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Article

A Comparative Study of Agroecological Intensification Across Diverse European Agricultural Systems to Assess Soil Structure and Carbon Dynamics

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Abstract: Continuous agricultural activities lead to soil organic carbon (SOC) depletion, and agroecological intensification practices (i.e., reduced soil disturbance and crop diversification) have been suggested as strategies to increase SOC storage. The study aims to assess the effect of agroecological intensification levels (lower (T1) and highest (T2)) on the soil C pool and aggregate stability and validate the correlation between different variables compared to the control (lowest/none (T3), where agroecological intensification was not applied. The C-stock, soil microbial biomass carbon (SMB-C), SOC, water extractable organic carbon (WEOC) in bulk soil, fine and coarse soil aggregates, and water-stable aggregates (WSA) were measured during maximum nutrient uptake in plants under diversified agroecological practices across different environmental conditions (core sites: Italy (CS1), France (CS2), Denmark (CS4), Spain (CS5), Netherlands (CS6), Lithuania (CS7), Turkey (CS8), and Belgium (CS9)). The soil aggregate stability varied among the CSs and treatments. At sites CS7 and CS9, WSA was higher in T1 and T2 compared to the control; a similar trend was observed at other sites, except CS1. SMB-C differed among the core sites, with the lowest value obtained in CS5 (52.3 µg g^{-1}) and the highest in CS6 (455.1 µg g^{-1}). The highest average contents of SOC and WEOC were obtained in bulk soil at CS2 (3.1 % and 0.3 g kg^{-1} respectively). Positive and statistically significant ($p < 0.001$) correlations were detected among all variables tested with SOC in bulk soil and WSA. This study demonstrates the significance of agroecological practices in improving soil carbon stock and optimizing plant–soil–microbe interactions.

Keywords: aggregate stability; agroecological intensification; microbial biomass carbon; soil carbon; water extractable organic carbon

1. Introduction

The interest in soil health is increasing globally due to its active role in sustainable agriculture, enhanced biodiversity, and ecosystem services. As soil biodiversity and resilience are susceptible to anthropogenic disturbances and climate change impacts, implementing effective agricultural management and conservation strategies becomes imperative [1]. The challenges of climate change to agriculture are changing and daunting, requiring innovative solutions. Adopting sustainable agroecological practices is vital for maintaining soil productivity and resilience while preserving key functions like carbon sequestration to support sustainable agriculture and mitigate climate change [2]. Simultaneously, the interactions between plants and soil biota are crucial to regulating soil organic carbon (SOC) dynamics and overall soil health. The diverse pedoclimatic conditions across Europe, combined with varying levels of agricultural intensification, have introduced numerous drivers, such as plant diversity, rhizosphere traits, soil mesofauna, microbial functional diversity, nutrient cycles (C, N, and P), and water availability, that influence critical soil functions in different cropping systems. For example, perennial plant cover and high-input annual crops can yield similar biomass within the same pedoclimatic context [3]. However, the perennial cover has the added benefit of preserving soil organic matter (SOM) and fertility while delivering essential regulating services like water purification and carbon storage. Similarly, incorporating perennial legumes into annual crop rotations offers multiple benefits. It increases the total and labile fractions of water-extractable organic carbon (WEOC) and enhances the potential for organic matter leaching and rapid microbial transformation of dissolved organic materials in soils [4]. In some agricultural settings, high-input annual crops, prevalent in many farming systems, frequently cause soil degradation, nutrient losses, and elevated GHG emissions by disrupting natural plant–soil interactions. Agroecological farming practices have been adopted as a promising pathway to address these challenges. This will encourage the shift in agricultural intensification toward beneficial practices that enhance critical soil functions such as a shift in microbial communities and the breakdown and re-synthesis of SOM. Agroecological practices have positively impacted soil functions, especially the efficiency of plant–soil biota interactions [5]. These practices optimize the synchronization between soil nutrient supply and plant demand, reduce nutrient losses and greenhouse gas emissions (GHG), and enhance carbon sequestration [3]. The agroecological practices include but are not limited to conservation tillage practices, ecological service crops (cover crops, intercropping), diversification strategies (rotations, multi-cropping, agroforestry), and organic inputs (compost, biochar, digestate). These strategies foster beneficial microbial associations, including symbiotic relationships with rhizobia [6]. Amongst the different soil indicators considered in soil health, the description of soil aggregate stability with soil C nutrient cycling is hugely important. Soil aggregate stability is an important physical indicator of soil health, as improvements in aggregate stability are related to reduced resistance against erosion [7]. The relevance of soil aggregate stability in maintaining soil structure remains critical due to the multiple functions played by micro- and macroaggregates [8–10]. The diverse pedoclimatic conditions across Europe, combined with varying levels of agricultural intensification, have introduced numerous drivers, such as plant diversity, rhizosphere traits, soil mesofauna, microbial functional diversity, nutrient cycles (C, N, and P), and water availability, that influence critical soil functions in different cropping systems. For example, perennial plant cover and high-input annual crops can yield similar biomass within the same pedoclimatic context [3]. However, the perennial cover has the added benefit of preserving soil organic matter (SOM) and fertility while delivering essential regulating services like water purification and carbon storage. Similarly, incorporating perennial legumes into annual crop rotations has multiple benefits.

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The impact of agroecological intensification on aggregate stability cannot be overemphasized. For instance, several studies have demonstrated that intensive tillage weakens soil structure and enhances soil erosion [11]. In contrast, no-till as a form of conservative agriculture reduces soil disturbance and strengthens the soil's physical attributes. The benefits have been well studied, with some of the benefits listed as the formation of larger aggregates [11], gradual build-up in soil organic carbon due to a slower rate of crop residue breakdown [12], and better soil productivity [13,14]. Additionally, managing cover crops as part of agroecological intensification levels can offer many advantages for the soil's physical, chemical, and biological characteristics, with improved aggregation forming a crucial part [15,16].

The complex interaction between soil structure and microbiome is crucial in stabilizing SOM. It is acknowledged that soil management practices significantly affect SOC [17,18]. This connection is further emphasized by implementing specific management practices, such as organic material inputs (digestate, livestock manures, composts, biosolids, etc.) [19,20]. SOM content and the stability of soil aggregates, being closely linked and mutually influential, serve as significant indicators of soil quality and degradation [21]. Furthermore, increased organic matter inputs enhance microbial activity and facilitate the synthesis of organic substances that bind soil particles into aggregates but also serve as a nutrient reservoir, promoting the formation of stable aggregates [22].

Considering the interlinked multiple factors affecting soil aggregate formation and distribution, there are questions about how to measure the effects of agroecological intensification on soil carbon cycling across varied EU pedoclimatic conditions. There is a need to identify the relevant and most sensitive drivers that can describe and enhance the productivity of agroecosystems in different cropping systems concerning soil carbon dynamics and aggregate stability. Hence, it was opined that agricultural intensification could shape soil structure and carbon cycling while promoting nutrient supply-plant demand synchrony and SOC persistence in soil. It was hypothesized that reducing soil disturbance and increasing plant diversity improve soil C retainment and soil aggregate stability. Therefore, this study aimed to assess the effect of the agroecological intensification, introduced in different EU agricultural cropping systems, on soil carbon (C) pool and aggregate stability. To achieve this, we tested a gradient of agricultural practices, ranging from the most intensive (intense tillage, monocropping, mineral fertilization) to the most disruptive ones (agroforestry, introduction of ecosystem service plants) in a network of long-term experiment sites representative of several pedoclimatic EU regions.

2. Materials and Methods

2.1. Experimental Design and Treatments

The experiment was carried out in 8 experimental fields/core sites across Europe—6 long-term experiments (LTE) and 2 recently established (Figure 1). The selected sites were representative of two crossed gradients of agroecological intensification: (i) a local-scale gradient and (ii) a European-scale gradient built in the frame of this study (Figure 1).

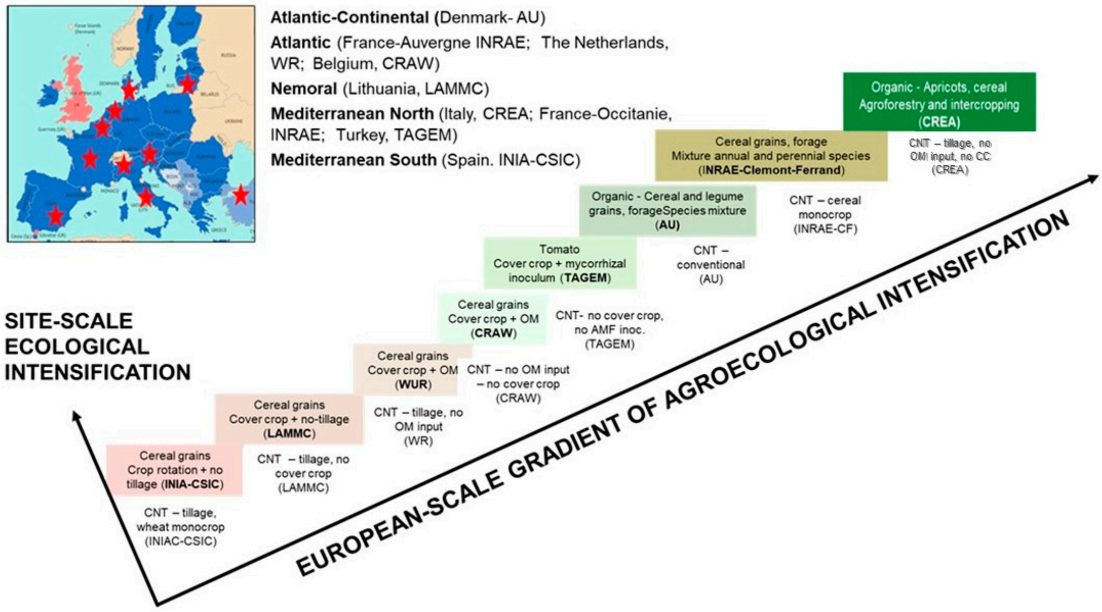

Figure 1. Selected sites are represented as (i) local-scale and (ii) European-scale gradients of agroecological intensification. See Table 1 for the full description of the experimental core sites.

Several criteria were considered for selection: (i) availability of long-term experiments sites—LTEs; (ii) introduction of crop diversity (cover crops, intercropping, agroforestry); (iii) reduction of soil disturbance (reduced tillage, no-tillage); (iv) organic inputs (plant residues, animal manure, composts, bioinoculants). Each sampled site includes a lower agroecological intensification level (T1), the highest agroecological intensification (T2), and the lowest/no agroecological intensification "control" site (T3), where agroecological intensification was not applied (tillage, monocropping, no service crop introduction, no organic inputs), and applied in four field replicates (Table 1), allowing the effect of site-scale ecological intensification on ecosystem variables to be tested.

To limit the immediate effect of management practices on soil sampling, samples were predominantly collected at different time intervals, representing the peak of green biomass (maximum nutrient uptake when soil functions as a C source) at the respective core sites. The soil samples were taken at a 0–20 cm depth range for soil aggregate stability, soil organic carbon (SOC), water-extractable organic carbon (WEOC), and soil microbial biomass C (SMBC). Macroaggregates are essential in organic matter stabilization, C accumulation, sequestration, and macroaggregate formation. Hence, in this study, we focused on the characterization of SOC and WEOC in coarse >1.0 mm (1.0–4.0 mm) and fine (0.25–1.0 mm) soil macroaggregates under different site conditions and agroecological intensification at the European-scale gradients. In all the eight core sites, three treatments (T1, T2, T3) × 4 blocks × 1 replicate/block = 96 soil samples. Each sample was made as a composite sample from 4 sub-samples collected within each plot. To ensure standardization and appropriate quality control measures, soil samples collected at a specified time in any core sites were immediately dispatched after following the standard protocol for each soil analysis to the receiving laboratory for further analysis. Additionally, for individual analysis, such as soil aggregate analysis, the sampling was carried out when the soil was of adequate moisture (normally moist—moisture close to field capacity) at sampling. If the soil is too wet or too dry, the soil structure can be damaged, and it would influence the results of the analysis. For SMB-C, freshly collected field samples were analyzed immediately.

Table 1. Treatment classifications in the experimental core sites.

Core Site	Experimental Location	Experimental Plot Size	pH	Soil Texture	Treatments	YFE	Sampling Time	Code	Fertilization Rate
					INC—compost, no-tillage, spontaneous cover			T1	Municipal waste compost 3% N: 4909 kg ha^{-1}, locally distributed
CS1 (ITALY)	Italy, Rome, 41°79′89″ N 12°57′21″ E	132 m^2	6.9	Sandy clay loam	ICC—compost, tillage, mixed cover crops	2017	April 2023	T2	Municipal waste compost 3% N: 4909 kg ha^{-1} locally distributed
					BAU—tillage, organic fertilizer, no CC (control)			T3	Commercial organic fertilizer 5% N: 3030 kg ha^{-1}, locally distributed
					GLN—grasses, legumes—new system			T1	None
CS2 (FRANCE)	France, Clermont Ferrand, 45°46′27.03″ N 3°8′31.02″ E	490.9 cm^2 (Mesocosms of 25 cm diameter).	6.1	Sandy clay loam	WGLN—spring wheat, grasses, legumes—new system	2016	May 2023	T2	None
					WN—spring wheat—new system (control)			T3	None
					LP + TR—*Lolium perenne* and white clover			T1	N 75, P 24, K 303 kg ha^{-1} y^{-1}
CS4 (DENMARK)	Denmark, Foulum, 56°30′ N, 9°34′ E altitude 45 m a.s.l.	18 m^2	5.8	Sandy loam	MS6—Six-species mixture	New Experiment	July 2022	T2	N 75, P 24, K 303 kg ha^{-1} y^{-1}
					LP—*Lolium perenne* (control)			T3	N 75, P 24, K 303 kg ha^{-1} y^{-1}
		250 m^2			No tillage, monocrop			T1	NPK 15:15:15 at 200 kg ha^{-1} at sowing and 200 kg ha^{-1} of ammonium nitrate 27% in spring.
CS5 (SPAIN)	Spain, Alcalá de Henares, 40°52′34″ N 3°12′42″ W		8.0	Loam	No tillage, rotation	1994	May 2023	T2	NPK 15:15:15 at 200 kg ha^{-1} at sowing and 200 kg ha^{-1} of ammonium nitrate 27% in spring.
					Minimum tillage, monocrop (control)			T3	NPK 15:15:15 at 200 kg ha^{-1} at sowing and 200 kg ha^{-1} of ammonium nitrate 27% in spring.
					VO—Vetch + oat			T1	None
CS6 (NETHERLANDS)	Netherlands, Wageningen, 51°59′41.9″ N, 5°39′17.5″ E	50 m^2	5.3	Loamy sand	VOR—Vetch + oat + radish	2016	April 2023	T2	None
					F—Fallow (control)			T3	None

Table 1. *Cont.*

Core Site	Experimental Location	Experimental Plot Size	pH	Soil Texture	Treatments	YFE	Sampling Time	Code	Fertilization Rate
CS7 (LITHUANIA)	Lithuania, Akademija, Kedainiai distr., 55°39′72″ N 23°86′11″ E	45 m^2	6.7	Loam	NT—No tillage without cover crops	2013	October 2022	T1	N–219; P–72; K–136; S–140
					NT + CC—No tillage + cover crops			T2	N–219; P–72; K–136; S–140
					T—Conventional tillage without cover crops (control)			T3	N–219; P–72; K–136; S–140
CS8 (TURKEY)	Turkey, Sanliurfa; 36°53′12.72″ N–38°55′15.04″ E	57.6 m^2	7.7	Clay	NFC—No fungi inoculum with cover crop	New Experiment	September 2023	T1	109.4 kg ha^{-1}yr^{-1}
					FC—Fungi inoculum with cover crop			T2	109.4 kg ha^{-1}yr^{-1}
					NFNC—No fungi inoculum without cover crop (control)			T3	109.4 kg ha^{-1}yr^{-1}
CS9 (BELGIUM)	Belgium, Gembloux, Latitude = 50.5606556, Longitude = 4.7264556, Altitude = 170 m	48 m^2	6.8	Silt loam	SBWB-FYM—Sugar beet, wheat, barley + farmyard manure	1959	April 2023	T1	Organic fertilization: 10000 kg ha^{-1} yr^{-1} (0.6% N—0.4% P—0.8% K)
					SBWB-CR—SBWB + crop res. restitution and cover crop			T2	Mineral fertilization: N–143; P–0; K–0
					SBWB—SBWB + residue exportation (control)			T3	Mineral fertilization: N—63; P–0; K–0

T1 = lower agroecological intensification, T2 = highest agroecological intensification, T3 = lowest/no agroecological intensification (control), YFE—year of field experiment establishment.

2.2. Soil Sampling and Preparation for the Aggregate Stability Analysis

The analysis procedure for the soil aggregate stability was carried out according to the method described in [23]. Soil sampling was carried out at the maximum crop demand and when the soil was sufficiently moist. Undisturbed soil monoliths were collected by flat shovel, approximately 5 cm × 15 cm (thickness and wideness) from a 0 to 20 cm depth. Immediately after the collection, each soil monolith was gently broken into ~1 cm^3 size soil clods (aggregates) by removing large stones, roots, straw, etc., and left at room temperature for approximately 1 month to air dry before the sieving procedure.

2.2.1. Dry Sieving Process

Air-dried soil samples (200 g) were weighed and sieved by the Retsch AS200 basic (Retsch GmbH, Haan, Germany) sieve shaker with a set of 8000, 5600, 4000, 2000, 1000, 500, and 250 µm mesh sizes. The sieving procedure proceeded for 2 min at the shaking amplitude of 60 rpm. The soil samples from the 1 mm sieve were analyzed for the wet sieving procedure. The aggregates from dry sieving were also used to maintain the SOC and WEOC content analysis with fine macroaggregates (0.25–1.0 mm fractions: from 0.5 mm and 0.25 mm sieves) and coarse macroaggregates (>1.0 mm (1.0–4.0 mm) fractions: from 2.0 mm and 1.0 mm sieves).

2.2.2. Wet Sieving Procedure by Ejkelkamp Apparatus

A 4 g sample of each 1.0–2.0 mm soil fraction (from 1 mm sieve) sample was weighed, placed on numbered 0.25 mm sieves, moistened with distilled water by hand fog-sprayer, and allowed to become adequately wet for about 15 min. After that, the samples with sieves were placed in the Ejkelkamp wet sieving apparatus (Eijkelkamp Soil & Water, Zevenaar, The Netherlands) apparatus, with numbered holes, and the cylinder was placed below the sieves according to numbers. A measurement of 100 mL of distilled water was added to each cylinder, the sieves with samples were immersed down to the cylinders with distilled water, and the apparatus was turned on for 3 min. at intervals for the sieving procedure. The non-stable aggregates are separated by this procedure and remain in the cylinders with distilled water. The sieves with water-stable soil aggregates were placed on the side of the apparatus hole and allowed to drain. Furthermore, an alkalic solution of 2 g sodium hexametaphosphate $(NaPO_3)_6/1$ L distilled water was prepared to separate the water-stable aggregates. New, numbered cylinders filled with 100 mL prepared solution were placed below the sieves according to the above-mentioned procedure and the sieving process for about 0.5–3 h, depending on the soil type. The sieving procedure lasts until there is no soil left, leaving only garbage and pebbles. After the sieving, all the cylinders are oven-dried at 110 °C for about 17–24 h (including an extra sample to control 100 mL alkalic solution without the soil). Before weighing, the cylinders are placed in an excicator or left in an oven to cool down. WSA was calculated with Equation (1).

$$\text{WSA}(\%) = \frac{(\text{WSA(g)}–\text{AC(g)})}{(\text{NSA(g)} + \text{WSA(g)}–\text{AC(g)})} * 100 \quad (1)$$

where WSA refers to water stable aggregates (g), NSA indicates non-stable aggregates (g), and AC is the alkalic control (g).

2.3. Soil Microbial Biomass Carbon Determination

The fumigation-extraction method was used to determine the soil's microbial biomass carbon from the soil samples (sieved with 2 mm mesh) that were collected from all the core sites at a depth of 0–20 cm [24]. A 20 g sample of the sieved soil was measured and fumigated by exposing the soil to the alcohol-free chloroform ($CHCl_3$) vapor in a sealed vacuum desiccator for 24 h. The fumigated soil was evacuated repeatedly in a clean, empty desiccator until the odor of chloroform ($CHCl_3$) was no longer detected and then further extracted with 0.5 M K_2SO_4 (soil (20 g): K_2SO_4 (80 mL) in a ratio 1:4) for 30 min by oscillating shaking at 200 rpm and then filtered through a Whatman No. 42 filter paper. The same procedure was applied to sieved soil samples (20 g) without exposure to alcohol-free chloroform to obtain unfumigated soil extracts.

Organic carbon content in the extracted samples was subsequently determined using the dichromate digestion method. Two mL of potassium dichromate ($K_2Cr_2O_7$ (66.7 mM) and 15 mL of the digestion mixture (2:1 conc. H_2SO_4:H_3PO_4 (*v*/*v*) was added to 8 mL of extract in a 250 mL conical flask. The mixture was gently refluxed for 30 min, cooled, and diluted with 20 mL distilled water. The excess $K_2Cr_2O_7$ was measured by back titration with ferrous ammonium sulfate solution (40.0 mM) using a 1.10-phenanthroline-ferrous sulfate complex $[Fe(C_{12}H_8N_2)_3]SO_4$ (25 mM) solution as an indicator. SMB-C was calculated from the differences in extractable organic carbon between the fumigated and non-fumigated soil samples with a conversion factor (KEC) of 0.38 [24].

2.4. SOC, WEOC, and C-Stock Concentrations in Fine Macroaggregates (0.25–1.0 mm) and Coarse Macroaggregates (>1.0 mm) of Soil

The SOC content in bulk soil and soil aggregate fractions was analyzed according to the Nikitin-modified Tyurin dichromate oxidation method using wet combustion at 160 °C. SOC measurement was performed using an automatic spectrophotometer Carry 50 at a wavelength of 590 nm, with glucose as a standard [25]. The WEOC determination was performed according to the methodology guided by SKALAR, using $C_8H_5KO_4$ as a standard. The soil prepared for the chemical analyses was dispensed with distilled water at a ratio of 1:5, and the extract was prepared by shaking, centrifugating for 15 min at 4500 rpm, and filtration. After that, the automated measurement procedure was performed based on the IR detection method following UV-catalyzed persulfate oxidation under a nitrogen environment (SKALAR, The Netherlands). Each soil sample was analyzed in triplicate after dry sieving with a 1 mm mesh size sieve, and the mean value was calculated.

For calculating the C-stock, the soil bulk density was determined by estimating the Soil volume weight (SVW). The sieved soil (<2 mm) was used to measure the SVW and then calculated as the ratio of the oven-dried (105 °C) soil mass to a known volume. A coarser fraction (stones, gravels) was weighed to derive bulk density.

2.5. Statistical Analysis

The observed data were statistically processed using R Studio 4.3.2 software [26]. The Shapiro–Wilk test for normality and Levene's test for homogeneity of variance were applied to each indicator separately for each core site. Based on these tests, indicators meeting both assumptions in a given CS were analyzed using ANOVA, followed by Tukey's HSD for post hoc comparisons. For indicators in CSs where normality or homogeneity assumptions were not met, Kruskal–Wallis tests were used, followed by Dunn's test for post hoc analysis. Pearson's correlation analysis was used to analyze the relationship between the C-stock, SMB-C, WSA, SOC, and WEOC data.

3. Results

3.1. Water-Stable Aggregates

The data distribution in the core sites was closely related to the European-scale gradient of agroecological intensification. The percentage of water-stable aggregates varied across the experimental sites and treatments (Figure 2). The lowest WSA value was determined at CS5 (Spain), while higher WSA values were observed at CS2 (France), CS1 (Italy), and CS4 (Denmark), respectively. Significant differences ($p < 0.05$) were found within the CS7 (Lithuania) and CS9 (Belgium) treatments, with the lowest WSA values in the control group in both treatments. In contrast, the WSA value at the CS1 (Italy, organically managed system) site was greater in the control treatment, where minimum tillage (0–10 cm) was applied, compared to T1 and T2 (no-tillage). Overall, significant differences were found between the level of agroecological intensification and the control at the following core sites: CS7 (Lithuania)—T1 = T2 > T3; CS9 (Belgium)—T2 and T3; and CS1 (Italy)—T1 = T2 < T3.

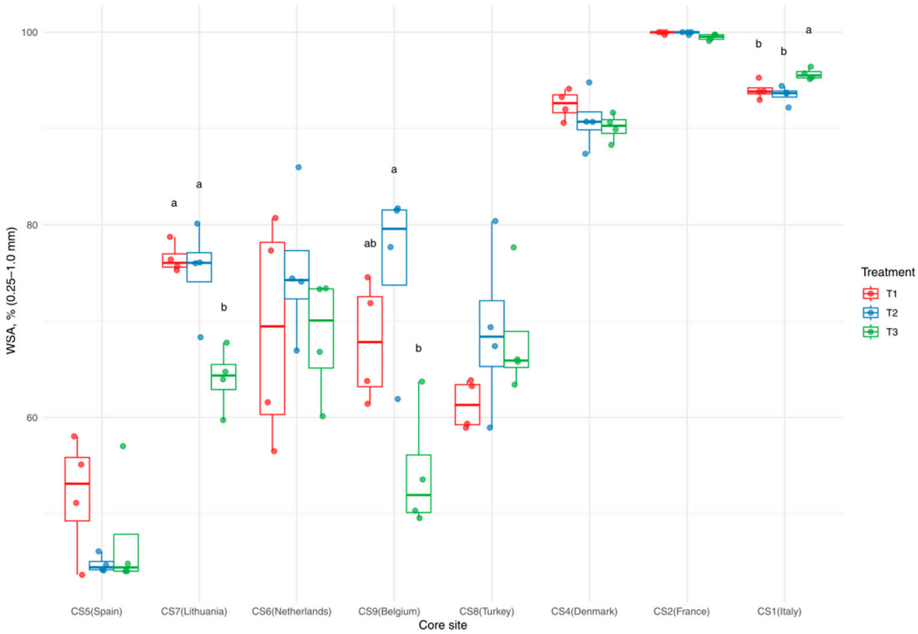

Figure 2. Amount of water stable aggregates (WSA) (%) within 0.25–1.0 mm soil fractions under diversified agroecological practices and different environmental conditions in tested sites. The sites are arranged according to European-scale gradients of ecological intensification (Figure 1). Each box plot represents the distribution of four replicates, showing the median, interquartile range, and data range (whiskers). The compact letters shown above each box plot indicate significant differences between treatments in each core site ($p < 0.05$) based on Tukey's HSD test or Dunn's test.

3.2. Soil Carbon Stock

The highest amount of C-stock in bulk soil (on average 49.6 t ha^{-1}) was observed at CS1 (Italy), followed by C-stock values in CS4 (Denmark) and CS6 (Netherlands) (Figure 3). The lowest C-stock (on average 27.3 t ha^{-1}) was found at site CS7 (Lithuania). Additionally, low carbon stocks of 27.7 t ha^{-1}, 31.3 t ha^{-1}, and 31.1 t ha^{-1} were found in CS5 (Spain), CS8 (Turkey), and CS9 (Belgium), respectively. Significant differences ($p < 0.05$) were found among the treatments in CS2 (T1 = T3 < T2) and CS9 (T1 = T2 > T3).

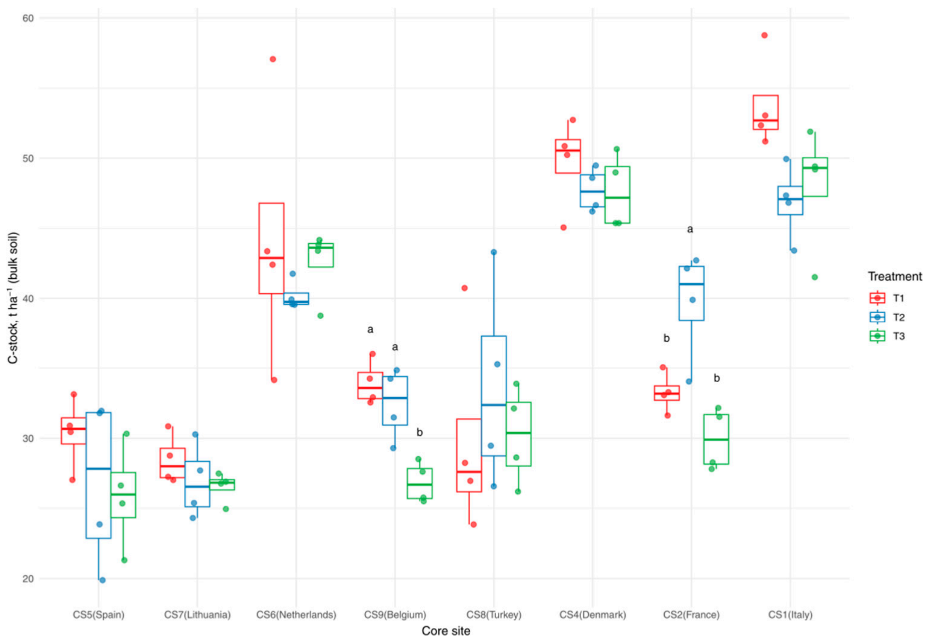

Figure 3. The C-stock (t ha^{-1}) in bulk soil, under diversified agroecological practices and different environmental conditions at experimental sites. The sites are arranged according to European-scale gradients of ecological intensification (Figure 1). Each box plot represents the distribution of four replicates, showing the median, interquartile range, and data range (whiskers). The compact letters shown above each box plot indicate significant differences between treatments in each core site ($p < 0.05$) based on Tukey's HSD test or Dunn's test.

3.3. Soil Microbial Biomass Carbon

The SMB-C, as shown in Figure 4, showed higher SMB-C values in CS6 (Netherlands), CS2 (France), and CS1 (Italy). The lowest SMB-C mean value (52.3 µg g^{-1}) was found in CS5 (Spain) under T3 while the highest SMB-C value reached 455.1 µg g^{-1} as observed in CS6 under T2. The study demonstrated the impact of diversified systems, especially when incorporating cover crops and external organic matter (farm manure, crop residue) on soil microbial biomass. The levels of agroecological intensification on SMB-C were evident, as treatments in CS4 T1 (Denmark) and CS9 T2 (Belgium) showed statistically significant differences ($p < 0.05$) compared to other treatments within their respective core sites. For CS4, the highest average SMB-C value (259.6 µg g^{-1}) was found in the T2 treatment with a six-species mixture. For CS9, the highest mean SMB-C value was 174.4 µg g^{-1} in the T2 treatment, having crop rotation of sugar beet, wheat, and barley incorporated with crop restitution and cover crop.

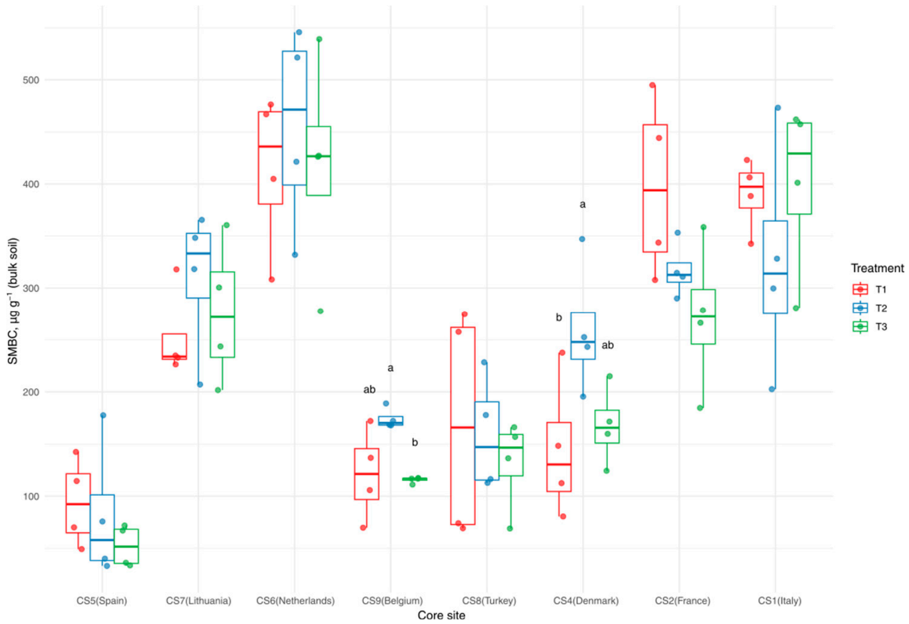

Figure 4. The soil microbial biomass carbon (SMB-C) (μg g^{-1}) in bulk soil, under diversified agroecological practices and different environmental conditions at experimental sites. The sites are arranged according to European-scale gradients of ecological intensification (Figure 1). Each box plot represents the distribution of four replicates, showing the median, interquartile range, and data range (whiskers). The compact letters shown above each box plot indicate significant differences between treatments in each core site ($p < 0.05$) based on Tukey's HSD test or Dunn's test.

3.4. Water-Extractable Organic Carbon in Bulk Soil and Aggregate Fractions

The WEOC in the bulk soil and its fractions (fine macroaggregates (0.25–1.0 mm) and coarse macroaggregates (>1.0 mm) of soil), showed variations across the scale of ecological intensification (Figure 5), highlighting the dynamics of labile soil carbon and the spatial variability of these properties. This variation underscores the instability, or lability, of soil WEOC.

The WEOC represents the smallest fraction of SOC as it is the most mobile and the quickest to mineralize. In the soil of the experimental areas, higher contents of average WEOC in the bulk soil were found in CS2 (France)—0.3 g kg^{-1} and CS1 (Italy)—0.3 g kg^{-1}, with the lowest average WEOC values found in CS4 (Denmark) and CS8 (Turkey). CS2, CS7 (Lithuania), and CS9 (Belgium) significantly differed between the treatments when considering the WEOC in bulk soil. For CS2, T1 was significantly different from T3 (control) and T2, while in CS7, T2 was significantly different from T3. Furthermore, in CS9, T1 and T2 are significantly different ($p < 0.05$) from T3. A deeper look into the fine (0.25–1.0 mm) and coarse macroaggregates (>1.0 mm) revealed a similar trend, except for the higher WEOC average values found in CS4 and CS8. In all the core sites considered, only CS2 (France) showed a difference statistically in fine macroaggregates, and coarse macroaggregates when considering the effects of agricultural practices between the treatments, with T1 significantly higher than T3. Furthermore, the WEOC fractions offered similar trends of lower levels in the organic matter pool in CS7, CS9, and CS6 (Netherlands). The benefits of cover crops on SMB-C were evident, as treatments with cover crops (T1 and T2) in CS4 and CS9 showed statistically significant differences ($p < 0.05$) compared to other treatments within their respective core sites.

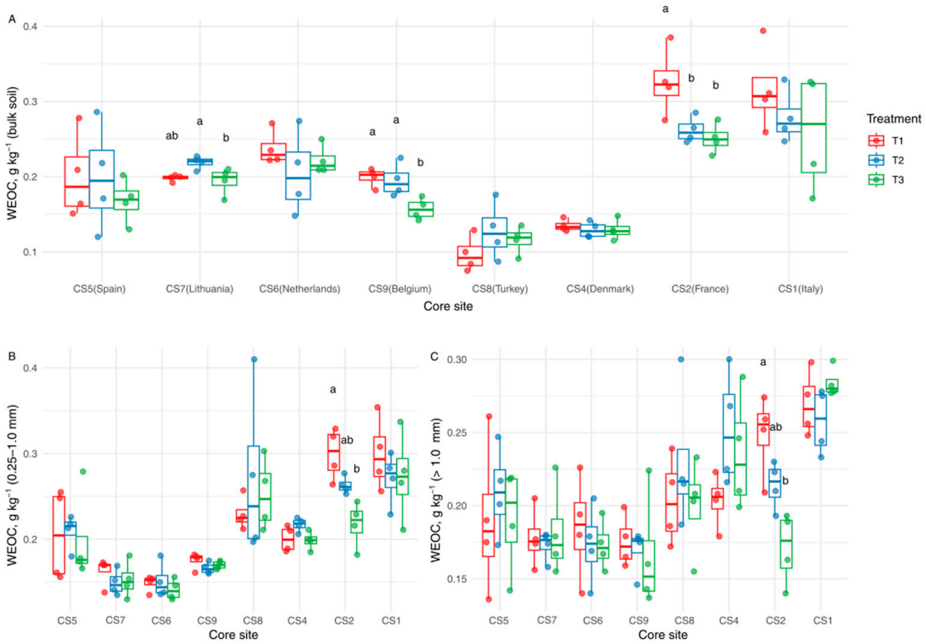

Figure 5. (**A**) The water-extractable organic carbon (WEOC), g kg^{-1} in bulk soil, (**B**) fine (0.25–1.0 mm), and (**C**) coarse (>1.0 mm) macroaggregates, under diversified agroecological practices and different environmental conditions at experimental sites. The sites are arranged according to European-scale gradients of ecological intensification (Figure 1). Each box plot represents the distribution of four replicates, showing the median, interquartile range, and data range (whiskers). The compact letters shown above each box plot indicate significant differences between treatments in each core site ($p < 0.05$) based on Tukey's HSD test or Dunn's test.

3.5. SOC in Bulk Soil, 0.25–1.0 mm, and >1.0 mm Soil Fractions

The mean soil SOC differed among sites at all analyzed fractions (Figure 6) following the decreasing order concerning SOC bulk soil (CS2 France > CS1 Italy > CS4 Denmark); SOC >1.0 mm (CS6 Netherlands > CS7 Lithuania > CS2 France); SOC 0.25–1.0 mm (CS2 France > CS1 Italy > CS8 Turkey). With respect to SOC in the bulk soil, significant differences were observed among the treatments in CS9, CS2, and CS1. In CS9, T1 and T2 treatments and, in CS2, T1 treatment were significantly different ($p < 0.05$) from T3 treatments. In CS1, a significant difference ($p < 0.05$) was observed between T1 and T2 treatments. Furthermore, in the SOC fraction in 0.25–1.0 mm, significant differences were found in CS2, CS5, and CS9 from the T1 and T3 treatments in the three core sites. Additionally, when considering >1.0 mm SOC fraction, CS9 was the only core site showing a statistically significant difference ($p < 0.05$) with higher values in T1 and T2 compared to T3.

3.6. Ratio Between SMB-C, SOC, and WEOC

The soil microbial quotient (SMQ), which is the ratio of SMB-C and SOC and the WEOC to SOC varied across the respective core sites, with the influence of agroecological intensification levels significant in selective sites (Table 2). The SMQ was significantly different ($p < 0.05$) in T1 and T2 treatments in CS4 (Denmark) and CS9 (Belgium), respectively (Table 2). For the the ratio of WEOC: SOC ratio in bulk soil, there were no significant differences observed among all the treatments in the core sites; however, the highest ratio quotient was found in CS5 (Spain) (Table 2). Additionally, WEOC to SOC in the soil fractions considered fine macroaggregates (0.25–1.0 mm) and coarse macroaggregates (>1.0 mm) exhibited variation depending on the level of agroecological intensification (Table 2). For both soil fractions, the ratio of WEOC:SOC was highest in CS5 (Spain). The WEOC:SOC ratio in the coarse macroaggregates showed a significant difference ($p < 0.05$) between T1 and T2 treatments in CS4 (Table 2).

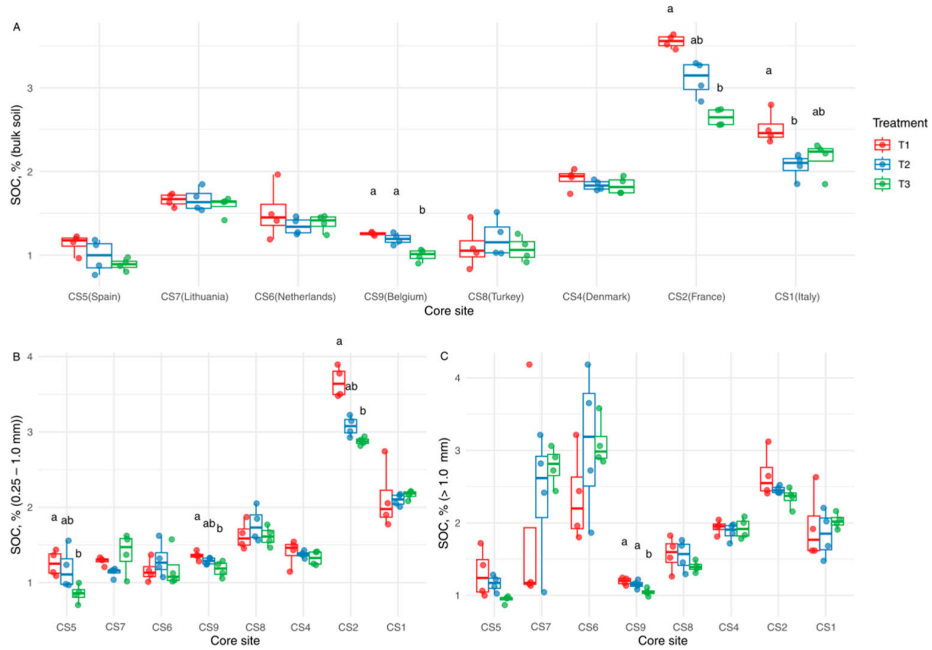

Figure 6. (**A**) SOC, % in bulk soil, (**B**) (0.25–1.0 mm) and (**C**) (>1.0 mm), under diversified agroecological practices and different environmental conditions at experimental sites. The sites are arranged according to European-scale gradients of ecological intensification (Figure 1). Each box plot represents the distribution of four replicates, showing the median, interquartile range, and data range (whiskers). The compact letters shown above each box plot indicate significant differences between treatments in each core site ($p < 0.05$) based on Tukey's HSD test or Dunn's test.

Table 2. The ratio of labile carbon to soil organic carbon.

Core Sites	Treatments	MeanS MQ (Bulk), %	Mean WEOC:SOC (Bulk), %	Mean WEOC:SOC (0.25–1 mm)	Mean WEOC:SOC (> 1 mm)
CS1 (ITALY)	T1	1.56 ± 0.12	1.25 ± 0.08	1.43 ± 0.07	1.43 ± 0.13
	T2	1.56 ± 0.22	1.36 ± 0.08	1.29 ± 0.07	1.42 ± 0.12
	T3	1.84 ± 0.13	1.20 ± 0.17	1.26 ± 0.12	1.40 ± 0.04
CS2 (FRANCE)	T1	1.12 ± 0.11	0.92 ± 0.70	0.82 ± 0.02	0.95 ± 0.09
	T2	1.03 ± 0.07	0.84 ± 0.02	0.86 ± 0.02	0.87 ± 0.04
	T3	1.03 ± 0.14	0.95 ± 0.14	0.76 ± 0.05	0.73 ± 0.07
CS4 (DENMARK)	T1	0.75 ± 0.16 b	0.71 ± 0.03	1.45 ± 0.15	1.05 ± 0.03 b
	T2	1.41 ± 0.16 a	0.70 ± 0.03	1.58 ± 0.05	1.34 ± 0.08 a
	T3	0.91 ± 0.09 ab	0.71 ± 0.02	1.51 ± 0.09	1.22 ± 0.09 ab
CS5 (SPAIN)	T1	0.83 ± 0.17	1.78 ± 0.26	1.65 ± 0.24	1.60 ± 0.37
	T2	0.78 ± 0.27	1.98 ± 0.21	1.82 ± 0.19	1.81 ± 0.13
	T3	0.58 ± 0.10	1.91 ± 0.23	2.32 ± 0.20	2.04 ± 0.22
CS6 (NETHERLANDS)	T1	2.90 ± 0.51	1.64 ± 0.24	1.30± 0.10	0.81 ± 0.10
	T2	3.42 ± 0.48	1.54 ± 0.26	1.17 ± 0.03	0.59 ± 0.07
	T3	3.02 ± 0.37	1.61 ± 0.09	1.22 ± 0.10	0.56 ± 0.02
CS7 (LITHUANIA)	T1	1.52 ± 0.11	1.20 ± 0.02	1.26 ± 0.04	1.22 ± 0.24
	T2	1.86 ± 0.21	1.32 ± 0.07	1.32 ± 0.06	0.88 ± 0.26
	T3	1.75 ± 0.23	1.22 ± 0.02	1.11 ± 0.07	0.67 ± 0.09
CS9 (BELGIUM)	T1	0.96 ± 0.17 b	1.59 ± 0.05	1.29 ± 0.02	1.47 ± 0.05
	T2	1.47 ± 0.08 a	1.64 ± 0.10	1.29 ± 0.03	1.47 ± 0.07
	T3	1.16 ± 0.05 ab	1.58 ± 0.12	1.45 ± 0.08	1.59 ± 0.19

T1 = lower agroecological intensification, T2 = highest agroecological intensification, T3 = lowest/no agroecological intensification (control), SMQ—soil microbial quotient, WEOC—water extractable organic carbon, SOC—soil organic carbon; CS—core site; The columns with different letters show the significant differences within the treatments in each core site.

3.7. The Relationship Between SMB-C, WSA, SOC, and WEOC

Pearson's correlation analysis showed a positive correlation between the WSA, and all C pool sources tested (SMB-C, C-stock in bulk soil, SOC, and WEOC in 0.25–1.0 mm and >1.0 mm soil aggregates) (Figure 7). C-stock in bulk soil positively correlated with SMB-C, SOC in bulk soil, and SOC in > 1.0 mm and WEOC in bulk soil, 0.25–1.0 mm, and >1.0 mm soil aggregates, respectively. Soil microbial biomass carbon also correlated with SOC in bulk soil and fractions and WEOC in bulk soil. SOC in >1.0 mm soil aggregates showed a correlation with SOC in bulk soil and 0.25–1.0 mm soil aggregates, and WEOC in bulk soil. WEOC in >1.0 mm soil aggregates correlated with WSA, C-stock in bulk soil, SOC in bulk soil, and in the 0.25–1.0 mm soil aggregates of WEOC and SOC.

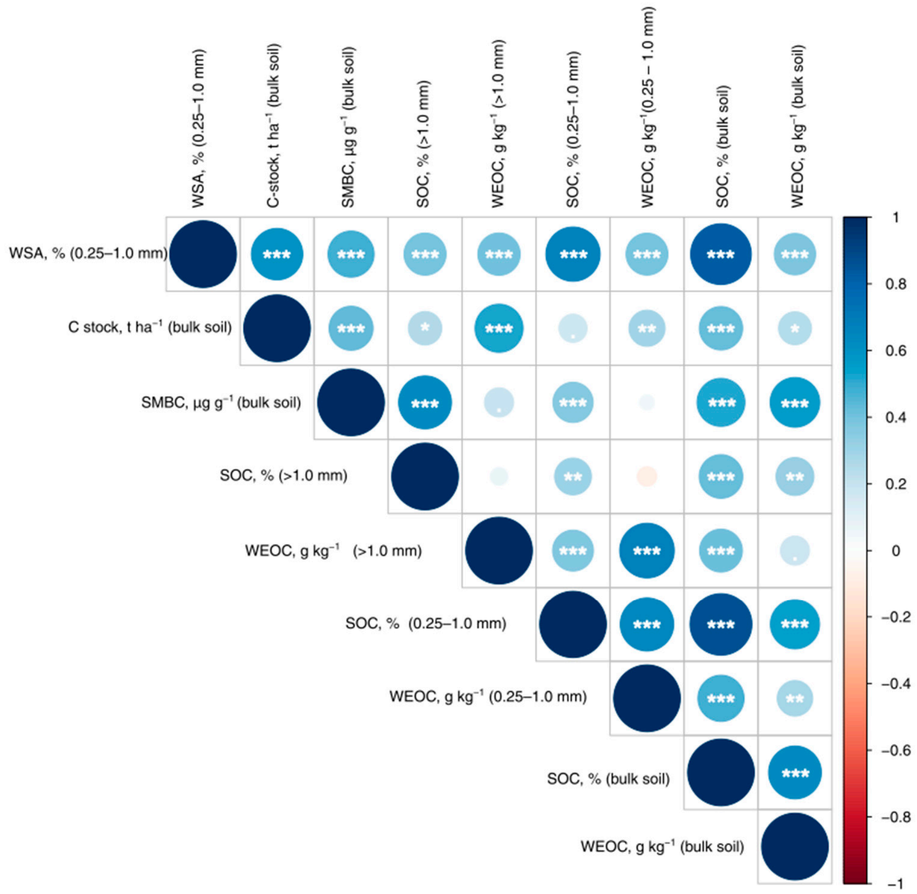

Figure 7. Pearson correlation analysis between the WSA, SOC, WEOC, C-stock, and SMB-C data. Significance codes: · $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

Soil health is assessed by examining its physical, chemical, and biological properties, which are highly influenced by agricultural practices with the attendant effects on soil functions. These soil functions are impacted by varying levels of agricultural intensification with different interplays in critical functions such as soil aggregate stability [27,28], carbon dynamics [29,30], and microbial interactions [31]. Agricultural practices that reduce soil disturbance and enhance organic matter inputs, such as those deployed at the experimental sites, contribute to soil carbon pools, support better soil structure, and increase soil carbon sequestration [32,33]. Equally, intensive practices that disrupt soil aggregates can lead to reduced soil stability and potentially hinder the long-term sustainability of agricultural systems [34,35]. These findings provide valuable awareness of the need for site-specific strategies that balance agricultural productivity in terms of SOC stability using agricultural intensification levels to sustain soil ecosystem services. Long-term field trials incorporating various soil health management practices were conducted to measure sensitive biological and physical soil parameters under diverse agroecological practices across different pedoclimatic conditions. These parameters included water-stable aggregates (0.25–1.0 mm WSA), carbon stock in bulk soil, SMB-C, SOC, and WEOC in both bulk soil and different soil aggregates (fine: 0.25–1.0 mm and coarse: >1.0 mm). In furtherance of these studies, several criteria were considered for selection: (i) introduction of crop diversity (cover crops, intercropping, agroforestry); (ii) reduction in soil disturbance (reduced tillage, no-tillage); (iii) organic inputs (plant residues, animal manure, composts, bioinoculants).

Generally, various agricultural practices are being employed as restorative management strategies to enhance soil fertility and increase soil carbon storage. However, understanding the integrated effects of these intensification levels on agricultural productivity becomes necessary with the increased interest in sustainable farming practices such as legumes, crop rotation, cover cropping, and no-till. Crop diversity in the study ranged from cereal–legume–grasses, which were classified as annual and perennial. The timing of the sampling periods, corresponding to maximum plant nutrient demand, varied across core sites and depended on pedoclimatic conditions and cover crop species. We define the minimum plant demand (MIN) as the period when the soil acts as a carbon sink just before cover crop sowing [3]. Conversely, the maximum plant demand (MAX) is when the soil functions as a carbon source. This happens at the full flowering stage of the cover crop, just before its termination. The tested ecological intensification context assessed various soil health practices simultaneously to identify the combination of practices that improve soil properties. The study revealed significant variability across sites with different soil health practices, highlighting the impact of varying levels of agricultural intensification. This aligns with an earlier study of long-term experiments across Europe and China, which reported that agricultural management practices significantly influenced soil quality indicators, with organic matter inputs positively affecting all measured indicators, most notably earthworm numbers, yield, SOM content, and soil aggregate stability, while pH effects varied by soil type [36]. The differences in soil physical metrics reflect the European-scale gradient of agroecological practices and underscore management intensity's critical role and sensitivity in shaping soil structure and stability. Specifically, the significantly average values of soil WSA and SMB-C were mainly related to conservative and sustainable practices such as no-tillage, legume incorporation, cover cropping, and external organic matter input (manure), which tend to promote soil structure. Manure, when applied to soil, is degraded by microbes, producing natural cement that enhances the formation of macroaggregates and large aggregates [37]. Similarly, the practice of cover crops and no-tillage highly induced changes in soil aggregate stability, infiltration rates, and microbial indicators over three years [38].

Furthermore, the marked differences in WSA were distinct in sites incorporating cover crops and manure (CS1 (Italy), CS7 (Lithuania), and CS9 (Belgium)), highlighting the effectiveness of these specific intensification levels in improving soil stability. Interestingly, at the organically managed site in Italy, the WSA value was more significant in the control treatment with minimum tillage than the no-tillage treatments (T1 and T2). This predicates that while reduced tillage has benefits, an optimal approach for maintaining soil structure may involve balancing tillage intensity with the input and management of external organic materials [39–41]. In contrast, the CS5 (Spain) site in the Mediterranean South climatic zone had lower WSA, SMBC, and carbon stock with no-tillage and crop rotation systems deployed. The results contradict the earlier claim that SOC stocks and aggregate stability can be improved by increasing residue inputs and reducing soil disturbance; practices typically associated with conservation management [42,43]. A more detailed explanation for the waning critical soil parameters in CS5 (Spain), in relation to SOC, may be attributed to the region's climatic conditions (Mediterranean climate). Rising temperatures from climate change negatively impact SOC storage by accelerating the decomposition of organic matter in the soil [44,45]. The gradual rise in temperature can thus be ascribed as the dominant factor behind the decline in SOC when suitable agricultural intensification levels are not deployed. This scenario highlights the negative impact of climate change in the Mediterranean region, where SOC levels are alarmingly low [46]. In contrast, core sites such as CS2 (France), CS4 (Denmark), and CS1 (Italy) with favorable climatic conditions, coupled with agricultural practices such as conservation tillage, agroforestry, crop rotation, cover crops, and those incorporating organic matter, promote greater soil stability and a tendency for enhanced SOC [42,47,48].

The multidimensional benefits of incorporating cover crops with manure were more pronounced as the fractions of labile SOC were higher in such core sites. Doyeni et al. [14] reported lower indices in soil physical properties (soil volumetric water content, electrical conductivity, and bulk density) in conventional tillage without other complementary management practices over a long-term period. Specifically, cover cropping positively impacted soil biological indicators, with treatments involving cover crops increasing microbial community abundance as characterized by increased SMB-C [49,50]. Cover cropping provides above- and below-ground plant biomass and root exudates that boost soil microbial growth and prevent rich topsoil from eroding [51]. These findings align with previous research, including meta-analyses, which have documented increases in soil carbon, microbial biomass, and organic matter dynamics following cover cropping [52,53]. Enhanced soil microbial activity under higher SOM triggered by cover cropping was reported in the literature [54]. Additionally, our results revealed significantly higher average carbon stock and SOC levels under cover cropping (Figures 3 and 6). This is consistent with previous studies on the positive impact of cover crops on SOC increase [53,55,56]. However, the size of its effect varies among the agroecological sites with the highest mean SOC values in CS2 (France) and CS1 (Italy). Site-specific factors like climate, soil types, cover crop type, root system, and agronomic management practices (e.g., crop management, fertilization, and resulting biomass production) are essential factors behind such a variable response to cover cropping. For example, agroecological intensification levels showed a significant increase in carbon stock and SOC at CS2 (France) with grasses and legumes incorporation and CS9 (Belgium) with crop rotation, organic fertilizers, and cover crops (Figures 3 and 6).

Soil labile organic carbon is the most active component of SOC. It consists of various forms of readily bioavailable organic carbon, such as WEOC, SMB-C, dissolved organic carbon, and other organic carbon fractions. SMB-C, which indicates how microbes respond to anthropogenic influence, tends to be sustained and enhanced with intensification levels that tilt toward conservative practices. Experimental sites with a higher intensity of combined cover crops and compost practices had enhanced SOC. Enhanced soil aggregate stability, increased organic matter, better nutrient distribution within WSA, and moisture conservation are some benefits that are more related to agroecological practices [37,57]. The active SOC fractions were more sensitive to the different soil management, with varying impacts on the active SOC. Applying and incorporating organic materials tend to increase soil organic carbon levels [19,58]. Likewise, the chemical composition of the organic amendments presents a key factor contributing to the higher SOC levels, thus playing a significant role in soil carbon stabilization and overall soil functions. Essential components like organic carbon and nitrogen in manure are crucial for managing various soil properties, including aggregate stability, soil moisture, porosity, texture, and pH [38]. Furthermore, increasing active SOC fractions and maintaining soil microbial communities can best be achieved with organic fertilizer inputs based on the results obtained and in similar agreement with related studies on the sustainable development of fertile and productive soils [59].

Overall, our results demonstrate that differences in bulk SOC soil were influenced by agroecological intensification practices such as no-tillage, cover crops, and manure incorporation, with no significant changes in bulk SOC levels observed among other practices. The significant relationships observed among SOM pools in soils across various agroecosystems are consistent with the documented strong interrelationships among SOM pools in other arable soils across different ecological regions [60,61]. The impacts of factors such as climate, inherent soil parameters, and management practices on SOC dynamics have been well reported [62]. Organic matter accumulation and nutrient availability typically strengthen the connections between WEOC, SMB-C, and SOC [63]. This is demonstrated by the higher ratios of SMQ, and WEOC:SOC, which provide key insights into microbial efficiency and the availability of readily decomposable carbon. A higher SMQ suggests more efficient microbial carbon turnover, with optimal values indicating healthier, resilient soils that support efficient carbon cycling [64]. Notwithstanding, our study, in alignment with Ren et al. [65] showed that the scale at which intensification levels and farming practices impacted these relationships was mostly limited compared to the impacts of climate and soil factors. The positive correlations among the carbon pools (SMB-C, SOC, WEOC, and WSA) underscore the strong connection between these intensification levels and soil carbon processes through soil aggregate stability. This implies that soil aggregation is vital for SOC storage and preservation, as it serves as a barrier between decomposers and SOC; however, this structure remains susceptible to management practices [66]. These findings further reinforce previous research indicating that the physical (WSA), chemical (WEOC), and microbiological (MBC) organic carbon pools are more sensitive to tillage disturbance than the total SOC [60].

5. Conclusions

This study emphasizes the complex relationship between different agroecological intensification levels and SOM stabilization across various environmental conditions. Agroecological intensification practices, such as cover cropping, reduced tillage, crop diversification, and organic matter inputs, are vital for improving soil carbon dynamics and aggregate stability, with WEOC and SMB-C serving as critical indicators of soil health. The differences in the results obtained across the studied site locations showed that soil carbon dynamics responded to the influence of environmental factors and management practices. Although variations in SOC, WEOC, and SMB-C were noted across the experimental sites, the strongest correlations were between SOC and WEOC, especially within the various soil aggregates. Furthermore, WSA is strongly and statistically correlated with SOC in bulk soil, fine aggregates (0.25–1.0 mm), and SMB-C, which highlights its importance in preserving soil structure and organic carbon pools. There is thus a strong argument pinpointing that practices promoting all these labile carbon pools significantly influence soil structure and carbon sequestration, enhance soil microbial activity, and, in effect, boost the sustenance of long-term soil health and resilience to climate variability. However, the impacts and roles of specific factors such as climate and soil types cannot be downplayed, as variations in environmental factors and management practices play a crucial role in shaping soil carbon dynamics and aggregate stability. Hence, consistent and concerted efforts must be implemented in sustainable agricultural strategies to ensure long-term soil health and carbon stability, particularly in ecological areas susceptible to climate change.

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