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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

Study plot	Farming management	Previous culture	Fertilization	Current culture	Geographic coordinates
OF1	Organic	Lucerne	Lucerne green manure	Corn	40°13’03’’N, 8°26’51’’W
OF2	Organic	Corn	Organic compost	Corn	
OF3	Organic	Corn	None	Corn	
CF	Conventional	Corn	Chemical fertilizer	Corn	40°13’14’’N, 8°28’36’’W

Soil preparation and analysis

For soil physic-chemical characterization, nine random core samples (0-20 cm; 250 cm^3) 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 CO_2 by infrared absorption spectrophotometry (Leco SC-144 DR) after combustion at 590°C (Rossell et al., 2001). Phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) were extracted with the Egnér-Riehm method (Balbino, 1968) and quantified by colorimetry after molecular absorption spectrophotometry (P_2O_5; Philips PYE Unicam SP6-350) and atomic absorption spectrophotometry with a flame atomizer (K_2O; Perkin Elmer Analyst 300). Total Kjeldahl nitrogen (ammonia (NH_3), ammonium (NH_4^+), and organic nitrogen) was quantified with the Bremmer method (Bremmer, 1979). The exchange cations K^+, Na^+, $Ca^{2+}$and Mg^{2+} 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 (105ºC, 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 ($CHCl_3$) 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 ($NaHCO_3$; 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 (r^2=0.998) was used to quantify P in the samples.

Carbon and nitrogen were extracted by adding 70 mL of K_2SO_4 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 4ºC.For carbon determination, 8 mL of the extract were digested at 100ºC during 45 minutes in 2 mL $K_2Cr_2O_7$ 0.066 M, 10 mL H_2SO_4 98% and 5 mL de H_3PO_4 88%. After cooling, 70 mL of deionized water and 4 diphenylamine drops ($C_{12}H_{11}N$) were added and the solution was titrated with a solution of 0,033 M $(NH_4)_2Fe(SO_4)_2.6H_2O$.For nitrogen determination, 20 mL of the extract were digested at 350ºC during 2 hours with 5 mg of a mixture of K_2SO_4, $FeSO_4.7H_2O$ e CuSO4 (25:4.5:1) and 7.5 mL of H_2SO_4 98%. After cooling, the Kjeldahl nitrogen was distilled (Tecator Kjeltec System 1026), recovered in 2% boric acid in the presence of bromocresol purple ($C_{21}H_{16}Br_2O_5S$) and methyl red ($(CH_3)_2NC_6H_4N$), and neutralized with 0,1 M HCl. Carbon and nitrogen were quantified using Eq. (S1):

$$\text{Extracted element}\left(\text{mg kg}^{-1}\right)=\frac{(\text{Vb-Vs})\times\text{M}\times\text{mEq}\times\text{V1}\times10^{6}}{\text{DM}\times\text{V2}} \qquad \textbf{(Equation S1)}$$

Where: Vb is the volume of titrant used for the blank, Vs 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, V1 is the volume of the extractant and V2 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}

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