t3, respectively) soil Se fertilization as sodium selenite. Data presented are mean  $\pm$  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 $\pm$ 2 C a	277 $\pm$ 3 B a	846 $\pm$ 7 A a
$\mu\text{g kg}^{-1}$	t2	239 $\pm$ 1 B a	241 $\pm$ 6 B b	288 $\pm$ 4 A b
	t3	243 $\pm$ 2 A a	235 $\pm$ 5 A b	256 $\pm$ 7 A c
TOC	t1	15.2 $\pm$ 0.2 A a	14.8 $\pm$ 0.3 A a	12.9 $\pm$ 0.4 B b
$\text{g kg}^{-1}$	t2	16.3 $\pm$ 0.1 A a	17.0 $\pm$ 0.1 A a	16.0 $\pm$ 0.5 A a
	t3	16.6 $\pm$ 0.3 A a	16.9 $\pm$ 0.8 A a	17.8 $\pm$ 0.2 A a
WEOC	t1	0.217 $\pm$ 0.025 A a	0.250 $\pm$ 0.019 A ab	0.282 $\pm$ 0.011 A a
$\text{g kg}^{-1}$	t2	0.219 $\pm$ 0.006 B a	0.288 $\pm$ 0.020 A a	0.234 $\pm$ 0.005 B a
	t3	0.227 $\pm$ 0.009 AB a	0.198 $\pm$ 0.011 B b	0.274 $\pm$ 0.026 A a
TN	t1	1.13 $\pm$ 0.09 B b	1.30 $\pm$ 0.01 AB a	1.36 $\pm$ 0.02 A a
$\text{g kg}^{-1}$	t2	1.31 $\pm$ 0.01 A a	1.34 $\pm$ 0.01 A a	1.32 $\pm$ 0.01 A ab
	t3	1.33 $\pm$ 0.01 A a	1.59 $\pm$ 0.30 A a	1.26 $\pm$ 0.01 A b
AvP	t1	20.4 $\pm$ 0.6 B b	27.3 $\pm$ 2.1 A a	33.6 $\pm$ 2.1 A a
$\text{mg kg}^{-1}$	t2	18.8 $\pm$ 0.5 B b	28.6 $\pm$ 1.9 A a	19.9 $\pm$ 1.0 B b
	t3	27.3 $\pm$ 1.4 A a	27.3 $\pm$ 1.1 A a	22.3 $\pm$ 1.4 A b
Cmic	t1	247 $\pm$ 18 A a	202 $\pm$ 38 A a	216 $\pm$ 7 A a
$\text{mg kg}^{-1}$	t2	228 $\pm$ 15 A a	237 $\pm$ 5 A a	214 $\pm$ 12 A a
	t3	258 $\pm$ 37 A a	215 $\pm$ 30 A a	218 $\pm$ 4 A a
$\Sigma\text{CO}_2\text{-C}$	t1	421 $\pm$ 44 A b	486 $\pm$ 35 A a	453 $\pm$ 15 A a
$\text{mg kg}^{-1}$	t2	388 $\pm$ 14 A b	440 $\pm$ 51 A a	476 $\pm$ 11 A a
	t3	605 $\pm$ 26 A a	462 $\pm$ 3 B a	503 $\pm$ 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:

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**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 <sup>a</sup>	Texture <sup>b</sup>	Structure <sup>c</sup>	Consistency and plasticity <sup>d</sup>	Roots <sup>e</sup>	Boundary <sup>f</sup>	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 <sub>1</sub> f	-	Skeleton (by volume): 5%; Ø < 1 cm

<sup>a</sup> moist and crushed, according to the Munsell Soil Color Charts.

<sup>b</sup> sc = silty clay

<sup>c</sup> 1 = weak, 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse; cr = crumb, abk = angular blocky, sbk = subangular blocky.

<sup>d</sup> m = moist, w = wet, fr = friable, fi = firm; s = sticky; vs = very sticky, ps = slightly plastic, p = plastic, vp = very plastic.

<sup>e</sup> 0 = absent, v<sub>1</sub> = very few, 1 = few, 2 = plentiful; vf = very fine, f = fine, m = medium, co = coarse.

<sup>f</sup> c = clear; w = wavy, s = smooth.