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# Soil Carbon Investigation in Three Pedoclimatic and Agronomic Settings of Northern Italy

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**Abstract:** Sustainable agricultural management is needed to promote carbon (C) sequestration in soil, prevent loss of soil fertility, and reduce the release of greenhouse gases. However, the influence of agronomic practices on soil C sequestration depends on the existing pedoclimatic features. We characterized the soils of three farms far away each other in the Emilia-Romagna region (Northern Italy): an organic farm in the Northern Apennines, a biodynamic farm, and a conventional farm on the Po Plain. The total, inorganic, and organic carbon in soil, as well as the distinct humic fractions were investigated, analyzing both the elemental and isotopic ($^{13}C/^{12}C$) composition. In soils, organic matter appears to be variously affected by mineralization processes induced by microorganisms that consume organic carbon. In particular, organic carbon declined in farms located in the plain (e.g., organic carbon down to 0.75 wt%; carbon stock$_{0-30 cm}$ down to 33 Mg/ha), because of the warmer climate and moderately alkaline environment that enhance soil microbial activity. On the other hand, at the mountain farm, the minimum soil disturbance, the cold climate, and the neutral conditions favored soil C sequestration (organic carbon up to 4.42 wt%; carbon stock$_{0-30 cm}$ up to 160 Mg/ha) in humified organic compounds with long turnover, which can limit greenhouse gas emissions into the atmosphere. This work shows the need for thorough soil investigations, to propose tailored best-practices that can reconcile productivity and soil sustainability.

**Keywords:** soil organic matter; sustainable agriculture; isotopic analyses; humic substances; soil management; carbon sequestration

## 1. Introduction

Soil organic matter (SOM) plays a crucial role in soil fertility, crop productivity, and agronomic sustainability, as well as in ecosystem stability and mitigation of climate change [1–4]. SOM represents the main organic carbon stock in terrestrial ecosystems, storing 1500 Pg of carbon (C), approximately twice as much as in the atmosphere and three times the amount in vegetation [5]. However, through soil respiration, which includes root and microbial respiration, C can be emitted in the atmosphere as carbon dioxide ($CO_2$), one of the main greenhouse gases (GHGs; [6]). The role of SOM as a source or sink of C depends on multiple factors, including the climatic conditions, physicochemical properties of soil, land cover, land use, and soil management [7–9]. In particular, soil management is believed to be a factor controlling soil C stocks and GHGs fluxes [10]. Unsustainable agricultural management, which promotes rapid SOM mineralization, causes the loss of organic carbon and an increase in GHG

emissions [11], whereas sustainable agricultural practices (e.g., conservation strategies) can increase the C content in the soil, minimizing the rising levels of atmospheric $CO_2$ [11–15]. However, it is important to emphasize that the influence of management on C sequestration in soil strongly depends on the soil characteristics and climate of the area [16]. The soil in Southern Europe is poor ($\leq$2%) or very poor ($\leq$1%) in organic C [17]. In order to counteract the global SOM decline, the initiative "4 per mille Soils for Food Security and Climate" was launched at COP21 to demonstrate that sustainable agricultural practices can increase the global SOM stock by 4 per 1000 (0.4%) per year as a compensation for the global emissions of anthropogenic GHGs [18]. However, as noted above, several factors affect SOM dynamics, and the ability of soil to reach this goal can differ among pedoclimatic and agronomic systems.

In this framework, the present study reports the results of the SaveSOC2 (Save Soil Organic Carbon) project, funded by the Rural Development Program (RDP) of the Emilia-Romagna region, to evaluate the quality and quantity of SOM and its potential to sequester C. This project evaluated distinct SOM pools, characterized by different turnover and permanence in soil (e.g., labile and stabile pools), in three farms located in different pedoclimatic settings of Emilia-Romagna, one of the northern Italian regions. The purpose was to test the hypothesis that soil management and environmental conditions affect SOM dynamics. For each study area, the inorganic (IC) and organic (OC) carbon fractions were measured and isotopically characterized in two soil layers (0–15 and 15–30 cm; top- and subsoil, respectively). The organic matter pools, namely, non-extractable organic matter and humic and fulvic acids, were separated using their relative solubility and characterized for chemical and isotopic composition to ascertain the sources and fate of the SOM [19–24]. Subsequently, the carbon stock at the 0–30 cm soil depth was calculated for each farm. This comprehensive approach allows an evaluation of SOM evolution, and thus assesses the C sequestration and/or GHG release from the soils under these management systems and environmental conditions.

## 2. Study Sites and Soil Sampling

Three farms in distinct pedoclimatic conditions were selected in the Emilia-Romagna region (Figure 1a): one in the Northern Apennines (Modena district) and two on the Po Plain (Ferrara district). Soil sampling was performed in summer 2017. At each farm, different agricultural practices were followed; therefore, sample collection was planned to be representative of each management zone. Soils characterized by different agricultural management at each farm were sampled by opening at least three minipits dug down to a 30-cm soil depth (Figure 2). For each field, material from the 0–15 and 15–30 cm layers was collected and thoroughly mixed, obtaining composite samples from each soil depth. Additionally, for both soil layers, undisturbed soil cores were taken using a stainless-steel corer (5.65 cm in diameter, 4 cm in height) for the determination of bulk density.

### The Investigated Farms

"I Rodi" (IR) is a certified organic mountain farm, covering an area of 8.5 ha in the Northern Apennines (44°13′20″ N, 10°47′37″ E; Figure 1b) at an altitude of ca. 700 m above sea level (a.s.l.), near the town of Fanano (in the province of Modena). The Apennine region has a mean annual precipitation of 920 mm, concentrated in the autumn and winter, while the summer is dry. The mean annual air temperature is 11.3 °C; July and August are the warmest months and January is the coldest. The IR farm is situated on deposits formed after multiple landslides in the surrounding mountains, where there is a marly-shaly-sandstone sedimentary succession of the early Miocene age outcrops [25,26] (Figure 1b). The IR farm specializes in the production of small fruits, in particular raspberries. The farmers complained about an area where plants were in distress and less productive with respect to other plants on the same farm. Therefore, three areas of investigation were selected: (i) a grassland field not used for raspberry production; (ii) a not very productive raspberry field; and (iii) a productive raspberry field. Every year, in spring (March/April), an organic fertilizer (liquid cattle manure) is spread on the cultivated fields at a dose of ca. 80 tons per hectare.
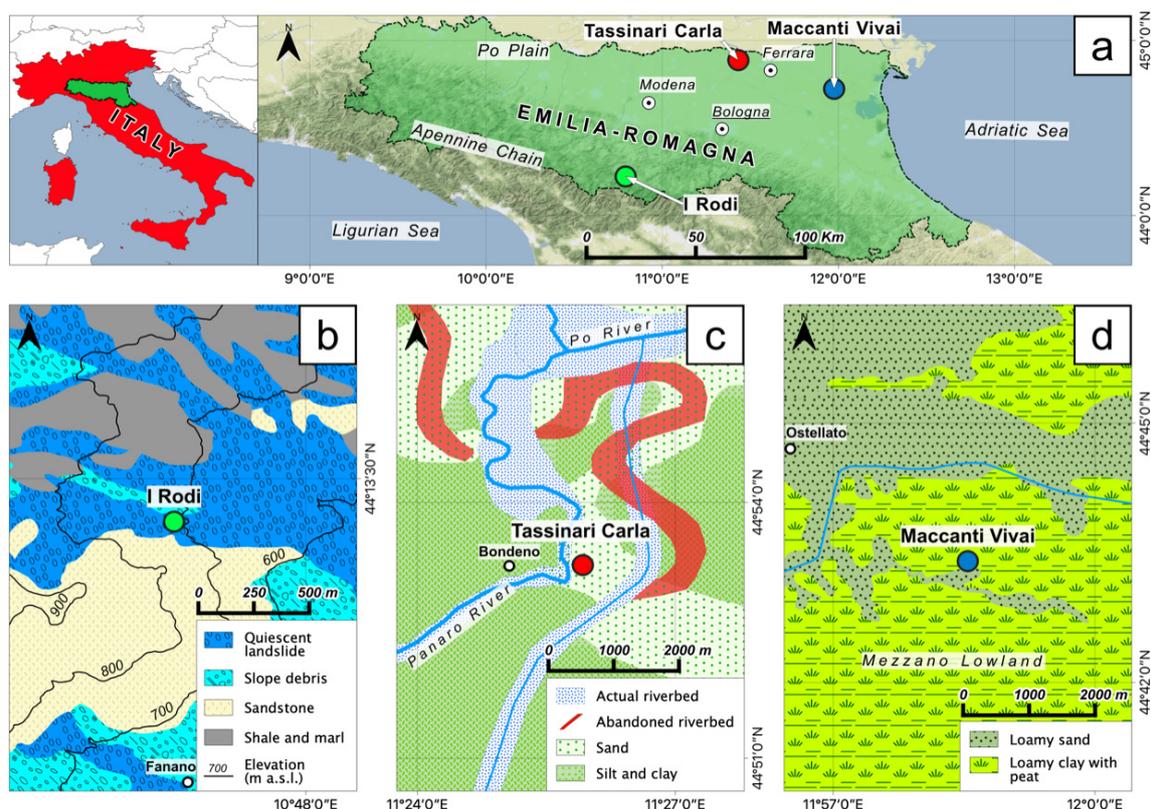
**Figure 1.** (**a**) Locations of the investigated farms within the Emilia-Romagna region (Northern Italy). Simplified geomorphological maps of the studied areas showing the location of (**b**) the I Rodi (IR) farm in the Apennines, as well as the (**c**) Tassinari Carla (BT) farm and (**d**) Maccanti Vivai (MV) farm on the Po Plain.



**Figure 2.** Sampling of soil layers (0–15 cm and 15–30 cm) from a minipit dug at one of the farms investigated by the SaveSOC2 project.

"Tassinari Carla" (BT) farm is a biodynamic organic farm, covering a surface of 3 ha, located on the Po Plain near the town of Bondeno, i.e., in the northwestern part of the province of Ferrara (44°53'16" N, 11°25'53" E; Figure 1c) at an altitude of ca. 8 m a.s.l. In this area, the climate is temperate. The mean annual precipitation is 666 mm, concentrated during the autumn; the mean annual air temperature is 24.5 °C. July and August are the warmest months and January is the coldest. The BT farm is situated on Holocene deposits characterized by sandy-silty textures related to ancient branches of the Po and Panaro rivers [27]. This farm produces fruit and vegetables (e.g., watermelon, apricots,

apples, strawberries, cherries, pumpkins, onions, and potatoes). For this work, seven fields were selected on the basis of the different crops and agronomic age: (i) a turfed orchard with organic management since 1992; (ii) an organic vegetable garden operating since 1992; (iii) a turfed orchard operating since 2007; (iv) an organic vegetable garden operating since 2007; (v) an organic vegetable garden operating since 1996, plowed during the sampling operation; (vi) a harrowed field prepared for potato crops; and (vii) a strawberry field operating since 1996. Annually, in spring, manure is spread on the soil of each field; in addition, a liquid fertilizer obtained through the maceration of plants and poultry manure diluted with water (1:10) is repeatedly sprayed on the soil of each field via a micro-irrigation system throughout the year.

"Maccanti Vivai" (MV) farm is a conventional farm covering a surface of 65 ha located in the easternmost sector of the Po Plain (44°43′27″ N, 11°58′16″ E; Figure 1d) at an altitude of ca. −3 m a.s.l., near the town of Ostellato (Province of Ferrara). The climate is temperate and similar to that of the BT farm. The "Maccanti Vivai" farm is situated on lacustrine deposits, characterized by the presence of peat layers enriched in organic matter [28] of the Mezzano Lowland, which was reclaimed in the 1960s from the former Comacchio lagoons [29]. The farm produces fruit saplings and in particular is a nursery center of multiplication of rootstocks. Soil sampling was focused on the distinct fields of the pear nursery, characterized by trees having a different age: (i) an orchard with one-year-old pear trees; (ii) an orchard with two-year-old pear trees; and (iii) an orchard with three-year-old pear trees. The fertilization of the fields in this farm is generally done using the following fertilizers: manure (usually in liquid form) in October (0.4 tons/ha), NPK + (Mg) + (S) compounds in spring (0.5 tons/ha), and urea (0.4 tons/ha) during plant growth.

## 3. Materials and Methods

### 3.1. Soil Sample Preparation, Textural Analysis, and Determination of Physicochemical Parameters

The composite soil samples were air-dried and sieved at 2 mm. Additionally, an aliquot of the <2 mm sample had been powdered in an agate mill. For the <2 mm soil fractions, the particle size distribution was investigated by the pipette method, after dispersion of the sample with a sodium hexametaphosphate solution [30] to determine the sand (0.05–2.0 mm), silt (0.02–0.05 mm), and clay (<0.02 mm) size fractions.

The pH value was determined potentiometrically in a 1:2.5 (w/v) soil:distilled water suspension with a Crison pH meter. The electrical conductivity (EC) was also performed in a 1:2.5 (w/v) soil:distilled water suspension with an Orion conductivity meter. In the powdered samples, the carbonate content was measured by volumetric analysis of the $CO_2$ released by a 6 M HCl solution [31].

### 3.2. EA-IRMS Analysis

The carbon and nitrogen content (reported in wt%) and their respective stable isotope ratios (expressed in ‰) were analyzed using an elemental analyzer (Vario Micro Cube, Elementar, Hanau, Germany) in line with an isotopic ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK) operating in continuous-flow mode at the Department of Physics and Earth Science of University of Ferrara (Italy). The combustion temperature can be varied for the extraction of different components having distinctive destabilization temperatures. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios (R) were expressed in δ notation (in ‰ units):

$$\delta = \left( \frac{R_{sam}}{R_{std}} - 1 \right) \times 1000$$

where $R_{sam}$ is the isotopic ratio of the sample and $R_{std}$ is the isotopic ratio of the international isotope standards Pee Dee Belemnite (PDB) and air $N_2$, for C and N, respectively [32]. Calibration was performed by measuring the Carrara Marble (calibrated at the Institute of Geoscience and Georesources of the National Research Council in Pisa) and synthetic sulfanilamide (provided by Elementar,

Hanau, Germany) every 15 samples. As defined by repeated analyses of selected samples (Table S1), the elemental compositions of C and N were characterized by an average standard deviation (1 sigma) of ±0.11 wt% and ±0.02 wt%, respectively, while the $\delta^{13}C$ and $\delta^{15}N$ values had an average standard deviation (1 sigma) of ±0.1‰ and ±0.3‰, respectively.

### 3.3. Discrimination of Soil Inorganic and Organic Pools

The elemental and isotopic composition of total (TC), inorganic (IC), and organic (OC) carbon was analyzed on the powdered samples following the analytical approach of Natali and Bianchini [33] and Natali et al. [34], which is based on the different thermal stabilities of the IC and OC pools. During sample preparation, any chemical pre-treatment of the samples was avoided, as acidification methods for carbonate removal may produce partial loss of organic matter, causing significant deviations in the related isotopic values (e.g., [35–37]). Up to 40 mg of powdered sample was wrapped in tin capsules and combusted via EA-IRMS according to the following analytical protocol:

- 950 °C for TC and N measuring;
- 950 °C for IC measuring, after removal of organic matter by preliminary combustion in a muffle furnace (550 °C for 12 h); the relative gravimetric loss (LOI) was also determined to correct the elemental concentration of the IC fraction;
- 550 °C for OC measuring.

### 3.4. Fractionation of Organic Matter Pools

The organic matter pools of the topsoil (i.e., 0–15 cm) were chemically separated in non-extractable organic matter (NEOM), humic (HA), and fulvic (FA) acids, according to Vittori Antisari et al. [38]. Ten grams of each soil sample were placed in 250 mL Teflon bottles and 100 mL of 0.1 M $Na_4P_2O_7$ plus a 0.1 M NaOH solution were added, and then shaken in a Dubnoff water bath at 65 °C for 24 h. After centrifugation at 7000 rpm for 20 min, the supernatant was separated from the mineral fraction, filtered at 0.45 μm with a Millipore vacuum filter, and subsequently acidified with 6M HCl to a pH < 2 to force HA precipitation. The HAs were isolated with centrifugation at 8000 rpm for 20 min and redissolved in a 0.5 M NaOH solution three consecutive times. The FA fraction was separated from the non-humic compounds using solid chromatography with polyvinylpyrrolidone (PVP), following the procedures of Ciavatta et al. [39]. The HA and FA fractions were purified using Spectra-por dialyzing membranes (MWCO 6000–8000 Da and 1000 Da, for HA and FA, respectively), and after dialysis the purified samples were freeze-dried. The residual SOM after the alkaline extraction represented the NEOM fraction, i.e., the non-alkaline extractable SOM. In order to remove any soluble residue from the NEOM fraction, NEOM was added with 100 mL of distilled water, shaken, centrifugated, and the excess water discarded. The washing of the NEOM fraction was repeated at least five times. The NEOM fraction was then oven-dried at 60 °C and powdered in an agate mill. The weight of the separated FA, HA, and NEOM fractions was recorded (i.e., $g_{fraction}/kg_{soil}$) and the C content and $\delta^{13}C$ values of each fraction were analyzed by combustion at 550 °C via EA-IRMS, according to the protocol described above. The amount of OC of each fraction (i.e., OC wt% in FA, HA, and NEOM) was transformed to g of OC per kg of soil (i.e., g $C_{fraction}/kg_{soil}$).

### 3.5. Soil Bulk Density and Organic Carbon Stock

The undisturbed soil cores collected from the top- and subsoil were oven-dried at 105 °C to a constant weight, and the soil bulk density was calculated as the ratio of the total dry weight to the total soil volume. For each investigated soil, the stock of OC in the 0–30 cm soil depth was thus calculated based on the OC concentration, soil thickness, and bulk density [40], as follows:

$$\text{OC stock (Mg/ha)} = \text{OC concentration (wt\%)} \times H \text{ (cm)} \times D \text{ (g/cm}^3)$$

where H is the soil layer thickness and D is the bulk density.

### 3.6. Statistical Analysis

The statistical analyses was done in R version 4.0.2 [41]. The analysis of variance (ANOVA) test was applied to every variable in order to determine the statistical differences among the farms. Principal component analysis (PCA) was used to exhibit the samples in an unsupervised pattern recognition map (score plot) and to identify the relationships between the samples and variables.

## 4. Results

### 4.1. Soil Texture and Physicochemical Properties

The physicochemical characteristics of the two soil layers (0–15 cm and 15–30 cm) of each investigated site of the three farms are reported in Table 1.

**Table 1.** Average values of the pH, electric conductivity (EC), CaCO₃, clay, sand, and silt content of the soil horizons (0–15 cm and 15–30 cm) of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm.

| Field | Depth (cm) | pH | EC ($\mu$S/cm) | CaCO$_3$ (g/kg) | Sand (g/kg) | Silt (g/kg) | Clay (g/kg) |
|---|---|---|---|---|---|---|---|
| IR farm | | | | | | | |
| Grassland | 0–15 | 6.8 | 115 | – | 203 | 665 | 132 |
| | 15–30 | 7.1 | 141 | – | 240 | 636 | 124 |
| Low-yield | 0–15 | 7.9 | 259 | 17 | 92 | 799 | 109 |
| | 15–30 | 8.0 | 250 | 25 | 94 | 784 | 123 |
| Productive | 0–15 | 7.0 | 293 | – | 114 | 761 | 126 |
| | 15–30 | 6.5 | 328 | – | 134 | 748 | 118 |
| BT farm | | | | | | | |
| Turfed orchard (since 1992) | 0–15 | 8.2 | 227 | 102 | 208 | 612 | 180 |
| | 15–30 | 7.9 | 195 | 124 | 173 | 637 | 190 |
| Vegetable garden (since 1992) | 0–15 | 8.3 | 227 | 93 | 101 | 798 | 201 |
| | 15–30 | 8.3 | 187 | 102 | 118 | 699 | 183 |
| Turfed orchard (since 2007) | 0–15 | 8.3 | 166 | 98 | 256 | 545 | 199 |
| | 15–30 | 8.5 | 141 | 104 | 246 | 539 | 215 |
| Vegetable garden (since 2007) | 0–15 | 8.4 | 138 | 87 | 230 | 562 | 208 |
| | 15–30 | 8.7 | 124 | 87 | 241 | 535 | 224 |
| Vegetable garden (since 1996) | 0–15 | 8.5 | 171 | 73 | 254 | 555 | 191 |
| | 15–30 | 8.3 | 197 | 71 | 276 | 556 | 168 |
| Harrowed | 0–15 | 8.4 | 156 | 73 | 356 | 451 | 193 |
| | 15–30 | 8.3 | 190 | 84 | 344 | 344 | 312 |
| Strawberry (since 1996) | 0–15 | 8.4 | 135 | 60 | 376 | 435 | 189 |
| | 15–30 | 8.4 | 134 | 60 | 345 | 469 | 186 |
| MV farm | | | | | | | |
| 1-year-old pear orchard nursery | 0–15 | 7.5 | 1397 | 35 | 148 | 679 | 173 |
| | 15–30 | 7.5 | 1816 | 35 | 151 | 669 | 181 |
| 2-year-old pear orchard nursery | 0–15 | 7.6 | 1552 | 43 | 149 | 661 | 190 |
| | 15–30 | 7.5 | 2017 | 43 | 124 | 706 | 171 |
| 3-year-old pear orchard nursery | 0–15 | 8.0 | 460 | 65 | 100 | 687 | 213 |
| | 15–30 | 7.7 | 950 | 72 | 94 | 736 | 170 |

The studied soils were loamy silt with high silt content, and only the BT harrowed and strawberry fields had loam texture, as shown in Figure S1. Comparatively lower pH values were detected in the soil of the IR farm in the Apennines than in the BT and MV farms located on the Po Plain. Within the IR

sites, only the low-yield field had moderately alkaline (7.9–8.0) soil due to the notable amount of $CaCO_3$ (17–25 g/kg). In the other IR fields, a soil pH close to neutrality (6.8–7.1) was detected, in accordance with the absence of carbonate. On the Po Plain, the BT soils showed the highest pH values (7.9–8.7), associated with a high concentration of carbonate (60–124 g/kg). The EC values varied greatly between sites, ranging from 115 to 2017 µS/cm; however, all the investigated soils were non-saline.

### 4.2. Soil Carbon and Nitrogen Elemental and Isotopic Composition

The average contents of TC, IC, OC, and N and the respective isotopic ratio in the 0–15 cm and 15–30 cm soil layers are reported in Table 2 and Figure 3.

The observed differences between the farms greatly exceeded the analytical errors for all the described parameters and therefore reflect real differences among soils developed under different pedoclimatic settings and management with distinct agronomic techniques. This statement is supported by the one-way ANOVA test, showing that the elemental and isotopic variables were highly affected by the location of the farm ($p < 0.0001$). According to the PCA investigation, there was great variability in elemental and isotopic C and N among the soils of the three farms (Figure S2a).

**Table 2.** Elemental and isotopic composition of total carbon (TC), inorganic carbon (IC), organic carbon (OC), and nitrogen (N) of the soil samples collected at depths of 0–15 and 15–30 cm in different fields of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm.

| Field | Depth (cm) | TC (wt%) | $\delta^{13}C_{TC}$ (‰) | IC (wt%) | $\delta^{13}C_{IC}$ (‰) | OC (wt%) | $\delta^{13}C_{OC}$ (‰) | N (wt%) | $\delta^{15}N$ (‰) |
|---|---|---|---|---|---|---|---|---|---|
| IR farm | | | | | | | | | |
| Grassland | 0–15 | 2.88 | – | 0.04 | – | 2.85 | −27.3 | 0.29 | 3.2 |
| | 15–30 | 1.82 | −26.3 | 0.05 | – | 1.77 | −27.0 | 0.22 | 3.9 |
| Low-yield | 0–15 | 2.87 | −22.8 | 0.40 | −3.2 | 2.47 | −26.1 | 0.32 | 4.4 |
| | 15–30 | 2.44 | −21.9 | 0.39 | −3.4 | 2.05 | −25.6 | 0.27 | 4.6 |
| Productive | 0–15 | 4.52 | −27.5 | 0.10 | – | 4.42 | −28.1 | 0.50 | 4.7 |
| | 15–30 | 4.27 | −27.5 | 0.11 | – | 4.16 | −28.2 | 0.47 | 4.6 |
| BT farm | | | | | | | | | |
| Turfed orchard (since 1992) | 0–15 | 2.76 | −9.8 | 1.63 | −1.2 | 1.14 | −22.0 | 0.14 | 5.5 |
| | 15–30 | 2.52 | −7.9 | 1.75 | −1.4 | 0.78 | −22.7 | 0.11 | 6.0 |
| Vegetable garden (since 1992) | 0–15 | 2.79 | −10.1 | 1.63 | −1.4 | 1.16 | −22.4 | 0.15 | 6.8 |
| | 15–30 | 2.85 | −10.2 | 1.71 | −1.4 | 1.14 | −23.7 | 0.16 | 6.3 |
| Turfed orchard (since 2007) | 0–15 | 2.62 | −10.7 | 1.62 | −2.9 | 1.01 | −23.3 | 0.13 | 7.1 |
| | 15–30 | 2.59 | −10.3 | 1.53 | −2.9 | 1.07 | −21.0 | 0.12 | 6.2 |
| Vegetable garden (since 2007) | 0–15 | 2.53 | −10.3 | 1.51 | −1.4 | 1.03 | −23.3 | 0.14 | 6.2 |
| | 15–30 | 2.44 | −9.0 | 1.51 | −1.1 | 0.94 | −21.8 | 0.12 | 6.8 |
| Vegetable garden (since 1996) | 0–15 | 2.19 | −11.1 | 1.30 | −1.9 | 0.89 | −24.6 | 0.12 | 7.0 |
| | 15–30 | 2.24 | −12.2 | 1.22 | −1.7 | 1.02 | −24.8 | 0.14 | 7.3 |
| Harrowed | 0–15 | 2.05 | −10.1 | 1.23 | −1.8 | 0.82 | −22.5 | 0.11 | 6.9 |
| | 15–30 | 2.04 | −10.3 | 1.30 | −1.7 | 0.75 | −25.6 | 0.11 | 5.8 |
| Strawberry (since 1996) | 0–15 | 2.03 | −12.1 | 1.16 | −2.9 | 0.88 | −24.4 | 0.13 | 6.8 |
| | 15–30 | 1.93 | −11.1 | 1.04 | −2.0 | 0.90 | −21.7 | 0.11 | 7.3 |
| MV farm | | | | | | | | | |
| 1-year-old pear orchard nursery | 0–15 | 8.08 | −24.2 | 0.42 | −6.7 | 7.66 | −25.1 | 0.64 | 3.8 |
| | 15–30 | 8.55 | −24.2 | 0.43 | −6.2 | 8.11 | −25.2 | 0.68 | 3.9 |
| 2-year-old pear orchard nursery | 0–15 | 7.80 | −23.4 | 0.69 | −5.5 | 7.11 | −25.2 | 0.67 | 5.5 |
| | 15–30 | 8.20 | −23.3 | 0.68 | −5.6 | 7.53 | −25.0 | 0.65 | 3.5 |
| 3-year-old pear orchard nursery | 0–15 | 7.67 | −22.4 | 0.95 | −5.4 | 6.73 | −24.9 | 0.64 | 5.8 |
| | 15–30 | 7.82 | −22.7 | 1.06 | −5.2 | 6.76 | −25.5 | 0.62 | 5.9 |
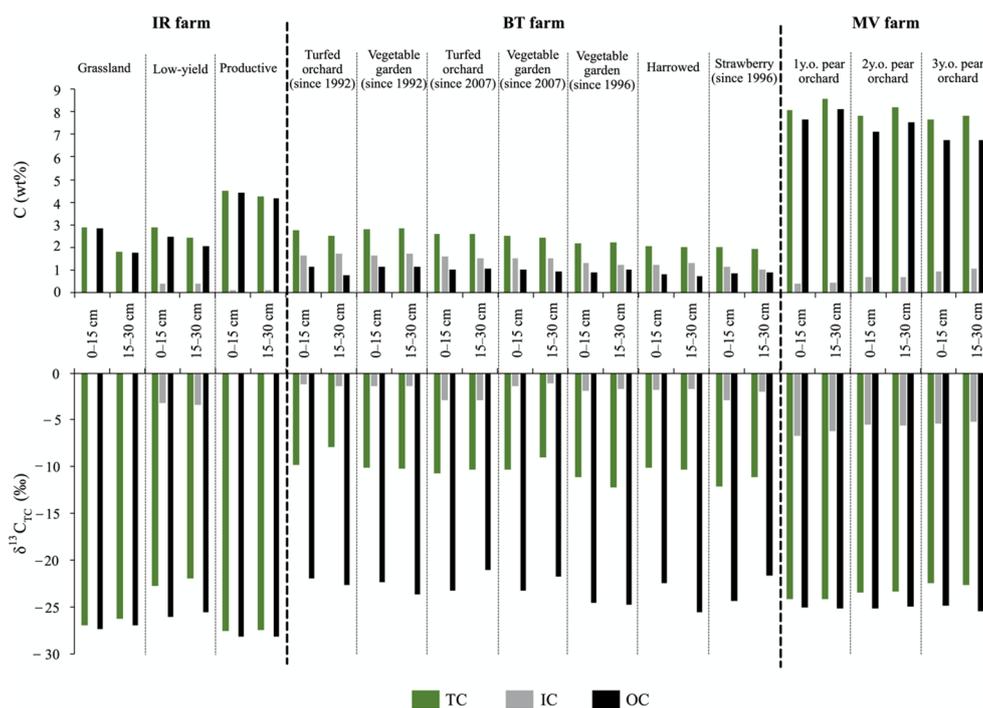
**Figure 3.** Elemental and isotopic composition of the total (TC), inorganic (IC), and organic (OC) carbon fractions of the soil samples collected at depths of 0–15 and 15–30 cm in different fields of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. Analytical uncertainties are in the order of 0.11 wt% for elemental carbon and 0.1‰ for $\delta^{13}C$, therefore they are negligible at the graphical scale.

For the IR farm, the TC contents varied from 1.82 wt% to 4.52 wt% and decreased with depth. The grassland and low-yield fields were characterized by TC contents lower than those of the productive field (grassland average: 2.35 wt%; low-yield average: 2.65 wt%; productive average: 4.39 wt%). In the IR fields, the $\delta^{13}C_{TC}$ values varied from −21.9‰ to −27.5‰, becoming less negative with depth for grassland and low-yield fields and remaining stable for the productive field. The grassland and productive fields had more negative $\delta^{13}C_{TC}$ values (grassland average: −26.6‰; productive average: −27.5‰) than the low-yield field (average: −22.3‰). The IC fraction was significant only in the low-yield field (average: 0.39 wt%) and irrelevant in the other IR fields (grassland average: 0.05 wt%; productive average: 0.10 wt%). Therefore, the $\delta^{13}C_{IC}$ was measurable only in the low-yield field (average: −3.3‰). In all IR fields, the OC contents decreased with depth. The OC fraction had a higher content and more negative isotopic value in the productive field (average OC: 4.29%; average $\delta^{13}C_{OC}$: −28.2‰) with respect to the grassland (average OC: 2.31%; average $\delta^{13}C_{OC}$: −27.2‰) and the low-yield (average OC: 2.26%; average $\delta^{13}C_{OC}$: −25.8‰) fields, in agreement with the TC trend. In the IR fields, the N contents varied from 0.22 wt% to 0.50 wt% and decreased with depth. On the contrary, for $\delta^{15}N$ there was no variation with soil depth. The cultivated fields recorded more positive $\delta^{15}N$ values (4.5‰) than the grassland field (3.5‰), plausibly reflecting the effect of organic fertilization.

For the BT farm, the variability of the TC contents was narrow, ranging from 1.93 wt% to 2.85 wt%. The strawberry field had the lowest TC contents (average: 1.98 wt%), while the vegetable garden since 1992 had the highest (average: 2.82 wt%). The isotopic C composition varied from −7.9‰ to −12.2‰ and significant variation was not observed along the depth, with the exception of one turfed orchard exhibiting a more negative value in the 0–15 cm layer than in the 15–30 cm layer. The vegetable garden since 1996 had the most negative $\delta^{13}C_{TC}$ values (average: −11.7‰), while the turfed orchard had the least negative (average: −8.9‰). Among the BT fields, the IC and OC contents showed little variation, ranging from 1.04 wt% to 1.75 wt% and from 0.75 wt% to 1.16 wt%, respectively. All the BT fields had similarly low negative $\delta^{13}C_{IC}$ values, with the turfed orchard since 2007 and the strawberry field exhibiting the most negative values (~−2.9‰). The turfed orchard since 2007 had the least negative

$\delta^{13}C_{OC}$ value and the harrowed field the most negative one. The N contents were similar among fields and along the soil profile (~0.13 wt%) and the $\delta^{15}N$ values range from 5.5‰ to 7.3‰. The turfed orchard since 1992 showed the lowest average N isotopic content (average: 5.8‰), while the vegetable garden since 1996 exhibited the highest one (average: 7.2‰). These comparatively high $\delta^{15}N$ values confirm the absence of synthetic fertilizations, as prescribed by organic protocols.

For the MV farm, the TC contents varied from 7.67 wt% to 8.55 wt%, increasing with depth and decreasing with the age of the pear trees (one-year-old average: 8.32 wt%; two-year-old average: 8.00 wt%; three-year-old average: 7.75 wt%). The range of $\delta^{13}C_{TC}$ was narrow, varying from −22.4‰ to −24.2‰. The $\delta^{13}C_{TC}$ values become less negative with the age of pear trees (one-year-old average: −24.2‰; two-year-old average: −23.4‰; three-year-old average: −22.6‰). Like TC, the OC content also decreased with the age of the pear trees. On the contrary, IC tended to increase with the age of pear trees, suggesting the precipitation of secondary (pedogenic) carbonates. Along the soil profile, the IC contents remained stable with depth, whereas those of the OC increased. Among the MV parcels, the $\delta^{13}C_{IC}$ values were more negative in the one-year-old pear trees field with respect to the other two fields, whereas $\delta^{13}C_{OC}$ had indistinguishable values. The N contents were similar among fields and along the soil profile (~0.65 wt%). On the contrary, the $\delta^{15}N$ values increased with the age of the pear trees (one-year-old average: 3.9‰; two-year-old average: 4.5‰; three-year-old average: 5.9‰).

### 4.3. Carbon Fractionation of Organic Matter

The C elemental and isotopic analyses of the NEOM, HA, and FA fractions for the 0–15 cm soil layer of each investigated field are reported in Table 3 and Figure 4.

One-way ANOVA showed that humic substances were highly affected by the location of the farm ($p < 0.001$). According to the PCA investigation, there is great variability in humic substances between the three farms (Figure S2b).

**Table 3.** The average carbon (C) elemental (grams of C of each fraction for kilograms of soil) and isotopic (‰) composition of the non-extractable organic matter (NEOM) and humic (HA) and fulvic (FA) acids from soil collected at a depth of 0–15 cm in different fields of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. The standard errors for C content are also reported.

| | NEOM | | | HA | | | FA | | |
|---|---|---|---|---|---|---|---|---|---|
| Field | C (g/kg) | | $\delta^{13}C_{NEOM}$ | C (g/kg) | | $\delta^{13}C_{HA}$ | C (g/kg) | | $\delta^{13}C_{FA}$ |
| IR farm Grassland | 29.87 | ±1.07 | −27.0 | 3.81 | ±0.14 | −28.0 | 2.49 | ±0.09 | −27.1 |
| Low-yield | 22.83 | ±0.82 | −26.4 | 2.00 | ±0.07 | −26.8 | 1.11 | ±0.04 | −26.0 |
| Productive | 31.67 | ±1.13 | −27.5 | 4.95 | ±0.18 | −28.5 | 1.93 | ±0.07 | −26.8 |
| BT farm Turfed orchard (since 1992) | 9.15 | ±0.42 | −23.8 | 0.23 | ±0.01 | −23.1 | 0.43 | ±0.02 | −22.8 |
| Vegetable garden (since 1992) | 11.62 | ±0.54 | −24.7 | 0.09 | ±0.00 | −25.1 | 0.42 | ±0.02 | −24.4 |
| Vegetable garden (since 2007) | 7.36 | ±0.34 | −24.0 | 0.13 | ±0.01 | −25.2 | 0.61 | ±0.03 | −25.0 |
| Vegetable garden (since 1996) | 6.55 | ±0.30 | −24.2 | 0.08 | ±0.00 | −24.9 | 0.42 | ±0.02 | −24.4 |
| Harrowed | 6.41 | ±0.30 | −24.6 | 0.08 | ±0.00 | −25.0 | 0.32 | ±0.01 | −24.5 |
| Strawberry (since 1996) | 7.62 | ±0.35 | −24.0 | 0.10 | ±0.00 | −25.1 | 0.44 | ±0.02 | −24.2 |
| MV farm 1-year-old pear orchard nursery | 52.87 | ±1.89 | −25.7 | 4.75 | ±0.17 | −25.8 | 1.82 | ±0.07 | −24.9 |
| 2-year-old pear orchard nursery | 48.36 | ±1.73 | −25.7 | 4.66 | ±0.17 | −25.7 | 0.90 | ±0.03 | −25.9 |
| 3-year-old pear orchard nursery | 37.98 | ±1.36 | −25.4 | 3.88 | ±0.14 | −25.5 | 1.22 | ±0.04 | −24.7 |
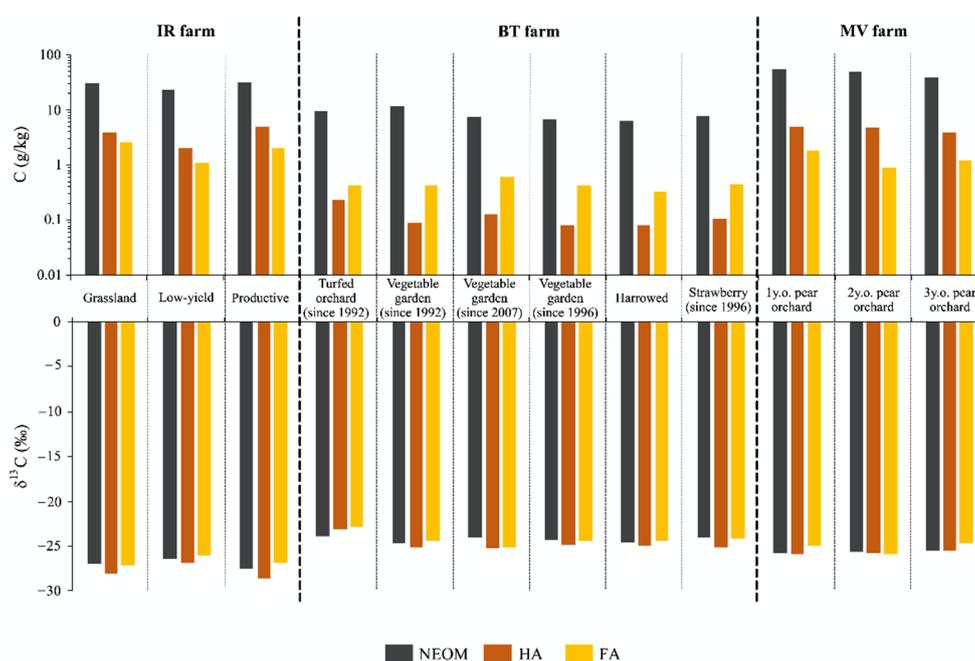
**Figure 4.** Average elemental (in logarithmic scale) and isotopic composition of the non-extractable organic matter (NEOM), humic (HA) and fulvic (FA) acids from soils collected at a depth of 0–15 cm in different fields of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. As evidenced by the standard errors reported for the carbon contents of humic substances in Table 3, and for the carbon isotopic analyses in Table S1, the analytical uncertainties are negligible at the graphical scale.

In all the IR fields, $C_{NEOM}$ was the predominant fraction, followed by $C_{HA}$ and then $C_{FA}$. Among the parcels, the low-yield field exhibited the lowest $C_{NEOM}$, $C_{HA}$, and $C_{FA}$ values (22.83 g/kg, 2.00 g/kg, and 1.11 g/kg, respectively) coupled with the less negative $\delta^{13}C_{NEOM}$, $\delta^{13}C_{HA}$, and $\delta^{13}C_{FA}$ isotopic ratios (−26.4‰, −26.8‰, and −26.0‰, respectively). Instead, in grassland and productive fields, humic substances had higher C contents and more negative isotopic ratios.

On the whole, in the BT fields $C_{NEOM}$ was the most abundant fraction, followed by $C_{FA}$ and then $C_{HA}$. Apart from that, within the BT fields it is not possible to delineate a clear trend among the C contents of the humic substances. The variability in $\delta^{13}C_{NEOM}$ (from −23.8‰ to −24.7‰), $\delta^{13}C_{HA}$ (from −23.1‰ to −25.2‰), and $\delta^{13}C_{FA}$ (from −22.8‰ to −25.0‰) is narrow. Even for the isotopic C ratios of the humic substances it is not possible to delineate a clear trend among the different fields of the BT farm.

In all the MV fields, the C content of NEOM widely prevails that of HA and FA. Among the MV fields, only the $C_{NEOM}$ and $C_{HA}$ contents decrease with the age of the pear trees (the $C_{FA}$ content of the two-year-old pear trees is higher than that of the three-year-old pear trees). In MV fields, the $\delta^{13}C$ values of the humic substances are nearly indistinguishable (~−25‰). Only the $\delta^{13}C_{FA}$ of the one- and three-year-old pear trees are ~1‰ less negative with respect to the other C isotopic values.

## 5. Discussion

### 5.1. Comparison of Carbon Pools among and within Farms

Comparing the three farms, the amount of TC increased in the order BT < IR < MV. In the IR and MV farms' soils, the C pools were mainly represented by OC, while at the BT farm IC was the most abundant fraction. The isotopic signature of the soil reflected the OC or IC predominance: the $\delta^{13}C_{TC}$ values of the IR farm and MV farm were more negative than those of the BT farm. This correlation is better displayed in Figure 5, showing the distribution of OC/IC with respect to $\delta^{13}C_{TC}$. The IR and

MV farm soil samples had the highest OC/IC ratios and the most negative $\delta^{13}C_{TC}$ values, whereas soil samples from the BT farm had the lowest OC/IC ratios and the least negative $\delta^{13}C_{TC}$ values.
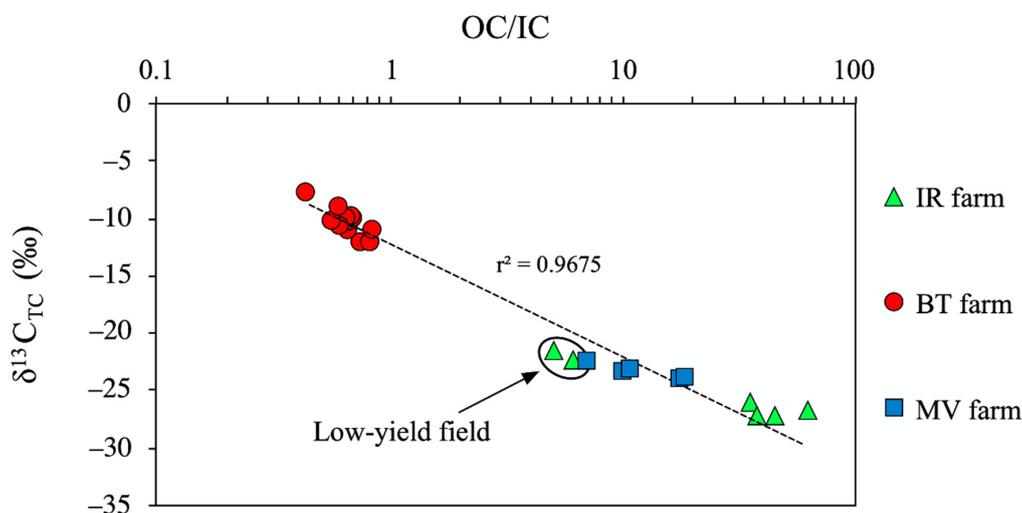


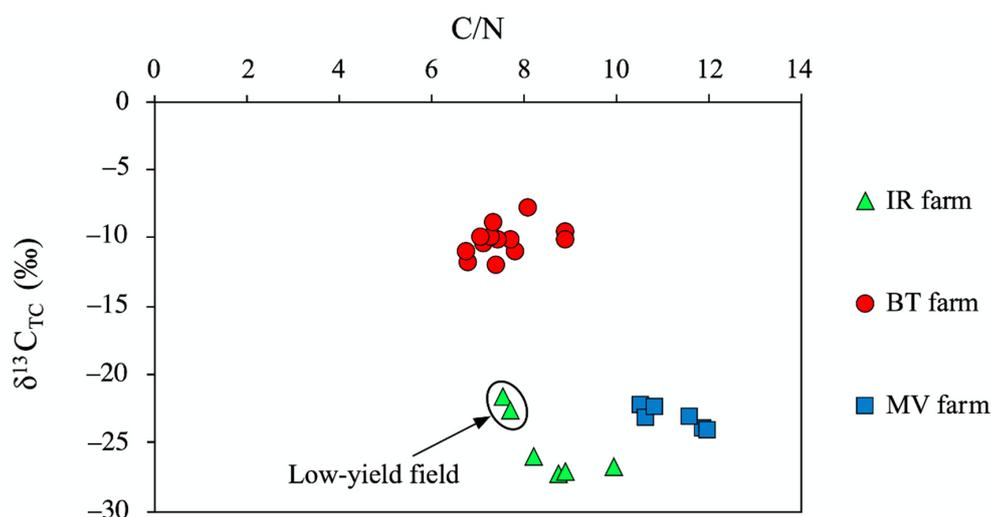**Figure 5.** The OC/IC ratio (in logarithmic scale) versus $\delta^{13}C_{TC}$ of soils from the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. The error bars are too small (with respect to the symbol size) to be reported, and the observed difference cannot be attributed to analytical artifacts.

For the MV and BT farms, the elemental and isotopic TC contents reflected the soil type of the investigated areas. The soil of the MV farm hosts peat layers, which are enriched in organic matter, and therefore in OC, whereas BT farm soils are calcareous, constituted mainly by IC. At the IR farm, there were instead differences in the relative distribution of C between the OC and IC fractions among the three investigated fields. In fact, among the IR soils the low-yield field had the lowest OC/IC ratios (5.2–6.2; Figure 5) and the least negative isotopic composition (from −21.9‰ to −22.8‰; Figure 5), which reflected the higher IC content, i.e., an appreciable presence of carbonates only in this field. Meanwhile, the extremely low IC contents of the grassland and productive fields led to a wide range of OC/IC ratio values (36.5–64.4; Figure 5). These ratios were coupled with more negative $\delta^{13}C_{TC}$ (−27.5‰ and from −26.3‰ to −27.0‰ for productive and grassland fields, respectively; Figure 5), confirming the marked predominance of the OC fraction.

Among the parcels of BT farm, there was a low variability in TC, IC, and OC contents and no trends can be observed. Among the three fields of the MV farm, the variabilities in TC, IC, and OC were also narrow. Despite this, in the MV fields the TC and OC values tended to decrease with the age of the pear trees, while IC showed the opposite trend. In fact, in Figure 5, the distribution of the MV farm soil samples is closely related to the age of the pear orchards: from the youngest orchard to the oldest one the OC/IC ratio decreases and the $\delta^{13}C_{TC}$ becomes less negative. This is due to the progressive (i) formation of pedogenic carbonates and (ii) consumption of organic matter (and OC), induced by plant growth that extracts nutritive elements from soils. In turn, this reflects the mineralization processes mediated by microorganisms that decompose the organic matter to use C, releasing soil nutrients for the supply of plants.

## 5.2. Effects of Pedoclimate Setting on the Soil C/N Ratio and Isotopic Signature

In soils, the proportion between OC and total N, expressed as the C/N ratio, can be used as an indicator of SOM quality and its expected decomposability [42]. The C/N ratio in agroecosystems is influenced by natural ecological processes and by climate, soil type, topography, vegetation type, and land use and management [43,44]. Therefore, C/N can be a sensitive index to discriminate the soil quality/health of the three farms of the SaveSOC2 project, considering their different pedoclimatic characteristics and agricultural practices. The BT farm recorded the lowest C/N range (6.8–8.9; Figure 6),

the MV farm recorded the highest one (10.6–12.0; Figure 6), and the IR farm's C/N range was between them (7.6–9.9; Figure 6). According to the C/N ranges, the organic matter mineralization processes variously affected the soil at all the investigated study sites, including the soils of the MV farm, whose C/N ratio was expected to be higher considering the abundance of SOM (and OC) in the area for the presence of peaty components. The conventional tillage used by the MV farm accelerated SOC mineralization, thus lowering the C/N ratios as C oxidation in the form of $CO_2$ was the dominant process [42,43,45].



**Figure 6.** The C/N ratio versus $\delta^{13}C_{TC}$ of soils from the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. The error bars are too small (with respect to the symbol size) to be reported, and the observed difference cannot be attributed to analytical artifacts.

Furthermore, the role of pH must be considered since the soils of the MV farm are slightly alkaline (pH: 7.5–8.0; Table 1 and Figure 7). In fact, according to several studies [9,46–49] in soil with acidic pH conditions, the SOM decomposition is inhibited, while in soil with a moderately alkaline pH the SOM decomposition is favored. Such a trend was also seen in the soils of the BT farm. Despite the BT farm avoiding conventional tillage, it recorded the lowest C/N ratio and the least negative $\delta^{13}C_{OC}$ (from −21.0‰ to −25.6‰; Figures 6 and 7), reflecting a higher degree of SOM transformation. This can be due both to the relatively warm climatic conditions that characterize the Po Plain, in turn increasing the mineralization rate [50–52], and to the moderately alkaline pH (7.9–8.7; Figure 7).

Unlike the other two farms, the IR farm is in a mountain setting, and the relatively lower temperature characterizing this environment tends to preserve SOM from decomposition [9]. For grassland and productive fields, the neutral pH (6.5–7.1; Figure 7) slows down the SOM decomposition rate through the inhibition of microbial activity. The C/N ratio (8.2–9.9; Figure 6) and $\delta^{13}C_{OC}$ (from −27.0‰ to −28.2‰) values support this interpretation. For the IR farm, the C/N ratio coupled with the $\delta^{13}C_{TC}$ values can be useful to discriminate the differences in soil quality among the distinct fields (Figure 6). In fact, during the decomposition of the organic matter, the decreasing C/N ratio proceeds in parallel with the isotopic fractionation, as microorganisms preferentially take the lighter $^{12}C$ from the SOM, leading to a highly $^{13}C$ enriched SOM fraction [53,54]. For the IR farm, the soil samples were distributed along a trend in which the C/N ratio decreases and $\delta^{13}C_{TC}$ becomes less negative from the productive (average: −27.5‰) and grassland (average: −26.6‰) fields to the low-yield field (average: −22.3‰). For the latter, the moderately alkaline pH (7.9–8.0) enhanced the SOM mineralization processes, decreasing the SOM contents and the bioavailability of the nutrients necessary to the plants. These trends were not observed for the BT or MV farm, probably due to the low variability in soil pH. As already observed, the $\delta^{13}C_{TC}$ signature in the BT and MV soils appeared to be mainly affected by the OC/IC proportion. In the first case (BT), the soil samples cluster around

similar less negative $\delta^{13}C_{TC}$ values (from −7.9‰ to −12.2‰), reflecting the isotopic signature of the calcareous nature of the soil. On the contrary, in the second case (MV), the range of $\delta^{13}C_{TC}$ of the MV soil samples was narrow and more negative (from −21.6‰ to −24.2‰), reflecting the predominant contribution of the organic matter (and OC) of the peat.
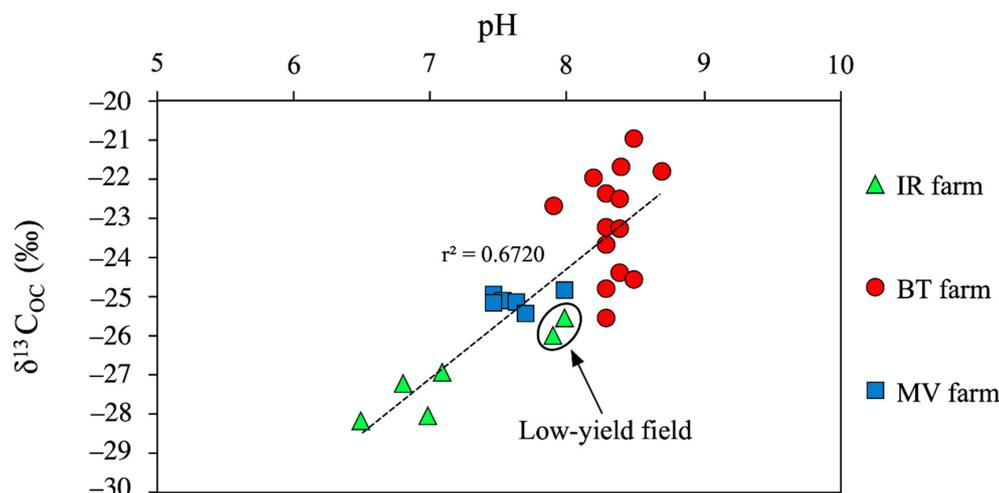


**Figure 7.** The pH versus $\delta^{13}C_{OC}$ of soils from the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. The error bars are too small (with respect to the symbol size) to be reported, and the observed difference cannot be attributed to analytical artifacts.

### 5.3. Effects of Pedoclimate Setting and Soil Management on Humic Substances

Humic substances are the largest constituents of SOM and their specific composition depends on several environmental factors, such as the climate, soil type, vegetation, and biodiversity of the microbial biomass, as well as on anthropic factors such as management practices [55]. For the studied soils, both the amount and isotopic signature of the C of the humic substances highlighted the great difference among the investigated farms, which are characterized by different pedoclimatic and agronomic settings.

In the IR farm soil, the distribution of C in the humic fractions in the topsoil followed this trend: NEOM > HA > FA. Therefore, the most abundant humic fractions were NEOM and HA, i.e., the C-compounds with a longer turnover. In comparison with the BT farm and MV farm, the humic substances of the IR farm had the most negative $\delta^{13}C$ values, indicating that the mineralization processes were particularly slow, probably inhibited by the colder climate and the less alkaline soil (see the discussion above). Comparing the three IR farm fields, the humic fractions of the low-yield field had the lowest C contents and the least negative $\delta^{13}C$ values, confirming that the mineralization rate was relatively higher here, probably due to the more alkaline conditions of the topsoil (Figure 7), as suggested by the relationships between pH and $\delta^{13}C_{OC}$ (Figure 7).

Among the investigated farms, the topsoil of the BT farm exhibits the lowest proportions of C in all the humic fractions, in accordance with the low OC amount. In addition, this is the only case-study where the HA/FA ratios are lower than 1 (0.1–0.5), due to the higher abundance of $C_{FA}$ (average $C_{FA}$: 0.44 g/kg) than $C_{HA}$ (average $C_{HA}$: 0.12 g/kg). According to the classical humification model, during humification processes, FAs are converted to HAs through condensation reactions [56–58]. Compared to FA, HA has a more complex structure, higher molecular weight and belongs to the highly stable humus [59]. Therefore, the HA/FA ratio reflects the soil humification degree [59,60]. At the BT farm, the low HA/FA ratio and the low abundance of highly stabilized organic matter (HA and NEOM) indicated that the humification processes were particularly scarce and SOM mineralization was dominant in relation to the relatively warm climate conditions and alkaline pH of the soil (see the discussion above). Besides the pedoclimatic causes, the management practices could have played an

important role in the loss of recalcitrant OC, triggering the priming effect; i.e., an increase in microbial SOM decomposition due to the continuous supply of easily available and labile C on soils [61–63]. The easily available C is used as source of energy by microorganisms, which in response mineralize less available and stabilized organic compounds, leading to a loss of C in soils [64]. In fact, at the BT farm, easily available labile C input was continuously supplied (in the form of liquid fertilizer, obtained by plant maceration) in all the investigated fields; as a consequence, the priming effect was triggered, and the topsoil recorded the lowest C contents in its humic fractions, preventing C storage in the most recalcitrant compounds (HA and NEOM). Likewise, the humic fractions were also characterized by the least negative isotopic signatures (average $\delta^{13}C_{NEOM}$: −24.2‰; average $\delta^{13}C_{HA}$: −24.7‰; average $\delta^{13}C_{FA}$: −24.2‰), as a consequence of the accelerated microbial metabolism.

Similar to the IR farm, the distribution of C in the humic fractions of the topsoil of MV followed this order: NEOM > HA > FA. However, unlike the IR farm, in all MV pear orchards, NEOM and HA had indistinguishable $\delta^{13}C$ values (~−25‰; Table 3; Figure 4), indicating that OC was transformed by the mineralization processes before being stabilized in the most recalcitrant C compounds. Therefore, despite this farm being located in an area with peat layers where organic matter is abundant, the OC neither increased nor stayed the same, but diminished over the years. This was a consequence of conventional tillage, which triggers oxidative processes favoring the degradation of organic matter. In addition, the slightly alkaline nature of the soil must be taken into account, as it probably further enhances the mineralization processes (see the discussion above).

Finally, it is important to note that NEOM was the most abundant humic fraction at all the investigated farms, independently of the pedoclimatic setting, soil management, and SOM contents. According to the literature [65–68], NEOM is the most stable humic molecule, as it is the most prone to create bonds with soil minerals, forming organic aggregates inaccessible to the decomposers, which lengthens the OC turnover in the soil.

### 5.4. Soil Organic Carbon Storage in Different Pedoclimatic and Agronomic Setting

As stated above, the OC storage in soil depends on several factors: climate conditions, soil characteristics (e.g., pH), and land use and management [9]. Therefore, in this work, which investigates the OC contents in agricultural soils of distinct pedoclimatic settings and land management types, different average OC stocks were recorded in the topsoil (0–30 cm) of the farms, increasing in the order BT < IR < MV (Table 4; Figure 8). Despite BT farm avoiding conventional tillage, its soil stored little OC (33.0–48.3 Mg/ha; Table 4; Figure 8). On the contrary, the IR farm, which practices similar management, had higher OC stocks in the soil (84.1–159.6 Mg/ha; Table 4; Figure 8). This gap is related to the different pedoclimatic conditions affecting the two areas. As discussed above, the mineralization rate of the BT soils is accelerated by the warmer climate typical of the Po Plain, and by the moderately alkaline pH of the soils. On the contrary, at the IR farm the colder climate and neutral pH of the soils tended to slow the C dynamics and, therefore, preserve the OC in the soil. Although the MV farm uses conventional tillage, it recorded very high average OC stock values (228.6–267.3 Mg/ha; Table 4; Figure 8) due to the abundance of organic matter in the form of peat layers in its soil.

Both the BT farm and MV farm showed limited differences between fields, whereas at the IR farm, the grassland and low-yield fields recorded lower average OC stocks (85.9 Mg/ha and 84.1 Mg/ha, respectively; Table 4; Figure 8) than the productive field (159.6 Mg/ha; Table 4; Figure 8). The relatively poor OC content (and OC stock) of the low-yield field with respect to the productive field was related to the moderately alkaline pH of its soil (Table 1), which enhanced the mineralization rate. Generally, the storage of OC increases from cropland to grassland [9,69,70], but, in this case, we observed the opposite trend. In fact, the IR farm avoids conventional tillage in favor of minimum tillage, which could preserve or even increase the OC in soil, reducing the disruption of soil aggregates, decreasing oxidative processes, and improving the aggregate stability.
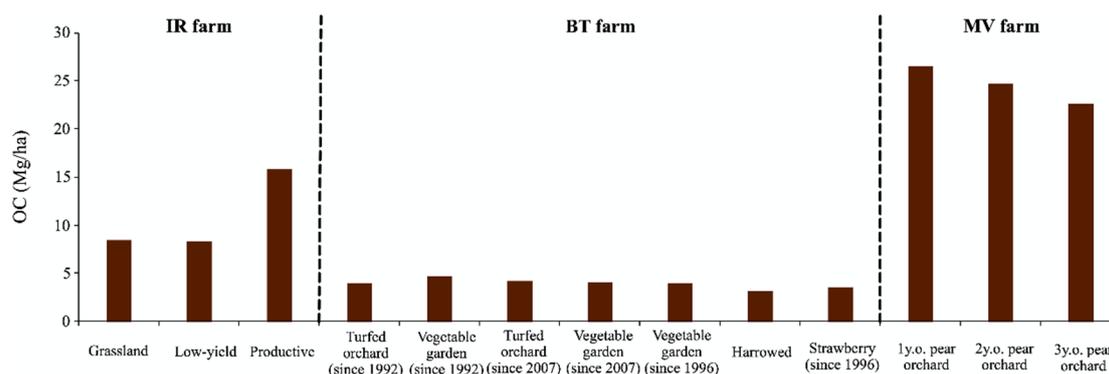
**Table 4.** Average organic carbon stock in soil collected at 0–30 cm in the different fields of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm. The respective bulk densities for each investigated area are also reported.

| Field | Bulk Density (g/cm$^3$) | OC Stock (Mg/ha) |
|---|---|---|
| **IR farm** | | |
| Grassland | | 85.9 |
| Low-yield | 1.24 | 84.1 |
| Productive | | 159.6 |
| **BT farm** | | |
| Turfed orchard (since 1992) | | 40.3 |
| Vegetable garden (since 1992) | | 48.3 |
| Turfed orchard (since 2007) | | 43.7 |
| Vegetable garden (since 2007) | 1.40 | 41.4 |
| Vegetable garden (since 1996) | | 40.1 |
| Harrowed | | 33.0 |
| Strawberry (since 1996) | | 37.4 |
| **MV farm** | | |
| 1-year-old pear orchard nursery | | 267.3 |
| 2-year-old pear orchard nursery | 1.13 | 248.0 |
| 3-year-old pear orchard nursery | | 228.6 |



**Figure 8.** Average organic carbon (OC) stock from soils collected at 0–30 cm in the different fields of the I Rodi (IR) farm, Tassinari Carla (BT) farm, and Maccanti Vivai (MV) farm.

## 6. Conclusions

The interest in soil organic carbon (SOC) and agricultural best-practices is growing at a global level, as carbon is crucial for maintaining soil quality and mitigating climate change. It must be taken into account that the potential C sequestration of sustainable soil management also depends on the soil type and climate features of the investigated areas.

This study gives an overview of the SOC dynamics of the three farms in the Emilia-Romagna region (Northern Italy) selected for their pedoclimatic settings and management. For the organic I Rodi farm, located in the Apennines, the minimum soil disturbance coupled with favorable pedoclimatic conditions (i.e., cold climate and neutral pH of soil) allowed for sequestration of C, mainly in the form of stable recalcitrant C compounds, limiting the mineralization rate (C/N: 7.6–9.9) and the decomposition of organic matter (OC: 1.77–4.42 wt%; C stock: 84–160 Mg/ha). On the other hand, agricultural soils of the biodynamic Tassinari Carla farm on the Po Plain were affected by a warmer climate and moderately alkaline soil pH, which enhance the mineralization rate (C/N: 6.8–8.9) and the organic carbon depletion (OC: 0.75–1.16 wt%; C stock: 33–48 Mg/ha), despite the adoption of minimum tillage and biological practices. At the Maccanti Vivai farm, conventional tillage triggers oxidative processes, enhancing the degradation of organic matter (OC: 6.73–8.11 wt%; C stock: 228.6–267.3 Mg/ha) and consequently lowering the C/N ratio (10.6–12.0), which should be much higher considering

the abundance of organic carbon in the form of peat layers. Therefore, this work evidenced that agriculture in mountainous areas, although difficult, should be promoted and encouraged. If properly managed (also preventing erosion), mountain soil represents an important C sink, thus having a high C sequestration potential. For the investigated farms located on the plain, where C preservation is critical, we suggest the following specific improvements: (1) the Tassinari Carla farm needs to define a strategy to enhance humification processes and convert labile organic C compounds into more stable ones (NEOM and humic acids), likely incorporating in the soil more recalcitrant organic matter resistant to microbial attack; (2) the Maccanti Vivai farm should practice conservation strategies, in order to curb the OC depletion.

In our view, elemental and isotopic analyses of all C fractions are useful to evaluate the current carbon stock of soil and the SOM evolution to assess the C sequestration and/or GHG release in relation to soil management. Similar studies should also be repeated in the future, possibly involving additional farms, to form a regional soil geochemical archive, a useful tool to identify representative trends in soil dynamics and their consequences for the environment.

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