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Changes in Soil Quality through Conservation Agriculture in North-Eastern Italy

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Abstract: Conservation Agriculture includes practices focused on the conservation and the restoration of main soil features, such as organic carbon content, structure, and biological diversity and activity. Our study was conducted in three farms in North-Eastern Italy in pairs of closely located fields to compare conservation agriculture (no tillage, cover cropping) with conventional agriculture. Differences in terms of soil enzymatic activity, such as FDA and β-glucosidase through spectrophotometric analyses, microbial biomass carbon and nitrogen contents, total organic carbon, and nitrogen contents with CNS Elemental Analyzer and soil arthropod community via the QBS-ar index were investigated. Enzymatic activities resulted to be readily and positively affected by conservation agriculture whereas total and microbial carbon, nitrogen contents, and microarthropod community seemed to be more dependent on the time factor. The responses to conservation agriculture differed between the three farms, pointing out that differences in soil features may drive the effectiveness of conservation management. N stock, maybe dependent on previous soil management, might be the key characteristic able to influence soil evolution in the studied conditions. The present results could be helpful to predict soil reaction to sustainable agriculture in short periods.

Keywords: crop rotation; no tillage; soil quality; QBS-ar; conventional/conservation agriculture; soil biodiversity

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1. Introduction

Conservation Agriculture (CA) is a system of agronomic practices initially conceived as a countermeasure to the US "Dust Bowl" phenomenon [1]. Since then, CA reached Europe starting from the 1970s with marked differences from country to country [2]. In particular, in Italy, the introduction and the dissemination of "Agricoltura Blu" principles [3] encouraged a larger diffusion among farmers [2].

The global evidence shows that CA is now a worldwide phenomenon. It is estimated that in 2015/16 CA covered 180 million hectares worldwide, representing 12.5% of total cropland [4]. Compared to other regions, Europe is lagging behind in terms of the adoption of CA with approximately 1.36 million ha (not including Russia) under CA, which corresponds to approximately 2% of the global arable cropland [5]. In Italy, in 2015, 380,000 hectares were reported to be managed according to CA guidelines [6].

In general, CA consists of: (1) reduction in tillage to achieve non inversion of the topsoil layer, involving controlled tillage seeding systems to disturb no more than 20–25% of the soil surface; (2) permanent soil cover by main and cover crops, also by crop residues, aiming at protecting the soil from wind and water erosion, while improving its physical, chemical, and biological properties; (3) crop rotations, including cover crops to help mitigate

disease, pest and weed problems, and to provide farmers with economically viable cropping options [7]. According to FAO, this approach improves productivity, profits, and food security while preserving and enhancing the resource base and the environment [8]. CA is deemed to be practiced only when all three principles are meticulously applied [9].

Despite long-term research on CA, many aspects are still debated with respect to its effects on crop yields [10,11], its influence on carbon sequestration [12], its applicability in different farming contexts [13], and its impact on soil biodiversity [7]. Advantages of CA including nutrient cycling, carbon sequestration, and soil biodiversity are quite variable, from positive, to neutral or even negative [14] depending on site-specific context, management, soil type, and climate [13]. To increase the adoption of CA worldwide, it is critical to adapt the system to specific climates, soil types, and communities [15].

Although soil organic matter (SOM) changes related to CA management are the most studied effects in the literature, soil biological quality and biodiversity also deserve research attention [16]. The contribution of soil organisms to decomposition, nutrient cycling, maintenance of soil physico-chemistry and structure properties, and as bioindicators of soil health is well recognized [17,18].

Microbial community access to organic matter is greater with tillage as organic residues are broken into smaller pieces, which increases the available surface area for microbial colonization [19]. On the other hand, the no-till soil microclimate is cooler and moister than in soils which undergo tillage [20]. Cover crops can minimize soil erosion, promote nutrient cycling, prevent water loss, and increase soil organic C and biological activity [21]. Finally, crop rotations influence the type and quantity of crop residues being returned to the soils; thus, shaping the microbial community. In a 3-year field study, Fiorini et al. [22] evaluated the effects of selected winter cover crops under NT on grain yield of maize and soybean, cumulative biomass, C, and N input to the soil, as well as soil C pools and biodiversity in an intensive crop production system. The authors suggested that the inclusion of extra-biomass amount into the soil with leguminous-based cover crops may positively affect soil biodiversity. Therefore, they suggested that properties of leguminous biomass could be considered efficient drivers to define the complexity of arthropod and earthworm communities. As reported in Pittarello et al. [23] main crops and cover crops can have an important and variable influence on microbial activity depending on the mix of species employed. Microbial biomass and enzymatic activities are commonly used as a tool for soil quality detection [24]. Among soil enzymatic activities, fluorescein diacetate (FDA) hydrolase and β-glucosidase are involved in the decomposition of complex organic compounds and are correlated with fungal and microbial biomass [25]. The activities of these enzymes have been widely used as response indicators of soil microbial communities to several environmental pressures [26]. Considering the complex interactions that take place in soil, microbial biomass is affected, directly and indirectly, by the soil fauna community. Soil fauna plays an important role in maintaining soil quality and health, as well as providing ecosystem services [27] through processes such as organic matter translocation, fragmentation and decomposition, nutrient cycling, soil structure formation and, consequently, water regulation [17,18]. Some groups are highly sensitive to changes in soil quality because they are adapted to specific soil conditions [28] and may be informative of soil quality alteration [29]. Beyond its important role in maintaining soil functionality and providing ecosystem services, soil fauna has been included in monitoring plans as indicators of soil health and biological quality [17].

The Po valley is the most exploited Italian area in terms of intensive agriculture and urbanization [30]. In this context, CA could mitigate the environmental impact of crop production, in particular in terms of soil dynamics such as organic carbon accumulation and changes in microbial populations [31]. In a previous study [32], results on soil organic carbon (SOC) evolution over a 3-year transition period from conventional to CA in three field experiments located in the low plain of the Veneto Region has been reported. The research highlighted that CA did not affect the overall SOC stock but altered its stratification within the profile by avoiding soil inversion and influencing the root growth and pattern.

In the same experimental fields, we determined soil enzyme activities, soil arthropod community (QBS-ar index), microbial biomass C and N over the same time frame.

As reported by Li et al. [33] although main trends are identified in terms of SOC accumulation and microbial community evolution, some factors make clear data interpretation difficult, such as variations in cropping intensity and crop species diversity. In particular, the effects of crop rotations and CA are still poorly explored in this region, while coupling soil enzyme activities with arthropod community to monitor soil biodiversity represents a novel approach. In the present paper, two hypotheses were explored: (I) Conservative Agriculture (CA) management can ameliorate soil biological parameters independently of the cultivated crop species and (II) standardized CA management applied to different farms in the same geographical area can give the same soil evolution and/or restoration outcomes in terms of soil biodiversity. To test these hypotheses, the same pool of crop and cover crop species were rotated in the same fields over three consecutive years, in three North-Eastern Italian Farms. The main objective was to monitor soil responses to the newly adopted conservative management starting from a conventional farming regime, with the secondary objective to correlate soil enzyme activities and arthropod community index.

2. Materials and Methods

2.1. Experimental Sites

The study was conducted in three farms located in North-Eastern Italy: 'Vallevecchia', placed on the Adriatic coast (45°38′350″ N 12°57′245″ E, −2 m m.a.s.l.), is characterized by a silty-clay/sandy-loam soil classified as Gleyic Fluvisols or Endogleyic Fluvic Cambisols [31]; 'Diana' and 'Sasse Rami', are located on the western, on the central (45°34′965″ N 12°18′464″ E, 6 m m.a.s.l.), and southern plain (45°2′908″ N 11°52′872″ E, 2 m m.a.s.l.), respectively. Both are characterized by Endogleyic Cambisols [34] silty-loam soil.

The average annual rainfall, typical of sub-humid climate, was 829 mm in Vallevecchia, 846 mm in Diana, and 673 mm in Sasse Rami. The highest rainfall peaks are in autumn (302, 241, and 187 mm, respectively) whereas the lowest in winter (190, 157, and 129 mm, respectively). Temperatures varied from a minimum of −0.1 °C in January to a maximum of 29.6 °C in July, from −0.9 to 29.3 °C and from −0.2 to 30.6 °C, respectively. Reference evapotranspiration (ETo) was 860, 816, and 848 mm, with a peak in July (4.9, 4.6, and 4.8 mm d^{-1}). ETo exceeded rainfall from May to September in Vallevecchia and Diana and from May to October in Sasse Rami.

Total organic carbon (TOC) and total nitrogen (TN) contents have been evaluated in the three farms in 2011, before the experimental period (Table 1) following the sampling scheme and analytic protocols described in the next paragraphs.

Table 1. Soil parameters of the three farms in 2011, localization of places, and climate parameters during the experiment.

	Latitude and Longitude	A.A.R.	A.T.R.	TOC	TN
		mm	°C	%	
Diana	45°34′965″ N 12°18′464″ E	846	−0.9/29.3	0.90 ± 0.04 a	0.13 ± 0.01 b
Sasse Rami	45°2′908″ N 11°52′872″ E	673	−0.2/30.3	1.03 ± 0.06 a	0.16 ± 0.01 a
Vallevecchia	45°38′350″ N 12°57′245″ E	829	−0.1/29.6	0.92 ± 0.06 a	0.12 ± 0.01 b

A.A.R = average annual rainfall. A.T.R = annual temperature range. Values are the total average of both CONV and CONS fields ± their standard errors. Different letters mean significant differences (Tuckey test $p < 0.05$).

2.2. Experimental Design

In 2011, field research started to assess agronomic and environmental effects of the Rural Development Programme (RDP), Measure 214—Sub-Measure I, 'Eco-compatible management of agricultural lands' protocol implementation. This measure was economically supported by the Veneto Region (Regione Veneto, Italy, 2013). Each farm was managed both following conservative agriculture (CONS) principles and following conventional

agricultural (CONV) practices for the area. Pairs of closely located fields (1–1.5 hectares in size), selected to be homogeneous in terms of pedological characteristics, were chosen in each farm, repeated for each crop (wheat, canola, maize, soybean). The whole experimental area was constituted by a total of 24 (2 treatments × 4 crops × 3 farms) rectangular (40 × 30 m) fields, with the CONS and CONV adjacent theses. The fields managed through Conventional Agriculture (CONV) were considered as the control.

CONV fields were ploughed at a 35 cm depth and crop residues were buried and seedbed prepared by <15 cm depth chiseling. In CONS fields, no-tillage was associated to cover crop suppression, sod seeding both for main and cover crops leaving on the surface all residues after harvesting.

Crop rotation was as follows in both CONV and CONS: wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.), maize (*Zea mays* L.), and soybean (*Glycine max* (L.) Merr.). In CONV, the soil remained bare between the main crops. Conversely, in CONS, the main crops were interspersed with sorghum (*Sorghum vulgare* Pers. Var. sudanense) during spring–summer, and a mix of vetch (*Vicia sativa* L.) and barley (*Hordeum vulgare* L.) in autumn–winter.

Both groups of fields were locally supplied with mineral fertilizers in pre-sowing of all crops, integrated with a side dressing treatment in maize and wheat. In agreement with the sub-measure protocol, cover crops were not fertilized.

The same amount of herbicide was applied for CONV and CONS depending on crop, according to IPM implementation. CONS winter cover crops were suppressed before spring seeding, adding *N*-(phosphonomethyl) glycine before spring seeding, whereas sorghum was terminated by shredding.

2.3. Soil Sampling

Sampling for TOC, TN, microbial biomass, enzyme activities, and soil arthropods extraction was performed in 2012, 2013, and 2014, in May in soils cultivated with soybean and wheat; in September for the soils cultivated with maize and oilseed rape. The two sampling periods were chosen to minimize soil disturbance.

Soil samples were collected in 6 positions per field according to a systematic sampling scheme in the 0–20 cm profile, using a soil auger. The same points were sampled in the three campaigns, identifying the positions using a GNNS with Real Time Kinematic (RTK) correction (precision of ca. 2 cm). For each experimental field, two bulked samples were obtained for enzyme activities analyses.

2.4. Total Organic Carbon and Nitrogen

A total of 24 samples were analyzed for TOC and TN in 2011, one bucked sample per field. A total of 144 samples were analyzed for the years 2012–2014. Samples were weighed, air-dried, and sieved at 2 mm for total C and N determination using the flash combustion method using a CNS Elemental Analyzer (Vario Max, Elementar Americas, Inc., Langenselbold, Germany); organic carbon was determined after removal of organic C with acid pre-treatment [32]. TOC and TN were calculated as a difference between total and inorganic values.

2.5. Microbial C and N and Enzymatic Activities Determination

A total of 144 samples were analyzed for microbial biomass, 2 bulked samples per field × 2 treatments (i.e., CONS and CONV) × 4 crops × 3 farms × 3 years (2012, 2013 and 2014).

The microbial biomass-C and -N (micr C and micr N) contents were determined by the fumigation-extraction method [35] as reported in [36]. For each sample three fumigated and three non-fumigated aliquots were analyzed. Fumigation was performed under vacuum in a glass desiccator containing 3 mL H_2O, 2 g of NaOH pellets and a beaker with 50 mL of chloroform. After 16 h of incubation in the dark, fumigated soil was transferred to a centrifuge tube with 0.5 mol L^{-1} K_2SO_4 at a 1:4 w/v ratio. After 30 min of rotatory

shaking at 120 rpm, the sample was centrifuged for 5 min at 6500× *g* and the supernatant filtered through Whatman no. 4 filters and kept in polyethene tubes at −20 °C until micr C and micr N determination. Micr C was measured by oxidation with potassium dichromate and titration with ammonium iron(II) sulfate [37]. Micr N was determined spectrophotometrically following the method of Cabrera et al. [38].

The fluorescein diacetate hydrolase (FDA) was assessed [39] on a 2 g soil sample shaken with 15 mL of 60 mmol L^{-1} potassium phosphate pH 7.6 buffer and 0.2 mL of 1000 g mL^{-1} FDA solution for 20 min at 30 °C. Then, 15 mL of 2:1 chloroform/methanol solution was added, and the contents were shaken thoroughly by hand before filtration (Whatman 2). The absorbance was measured spectrophotometrically at 490 nm on the filtrate. The concentration was expressed in g of fluorescein diacetate released g^{-1} dry soil h^{-1}.

β-glucosidase (EC 3.2.1.21, β-D-glucoside glucohydrolase) activity was determined according to official Italian methods for soil analysis [40] and reported as µg p-nitrophenol (pNP) g^{-1} dry soil. For the determination of β-glucosidase activity, 4 mL of modified universal buffer (MUB), pH 6, and 1 mL of substrate p-nitrophenyl-b-D-glucopyranoside (PNG) 0.025 mol L^{-1} were added to 1 g of soil and incubated at 37 °C for 1 h. The reaction was stopped by adding 1 mL 0.5 mol L^{-1} $CaCl_2$ and 4 mL 0.1 mol L^{-1} TRIS-NaOH pH 12 buffer [41]. The p-nitrophenol (pNP) formed in β-glucosidase activity was determined by spectrophotometry at 400 nm. Enzyme activities were determined in triplicate on fresh moist sieved (<2 mm) soils within 10 days of sample collection. Control assays were performed by adding the substrate after the reaction was stopped and before filtration of the soil suspension. All data were corrected to soil dry weight by means of soil moisture content data. Standard error within triplicate measures was always below 5%.

2.6. Soil Arthropod Extraction and QBS-ar Application

For the soil arthropod study, soil samples (dimensions 10 cm × 10 cm × 10 cm) were collected in 6 RTK referenced points of the field, placed in plastic bags, kept oxygenated, and brought to the laboratory. A total of 144 samples per year (2012, 2013, and 2014) were analyzed for arthropod extractions: 6 samples per field × 2 treatments × 4 crops.

In the lab, soil arthropods were extracted with the Berlese-Tüllgren extractor [42]. Following the QBS-ar protocol, an ecomorphological score (EMI) was assigned to each taxon found, ranging between 1 and 20 depending on their adaptation to soil (1: low adaptation; 20: maximum adaptation) [29,42]. For each replicate, the QBS-ar value was calculated as the result of the sum of the highest EMI values for each taxon [29] considering that QBS-ar is based on the different adaptation level to soil of edaphic arthropods [27,29]. QBS-ar has been applied in different land uses as bioindicator of soil health, e.g., during restoration processes [43]; in natural and degraded soils in Mediterranean areas [44]; and in roadside anthropogenic soils [45]. Furthermore, it can be used as an indicator of anthropogenic impact on soil resources caused by land use practices [46].

2.7. Statistical Analysis

One-way repeated measures ANOVA was performed to compare treatments among the sampling periods; Tukey's t test was performed for post-hoc comparisons. Levene and Mauchly's tests were applied to check normality, homoscedasticity, and sphericity, to ensure that assumptions of the model were met. All these procedures were performed using IBM SPSS 27.0 (IBM Corp., Armonk, New York, NY, USA, 2020).

3. Results

3.1. Total Organic Carbon and Nitrogen

Considering the three farms at time 0 (2011) separately, TOC percentages were comprised between 0.90% and 1.03%, and TN between 0.12% and 0.16% (Table 1). Statistical analysis highlighted a significant difference: Sasse Rami showed higher TN percentage compared to Diana and Vallevecchia farms (Table 1).

The aggregated dataset from the three farms (Table 2) shows the following scenario: TOC and TN were not significantly influenced by Management, but the interaction between Management and Time affected TN significantly.

Table 2. Aggregated data of macronutrients, enzymatic activities, BF, and QBS-ar rate.

						p Value		
Parameters	**Management**	**2012**	**2013**	**2014**	**Average**	**M**	**T**	**M ∗ T**
TOC (%)	CONS	0.999 ± 0.04	0.991 ± 0.04	0.961 ± 0.05	0.984 ± 0.02	0.305	0.533	0.487
	CONV	0.924 ± 0.04	0.886 ± 0.04	0.914 ± 0.05	0.908 ± 0.03			
TN (%)	CONS	0.131 ± 0.01	0.125 ± 0.01	0.122 ± 0.01	0.126 ± 0.004	0.560	0.646	0.020 *
	CONV	0.119 ± 0.01	0.119 ± 0.01	0.125 ± 0.01	0.121 ± 0.003			
Microbial C (mg kg_{ds}^{-1})	CONS	205.5 ± 21.8	122.7 ± 8.7	199 ± 19.2	175.7 ± 10.9	0.423	<0.0001 *	0.723
	CONV	174.5 ± 20.9	105.1 ± 7.1	193 ± 26.7	157.5 ± 12.2			
Microbial N (mg kg_{ds}^{-1})	CONS	14.9 ± 1.6	11.5 ± 1.1	19.4 ± 1.9	15.3 ± 1	0.413	<0.0001 *	0.597
	CONV	13.8 ± 1.4	11.5 ± 1.8	16.7 ± 1.4	14 ± 0.9			
FDA (μg FDA g_{ds}^{-1} h^{-1})	CONS	0.94 ± 0.09	1.127 ± 0.09	0.99 ± 0.07	1.02 ± 0.05	0.007 *	0.224	0.534
	CONV	0.74 ± 0.10	0.79 ± 0.07	0.79 ± 0.11	0.77 ± 0.05			
β-glucosidase (μg PNP g_{ds}^{-1} h^{-1})	CONS	79 ± 6.9	85.4 ± 4.9	88.7 ± 7.5	84.4 ± 3.7	0.002 *	0.042 *	0.977
	CONV	52 ± 4.5	59 ± 2.9	63.4 ± 3.1	58.1 ± 2.1			
BF (Number of biological forms)	CONS	8.8 ± 0.4	7.8 ± 0.3	7.6 ± 0.3	8.1 ± 0.2	0.055	0.094	0.214
	CONV	7.1 ± 0.4	6.4 ± 0.3	6.8 ± 0.3	6.8 ± 0.2			
QBS.ar (Highest EMI scores summary)	CONS	111.6 ± 5	82.1 ± 3.5	81.5 ± 3.5	92.4 ± 2.6	0.103	<0.0001 *	0.160
	CONV	88.9 ± 4.6	71.6 ± 4.2	75.1 ± 4.2	79.7 ± 2.6			

TN = Total Organic Nitrogen; TOC = Total Organic Carbon, both reported in % (*w*/*w*). Microbial C and N are measured through mg kg^{-1} of dry soil. β-glucosidase and FDA activities are reported as μg of p-nitrophenol (pNP) g^{-1} dry soil released per hour and μg of fluorescein-diacetate (FDA) g^{-1} dry soil released per hour, respectively. BF = number of biological forms among microarthropods encountered. QBS-ar = soil biological quality index. T = Time factor; M = Management factor; T ∗ M: interaction between Time and Management. Significant *p* values are reported with asterisk. $p < 0.05$. Values are reported ± standard errors.

Considering the three farms separately (Tables 3–5), only TN Vallevecchia farm was significantly influenced by Time and by the interaction of Management and Time (Table 5).

Table 3. Soil evolution in Diana farm.

			Diana			M	T	M ∗ T
	Management	**2012**	**2013**	**2014**	**M Average**		***p* Value**	
TOC	CONS	0.92 ± 0.05	0.89 ± 0.06	0.93 ± 0.06	0.91 ± 0.03	0.135	0.182	0.519
	CONV	0.81 ± 0.02	0.75 ± 0.04	0.88 ± 0.05	0.81 ± 0.02			
	T average	0.86 ± 0.03	0.82 ± 0.04	0.90 ± 0.04				
TN	CONS	0.13 ± 0.01	0.13 ± 0.00	0.14 ± 0.01	0.13 ± 0.003	0.1506	0.2783	0.428
	CONV	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.01	0.12 ± 0.002			
	T average	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.01				
Microbial C (mg kg_{ds}^{-1})	CONS	217.5 ± 21.9	150.1 ± 8.7	242.5 ± 23.4	203.4 ± 13.3	0.5617	0.0001 *	0.2243
	CONV	221.1 ± 39.5	128.5 ± 8.9	181.9 ± 36.6	177.2 ± 19.1			
	T average	219.3 ± 21.8	139.3 ± 6.6	212.2 ± 22.4				
Microbial N (mg kg_{ds}^{-1})	CONS	17.5 ± 4	10.6 ± 2.2	21.6 ± 1.6	16.6 ± 1.8	0.9713	0.0001 *	0.9132
	CONV	17.2 ± 1.9	11.4 ± 2.2	20.7 ± 2.5	16.4 ± 1.5			
	T average	17.4 ± 2.1	11 ± 1.5	21.2 ± 1.4				
FDA (μg FDA g_{ds}^{-1} h^{-1})	CONS	1.007 ± 0.15	1.59 ± 0.11	1.10 ± 0.15	1.23 ± 0.09	0.0936	0.0028 *	0.3637
	CONV	0.729 ± 0.13	1.09 ± 0.08	0.97 ± 0.18	0.93 ± 0.08			
	T average	0.868 ± 0.10	1.34 ± 0.09	1.04 ± 0.12				
β-glucosidase (μg PNP g_{ds}^{-1} h^{-1})	CONS	88.5 ± 11.9	101.5 ± 4.3	65.4 ± 7.9	85.1 ± 5.7	0.1121	0.012 *	0.0013 *
	CONV	60.1 ± 4.9	68.1 ± 4.2	71 ± 3.1	66.4 ± 2.5			
	T average	74.3 ± 7.2	84.8 ± 5.2	68.2 ± 4.2				

Table 3. *Cont.*

		Diana				M	T	M ∗ T
	Management	2012	2013	2014	M Average		*p* Value	
BF (Number of biological forms)	CONS	8.42 ± 0.46	7.5 ± 0.39	7.58 ± 0.42	7.83 ± 0.25	0.0941	0.2730	0.4371
	CONV	7.13 ± 0.52	6.5 ± 0.57	7.5 ± 0.54	7.04 ± 0.31			
	T average	7.77 ± 0.36	7 ± 0.35	7.54 ± 0.34				
QBS.ar (Highest EMI scores summary)	CONS	113.7 ± 6.8	84 ± 4.3	83.8 ± 5.2	93.8 ± 3.6	0.4850	0.0001 *	0.3117
	CONV	96.9 ± 7.4	77.7 ± 7	84.9 ± 6.3	86.5 ± 4.1			
	T average	105.3 ± 5.1	80.8 ± 4.1	84.4 ± 4				

β-glucosidase and FDA activities are reported as µg of p-nitrophenol (pNP) g^{-1} dry soil released per hour and µg of fluorescein-diacetate (FDA) g^{-1} dry soil released per hour, respectively. T average = average of the year; M average = average of the treatment. T = Time factor; M = Management factor; T ∗ M: interaction between Time and Management. Significant *p* values are reported with asterisk. $p < 0.05$. Values are reported ± standard errors.

Table 4. Soil evolution in Sasse Rami farm.

		Sasse Rami				M	T	M ∗ T
	Management	2012	2013	2014	M Average		*p* Value	
TOC	CONS	1.12 ± 0.07	1.08 ± 0.07	1.08 ± 0.08	1.09 ± 0.04	0.5707	0.3533	0.9787
	CONV	1.05 ± 0.05	0.99 ± 0.06	1.01 ± 0.07	1.02 ± 0.03			
	T average	1.09 ± 0.04	1.03 ± 0.05	1.05 ± 0.05				
TN	CONS	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.6047	0.8521	0.2332
	CONV	0.13 ± 0.01	0.14 ± 0.01	0.141 ± 0.01	0.14 ± 0.01			
	T average	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01				
Microbial C (mg kg_{ds}^{-1})	CONS	304.2 ± 30.3	145.7 ± 9.5	178.4 ± 20.7	209.4 ± 18.7	0.1201	<0.0001 *	0.0806
	CONV	203.6 ± 37.7	108 ± 8.2	182.9 ± 22.7	164.9 ± 16.6			
	T average	253.9 ± 26.7	126.9 ± 7.7	180.7 ± 14.9				
Microbial N (mg kg_{ds}^{-1})	CONS	15.1 ± 2.3	14.1 ± 1.7	24.7 ± 4.4	18 ± 2	0.3158	0.037 *	0.5138
	CONV	14.9 ± 3	12.9 ± 3.9	17.9 ± 2.1	15.2 ± 1.8			
	T average	15 ± 1.8	13.5 ± 2.1	21.3 ± 2.5				
FDA (µg FDA g_{ds}^{-1} h^{-1})	CONS	0.63 ± 0.13	0.72 ± 0.08	0.96 ± 0.12	0.77 ± 0.07	0.077	0.0013 *	0.2045
	CONV	0.29 ± 0.03	0.44 ± 0.06	0.46 ± 0.13	0.39 ± 0.05			
	T average	0.46 ± 0.08	0.58 ± 0.06	0.71 ± 0.11				
β-glucosidase (µg PNP g_{ds}^{-1} h^{-1})	CONS	83.9 ± 10.1	90.5 ± 8.2	125.2 ± 9.6	99.9 ± 6.4	0.002 *	0.0003 *	0.0201 *
	CONV	39.5 ± 4.6	59.2 ± 5.2	56.3 ± 4	51.7 ± 3.1			
	T average	61.7 ± 7.9	74.9 ± 6.2	90.8 ± 10.2				
BF (Number of biological forms)	CONS	9.92 ± 0.68	8 ± 0.52	9.06 ± 0.43	9.08 ± 0.35	0.0418 *	0.1281	0.4998
	CONV	6.96 ± 0.64	6.56 ± 0.4	6.35 ± 0.46	6.66 ± 0.31			
	T average	8.44 ± 0.51	7.28 ± 0.34	7.71 ± 0.39				
QBS.ar (Highest EMI scores summary)	CONS	134.1 ± 9.3	86.4 ± 6.9	106.7 ± 5.6	111.7 ± 5.3	0.0399 *	<0.0001 *	0.4808
	CONV	94.9 ± 8.5	67.1 ± 6.4	70.1 ± 5.9	79.3 ± 4.6			
	T average	114.5 ± 6.9	76.8 ± 4.9	88.4 ± 5.1				

β-glucosidase and FDA activities are reported as µg of p-nitrophenol (pNP) g^{-1} dry soil released per hour and µg of fluorescein-diacetate (FDA) g^{-1} dry soil released per hour, respectively. T average = average of the year; M average = average of the treatment. T = Time factor; M = Management factor; T ∗ M: interaction between Time and Management. Significant *p* values are reported with asterisk. $p < 0.05$. Values are reported ± standard errors.

3.2. Microbial C and N and Enzymatic Activities

Considering the aggregated dataset from the three farms (Table 2), microbial C was comprised between 105.1 mg kg_{ds}^{-1} in CONV 2013 and 205.5 mg kg_{ds}^{-1} in CONS 2012, while microbial N ranged between 11.5 mg kg_{ds}^{-1} in CONS/CONV 2013 and 19.4 mg kg_{ds}^{-1} in CONS 2014. Both microbial C and N were influenced only by the time factor (T) (Table 2). This result emerged also when the three farms were considered separately (Tables 3 and 4), except for Vallevecchia farm for microbial N (Table 5).

FDA showed higher values in CONS for all the three years when compared with CONV, and it was comprised between 0.74 µg FDA g_{ds}^{-1} h^{-1} in CONV 2012 and 1.127 µg FDA g_{ds}^{-1} h^{-1} in CONS 2013. Management significantly affected this parameter. β-glucosidase activity, comprised between 52 µg PNP g_{ds}^{-1} h^{-1} in CONV 2012 and 88.7 µg

PNP g_{ds}^{-1} h^{-1} in CONS 2014, was significantly increased by CONS management and affected by Time.

Table 5. Soil evolution in Vallevecchia farm.

		Vallevecchia				M	T	M ∗ T
	Management	**2012**	**2013**	**2014**	**M Average**		***p* Value**	
TOC	CONS	0.95 ± 0.08	1.01 ± 0.06	0.87 ± 0.09	0.94 ± 0.05	0.1052	0.7775	0.719
	CONV	0.91 ± 0.10	0.91 ± 0.09	0.85 ± 0.13	0.89 ± 0.06			
	T average	0.93 ± 0.06	0.96 ± 0.05	0.86 ± 0.08				
TN	CONS	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.1 ± 0.01	0.7952	0.0248 *	0.0448 *
	CONV	0.108 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01			
	T average	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01				
Microbial C (mg kg_{ds}^{-1})	CONS	94.7 ± 10.5	72.2 ± 6.5	176 ± 47.3	114.3 ± 18.1	0.7395	0.0009 *	0.827
	CONV	98.6 ± 9.4	78.9 ± 13.2	214.3 ± 71.1	130.6 ± 26.3			
	T average	96.7 ± 6.8	75.5 ± 7.1	195.2 ± 41.5				
Microbial N (mg kg_{ds}^{-1})	CONS	12 ± 1.5	9.8 ± 1.5	12 ± 1.2	11.3 ± 0.8	0.6284	0.6647	0.7023
	CONV	9.4 ± 1.7	10.3 ± 3.2	11.5 ± 1.7	10.4 ± 1.3			
	T average	10.7 ± 1.2	10 ± 1.7	11.7 ± 1				
FDA (μg FDA g_{ds}^{-1} h^{-1})	CONS	1.17 ± 0.15	1.07 ± 0.06	0.92 ± 0.08	1.05 ± 0.06	0.5321	0.0779	0.4961
	CONV	1.20 ± 0.12	0.84 ± 0.06	0.92 ± 0.20	0.99 ± 0.08			
	T average	1.18 ± 0.09	0.95 ± 0.05	0.92 ± 0.10				
β-glucosidase (μg PNP g_{ds}^{-1} h^{-1})	CONS	64.7 ± 13.1	64.1 ± 6.6	75.4 ± 10.5	68.1 ± 5.9	0.4356	0.2141	0.9043
	CONV	56.3 ± 10.8	49.6 ± 3.9	62.9 ± 7.5	56.3 ± 4.5			
	T average	60.5 ± 8.3	56.8 ± 4.2	69.2 ± 6.4				
BF (Number of biological forms)	CONS	8.13 ± 0.61	8.04 ± 0.72	6.67 ± 0.37	7.61 ± 0.34	0.9548	0.1580	0.0637
	CONV	7.08 ± 0.66	6 ± 0.62	5.9 ± 0.74	6.54 ± 0.41			
	T average	7.6 ± 0.45	7.36 ± 0.54	6.44 ± 0.34				
QBS.ar (Highest EMI scores summary)	CONS	86.8 ± 7.2	77 ± 7	61.5 ± 3.8	75.1 ± 3.7	0.8298	0.0337 *	0.0144 *
	CONV	75 ± 7.5	66.3 ± 7.8	60.1 ± 10.3	69.5 ± 4.9			
	T average	80.9 ± 5.2	73.4 ± 5.4	61.1 ± 4				

β-glucosidase and FDA activities are reported as μg of p-nitrophenol (pNP) g^{-1} dry soil released per hour and μg of fluorescein-diacetate (FDA) g^{-1} dry soil released per hour, respectively. T average = average of the year; M average = average of the treatment. T = Time factor; M = Management factor; T ∗ M: interaction between Time and Management. Significant *p* values are reported with asterisk. $p < 0.05$. Values are reported ± standard errors.

The results of each farm show that significant differences are not equally distributed (Tables 3–5). Time and/or the interaction of Management per Time significantly influenced β-glucosidase activity and FDA in Diana and Sasse Rami farms (Tables 3 and 4). In this farm, β-glucosidase was affected also by Management. Vallevecchia did not show to be affected by both Management, Time, and by the interaction between these two parameters (Table 5).

3.3. Soil Arthropod and QBS-ar Index

The number of biological forms (BF) was not affected neither by Management nor by Time (Table 2) considering the overall dataset, but some differences emerged when the three farms were considered separately and only Sasse Rami was influenced by Management, showing higher values in CONS when compared with CONV (Table 4).

Differently from FB, QBS-ar index was influenced by the Time factor (Table 2) considering all data together, and some similitudes emerged between the three farms, all three being influenced by Time. In addition, Sasse Rami showed to be influenced by Management (Table 4), in favor of CONS, and Vallevecchia by the interaction between Management per Time (Table 5).

4. Discussion

Results for SOM parameters (Table 2) such as TOC and TN were not significantly influenced in the three years of CONS management, consistent with the data reported by Muscolo et al. [47] while Rakesh et al. [48] in a 4-year-long experiment found a significant TOC improvement compared to conventionally managed soils. Microbial biomass and soil

enzymatic activities were found to be more reliable factors to depict the soil evolution both in short periods [23,49] and long periods [50]. The significant increase in β-glucosidase activity in CONS indicated an improvement in carbon cycle and the significant differences between the managements suggest an important role of cover crops in the soil evolution under conservative management. Weerasekara et al. [51] found that cover crops increased β-glucosidase activity significantly while TOC and TN significantly decreased, that is the same trend found in our experiment although without significant decreases across the years for the last two parameters. As an average of all farms, FDA values were significantly higher in CONS than in CONV, indicating a higher microbial activity and indirectly confirming the higher microbial biomass detected in CONS [14,52].

Despite the small differences in soil texture among the farms, the present study also showed differences in response to management among the three farms: samples from Sasse Rami showed significant differences in β-glucosidase activity, BF, and QBS-ar, whereas in Diana farm only β-glucosidase was affected by the different management and in samples from Vallevecchia TN content was significantly different (Tables 3–5). Being the C stock similar in the three farms in terms of TOC, these different performances could be explained by Sasse Rami highest TN content (Table 1) in 2011. N could be the limiting factor for the development of microbial community and can influence the responses to the management change [53]. This means that the previous soil history could exert an important role in accelerating soil evolution under conservative agriculture management despite the homogeneity of other important parameters. As reported by Castle [54], nitrogen is a limiting factor for the microbial community in primary succession; this can be compared with exploited agricultural soils after crop harvest. This implies that soil quality improvement, in terms of microbial biomass, expected for conservative agriculture might be postponed or reduced in the situations where nitrogen is most needed. A second factor to be considered to explain different results between the farms could be the local microclimate: forest, sea or lake proximity, and wind streams could affect soil temperature and moisture and consequently its evolution.

The β-glucosidase activity was the parameter better responding to the changes in management practices and, in our opinion, is the best parameter of all to measure intensity of soil evolution; indeed, it is related to fungi activity indicating the degree of biomass replacement and soil quality [55].

Considering arthropod community detected by the application of biological forms (BF) and the QBS-ar index (Tables 2–4), time seems to have a greater effect than management, although the conservation system generally provides better conditions for soil fauna, often interacting with crop type [17], indeed in a two-year-long trial, de Oliveira et al. [56] did not found any difference between conservative and conventional agriculture in sugarcane plantations, suggesting the period considered was too short to find differences. QBS-ar in 2013 showed a lower value when compared to 2012 and 2014 in all the three farms, but it is in line with what was observed by [18] in a multidisciplinary study carried out over four years in Northern Italy on a silt loam under continuous maize. In three farms (in the Emilia-Romagna region, Italy), managed with both conventional and conservation practices (the last ones with and without sub-irrigation systems), monitored from 2014 to 2017, Menta et al. [16] found that soil arthropod communities varied among farms, although most differences were found among crops depending on management practices. Nonetheless, conservation systems and a wider reduction in anthropogenic practices provided better conditions for soil fauna, enhancing QBS-ar.

5. Conclusions

Also in a short period, conservation agriculture can start a process of soil quality restoration both for microbiological activity and soil fauna diversity, while in the short term, soil organic matter is not a reliable parameter to evaluate soil changes. Among the biological parameters, β-glucosidase was the most affected by the management change, probably due to its sensitivity to biomass decomposition and nutrient cycling.

The present results could be helpful to predict soil reaction to conservation agriculture in short periods. Furthermore, this work can contribute to complete the continuously increasing dataset of CA effects on agricultural soil evolution around the world. The reported scenario depicts the carbon sequestration and microbial activity dynamics, with consequences on soil microfauna, in a *ceteris paribus* condition across the years: this is a piece in "the puzzle" of the worldwide variable pathway of agricultural soil evolution, useful to refine future global strategies in soil management.

Future research should monitor soil biological parameters coupled with soil faunal communities, in relation to nutrient stocks, in longer experimental periods, to confirm the trends evidenced in the present short-time trial.

In conclusion, the present study demonstrated that the same soil management, soil characteristics, crop pool, rotation, and climate do not guarantee the same evolutionary pathway of soils in different farms. N stock, maybe dependent on previous soil management, might be the key characteristic able to influence soil evolution in the studied conditions although not investigated microclimate conditions could have a role in this issue.

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