Supplement of

Effects of agricultural practices on soil and microbial biomass carbon, nitrogen and phosphorus content: a preliminary case study

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Methods

Study sites

Table S1 Description of the four soil plots located at Coimbra, central Portugal

<table>
<thead>
<tr>
<th>Study plot</th>
<th>Farming management</th>
<th>Previous culture</th>
<th>Fertilization</th>
<th>Current culture</th>
<th>Geographic coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>OF1</td>
<td>Organic</td>
<td>Lucerne</td>
<td>Lucerne green manure</td>
<td>Corn</td>
<td>40°13’03’’N, 8°26’51”W</td>
</tr>
<tr>
<td>OF2</td>
<td>Organic</td>
<td>Corn</td>
<td>Organic compost</td>
<td>Corn</td>
<td></td>
</tr>
<tr>
<td>OF3</td>
<td>Organic</td>
<td>Corn</td>
<td>None</td>
<td>Corn</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>Conventional</td>
<td>Corn</td>
<td>Chemical fertilizer</td>
<td>Corn</td>
<td>40°13’14”N, 8°28’36”W</td>
</tr>
</tbody>
</table>

Soil preparation and analysis

For soil physic-chemical characterization, nine random core samples (0-20 cm; 250 cm³) were collected in each of the four plots and bulked to produce a composite sample per plot. The samples were homogenized, oven-dried (28°C with ventilation, 24 h), grounded and sieved (2 mm) to separate the fine particle fraction for analysis. Soil moisture was determined gravimetrically after drying at 105°C for 24 h, textural fractions (coarse sand: 2-0.2 mm; fine sand: 0.2-0.02 mm; silt: 0.02-0.002 mm; clay: <0.002mm) were separated by sedimentation, soil pH was measured in water (1:2.5). Total organic carbon was quantified as CO₂ by infrared absorption spectrophotometry (Leco SC-144 DR) after combustion at 590°C (Rossell et al., 2001). Phosphorus pentoxide (P₂O₅) and potassium oxide (K₂O) were extracted with the Egnér-Riehm method (Balbino, 1968) and quantified by colorimetry after molecular absorption spectrophotometry (P₂O₅; Philips PYE Unicam SP6-350) and atomic absorption spectrophotometry with a flame atomizer (K₂O; Perkin Elmer Analyst 300). Total Kjeldahl nitrogen (ammonia (NH₃), ammonium (NH₄⁺), and organic nitrogen) was quantified with the Bremmer method (Bremmer, 1979). The exchange cations K⁺, Na⁺, Ca²⁺ and Mg²⁺ were quantified by atomic absorption spectrophotometry with a flame atomizer (Chapman, 1979).

Soil and microbial biomass CNP extraction and quantification

Microbial biomass C, N and P were assessed by the chloroform fumigation-extraction (CFE) technique (Jenkinson and Powlson, 1976; Brookes et al., 1982, 1985a, 1985b; Vance et al., 1987). The composite samples were homogenized; sieved (2 mm) and all visible organic fragments were removed. Moisture was determined as the difference between fresh mass and
oven-dry mass (105°C, 24 h). The samples (100 g) were brought to 50% field water holding capacity and four subsamples of 25 g were allocated to 250 mL individual vials. One mL chloroform (CHCl₃) was added to two subsamples (fumigation); the vials were hermetically closed and incubated in a dark oven (Cassel EI-3) at 25°C during 24 h. Four additional samples incubated in the same conditions but without soil were used as controls. All samples were evaporated under an extraction hood for 6 h to allow total elimination of the chloroform (Brookes et al., 1982). From each subsample, 2 g of soil were used to determine phosphorus and 23 g were used to determine organic carbon and nitrogen.

Phosphorus was extracted by adding 30 mL of 0.5 M sodium bicarbonate (NaHCO₃; pH 8.5) to the 2 g of soil, the sample was agitated (180 rpm, 1 h) and decanted (1 h). The supernatant was filtered (filter paper) and 2 mL of 10 M hydrochloric acid (HCl) were added to destroy humic material. The mixture was agitated for 30 minutes and filtered again to separate the humic acids (Olsen & Sommers, 1982). Phosphorus was quantified by colorimetry using the method described by Murphy & Riley (1962). Color intensity was measured on a molecular absorption spectrophotometer (Philips PYE Unicam SP6-350) at 782 nm. Standard phosphate solutions (0, 0.25, 0.5, 1.0, 1.5 and 2.5 mg P L⁻¹) were prepared as above and a calibration curve, obtained from the linear regression between the concentration of P in the standard and absorbance (r²=0.998) was used to quantify P in the samples.

Carbon and nitrogen were extracted by adding 70 mL of K₂SO₄ 0.5 M to the 23 g subsample, the mixture was agitated (180 rpm, 1 h), decanted (1 h), the supernatant was filtered (filter paper) and the extract was kept at 4°C. For carbon determination, 8 mL of the extract were digested at 100°C during 45 minutes in 2 mL K₂Cr₂O₇ 0.066 M, 10 mL H₂SO₄ 98% and 5 mL de H₃PO₄ 88%. After cooling, 70 mL of deionized water and 4 diphenylamine drops (C₁₂H₁₁N) were added and the solution was titrated with a solution of 0.033 M (NH₄)₂Fe(SO₄)₂·6H₂O. For nitrogen determination, 20 mL of the extract were digested at 350°C during 2 hours with 5 mg of a mixture of K₂SO₄, FeSO₄·7H₂O e CuSO₄ (25:4.5:1) and 7.5 mL of H₂SO₄ 98%. After cooling, the Kjeldahl nitrogen was distilled (Tecator Kjeltec System 1026), recovered in 2% boric acid in the presence of bromocresol purple (C₂₁H₁₆Br₂O₅S) and methyl red ((CH₃)₂NCl·H₂N), and neutralized with 0.1 M HCl. Carbon and nitrogen were quantified using Eq. (S1):
Extracted element (mg kg$^{-1}$) = $\frac{(V_b-V_s) \times M \times mEq \times V_1 \times 10^6}{DM \times V_2}$  (Equation S1)

Where: $V_b$ is the volume of titrant used for the blank, $V_s$ is the volume of titrant used for the sample, $M$ is the molarity of the titrant, mEq is the milliequivalent weight of organic carbon (0.003) or nitrogen (0.014), $DM$ is the dry mass of soil, $V_1$ is the volume of the extractant and $V_2$ is the volume of the sample.

Microbial carbon, nitrogen and phosphorus were assessed as the difference between the fumigated (soil and intracellular organic elements) and the non-fumigated samples (soil organic elements). All values were expressed as mg soil kg$^{-1}$

References


