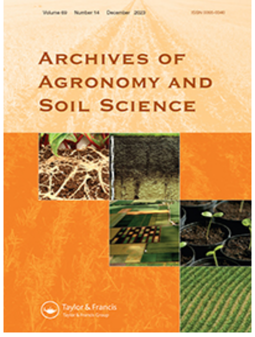

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Effects of fertilizer produced from agro-industrial wastes on the quality of two different soils

Federica Marra, Angela Maffia, Francesco Canino, Carmelo Greco, Carmelo Mallamaci and Muscolo Adele

Department of AGRARIA, Mediterranea University, Reggio, Italy

ABSTRACT

A newly developed sustainable fertilizer, known as SB, was created by blending organic and mineral components using agro-industrial waste, sulphur, and orange residue, bound together with bentonite. It was extensively tested on two distinct soils with different chemical and biological properties, comparing its effectiveness to traditional chemical (NPK) and organic (horse manure, HM) fertilizers, with unfertilized soil as a control (CTR). The introduction of SB did not alter soil texture but significantly impacted soil chemistry and biology. It positively influenced the labile fraction of soil organic matter, resulting in a 15% increase in soil microbial biomass, total phenolic content, cations, bacterial colonies, and enzyme activities, with varying effects depending on soil characteristics. SB demonstrated a beneficial effect on both soil types, with optimal concentrations determined to be 2.8 for Motta and 4.2 for Lazzaro, highlighting the importance of soil characteristics in fertilizer effectiveness. In conclusion, SB represents a promising innovation for transitioning from traditional agriculture to a more sustainable and circular approach, offering economic and environmental benefits by reducing waste disposal costs and decreasing reliance on mineral fertilizers in line with circular economy principles. This study emphasizes the need to consider soil properties when optimizing fertilizer use.

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Fertilizer production; soil enzyme activities; soil quality; soil fertility; sulphur-based fertilizer

Introduction

The global increase in population has led to a surge in worldwide food demand, significantly impacting soil due to intensive tillage and excessive fertilization practices. These practices, often irreversible, disrupt the delicate ecological balance of soil by affecting nutrient cycles, nutrient availability, and soil chemical properties. In addition to the pressing need to boost crop productivity, there is an equally urgent requirement to enhance the sustainability of the agricultural supply chain by reducing the reliance on agrochemicals and mineral fertilizers, aligning with the European Union's (EU) Green Deal program. Notably, this includes compliance with the Farm to Fork Strategy and the EU Biodiversity Strategy for 2030, both of which revolve around critical issues related to climate, the environment, and agriculture. These strategies envisage substantial reductions in pesticides, fertilizers, and antibiotics, accompanied by a significant upswing in organic farming. The overarching objective is to transition to a food chain grounded in circular bio-economy principles, reducing food waste and losses while

CONTACT Muscolo Adele amuscolo@unirc.it Department of AGRARIA. Mediterranea University, Feo di Vito 89124, Reggio, Italy

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embracing organic agriculture. The utilization of organic fertilizers to preserve soil fertility has long been a fundamental tenet of sustainable agriculture. Recently, there has been an increasing focus on organic fertilizer production from waste materials, aligning with the European Commission's goal of achieving a 30% reduction in non-renewable resource usage by recycling them into fertilizers. This concept of circularity underscores the repurposing of by-products, marking a shift from a fossil-based economy to a bio-economy, with a paramount emphasis on nutrient recovery to mitigate the high energy costs associated with mineral fertilizer production processes. Crucially, the agri-food industry consistently generates organic wastes, with their quantity expected to reach 3.4 billion tonnes in the near future. If left to languish in landfills, these waste materials can give rise to significant local and global environmental issues. These include the emission of greenhouse gases, soil contamination, pollution of local water sources, and the eutrophication of riverbeds and freshwater reserves due to an excess of nitrogen. Incorporating these waste products into agricultural practices can play a pivotal role in recycling vital plant nutrients. However, it's important to note that the impact of these fertilizers on soil properties can vary widely based on the specific soil type, environmental conditions, and the type of fertilizer used, each exerting a different level of effectiveness in enhancing soil productivity.

It's worth emphasizing that the use of chemical fertilizers is not advisable, as it can lead to several soil-related issues, such as soil compaction and degradation. Instead, a general recommendation is to increase the amount of soil organic matter (SOM) as an efficient means to enhance soil quality for sustainable agricultural production. SOM is widely acknowledged for its ability to improve soil quality and boost crop productivity. It achieves this by creating soil aggregates that enhance soil stability and by stimulating the activities of soil microorganisms. SOM also serves as a carbon source, which, through the mineralization process, results in an increased availability of essential nutrients for plant mineral nutrition. Furthermore, organic fertilization has garnered significant attention because its application promotes the biodiversity of soil bacteria. This microbial diversity not only drives secondary metabolic processes but also stimulates primary productivity (Shang et al. 2020). The reuse of waste materials for agricultural purposes, particularly citrus waste, has the potential to enhance soil quality by enriching soils with beneficial and effective microbes and nutrients (Corti et al. 2012). Microbial biomass plays a pivotal role in breaking down complex biomolecules into simpler forms, facilitating easier uptake by plants. Given the challenges posed by resource scarcity and waste disposal, the principles of the Circular Economy demand that waste management and the utilization of waste materials for sustainable raw material use be addressed comprehensively. Additionally, it's important to note that sulphur, the fourth most critical nutrient after nitrogen, tends to be deficient primarily in high-yield, arid, semiarid, and desertified soils (Yesmin et al. 2021). To enhance soil biodiversity and functionality through proper fertilization, the incorporation of sulfur into organic fertilizers derived from agricultural waste presents an avenue to bolster the soil's nutrient reservoirs while aligning with the principles of the circular economy, especially when utilizing reclaimed sulphur. Given its compatibility with other fertilizers and its suitability for early-stage and intensive plant growth, sulphur supplementation holds promise. Building upon these insights, the primary objective of this study was to assess the impact of a novel fertilizer composed of sulphur-bentonite and orange residue in open field conditions on different soils characterized by varying chemical and biochemical properties. This investigation encompassed varying concentrations of the fertilizer, with comparative evaluations against chemical fertilizer (NPK) and horse manure (HM). As a control, unfertilized soil (CTR) was also included. The central focus of this study revolved around the influence of soil characteristics on fertilizer effectiveness. Recognizing the paramount role soil attributes play in nutrient availability, pH balance, nutrient uptake, water retention, environmental consequences, and overall plant vitality, it is imperative to consider these factors when selecting a fertilizer. Neglecting soil characteristics during fertilizer selection can result in suboptimal nutrient utilization, impaired plant growth, and potential harm to the environment. Therefore, the investigation delved into the following aspects: 1) the fertilizer's impact on soil chemical properties; 2) the extent to which the new fertilizer affected soil quality, encompassing nutrients, soil enzymes, fungi, bacteria, and actinomyces; and 3) the influence of specific soil characteristics on the fertilizer's efficacy, all with the aim of elucidating changes in the quality and functionality of the two soils.

Materials and methods

Fertilizer production

Steel Belt System s.r.l. developed fertilizer in tablet of 3/4 mm as described in Muscolo et al. (2017, 2019). Sulphur was mixed with bentonite and orange rest of food industry (O). Elemental S was the principal component of fertilizer (Muscolo et al. 2020). The fertilizer was tested for pathogens (*total coliforms, faecal coliforms, salmonella spp and Escherichia coli*) and heavy metals to prevent unhealthy and dangerous effects on soil (Ben Said et al. 2017; Muscolo et al. 2021). Results evidenced absence of pathogens and heavy metals (Muscolo et al. 2021).

Soil treatment

The experiment was carried out in two soils differing for chemical and biological properties. A sandy-loam soil belonging to Cambisol (WRB, 2022) located in Motta San Giovanni, Loc. Liso, Italy (37.9991° N. 15.6999° E) arbitrarily named Motta, and a sandy clay loam soil belonging to Alisol (WRB, 2022) located in Lazzaro 37.9724° N. 15.6657° (arbitrarily named Lazzaro) were used for the experiments. Textural class of the two soils were identified using the Food and Agriculture Organization of the United Nations (FAO) soil classification system (FAO 2007).

The soils were amended with four doses of the new fertilizer and precisely with 476 kg S ha^{-1} (SB, 1.4), 952 kg S ha^{-1} (SB, 2.8), 1428 kg S ha^{-1} (SB, 4.2) and 1904 kg S ha^{-1} (SB, 5.6). The different doses were chosen on the basis of literature data on the quantity of pure sulphur that is normally used in respect to soil texture, which range from 2200 kg S ha^{-1} to 3300 kg S ha^{-1} in sandy or clay soil, respectively (Severson and Shacklette 1988; Muscolo et al. 2017). Soil no fertilized was used as control (CTR), nitrogen: phosphorous: potassium (NPK, 20/10/10) as chemical fertilizer and horse manure (HM, 4.3 q/ha) as organic fertilizer. Soils were divided in plots of 1 m square each and fertilized. Each treatment was replicated six folds. The experiment was arranged in a randomized complete block design, the parcels were six for each treatment. The experiments lasted six months and the results are the average of three independent experiments. During the experiment, the plots were irrigated to keep 70% of the field capacity for the vitality of soils, soil water content was monitored through a direct read soil pH/moisture meter – R181.

Soil chemical analysis

Soil texture was detected following Bouyoucos (1962) method. Electric conductibility (EC) was tested in 1:5 soil/water suspension, after stirring at 15 rpm for 1 h. EC was detected by Hanna instrument conductivity meter; pH was determined in soil/solution ratio 1:2.5 with a glass electrode. Organic carbon was tested with Walkley and Black (1934) methodology. Total nitrogen (TN) was assessed with Kjeldahl method (1883). C/N was quantified as a carbon:nitrogen ratio. Water soluble phenols were extracted and analysed as described by Kaminsky and Muller (1978) and monomeric and polyphenols were determined with Box (1983) method, using tannic acid as standard. The concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (μg TAE g^{-1} D.W.). Cation Exchange Capacity was analysed with barium chloride method (Hendershot and Duquette 1986). Cations and anions were detected with ion chromatography (DIONEX ICS-1100), as described in Muscolo et al. (2022).

Soil biological analysis

For the detection of microbial biomass carbon (MBC) the chloroform fumigation-extraction procedure was used (Vance et al. 1987) on fresh soil. Fumigated and unfumigated soil sample extracts were used to detect soluble organic C (Walkley and Black 1934).

To detect bacteria, fungi and actinomycetes 10 grams of each soil sample were extracted with 95 mL of 0.1% (w/v) solution of sodium pyrophosphate. Soil extract solutions were diluted (10^{-1} to 10^{-7}) and the shares were plated on agarized culture media, each specific for bacteria or fungi or actinomycetes (Elliot and Des Jardin 1999). Colony forming units (CFU) for each microorganism were counted as reported in Picci and Nannipieri (2003) and Eaton et al. (2005).

Fluorescein diacetate hydrolase (FDA) activity was determined according to the method of Adam and Duncan (2001).

Dehydrogenase (DHA) activity was assessed with Von Mersi and Schinner (1991) method.

Catalase activity (CAT) was detected assessing the absorbance during the transformation of H_2O_2 to oxygen and water (Muscolo et al. 2017). The decrease in the absorbance was measured at 240 nm, using the extinction coefficient of 39.4 M^{-1} cm^{-1}.

Protease activity was detected as reported in Muscolo et al. (2017).

Urease activity was determined as reported in Kandeler and Gerber (1988) with few modifications described in Sidari et al. (2008). Ammonium concentrations were determined at 690 nm by using a calibration curve. The results are reported as μg N-NH_4 g^{-1} d^{-1} 3 h^{-1}

Beta-glucosidase activity was assessed following Eivazi and Tabatabai (1988) method and the results have been expressed as μg of para-nitrophenol (p-NP) g h^{-1}

Statistical analysis

Analysis of variance was used for all the data sets. One-way ANOVA with Tukey's Honestly. Significant difference tests for analysing the effects of fertilizers on each of the parameters measured were used. ANOVA and T-test were done with SPSS software. The effects were significant at $p \leq 0.01$. To analyse the relationships among the different fertilizers and the soil parameters in the two different sites, Principal Component Analysis (PCA) was used.

Results and discussion

Soil chemical and biochemical characteristics of unamended soils

The selection of the two soils was deliberate, considering their distinct chemical and biological properties as detailed in Table 1. The Cambisol in Motta San Giovanni (CTR) exhibited a sandy-loam texture, comprising 65% sand, while the Allisol in Lazzaro (CTR) presented a sandy-clay-loam texture with 50% sand, 23% clay, and 27% silt. Notably, there were no significant disparities in pH and electrical conductivity between the two soils. In terms of organic content, Motta soil boasted a higher organic carbon content (1.98%) and total

Table 1. Chemical and biochemical properties of soil before the experiment located in Motta and Lazzaro. Soil texture (percentage of sand, silt and clay); pH$_{H2O}$ in water and pH$_{KCl}$ in potassium chloride; EC = electric conductivity (μS cm^{-1}); TP = total phenols (μg TAE g^{-1} ds): OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (μg C g^{-1} soil); CEC = cation exchange capacity (cmol$^{(+)}$ kg^{-1}), dehydrogenase, (DHA), fluorescein diacetate hydrolase (FDA), Catalase (CAT).

	Motta	Lazzaro
Sandy	65 ± 10^{a}	50 ± 1^{a}
Clay	12 ± 4^{a}	23 ± 0.8^{a}
Silt	23 ± 3^{a}	27 ± 0.9^{a}
Texture	**Sandy-loam**	**Sandy-clay loam**
pH $_{(H2O)}$	8.4 ± 0.1^{a}	8.4 ± 0.1^{a}
pH $_{(KCl)}$	6.91 ± 0.1^{a}	6.96 ± 0.1^{a}
EC	301 ± 10^{a}	301 ± 8^{a}
TP	280 ± 12^{b}	327 ± 12^{a}
MBC	845 ± 12^{b}	1122 ± 22^{a}
CEC	28 ± 1^{a}	11 ± 2^{b}
OC	1.98 ± 0.5^{a}	1.4 ± 0.2^{a}
TN	0.20 ± 0.02^{a}	0.16 ± 0.02^{a}
C/N	9.9 ± 1.5^{a}	8.8 ± 1.3^{a}
OM	3.4 ± 0.6^{a}	2.4 ± 0.3^{a}
ß-glucosidase	514 ± 6^{a}	208 ± 5^{b}
Protease	148 ± 7^{b}	166 ± 5^{a}
Urease	350 ± 12^{a}	253 ± 17^{b}
FDA	10 ± 1^{a}	10 ± 1^{a}
DHA	5.3 ± 1^{a}	5.2 ± 1^{a}
CAT	3.7 ± 1^{a}	1.2 ± 0.5^{b}
Bacteria colony	1 ± 0.07^{b}	163 ± 17^{a}
Fungi colony	30 ± 1^{a}	36 ± 2^{b}
Actinomycetes	48 ± 3^{b}	65 ± 5^{a}
Calcium	3.1 ± 0.3^{a}	1.9 ± 0.1^{b}
Magnesium	1.8 ± 0.2^{a}	2.2 ± 0.4^{a}
Potassium	1.2 ± 0.1^{b}	4.0 ± 0.3^{a}
Ammonium	15 ± 1^{b}	50 ± 5^{a}
Sulphate	44 ± 2^{a}	48 ± 2^{a}

nitrogen content (0.20%), along with a greater cation exchange capacity compared to Lazzaro soil, which had 1.4% organic carbon, 0.16% total nitrogen, and a lower cation exchange capacity. Conversely, Lazzaro soil exhibited a larger microbial biomass C (Table 1) and total phenol content. Furthermore, Lazzaro soil contained a more extensive array of anions and cations than Motta soil (Table 1). In relation to biological attributes, the activities of FDA and DHA displayed a similar trend in both soils, while catalase activity was notably higher in Motta soil (Table 1). The composition and dynamics of soil organic matter, especially the balance between its stable (humic substances) and labile components, have a direct correlation with nutrient release (Zanin et al. 2019) and, consequently, soil fertility and quality (Gerke 2022). Our data underscored that Lazzaro soil harbored a richer pool of nutrients, owing to a higher microbial biomass, as well as a greater abundance of bacteria and fungi (Table 1). These microorganisms play pivotal roles in nutrient cycling, aligning with previous studies by Prosser (2007) and Shay et al. (2015). These findings are further supported by the reduced quantity of organic matter in Lazzaro soil, attributed to the substantial mineralization driven by the numerous colonies of fungi and bacteria (Hicks et al. 2021), which are the primary producers of soil enzymes (Baćmaga et al. 2021). Soil microbial biomass and enzymatic activities, particularly hydrolase activities, are intimately involved in organic matter turnover and nutrient cycling, making them sensitive indicators of soil fertility (Sekaran et al. 2021). Collectively, the data from biochemical and biological parameters underscore the disparities between the two soils, with Lazzaro soil emerging as the more fertile substrate. These variations in biochemical parameters served as a basis for investigating the impacts of different fertilization practices, encompassing organic, chemical, and organic-mineral amendments, on soil quality and health.

Soil characteristics 6 months after treatments with the different fertilizers

No changes in textural class was observed in soils six months after the treatments with the different fertilizers in both locations in respect to CTR (Tables 2 and 3).

These findings confirm that texture is a soil property that remains relatively resistant to change. Fertilization, whether with organic or mineral fertilizers, primarily affects the

Table 2. Chemical and biochemical properties of soil located in Motta San Giovanni, 6 months after treatments with the different fertilizers. CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulfur bentonite + orange residue. Soil texture (percentage of sand, silt and clay); pH_{H2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (μS cm^{-1}); TP = total phenols (μg TAE g^{-1} ds): OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (μg C g^{-1} soil); CEC = cation exchange capacity ($cmol^{(+)}$ kg^{-1}).

	CTR	NPK	HM	SB 1.4	SB 2.8	SB 4.2	SB 5.6
Sandy	65 ± 10^a	65 ± 12^a	65 ± 11^a	65 ± 12^a	65 ± 9^a	65 ± 12^a	65 ± 11^a
Clay	12 ± 4^a	12 ± 2^a	12 ± 3^a	12 ± 2^a	12 ± 2^a	12 ± 4^a	12 ± 3^a
Silt	23 ± 3^a	23 ± 2^a	23 ± 4^a	23 ± 1^a	23 ± 2^a	23 ± 2^a	23 ± 3^a
Texture	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam
pH $_{(H2O)}$	8.43 ± 0.1^a	8.47 ± 0.2^a	8.46 ± 0.1^a	8.41 ± 0.2^a	8.43 ± 0.1^a	8.12 ± 0.1^b	8.19 ± 0.1^b
pH $_{(KCl)}$	6.94 ± 0.1^a	7.01 ± 0.1a	6.99 ± 0.1^a	6.97 ± 0.2^a	7.04 ± 0.1^a	7.01 ± 0.2^a	7.03 ± 0.1^a
EC	302 ± 10^a	301 ± 8^a	297 ± 12^a	296 ± 10^a	302 ± 13^a	267 ± 12^a	278 ± 10^a
TP	282 ± 12^b	320 ± 10^b	315 ± 10^b	280 ± 10^b	332 ± 15^{ab}	352 ± 20^a	357 ± 20^a
MBC	835 ± 12^c	798 ± 15^d	997 ± 12^a	845 ± 15^c	912 ± 12^b	933 ± 14^b	923 ± 16^b
CEC	27.8 ± 1^a	28 ± 1.5^a	30 ± 0.8^{ab}	31 ± 12^{ab}	38 ± 2^a	36 ± 1^a	36 ± 1.4^a
OC	1.98 ± 0.5^a	1.69 ± 0.3^a	2.15 ± 0.4^a	1.92 ± 0.5^a	2.24 ± 0.3^a	2.24 ± 0.5^a	2.08 ± 0.5^a
TN	0.20 ± 0.02^a	$.23 \pm 0.02^a$	0.21 ± 0.03^a	0.19 ± 0.01^a	0.15 ± 0.01^b	0.12 ± 0.02^b	0.10 ± 0.03^b
C/N	9.9 ± 1.5^c	7.39 ± 1.4^d	10.1 ± 1.3^c	10.2 ± 1.7^c	15.3 ± 1^b	20 ± 1.5^a	20.9 ± 1.5^a
OM	3.42 ± 0.6^a	2.91 ± 0.5^a	3.70 ± 0.5^a	3.30 ± 0.6^a	3.85 ± 0.5^a	3.85 ± 0.4^a	3.57 ± 0.3^a

Table 3. Chemical and biochemical properties of soil located in Lazzaro, 6 months after treatments with the different fertilizers. CTR = control, soil without fertilizer; NPK= nitrogen:phosphorous:potassium; HM = horse manure; SB = sulfur bentonite + orange residue (at different concentrations (1.4; 2.8; 4.2 and 5.6). Soil texture (percentage of sand, silt and clay); pH_{H2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (μS cm^{-1}); TP = total phenols (μg TAE g^{-1} ds): OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (μg C g^{-1} soil); CEC = cation exchange capacity ($cmol^{(+)}$ $kg^{-1.}$).

	CTR	NPK	HM	SB 1.4	SB 2.8	SB 4.2	SB 5.6
Sandy	50 ± 1^a	50 ± 2^a	50 ± 3^a	50 ± 4^a	50 ± 2^a	50 ± 3^a	50 ± 2^a
Clay	23 ± 0.8^a	23 ± 1^a	23 ± 0.9^a	23 ± 0.5^a	23 ± 0.8^a	23 ± 0.7^a	23 ± 0.6^a
Silt	27 ± 0.9^a	27 ± 1.2^a	27 ± 0.9^a	27 ± 1.8^a	27 ± 1.5^a	27 ± 1.7^a	27 ± 0.9^a
Texture	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam
pH (H_2O)	8.47 ± 0.1^b	8.41 ± 0.2^b	8.39 ± 0.2^b	8.07 ± 0.1^a	8.08 ± 0.4^a	8.04 ± 0.1^a	8.02 ± 0.1^a
pH (KCl)	6.99 ± 0.1^a	7.03 ± 0.2^a	6.99 ± 0.1^a	7.16 ± 0.2^a	7.17 ± 0.1^a	6.24 ± 0.2^b	6.52 ± 0.3^a
EC	301 ± 10^a	278 ± 13^a	299 ± 14^a	230 ± 10^b	233 ± 15^b	$229 \pm 13\ ^b$	222 ± 10^b
TP	332 ± 12^c	337 ± 10^c	365 ± 14^b	355 ± 12^b	398 ± 13^a	392 ± 11^a	352 ± 12^{ab}
MBC	1132 ± 22^c	1100 ± 18^c	1190 ± 16^b	1198 ± 12^b	1212 ± 12^b	1233 ± 12^b	1298 ± 12^a
CEC	11 ± 2^a	11 ± 1.5^a	14 ± 1.5^a	15 ± 0.9^a	16 ± 1.5^a	15 ± 1.5^a	14 ± 1^a
OC	1.4 ± 0.2^a	1.3 ± 0.1^a	1.5 ± 0.2^a	1.4 ± 0.2^a	1.4 ± 0.1^a	1.4 ± 0.1^a	1.4 ± 0.2^a
TN	0.16 ± 0.02^a	$.13 \pm 0.02^a$	0.12 ± 0.02^a	0.08 ± 0.02^a	0.12 ± 0.02^a	0.08 ± 0.02^a	0.08 ± 0.02^a
C/N	8.8	1.3	12.6	16.6	11.5	16.3	16.5
OM	2.4 ± 0.3^a	2.2 ± 0.2^a	2.6 ± 0.3^a	2.4 ± 0.3^a	2.4 ± 0.2^a	2.4 ± 0.3^a	2.4 ± 0.2^a

distribution ratio of soil aggregates due to its influence on soil carbon dynamics (Niu et al. 2022). Notably, there was a decrease in pH only in the presence of sulphur-based fertilizers at high concentrations, underscoring sulphur's role as an acidifying soil amendment (Mehdi et al. 2019). In Motta soil, this decrease in pH was observed only in water, not in KCl, indicating that the effects of sulphur-based fertilizers were more related to the acidification of the soil solution in circulation than to the reserve acidity in the colloids. Conversely, in Lazzaro soil, a reduction in pH values, both in water and KCl, was noted in soils treated with sulphur-based fertilizers (Table 3). These results can be correlated with some observed differences between the two soils, particularly the high level of organic matter detected in Motta soil (Table 2). This organic matter, as demonstrated by Dvořáčková et al. (2022), has the potential to act as a buffer against soil acidification due to its cation-binding capacity. This is further supported by the higher cation exchange capacity (CEC) observed in Motta soil due to its greater organic matter content (Table 2). These findings align with prior research by Solly et al. (2020), which established a correlation between CEC, organic matter content, and soil buffering capacity. The electrical conductivity (EC) in the untreated soils (CTR) was similar in both locations (Tables 2 and 3). In Motta, fertilization did not have a significant impact on EC. In contrast, in Lazzaro, EC decreased in the presence of sulphur-based fertilizers. Total phenols, precursors of humic substances that serve as a rich carbon source for microbial biomass and potent antioxidants (Min et al. 2015), increased in soils treated with sulphur-based fertilizers in comparison to other treatments in both locations (Tables 2 and 3). These findings align with the greater microbial biomass carbon (MBC) and higher C/N values observed in soils treated with sulphur-based fertilizers, indicating a distinct trend in organic matter dynamics between the two soils. Among the detected cations, magnesium and calcium were found in higher concentrations in soils fertilized with sulphur-bentonite compared to other fertilizer types in both locations (Figure 1a and b). The sulphur content in the soil may indirectly influence the uptake levels of other nutrients. Research has shown that sulphur has a positive effect on calcium, leading to an increase in total phosphorus and magnesium levels, while reducing potassium content (Skwierawska et al. 2016). The observed differences can be attributed to the CEC value, which was highest in the presence of sulphur-bentonite (SB), capable of retaining more cations, especially bivalent ones, compared to monovalent ions (Elbaalawy et al. 2023). Nitrate levels in the presence of sulphur bentonite were similar to those with horse manure (HM) and NPK, and they slightly increased with higher SB concentrations, while ammonium levels decreased, likely because monovalent ions were less retained by soil exchange sites compared to bivalent ions.

Furthermore, the higher abundance of these cations may also result from the organic matter mineralization process, coupled with the formation of sulphate by soil microorganisms. Calcium and magnesium sulphate, in particular, play a role in retaining calcium and magnesium cations in the soil, preventing their precipitation at the prevailing soil pH, as corroborated by previous studies (Skwierawska et al. 2008). Among the anions, sulphate, malate, and phosphate were found in the greatest abundance in both Motta and Lazzaro soils treated with sulphur-based fertilizers (Figure 2a and b).

DHA, serving as an enzyme marker for oxidative activity in the soil, exhibited higher levels in soils treated with SB in both locations (Figure 3a and b). FDA displayed a similar trend to DHA in Motta, although it increased only at the lowest SB concentrations (Figure 3c and d). In contrast, no significant changes were observed for FDA in Lazzaro soil among the treatments. FDA reflects the activity of various hydrolytic enzymes, including esterases, proteases, and lipases (Nikaeen et al. 2015), and can serve as an indicator of soil biological activity, as noted by Komilis et al. (2011). Catalase, an antioxidant enzyme that increases in soil under stress

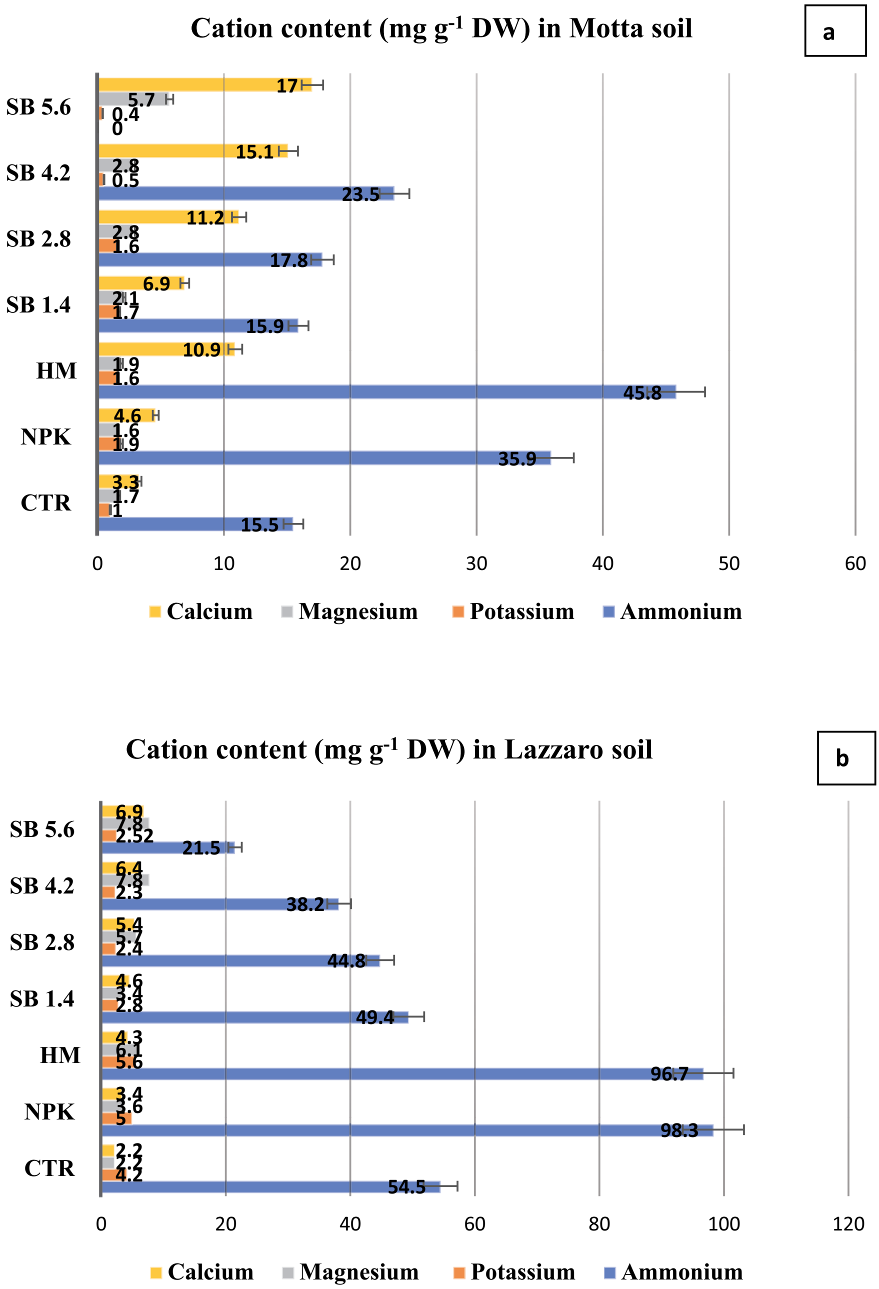


Figure 1. Cation content in Motta San Giovanni (a) and Lazzaro (b) soils, 6 months after the amendment with NPK = nitrogen: phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6) and CTR = control, soil without fertilizer. Data are the means of six independent experiments and bars represent the standard error of the parameters analysed. Data are the means of three replicates (*n* = 18) ± standard errors.

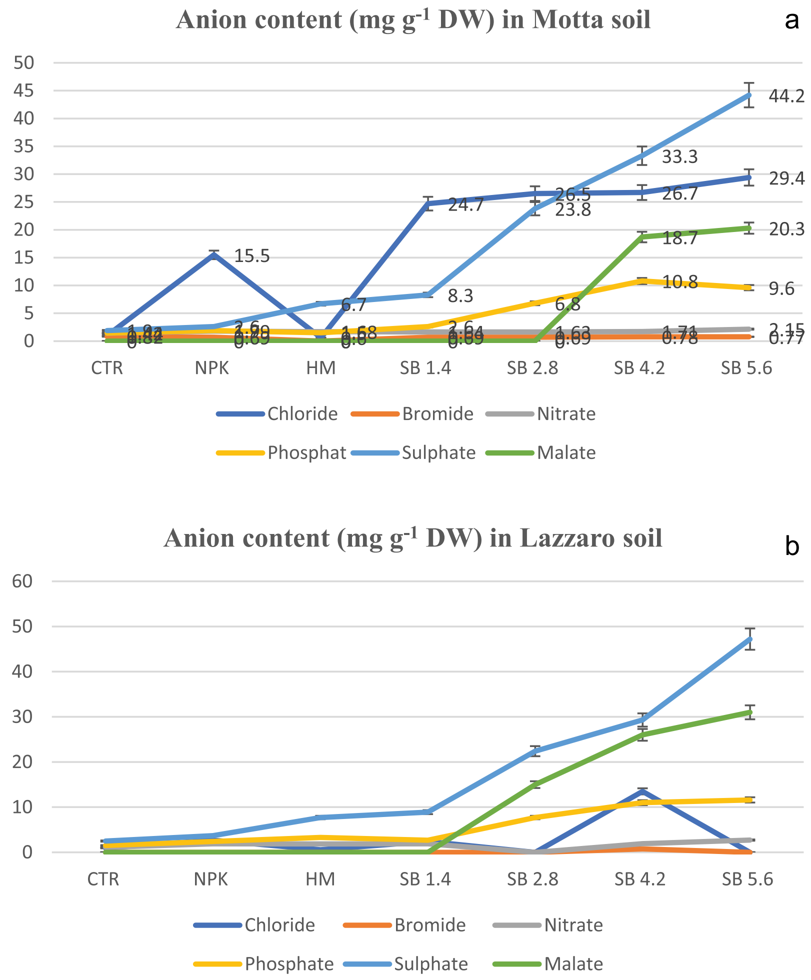


Figure 2. Anion content in Motta San Giovanni (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers. NPK= nitrogen:phosphorous:potassium; HM= horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6) and CTR= control, soil without fertilizer. Data are the means of six independent experiments and bars represent the standard error of the parameters analysed. Data are the means of three replicates ($n = 18$) ± standard errors.

conditions, protecting against oxidative damage by converting hydrogen peroxide into water and oxygen, decreased in SB-treated soils compared to the other treatments in both locations (Figure 3e and f). This suggests that the new fertilizer, at all concentrations, did not impose stress on the soil environment.

β-glucosidase, a key enzyme in the decomposition of litter components associated with the carbon cycle, showed greater activity in the NPK treatment in Motta soil and in the SB treatments at 1.4 and 2.8 in Lazzaro soil (Table 4). Protease, responsible for the hydrolytic degradation of proteins, a crucial step in the nitrogen cycle, followed a similar trend to β-glucosidase in Motta soil, while in Lazzaro soil, it peaked in the presence of SB 4.2 (Table 4). Urease activity was highest in Motta soil when fertilized with NPK and in Lazzaro soil with HM and SB 2.8 (Table 4). The trends observed in fungi, bacteria, and actinomycetes mirrored those of the enzymes (Figures 4 and 5). Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Reduced urease activity can positively affect soil by preventing excessive urea hydrolysis, which could lead to ammonia loss through volatilization or rapid nitrification,

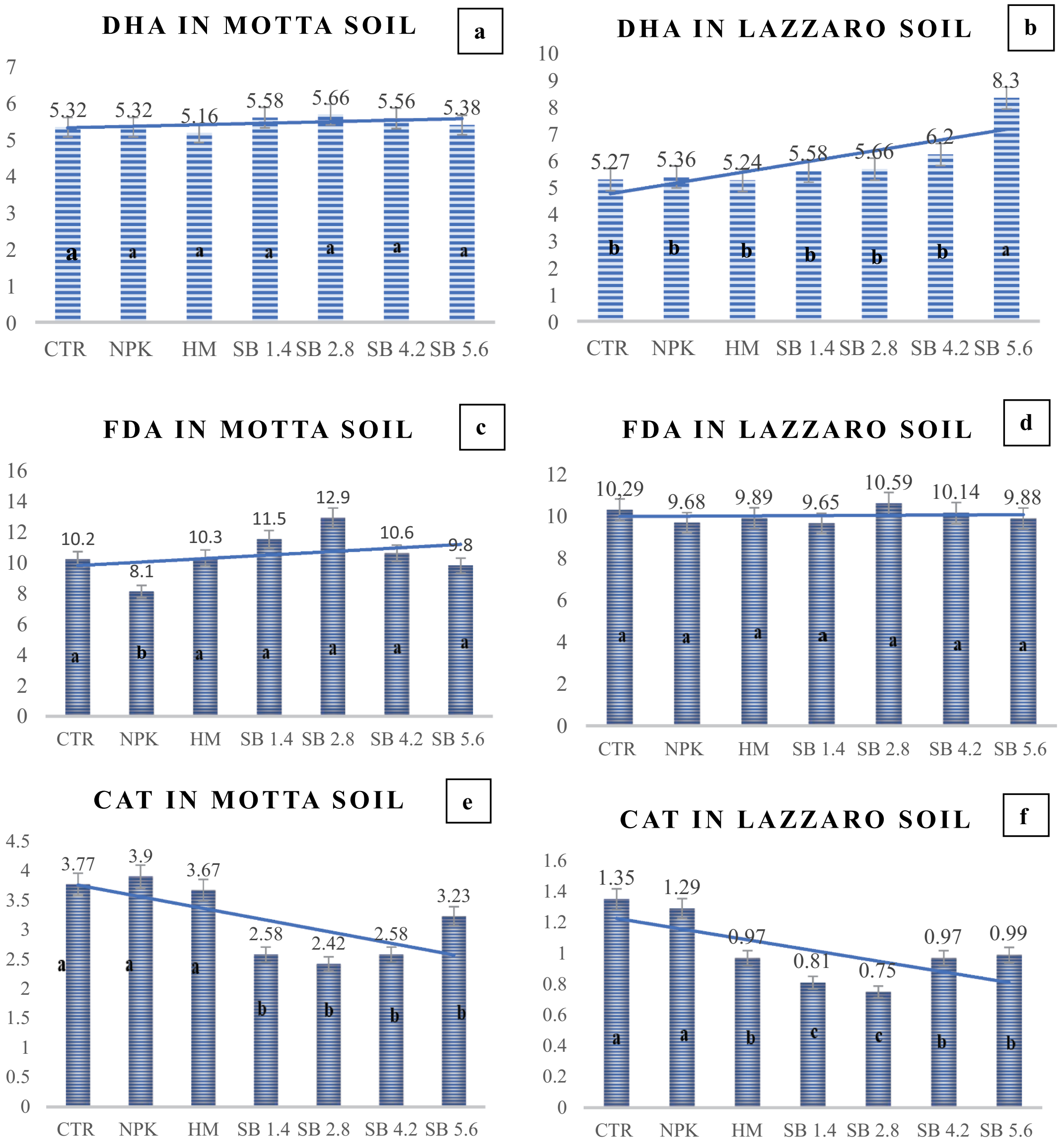


Figure 3. (a, b, c, d, e, f). dehydrogenase, (DHA), fluorescein diacetate hydrolase (FDA) Catalase (CAT) activities in Motta San Giovanni (a, c, e) and Lazzaro soils (b, d, f) 6 months after the amendment with the different fertilizers: CTR= control, soil without fertilizer; NPK= nitrogen:phosphorous:potassium; HM= horse manure; SB = sulphur bentonite + orange residue(at different concentrations: 1.4; 2.8; 4.2 and 5.6). Data are the means of six independent experiments and bars represent the standard error of the parameters analysed. Data are the means of three replicates (*n* = 18) ± standard errors.

followed by nitrate loss via leaching. This decrease in urease activity may be attributed to a shift in the microbial population, where the presence of sulfur has been reported to increase the percentage of sulphate-producing bacteria within the total bacterial population, at the expense of bacteria involved in nitrate and ammonium production, as indicated by Bouranis et al. (2019). Principal Component Analysis (PCA) revealed that ammonium and potassium, as expected, correlated with NPK and HM in both soils (Figure 6a and b). In contrast, calcium was

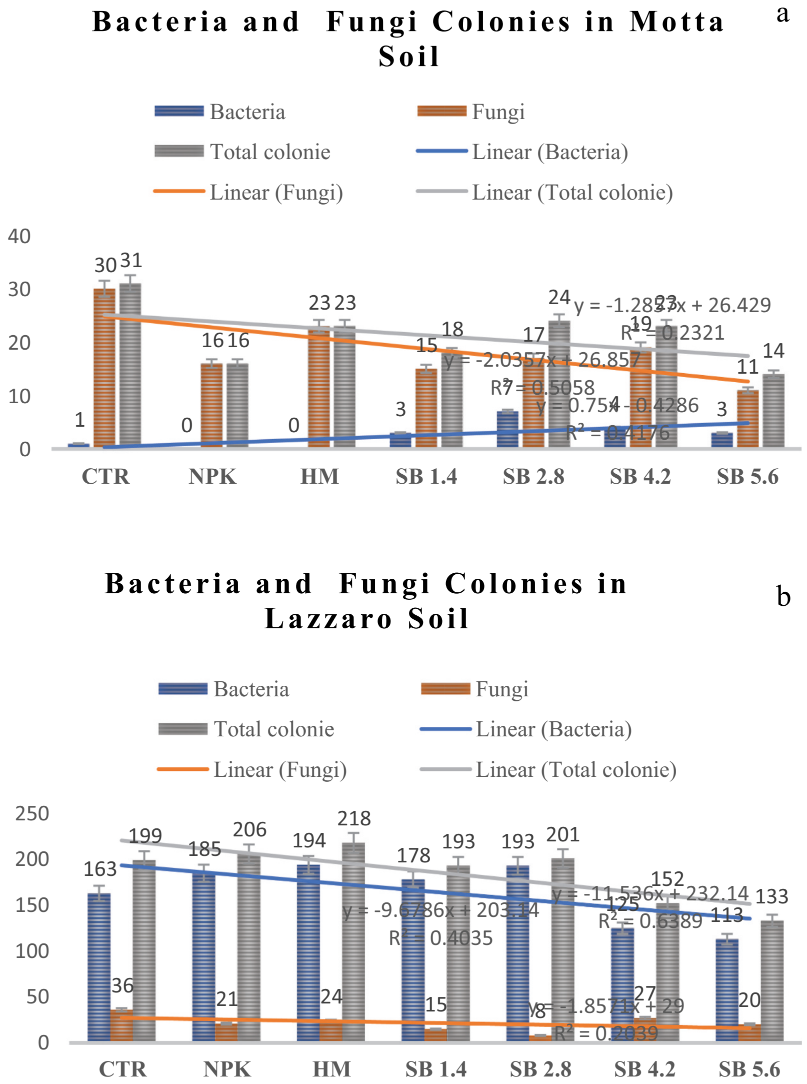


Figure 4. (a, b). bacteria (UFC 10^{-3}) and fungi (UFC 10^{-2}) colonies in Motta San Giovanni and Lazzaro soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM= horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6). Data are the means of six independent experiments and bars represent the standard error of the parameters analysed. Data are the means of three replicates (*n* = 18) ± standard errors.

Table 4. *SS*-glucosidase, protease and urease activities detected in Motta and Lazzaro soils 6 months after treatments with the different fertilizers. CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulfur bentonite + orange residue (at different concentrations 1.4; 2.8; 4.2 and 5.6).

	MOTTA			Lazzaro		
	ß-glucosidase	Protease	Urease	*ß*-glucosidase	Protease	Urease
CTR	514 ± 9^{e}	148 ± 9^{b}	350 ± 6^{d}	208 ± 6^{c}	166 ± 8^{b}	253 ± 9^{b}
NPK	709 ± 9^{a}	168 ± 5^{a}	403 ± 9^{a}	249 ± 10^{b}	158 ± 9^{b}	251 ± 5^{b}
HM	669 ± 11^{b}	168 ± 5^{a}	381 ± 7^{b}	209 ± 5^{c}	162 ± 8^{b}	273 ± 6^{a}
SB 1.4	559 ± 9^{d}	144 ± 5^{b}	319 ± 9^{e}	286 ± 7^{a}	165 ± 5^{b}	249 ± 9^{b}
SB 2.8	616 ± 11^{c}	176 ± 5^{a}	363 ± 8^{c}	291 ± 9^{a}	176 ± 11^{b}	265 ± 8^{a}
SB 4.2	648 ± 12^{b}	154 ± 7^{b}	267 ± 6^{f}	237 ± 7^{b}	210 ± 9^{a}	230 ± 7^{c}
SB 5.6	516 ± 11^{e}	154 ± 6^{b}	261 ± 9^{f}	244 ± 9^{b}	162 ± 5^{b}	197 ± 8^{d}

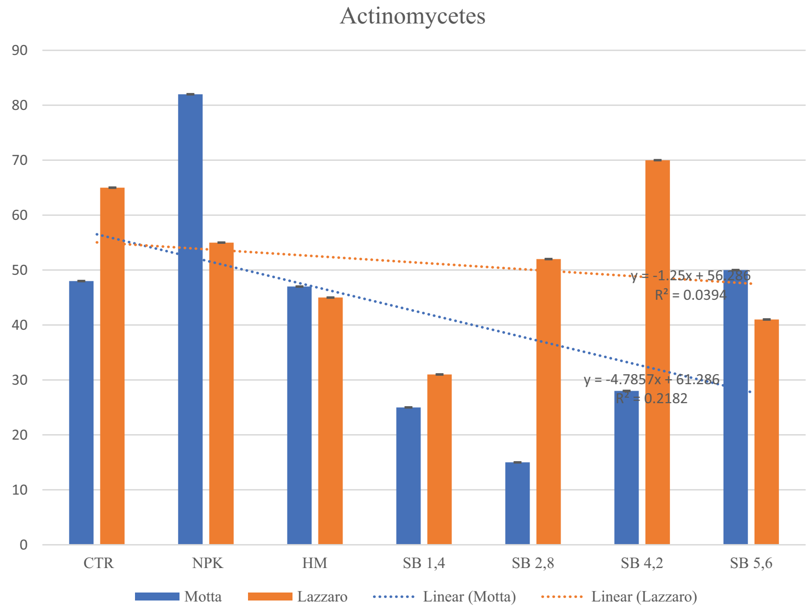


Figure 5. Actinomycetes detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).

correlated only with SB. Magnesium in Motta soil showed a significant correlation with SB 4.2 fertilization (Figure 6a), while in Lazzaro soil, it was linked to HM (Figure 6b).

Anions exhibited correlations with SB fertilizations in both soils, with a few exceptions such as SB 1.4 in Motta and SB 1.4 and 2.8 in Lazzaro (Figure 7a, b). The PCA analysis concerning enzymes supported these observations, highlighting that protease, urease, and beta-glucosidase in Motta soil correlated with HM and NPK, while FDA and DHA were associated with SB 2.8 and 4.2. Catalase (CAT) exhibited a correlation only with the control (CTR) (Figure 8a). In Lazzaro soil, CAT correlated with NPK, HM, and CTR, while protease and beta-glucosidase correlated with SB 1.4, 2.8, and 4.2. FDA did not exhibit a correlation with any treatment, and DHA correlated only with SB 5.6 (Figure 8b). These findings highlighted significant variations in soil enzyme activities, primarily influenced by soil characteristics such as pH, cation exchange capacity (CEC), organic matter content, and microbial biomass, rather than fertilizer type. Differences in correlations with fertilizations were also observed for fungi, bacteria, and actinomycetes (Figure 9a and b). Bacteria in Motta soils were influenced by SB 2.8 and 4.2 (Figure 9a). Fungi correlated with HM, while actinomycetes were linked to NPK (Figure 9 A). In Lazzaro soil, bacteria were influenced by SB 2.8, NPK, and HM, while fungi and actinomycetes remained unaffected by the fertilization treatments (Figure 9b). These PCA results aligned with those for enzymes and confirmed that FDA and DHA, as indicators of soil biological activity, exhibited correlations with bacteria following a similar trend. In summary, the findings revealed that SB had a positive impact on both soils, albeit to varying degrees, influencing different soil properties. The concentrations at which maximum effectiveness was observed were 2.8 for Motta and 4.2 for Lazzaro, underscoring once again the pivotal role of soil characteristics in shaping the decomposition pathway of external substances introduced into the soil.

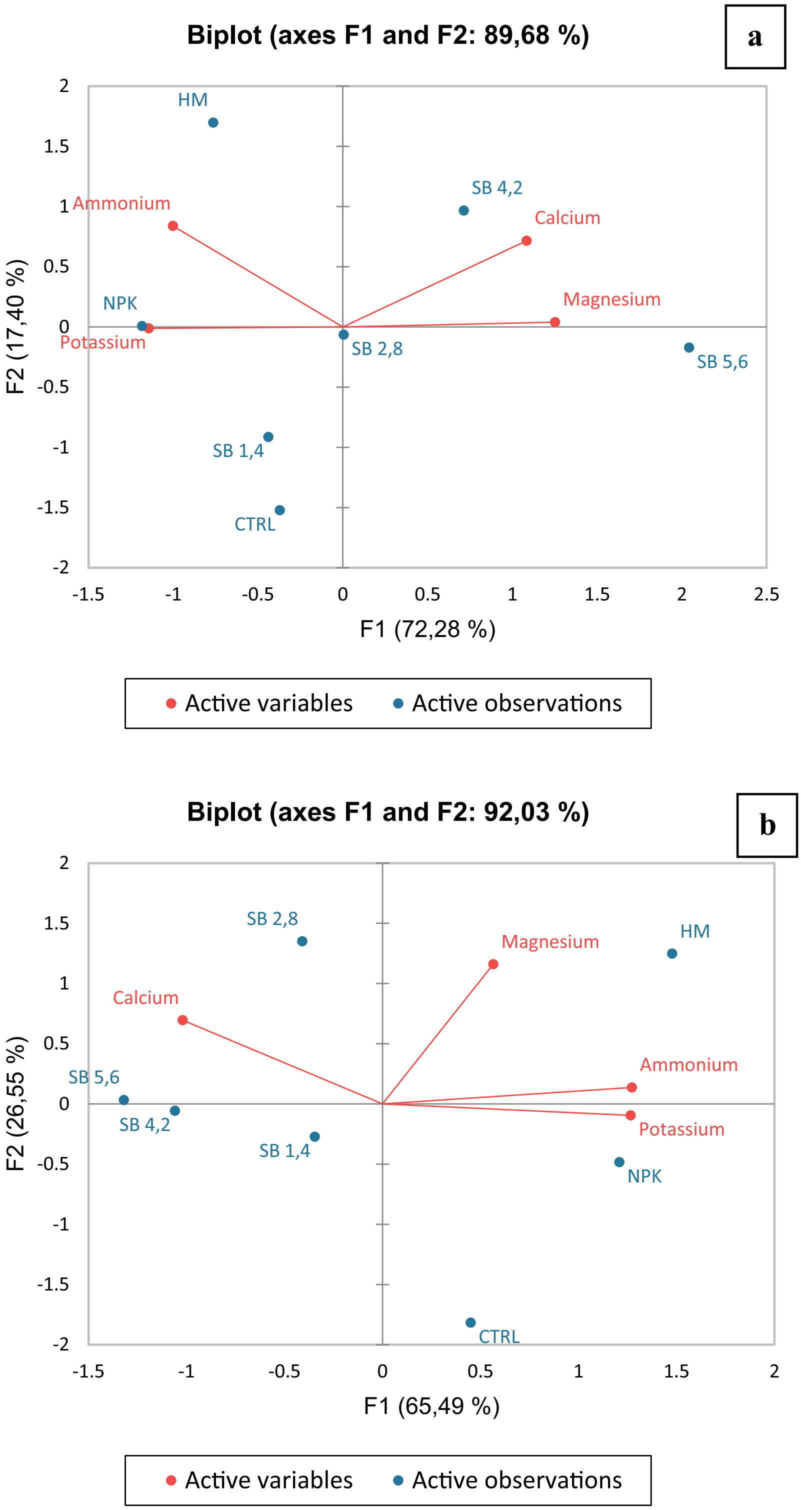
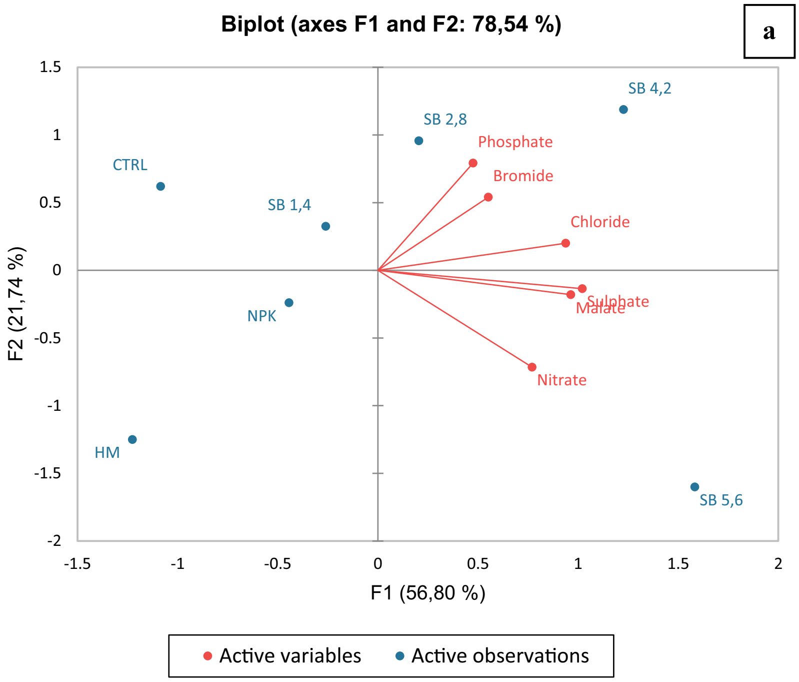


Figure 6. (a, b). PCA (principal component analysis) diagram of cations detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).



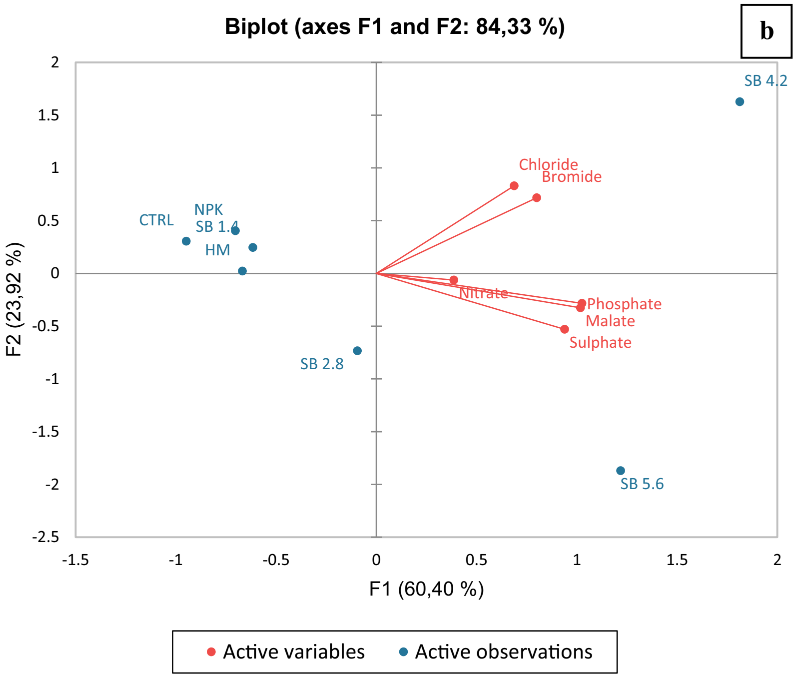


Figure 7. (a, b). PCA (principal component analysis) diagram of anions detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).

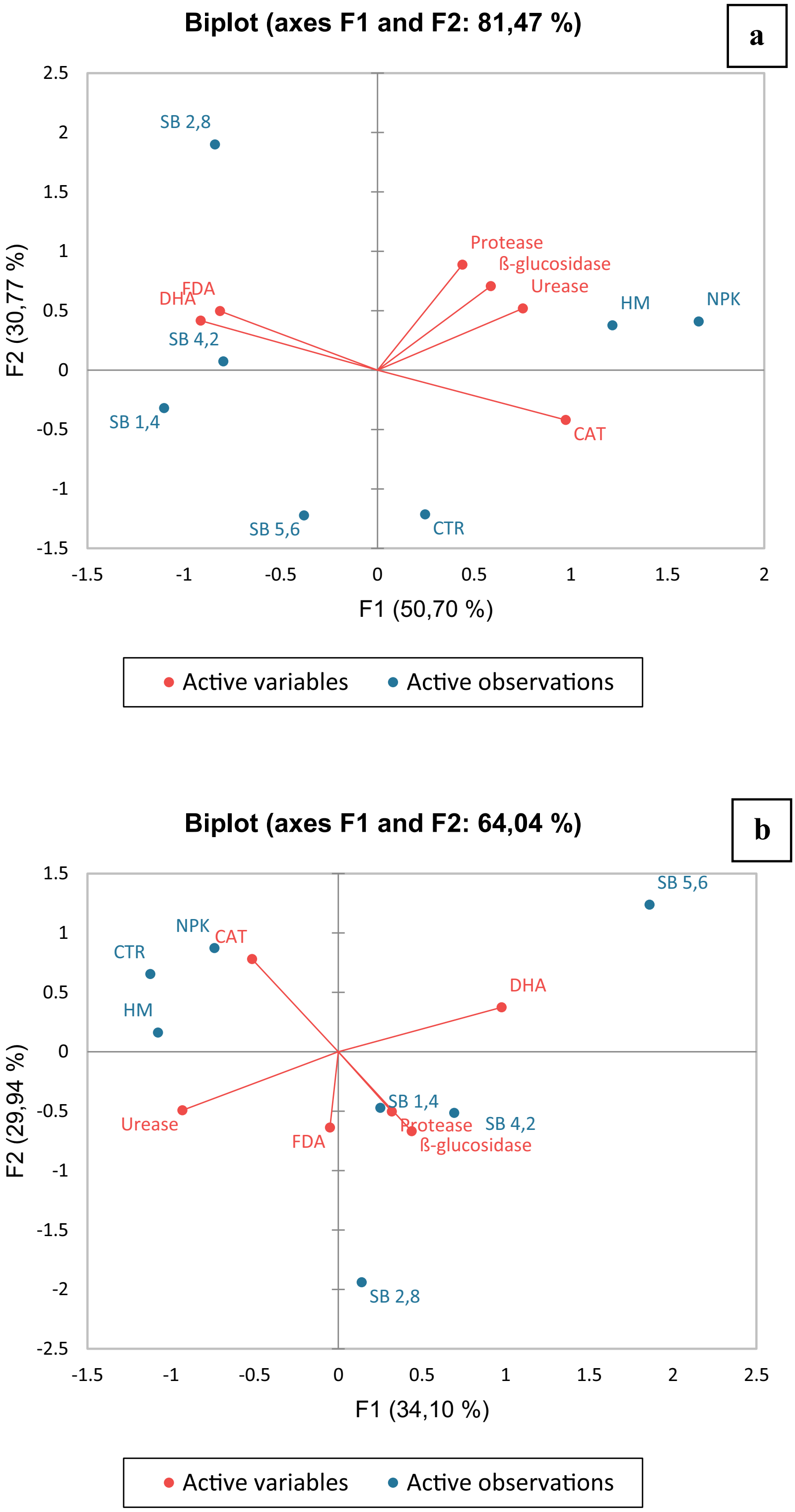
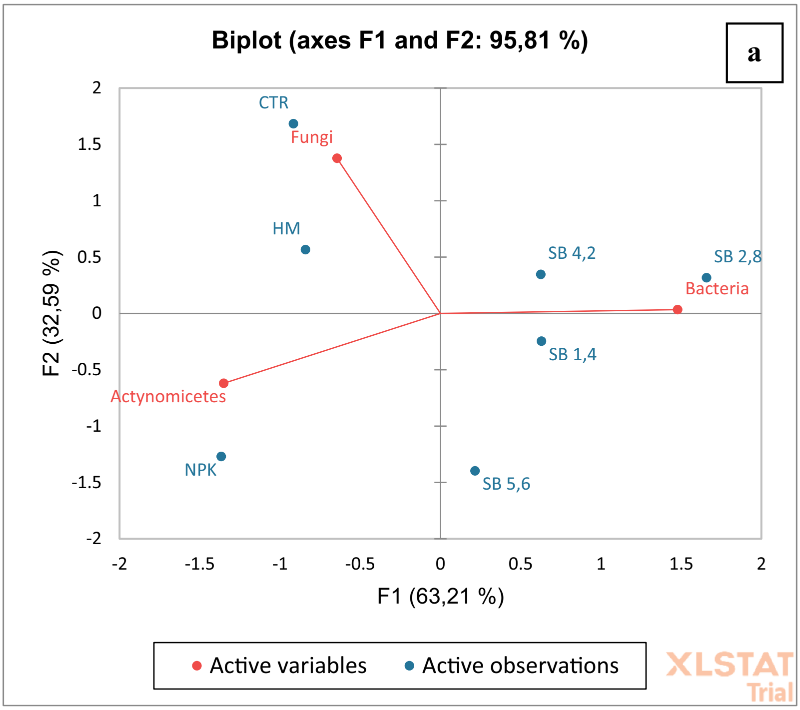


Figure 8. (a, b). PCA (principal component analysis) diagram of enzymes detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK= nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).



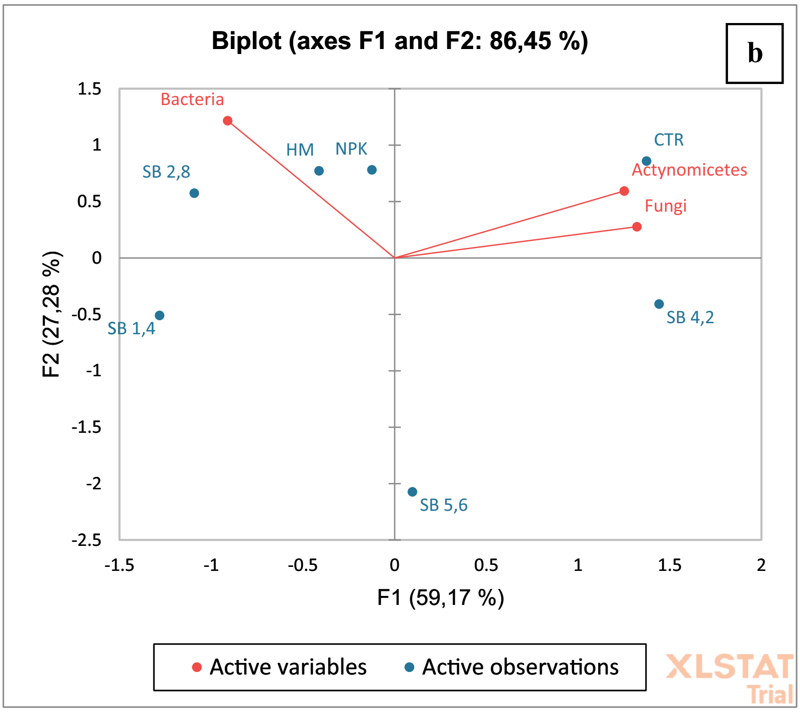


Figure 9. (a, b). PCA (principal component analysis) diagram of bacteria, fungi and actinomycetes found in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).

Conclusions

This study stands as an innovative contribution, shedding light on the pivotal role that soil properties play in shaping the trajectory of fertilization and the long-term efficacy of fertilizers. Within the realm of soil organic matter (SOM), labile organic carbon emerges as a critical component, encompassing microbial biomass carbon (MBC) and enzymes. Among the various soil properties, these components were found to exert the most pronounced influence on fertilizer effectiveness. However, it’s worth noting that the impact of the new fertilizer on the soil ecosystem, although varying in magnitude, consistently yielded positive outcomes in both soil types. In essence, this study underscores the potential benefits of transforming industrial and agricultural waste into fertilizers, offering both economic and environmental advantages by reducing waste disposal costs and lessening the reliance on mineral fertilizers in line with circular economy policies and strategies. The results unequivocally demonstrate an enhancement in soil quality when sulphur-based tablets are employed, surpassing the efficacy of commonly used organic and inorganic fertilizers. This holds particular significance in contemporary agriculture, especially within the organic farming paradigm, where continued dependence on traditional fertilizers is discouraged. In contrast to prior studies, this research accentuates the importance of characterizing soil properties to optimize the efficiency of fertilizer utilization.

Disclosure statement

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ORCID

Muscolo Adele http://orcid.org/0000-0002-0439-1614

Author contributions

All authors discussed the results and commented on the manuscript. Muscolo Adele designed the project, wrote the manuscript, review & editing it. Federica Marra worked in the laboratory, carrying on soil chemical analyses. Francesco Canino and Beatrix Petrovicova worked in the laboratory carrying on the biochemical soil analysis. Mallamaci Carmelo, Greco Carmelo carried on the field experiments.

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