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Microbial Indices to Assess Soil Health under Different Tillage and Fertilization in Potato (*Solanum tuberosum* L.) Crop

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Abstract: Intensive agronomic practices such as deep and repeated tillage and applying high mineral fertilization rates to improve crop yields have gradually determined soil resource degradation. A study was carried out over a two-year period (2015 and 2016) to assess effects of tillage (plough; subsoil; and spading) and fertilization (mineral vs. organic) on soil health relative to carbon and nitrogen dynamics in potato crop in the Mediterranean environment. Microbial indices could be successfully used as tool for assessing soil health in terms of predictors and indicators of carbon sequestration and nitrogen availability. The microbial quotients, calculated as percentage of the microbial-C to total organic C ($C_{mic}:C_{org}$), was significantly higher in subsoiling than in plowing and spading soil tillage, and higher in 2016 (3.19%) than 2015 (1.72%). The activity of enzymes involved in C cycle was significantly higher in subsoiling and spading than in plowing, while acid phosphatase was positively affected by spading and arylsulfatase increased with plowing. The whole enzyme activity expressed as synthetic enzymatic index (SEI) was positively affected by subsoiling and plowing in 2015 (4254) compared to spading tillage (3934). A general decrease in soil enzyme activity in 2016 than 2015 was observed. The subsoiling in potato crop favored the immobilization of carbon and nitrogen during the wet spring–summer period. Conversely, the plowing favored the mineralization process when the spring–summer period became more dried.

Keywords: soil health; organic fertilization; soil tillage; potato crop; sustainable agriculture

1. Introduction

Nowadays, the adoption of intensive agricultural practices, such as deep and frequent tillage associated with high mineral fertilization rate aimed to improve crop yields ensure food and human nutrition, have gradually led to the degradation of the soil resource [1]. Soil health is defined primarily from an agricultural perspective as "the soil's fitness to support crop growth without becoming degraded or otherwise harming the environment" [2]. Based on this definition, agricultural soils are considered a complex and dynamic component and represent an essential non-renewable resource of the agro-ecosystems due to their benefits and functions played in the agricultural systems, therefore, their management should be a cause of intense deliberation. From an ecological point of view, more attention is currently related to soil quality indicators that consist of a range of physical, chemical, and biological characteristics based on well specified soil ecosystem functions played by soils and its components, such as nutrient cycling, soil structure and stability, and soil microbial biodiversity [3–5].

Agro-ecosystem management affects positively or negatively the soil characteristics, in terms of physical, chemical, biological modifications, depending on the agronomic tech-

niques applied. Soil tillage determines variation in bulk density and porosity which affect yield and nutrient content of sweet potato. In addition, fertilization with poultry manure or NPK increases potato tuber yield, but the soil characteristics are better with organic fertilization than chemical ones [6]. The adoption of conservation soil tillage agronomic techniques can save energy, protect the soil, and contribute to reducing greenhouse gas emissions. The soil tillage operated with different machineries could affect positively the water holding, increasing the incorporation of crop residues in the soil surface with higher water content in the upper soil layer and lower evapotranspiration, lower soil temperature, and more stable soil aggregates, protection of soil erosion, nutrients and organic C accumulation near the soil surface, nitrogen leaching decreased [7]. Most of the crop cultivation came with intensive tillage operations using plow and disk harrow as primary and secondary tillage equipment with multiple fields passes to create a suitable seedbed for crop cultivation [8]. Although soil tillage had a positive influence on crop performance, intensive tillage could cause adverse effects on soil fertility [9]. Since tillage fractures the soil, it disrupts soil structure, leading to poor water-infiltration while accelerating surface runoff and soil erosion, reducing root growth [10]. Currently, reduced tillage practices aimed to reduce soil disturbance is considered one of the most effective agricultural practices to improve soil health and address environmental benefits of agro-ecosystems [11–13].

Organic fertilization combined with reduced soil tillage can contribute to contrast the soil degradation such as the decreasing of soil organic matter, pH alteration, cations and organic matter compounds leaching and at the same time stimulating effect on arbuscular mycorrhizal fungi [14]. In this context, the overuse of mineral fertilizers, especially nitrogen (N) could negatively affect soil quality and microbial community structure [15]. Therefore, optimal N rate and its management in the distribution are particularly important when conservation tillage practices are adopted, to avoid reduced efficiency and dangerous loss of nutrients in the environment [16–18]. The use of organic fertilizers in potato crop can determine a better use of nutrients and reduce the crop requirement of mineral fertilizers, consequently, affecting the increasing of tuber yield and their nutritional value [19].

Soil microbiological and biochemical indicators can be used to assess soil health, which has been defined as the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA-NRCS). In fact, soil microbial communities, considered an important indicator of soil health and quality, are extremely sensitive to both agricultural practices [20]. The role of the microbial fraction in mediating soil processes, and their relatively high rate of turnover, logically suggests that microbial fraction could be a sensitive indicator and early predictor of changing soil organic matter processes [21]. Soil health depends on soil biodiversity established by many soil properties and environmental conditions; therefore, its assessment in agro-ecosystem requires the selection of the most sensitive biological indicators to management practices [22]. The proper approach in defining soil health indicators with respect to nutrients cycling function must be holistic, but at the same time simple, so that the evaluation of soil management impact (e.g., tillage and fertilization) on soil health in a short-term experiment should consider at least the mineralization–immobilization as the two main processes regulating C storage and nutrient availability [23]. Among all soil biological properties, microbial biomass and soil enzyme activities prove to be sensitive indicators of soil health as they are measurements which rapidly respond to changes due to different management and environmental factors [24].

A better understanding of these processes could be used to improve our knowledge on soil health conditions as consequences of soil tillage and fertilizer source adopted and, thus, to support the adoption of sustainable management practices in agro-ecosystems. This study hypothesized that reduced tillage practices and organic fertilizer source applied to potato crop determine the development of soil microbial response toward improved soil health. The aim of this study was to assess the effects of tillage (plough; subsoil; spading) and fertilization (mineral vs. organic) on soil health with respect to carbon and nitrogen dynamic in potato crop in Mediterranean environment. In this study soil health was

assessed using microbial indices as indicators of mineralization–immobilization processes as predictors of carbon sequestration and nitrogen availability in a longer period.

2. Materials and Methods

2.1. Experimental Site and Treatments

The trials were established at the Experimental Farm of the University of Tuscia (Viterbo), located approximately 80 km North of Rome (45°25′ N, 12°04′ E). The study was carried out over a two-year period (2015 and 2016 cropping seasons). The area is a typically Mediterranean climate. The climate is characterized by warm-dry summers and cool-moist winters. Historical climatic data show a mean annual rainfall of 752 mm, most of it in the period October–May, and a mean annual temperature of 14 °C, the lowest mean monthly temperature of 1.6 °C in January and the highest mean monthly temperature of 30.8 °C in August. The data of minimum and maximum temperature and the amount of rainfall were collected from the meteorological station located approximately 100 m from the experimental fields. The total precipitation was higher in 2016 than 2015 and tended to be differently distributed between the potato growing seasons. The precipitations in 2015 were mainly concentrated in the first month after potato sowing, while in 2016 were mainly distributed in the period May–June.

The monthly aridity indexes (AI) as the (precipitation)/(temperature ratio+10)) were calculated as reported by Mancinelli et al. [25] throughout the study periods and are reported in Figure 1.
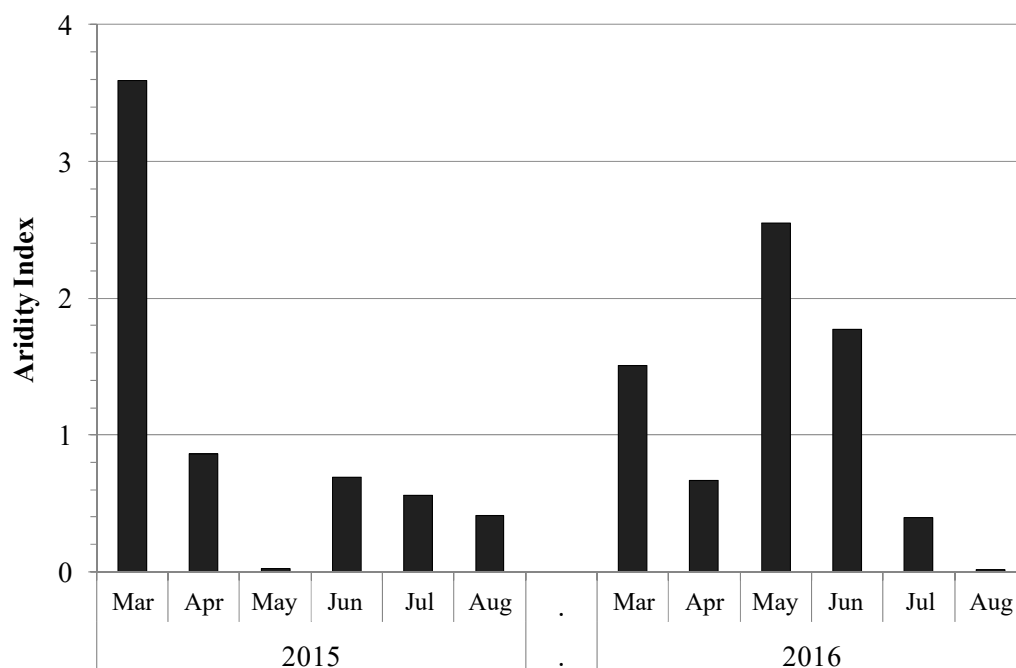

Figure 1. Aridity index throughout the periods of study in 2015 and 2016.

The soil at the experimental site is of volcanic origin and classified as *Typic Xerofluvent* with the particle size distribution was 76.3% sand, 13.3% silt, and 10.4% clay, and the total organic C content 0.97%, the total organic N content 0.12%, and pH (H_2O) 6.9.

A cropping system based on a 2-year crop rotation [durum wheat (*Triticum durum* Desf.)—potato (*Solanum tuberosum* L.)], was established in 2013 to compare soil tillage techniques and fertilizer sources. So, the treatments consisted of three tillage systems up to 20 cm of the soil layer (mould-board plow (hereafter called P); subsoiler (hereafter called R); and spading machine (hereafter called S)) and two fertilizer sources (mineral fertilization (hereafter called M) as performed in conventional farming and organic fertilization (hereafter called O) using municipal organic waste). The applied experimental design

was a randomized complete block with three replications. Both crops in rotation were simultaneously cropped in each year; therefore, the experimental field included 36 plots (2 crops $\times$ 3 soil tillage $\times$ 2 fertilizer source $\times$ 3 blocks). Each experimental plot was 60 m^2 (6 $\times$ 10 m) and they were separated by 3 m wide alleys to allow for equipment operations.

The field setup and crop management are in detail reported by Mancinelli et al. [11], therefore here ahead are very briefly described only. Before seeding the potato tubers (Monalisa variety) in April the soil was tilled at 20 cm depth according to the selected treatments. The mineral fertilizer was applied twice, the first time at potato seeding (100 kg ha^{-1} of P$_2$O$_5$, 50 kg ha^{-1} of K$_2$O and 100 kg ha^{-1} of N) and the second time after six weeks, at hilling stage (70 kg ha^{-1} of N). The organic fertilization was totally applied at planting after soil tillage and before seedbed preparation (18 Mg ha^{-1} of mature organic waste). Irrigation water by sprinkler irrigation system was applied when soil moisture reached 65% of the soil field capacity in each experimental year and stopped two weeks before tuber harvesting.

2.2. Soil Sampling

Soil samplings were carried out during the potato crop cycle period in both years (2015 and 2016). Five soil samples per plot were randomly taken in the central area and mixed in order to obtain a uniform sample. The samples were carried out by taking the soil at a depth of 0–20 cm in each plot. The soil sample was dried at air, sieved with a 2 mm mesh sieve and stored at 4 °C temperature. Subsequently, the soil analyses were performed.

2.3. Soil Analysis and Microbial Indices

Total organic carbon (TOC) and nitrogen (TN) contents were determined using the dry combustion method with Thermo Soil NC—Flash EA1112 Elemental Analyzer (Thermo Fisher Scientific, Bath, UK). Each sample was pre-treated with a 10% HCl solution to eliminate carbonates. Microbial biomass carbon (C$_{mic}$) and nitrogen (N$_{mic}$) were determined according to the fumigation–extraction method [26], using the TOC-V CSN and TNM-1 analyzer (Shimadzu, Japan). Microbial quotients were calculated as percentage of the microbial-C to total organic C (C$_{mic}$/C$_{org}$) and microbial-N to total N. The labile carbon (LC) and labile nitrogen (LN) were measured were determined using the same equipment on non-fumigated samples and expressed as a percentage of total organic C (LC/TOC) and N (LN/TN), respectively.

The following hydrolytic enzymes, known to be a part of soil biogeochemical cycles of C, N, P and S [27], were analyzed: for carbon β-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), xylosidase (EC 3.2.2.27) and cellobiohydrolase (EC 3.2.1.91); for nitrogen chitinase (EC 3.2.1.30), for phosphorus acid-phosphatase (EC 3.1.3.2); for sulphur arylsulphatase (EC 3.1.6.1). Finally, the butyrate esterase (EC 3.1.1.1) was analyzed as a proxy of intracellular activity [28]. The selected enzymes activity offers a general overview of soil organic matter mineralization process subjected to the different agronomical practices applied. Enzyme activities were determined using a microplate assay [29] with fluorogenic substrates (4-MUF-β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-β-d-glucoside, 4-MUF-α-d-glucoside, 4-MUF-phosphate, 4-MUF-sulphate, 4-MUF-7-β-d-xyloside and 4-MUF-butyrate as substrates). Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent, Thermo Fisher Scientific, Waltham, MA, USA) and readings were taken after 0, 30, 60, 120 and 180 min of incubation at 30 °C [30]. The whole enzyme activities were expressed as Synthetic Enzymatic Index (SEI) [31]. The SEI was calculated as the sum of 8 enzyme activities (β-glucosidase, α-glucosidase, xylosidase, cellobiohydrolase, chitinase, acid-phosphatase, arylsulphatase, butyrate esterase) and was expressed per unit of soil total organic carbon (SEI/TOC) or per unit of soil microbial biomass carbon (SEI/C$_{mic}$) [15]. The microbial quotients and the enzyme specific activities (enzyme activity per unit of C$_{mic}$) were used as valid indicators of the microbial efficiency in the use of energy and available nutrients [32].

2.4. Data Analysis

All data were subjected to statistical analysis by using the JMP statistical software package 4.0 (JMP Statistical Discovery, Cary, NC, USA).

The analysis of variance (ANOVA) was conducted for the two-year period applying a randomized complete block design with three blocks. A two-way factorial experimental design was performed for measured characteristics where the soil tillage and fertilization source were the treatments, and the year was considered as a repeated measure [33,34]. Means were compared adopting the Fisher's protected least significant difference (LSD) at $p < 0.05$. Correlations between C, N pools and enzyme activities of soil were performed.

3. Results

In this study no significant differences on total organic carbon and total nitrogen were registered among tillage treatments or between organic and mineral fertilization (data not shown). Conversely, the microbial quotients expressed as a percentage of microbial carbon and nitrogen to soil total organic carbon (C_{mic}/C_{org} and nitrogen (N_{mic}/N_{tot}) varied according to the cropping season x soil tillage ($p < 0.05$) and cropping season x fertilization source ($p < 0.05$, Table 1). The C_{mic}/C_{org} was significantly higher in subsoiling than in plowing and spading soil tillage regime (on average 2.66 vs. 2.35%, respectively), also showing values on average higher in the 2016 season than 2015 cropping season (on average 3.19 vs. 1.72%, respectively). In 2015 cropping season, the N_{mic}/N_{tot} was the highest in plowing followed by subsoiling and spading (2.10, 1.84 and 1.36%, respectively), while in 2016 it was high in subsoiling (3.20%), intermediate in plowing (3.96%) and low in spading (2.34%, Table 1). Regarding the fertilization source, both C_{mic}/C_{org} and N_{mic}/N_{tot} subjected to organic and mineral fertilization revealed an opposite effect in 2015 and 2016 cropping seasons, showing an increase with mineral in 2015 and with organic fertilization in 2016 (Table 1).

Table 1. Microbial quotients expressed as percentage of microbial-C to total organic carbon (C_{mic}/C_{org}) and microbial-N to total nitrogen (N_{mic}/N_{tot}).

	C_{mic}/C_{org}				N_{mic}/N_{tot}			
	———————————— % ————————————							
	2015		**2016**		**2015**		**2016**	
P	1.69	bB	3.01	bA	2.10	aB	2.96	bA
R	1.81	aB	3.51	aA	1.84	bB	3.20	aA
S	1.65	bB	3.04	bA	1.36	cB	2.34	cA
M	1.74	aB	3.02	bA	1.88	aB	2.54	bA
O	1.69	bB	3.35	aA	1.65	bB	3.13	aA

Values belonging to the same treatment with different letters in columns for tillage or fertilization effects (lower case letter), and in rows for year effect (upper case letter) are statistically different according to LSD (0.05). P = Plowing; R = Subsoiling; S = Spading; M = Mineral; O = Organic.

The activity of enzymes involved in C cycle was significantly higher in subsoiling and spading than in plowing (Figure 2A). Conversely, acid phosphatase was positively affected by spading (Figure 2B), and arylsulfatase increased with plowing (Figure 2C). No effect of tillage was registered on butyrate esterase (Figure 2D). The organic fertilization source showed higher results compared to the mineral fertilization source for the activity of enzymes involved in C cycle (on average 968 vs. 881 nmol MUF g^{-1} h^{-1}, respectively, Figure 2A), acid phosphatase (on average 423 vs. 393 nmol MUF g^{-1} h^{-1}, respectively, Figure 2B), arylsulphatase (on average 67 vs. 63 MUF g^{-1} h^{-1}, respectively, Figure 2C) and butyrate esterase (on average 1798 vs. 1592 nmol MUF g^{-1} h^{-1}, respectively, Figure 2D). The whole enzyme activity expressed as synthetic enzymatic index (SEI) was affected by cropping season x soil tillage and cropping season x fertilization source ($p < 0.05$, Table 2). The SEI was positively affected by subsoiling and plowing in 2015 (on average 4254 nmol MUF g^{-1} h^{-1}) compared to the spading tillage (3934 nmol MUF g^{-1} h^{-1}).

During the 2016 cropping season was observed a general decrease in soil enzyme activity than 2015 cropping season (2036 vs. 4147, respectively), even if the SEI values was greater in spading followed by subsoiling and plowing soil tillage (on average 2290, 2067 and 1752 nmol MUF g^{-1} h^{-1}, respectively; Table 2). Moreover, in both cropping seasons, organic fertilization sources showed a significant positive effect on soil enzyme activity compared to the mineral sources (on average 3254 vs. 2929 nmol MUF g^{-1} h^{-1}, respectively; Table 2).
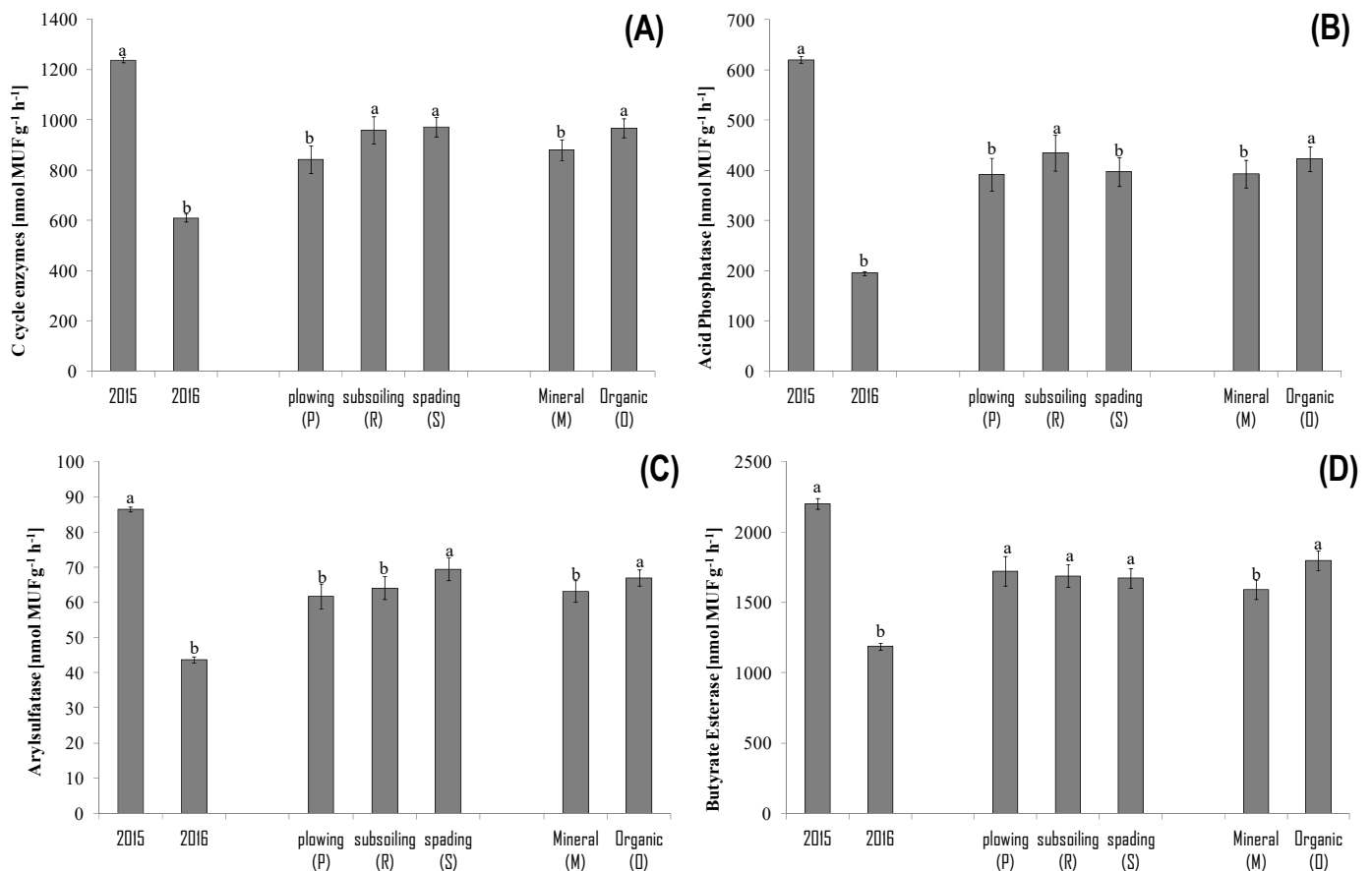

Figure 2. Soil enzyme activity in the main treatments (year of experiment, tillage and fertilization): (**A**) enzyme activities involved in C cycle, (**B**) acid phosphatase, (**C**) butyrate esterase, and arylsulfatase (**D**). Different letters indicate significant differences ($p < 0.05$), bars are standard errors ($n = 3$).

The Synthetic Enzymatic Index expressed per unit of microbial biomass (SEI/C_{mic}) was greater in 2015 than 2016 potato cropping season (on average 16.9 vs. 5.3 nmol MUF mg C_{mic}^{-1} h^{-1}, respectively) and, in 2015, it tended to be higher in spading followed by plowing and subsoiling (16.90, 19.96 and 15.85 nmol MUF mg C_{mic}^{-1} h^{-1}, respectively), while in 2016 it was high in spading (6.18), intermediate in plowing (5.21) and low in subsoiling (4.43), respectively (Table 2). No significant differences for SEI/C_{mic} were recorded between fertilization treatments (organic vs. mineral) in both years (Table 2).

Although the percentage of labile carbon form (LC/TOC) was higher in 2016 compared to 2015 potato cropping seasons (on average 0.325 vs. 0.185%, respectively), the data showed similar values among the various applied soil tillage (Table 3). Moreover, the LC/TOC was greater in organic than mineral fertilization sources in 2016 (0.338 vs. 0.313%, respectively), while no differences were detected between the fertilization sources in 2015 cropping season. Conversely, the labile nitrogen form (LN/TN) was affected by soil tillage in both potato cropping seasons showing the greater values in plowing compared to subsoiling and spading soil tillage regime (on average 0.443 vs. 0.385%, respectively).

The LN/TN ratio was generally similar in 2015 and 2016 cropping seasons, except in plowing treatment where it was higher in 2016 than 2015 (0.484 vs. 0.402%, respectively; Table 3). In addition, the labile nitrogen was significantly higher in mineral fertilization than organic one regardless of the cropping season (Table 3). Finally, no significant correlation was registered between C_{mic} and total organic carbon, as well as N_{mic} and total nitrogen. Conversely, the total amount of organic carbon and nitrogen were positively correlated with soil enzyme activities (Table 4). Moreover, microbial biomass and labile carbon were negatively correlated with single enzyme activities and SEI/C_{mic}, while labile nitrogen was positively correlated with β-glucosidase, acid phosphatase, arylsulfatase and SEI/C_{mic} (Table 4).

Table 2. Synthetic Enzymatic Index on soil mass base (SEI) and per unit of microbial carbon (SEI/C_{mic}).

	SEI ($nmol\ MUF\ g^{-1}\ h^{-1}$)			
	2015		**2016**	
P	4281.04	aA	1752.74	cB
R	4227.36	aA	2067.82	bB
S	3934.38	bA	2290.27	aB
M	4040.94	bA	1818.40	bB
O	4254.25	aA	2255.49	aB
	SEI/C_{mic} ($nmol\ MUF\ mg\ C_{mic}^{-1}\ h^{-1}$)			
	2015		2016	
P	17.96	aA	5.21	abB
R	15.85	cA	4.43	bB
S	16.90	bA	6.18	aB
M	16.78	aA	5.32	aB
O	17.02	aA	5.23	aB

Values belonging to the same treatment with different letters in columns for tillage or fertilization effects (lower case letter), and in rows for year effect (upper case letter) are statistically different according to LSD (0.05). P = Plowing; R = Subsoiling; S = Spading; M = Mineral; O = Organic.

Table 3. Labile carbon expressed as percentage to total organic carbon (LC/TOC) and labile nitrogen expressed as percentage to total nitrogen (LN/TN).

	LC/TOC				**LN/TN**			
	2015		**2016**		**2015**		**2016**	
P	0.182	aB	0.333	aA	0.402	aB	0.484	aA
R	0.187	aB	0.320	aA	0.376	bA	0.395	bA
S	0.186	aB	0.323	aA	0.384	bA	0.383	bA
M	0.187	aB	0.313	bA	0.413	aA	0.460	aA
O	0.183	aB	0.338	aA	0.361	bA	0.381	bA

Values belonging to the same treatment with different letters in columns for tillage or fertilization effects (lower case letter), and in rows for year effect (upper case letter) are statistically different according to LSD (0.05). P = Plowing; R = Subsoiling; S = Spading; M = Mineral; O = Organic.

Table 4. Pearson's correlation coefficient between C, N pools and enzyme activities of soil. TOC = Total organic carbon; TON = Total organic nitrogen; C_{mic} = Microbial carbon; N_{mic} = Microbial nitrogen; C_{mic}/C_{org} = Microbial quotients expressed as percentage of microbial-C to total organic carbon; SEI/C_{mic} = Synthetic Enzymatic Index on soil mass base per unit of microbial carbon.

	TOC	TON	C_{mic}	N_{mic}	C_{mic}/C_{org}	Labile C	Labile N
C_{mic}	ns	ns	-				
N_{mic}	ns	ns	0.77 ***	-			
C_{mic}/C_{org}	−0.64 **	−0.50 *	0.97 ***	0.76 ***	-		
Labile C	−0.58 *	ns	0.81 ***	0.76 ***	0.87 ***	-	
Labile N	ns	ns	−0.60 *	ns	−0.56	ns	-
Cellobioidrolase	0.57 *	0.54 *	−0.77 ***	−0.54 *	−0.80 ***	−0.81 ***	ns
Chitinase	0.70 **	0.73 **	−0.76 ***	ns	−0.81 ***	−0.76 ***	ns
β-glucosidase	0.61 *	0.69 **	−0.85 ***	−0.58 *	−0.86 ***	−0.80 ***	0.50 *
α-glucosidase	ns	ns	ns	0.58 *	0.54	0.59 *	ns
Acid Phosphatase	0.64 **	0.66 **	−0.86 ***	−0.55 *	−0.89 ***	−0.85 ***	0.50 *
Arylsulfatase	0.66 **	0.72 **	−0.84 ***	−0.56 *	−0.88 ***	−0.82 ***	0.55 *
Xylosidase	0.67 **	0.70 **	−0.82 ***	−0.57 *	−0.85 ***	−0.80 ***	ns
Butyrate esterase	0.67 **	0.63 **	−0.71 **	ns	−0.78 ***	−0.79 ***	ns
SEI/C_{mic}	0.65 **	0.56 *	−0.91 ***	−0.66 **	−0.95 ***	−0.90 ***	0.50 *

The significance level is *, **, ***, or ns, significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, or $p > 0.05$, respectively.

4. Discussion

In this study, as expected, tillage and fertilization of potato crop did not affect the total organic carbon (TOC) and nitrogen (TON) content of soil, while the soil microbial indices and labile C and N pools resulted in very sensitive indicators of soil health variation. Even if no significant correlation between enzyme activity and total C and N content were registered, a positive correlation was found between microbial quotient or soil enzyme activity and soil labile nitrogen pool, while a negative coefficient was registered with labile carbon pool. These results confirm that microbial quotient and enzyme activity can be used as sensitive indicators of soil carbon and nitrogen dynamics after tillage and fertilization practices. In fact, it is known that microbial biomass content in a soil health assessment is useful for consistently monitoring C stock changes over time, as they are affected by agricultural management history, but relatively insensitive to recent inputs and weather changes [35]. The obtained results also showed significantly different response of soil microbial indices, such as microbial quotients and specific enzyme activity, to tillage and fertilization in the two years of experiment. It is well known that the soil microbial indices show variability over the growing season. In the different fertilization applications in terms of quantity and typology, in the methods adopted for the tilling of the soil. According to Lazcano et al. [36], the addition of organic fertilizer sources characterized by high moisture, available forms of N and C could affect the development of soil microorganisms confirming that soil organic mass added to the agro-ecosystems represent a key factor for the function and quality of soil [37–39]. Indeed, soil microorganisms react differently to the soil changes caused by the fertilizer application, especially with modification of microbial community and variation of total microbial biomass [33,34]. Similarly, Heidari and colleagues [40] showed that tillage operations play an important role in soil biological properties. Overall, sustainable approach to soil tillage is mainly oriented toward reduced tillage intensity as adopted in the minimum tillage strategy because it improves soil structure, aggregate stability, and microbial diversity [40]. Sun et al. [41] observed that reduced tillage combined with organic fertilization appears to be an effective agricultural strategy that enhances microbial biomass, microbial residues and bacterial and fungal abundances. In addition, microbial residues as a fraction of soil organic carbon have higher sensitivity to farming management than to soil tillage. Additionally, Chen et al. [42] observed that soil tillage affected soil microbial indices at lower level than organic input. However, Choudhary et al. [43] highlighted that the continuous distribution of a balanced

fertilization adopting organic manure combined with mineral fertilizers enhanced soil microbial indices.

An enrichment of soil enzymatic activity is usually expected in response to the following: (i) increased microbial release of extracellular enzymes and/or (ii) improved soil environment by changes of physico-chemical properties [44]. In our study, the specific enzyme activities showed positive response to the plowing and suggests that the increase in hydrolytic enzymes was probably more related to higher microbial physiological capacity than to a larger biomass [32]. Conversely, in subsoiling, the increased enzymatic activity was much related to the increased amount of microbial biomass. Plowing, as with other tillage operations, produces greater aeration that incorporates previous crop residues and fertilizers, thus stimulating microbial activities and, thus, boosts the mineralization process of soil organic matter affecting soil biogeochemical processes [45]. The enzyme activities expressed per unit of microbial biomass allow to assess the metabolic *status* of the microbial community as well as variations in the stabilized extracellular enzyme activity, thus integrating the information obtained using the C_{mic}:C_{org} [46]. Indeed, soil enzymes represent the active part of soil organic components and are fully involved in all biochemical processes that happen in the soil environments, and are therefore frequently used as indicators of soil quality and agro-ecosystem functions [20]. It is known that substrate availability is the main factor influencing both the size and activity of the soil microflora [47]. On the other hand, Carter [48] found that a wheat-fallow system reduced microbial biomass, while 4-year zero tillage increased microbial biomass compared to shallow tillage and also 3 years of direct drilling caused an increase compared to a cultivated system. He concluded that microbial biomass responded rapidly to the changes in tillage and soil management. Kabiri et al. [49], reported that soil microbial attributes are more sensitive to tillage practices and soil disturbance than soil organic matter content. In addition, soil microbial biomass and enzyme activity were increased independently to microbial activity, and they concluded that soil microbial attributes can be useful indicators of tillage-induced changes. Even if changes in organic carbon could not be registered in a short-term experiment, the microbial biomass was modified by subsoiling, suggesting a trend towards an immobilization of organic C in a longer period. In the 2016 potato cropping season, the increase in soil microbial quotients reflects the larger organic substrates availability for microbial growth and immobilization into microbial biomass. In addition, the decrease in specific enzyme activity suggested a reduction in the mineralization process by lower biochemical activity per unit of microbial biomass [50]. Moreover, the climatic conditions, registered in the spring–summer period of the two years, significantly affected the soil microbial biomass and activity as indicators of soil health changing carbon and nitrogen dynamics.

Since a different behavior of SEI/C_{mic} was observed between 2015 and 2016 by the different climatic trend during crop cycle, the soil biochemical activity was more sensitive than microbial quotients showing different impacts of tillage on nutrient cycling with respect to climatic conditions in spring–summer period. In particular, the obtained results suggested a positive response of microbial activity to hot and dry spring–summer period, while the amount of microbial biomass was more affected by warm and wet conditions. Moreover, among soil tillage, plowing and spading increased soil enzymatic activity per unit of microbial biomass because the physiological capacity of soil microorganisms was enhanced [32], while microbial quotients were reduced. In fact, it subsists a relation between enzymatic activities and soil tillage method applied. As reported by Bielińska and Mocek-Płóciniak [51], the enzymes tests can be good indicators depending on the employed tillage system and the enzymes stimulation activity determines changes in soil chemical conditions. Those results suggested a more intense degrading activity and lower microbial biomass growth under plowing and spading during drier season. As reported by Franzluebbers et al. [52], soil under conventional tillage experienced greater seasonal variation in potential mineralization, showing a sensitive response to soil temperature and moisture. Since tillage may induce changes in environmental conditions such as a decline in the proportion of

water-stable soil macroaggregates, moisture and temperature [24,53], a more sensitive tillage disturbance may occur during harder season to soil microbial biomass.

5. Conclusions

In this study, the adoption of microbial indices resulted in sensitive indicators of soil health and carbon dynamic changes under different tillage and fertilization in potato cropping systems. Overall, organic fertilization modified the intensity of microbial quotient and specific enzyme activity representing a key factor for improving the soil health. Therefore, it is conceivable that the increased use of organic fertilizer also in combination with mineral fertilizer could represent an environmentally friendly practice to improve the sustainability of agro-ecosystems. Similarly, the results suggest that soil tillage regime modified nutrient availability. In fact, subsoiling in the potato crop favored the immobilization of carbon and nitrogen in a Mediterranean climate under wet spring–summer period, showing the need of mineral fertilization for improving nutrient availability for potato crop. Conversely, the adoption of plowing tillage, which favored the mineralization process when the spring–summer period became more dried, should be combined with organic fertilization to avoid an excessive mineralization of soil organic matter and, therefore, a pauperization of soil health.

Author Contributions: S.M., E.R. and R.M. edited the Special Issue entitled Soil Quality and Health to Assess Agro-Ecosystems Services and equally contributed to the preparation of the manuscript. Conceptualization, S.M., E.R. and R.M.; methodology, S.M., E.R. and R.M.; software, R.M. and E.R.; validation, S.M., E.R., R.M., M.A. and V.P.; formal analysis, R.M. and E.R.; investigation, R.M. and E.R.; resources, R.M. and S.M.; data curation, R.M.; writing—original draft preparation, S.M., E.R. and R.M.; writing—review and editing, S.M., E.R., R.M., M.A. and V.P.; visualization, R.M.; supervision, R.M. and S.M.; project administration, R.M. and S.M.; All authors have read and agreed to the published version of the manuscript.

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