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Soil Biochemical Indicators and Biological Fertility in Agricultural Soils: A Case Study from Northern Italy

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Abstract: Industrial farming without considering soil biological features could lead to soil degradation. We aimed to evaluate the biochemical properties (BPs) and biological fertility (BF) of different soils under processing tomato cultivation; estimate the BF through the calculation of a simplified BF index (BFIs); determine if the crop was affected by BP and BF. Three farms were individuated in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Italy. Soil analysis included total and labile organic C, microbial biomass-C (Cmic) and microbial respiration measurements. The metabolic (qCO₂), mineralization (qM) and microbial (qMIC) quotients, and BFIs were calculated. Furthermore, plant nutrient contents were determined. The low Cmic content and qMIC, and high qCO₂ found in MEZ soils indicate the occurrence of stressful conditions. The high qMIC and qM, and the low qCO₂ demonstrated an efficient organic carbon incorporation as Cmic in MO soils. In RA soils, the low total and labile organic C contents limited the Cmic and microbial respiration. Therefore, as confirmed by the BFIs, while MO showed the healthiest soils, RA soils had an inefficient ecophysiological energy state. However, no effects on plant nutrient contents were observed, likely because of masked by fertigation. Finally, BP monitoring is needed in order to avoid soil degradation and, in turn, crop production decline.

Keywords: soil quality; microbiological indicators; biofertility; intensive farming; cropland

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1. Introduction

Anthropogenic actions deeply influence processes occurring in the Earth's critical zone (CZ), and a typical example is what is observed in agroecosystems where soils are processed for crop production. In fact, soil features reflect parent rock and climatic characteristics but also the effect of biological activities, including those triggered by man-made actions. These features are important to understand the soil fertility, i.e., the soil attitude to sustain crop productivity, a characteristic that has to be maintained to carry sustainable agricultural practices. In this view, studies must be carried out with a multidisciplinary approach and there is a need to develop analytical methods that measure biogeochemical indexes for a detailed CZ understanding.

In industrialized countries, a large part of the soil suitable for cultivation is used for intensive crop production [1]. Agricultural production must, however, consider its sustainability in order to reduce soil degradation processes, such as soil erosion, loss of fer-

tility and biodiversity, accumulation of agrochemicals and salinization [2–5]. To date, conventional and industrial agriculture are considered unsustainable because they are eroding natural resources (i.e., soil and water) faster than the capacity of ecosystem to regenerate them.

Soil microbial biomass and its activity are driving forces for the cycling of nutrients in agricultural and natural ecosystems, soil processes and plant performance [4,6], and soil microbial biomass, together with soil organic carbon, is largely related to different soil ecological functions (e.g., sustain crops, accumulate and filter water, act as carbon sink).

Soil variability and specific environmental conditions may, however, control changes in soil processes and, consequently, soil microbial variations [6]. In fact, the considerable effects of pedological and climatic conditions on soil microbial biomass and activity have been documented [7,8].

The prompt response of soil microbial populations to changes in soil properties and land use (e.g., [8–11]) pressed the scientific community to focus on microbial biomass and activity as accurate and sensitive indicators of the fitness of soil to perform ecological functions [12]. In this context, several papers used soil biochemical properties to monitor soil fertility, (e.g., [12,13]), and degradation, (e.g., [14–16]), in agricultural lands. Widdig et al. [17], in an experiment conducted on six grassland sites from South Africa, the USA and the UK, found a reduction in soil microbial respiration and growth in soils added with nitrogen. Nunes et al. [18], in an investigation conducted in lands with different degradation degree in Northeast Brazil, showed that soil degradation, through the organic C loss, reduces both soil microbial biomass and activity. However, although preservation and improvement of soil biochemical parameters are signs of better soil ecological functions, in agricultural ecosystems, these ecological indicators have to face up with crop yields. From this point of view, previous studies highlighted the pivotal role of soil microbial community to sustain plant growth and yield [19–22].

Thus, the strong connection between soil edaphic properties and crop production through the intermediary role of the soil microbial community would suggest the use of biochemical properties as a useful tool to evaluate if a soil can be suitable for the cultivation of a given crop without having a negative impact on soil ecological functions. In this sense, we hypothesized that (i) the biochemical properties and related index of biological fertility are prompt and valuable indicators of soil stress conditions caused by industrial agriculture; (ii) soils with low biological fertility could negatively impact crop yield and quality.

Therefore, the aims of this study were (i) to evaluate the biochemical properties (i.e., microbial biomass and activity) and biological fertility of soils with contrasting characteristics under processing tomato cultivation; (ii) to estimate the soil biological fertility through the calculation of a simplified biological fertility index; (iii) to determine if the quality of the crop production (i.e., nutrients content in plants) was affected by soil biochemical properties and biological fertility.

2. Materials and Methods

2.1. Study Areas and Experimental Layout

The selected study areas were located in the eastern Padania Plain, and specifically in Modena (MO sites), Ferrara (MEZ sites) and Ravenna (RA sites) provinces of Emilia Romagna region, Italy (Figure 1). These sites were selected for their high representativeness of the Padania Plain of Emilia Romagna region, which is characterized by lands addressed for industrial crops.

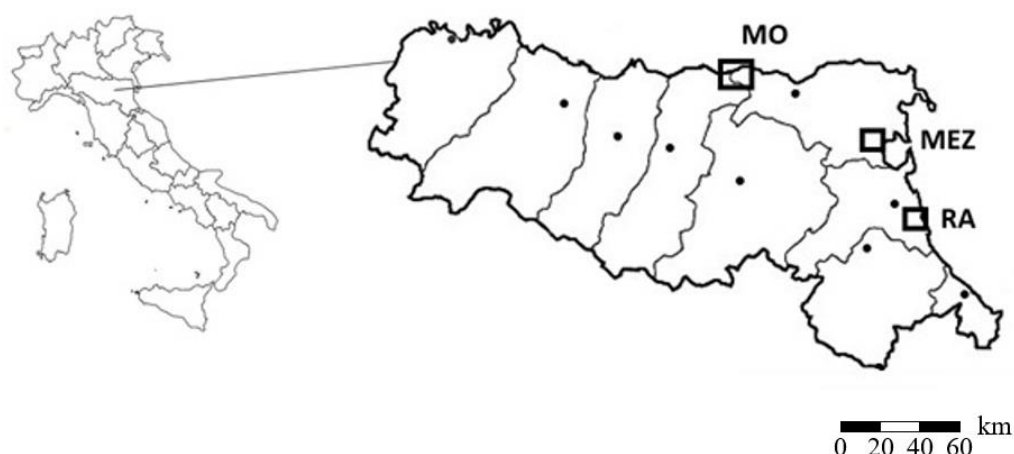


Figure 1. Map of Italy with magnification of the Emilia Romagna region and indication of the study sites.

The MO soils are located in the northernmost area and developed on silt and clay sediments of the Po River in the area between river course and the Apennines chain; the soils are classified as Irragic Vertisols [23]. The MEZ soils are located in a lowland reclaimed for agricultural purpose in the 1950s and are characterized by the common presence of peaty outcrops; the soils are classified as Thionic Sapric Histosols [23]. The RA soils are in the southernmost area consisting of silt and sand fluvial deposits flowing from the Apennines chain; the soils are classified as Endogleyic Fluvic Cambisols [23].

Temperature and precipitation were monitored during 2015 cultivation period through three climatic stations located in Guagnino (MEZ), San Felice sul Panaro (MO) and San Pietro in Vincoli (RA). For the considered year, the total annual precipitation was 621, 630 and 909 mm for MO, MEZ and RA, respectively. In the three sites, the minimum average annual temperature was recorded on February and ranged between 1.9 and 2.6 °C. The maximum average annual temperature was recorded in July and ranged between 22.8 and 24.0 °C. Finally, the mean annual temperatures were 13.3, 13.3 and 12.9 °C for MO, MEZ and RA, respectively. The climate data are in agreement with those recorded in the fifteen-year period (2001–2015) by the regional service in the climatic station located in Guagnino and Finale Emilia for MEZ and MO sites, respectively, and San Pietro in Trento and San Pancrazio for RA site [24].

For each study area, farms with similar land management were selected. In particular, 3 farms for MO, 3 for MEZ and 2 for RA were individuated. In each farm, 3 processing tomato fields (2.5 ha each) were chosen. In the fields, the tomato (*Solanum lycopersicum* L.) crop, which is a widely distributed crop of the three study areas, has been cultivated as monoculture since 2014 and was previously used for maize cultivation.

In the investigated year, the fields were thoroughly rototilled at a depth of 20 cm and then the tomato seedlings (hybrid Heinz 1015 F1) were transplanted within the first week of May (with a few days of variation among the fields). The planting density was 45,000 plants per hectare with row spacing of 0.5 m and plant spacing of 0.4 m. During the growing season, no more tillages were performed, while weed and pest controls were carried out according to the integrated crop management specifications of Emilia Romagna Region [25]. Plants were watered through drip irrigation lines and nutrients were provided through fertigation. Roughly, the amounts of nitrogen, phosphorus and potassium provided to the soils were 236, 167 and 62 kg ha⁻¹, respectively, for MO, 260, 160 and 150 kg ha⁻¹, respectively, for RA and 94, 117 and 174 kg ha⁻¹, respectively, for MEZ (Table S1 of the Supplementary Materials). The higher amounts of nitrogen provided in MO and RA

compared to MEZ, while the higher amounts of potassium provided in RA and MEZ comparing to MO were to counterbalance the lower amounts of those nutrients in the selected soils.

2.2. Soil and Tomato Plant Sampling

Two weeks after the tomato transplanting, in each field, soil sampling was carried out by opening two minipits down to 20 cm (i.e., soil samples at 0–20 cm) and by drilling the bottom part of the minipit down to 40 cm by auger (i.e., soil samples at 20–40 cm). For each field, the samples collected at each soil depth were mixed to obtain composite samples of 0–20 and 20–40 cm.

At harvesting, five randomly selected tomato plants and their rhizospheric soil (the soil still adherent to the roots after gently shaking and removing the loosely attached soil) were collected for each field. Once in the laboratory, the rhizospheric soil was separated from the plant roots and the organs of tomato plants (i.e., young and old leaves, stems, roots and tomato fruits) were separated from each other.

The composed 0–20 and 20–40 cm soil samples and the rhizosphere soils were air dried, sieved to 2 mm and an aliquot was finely ground. The tomato fruits were freeze-dried, while the other plant parts were dried at 60 °C for 48 h. All plant samples were then finely ground.

2.3. Soil and Tomato Plant Analyses

2.3.1. Soil Physicochemical Properties

The pH was determined potentiometrically in a 1:2.5 soil:water suspension (w/v). The electrical conductivity (EC) was measured in a 1:5 soil:water suspension (w/v). The cation exchange capacity (CEC) and the exchangeable cations were determined after exchange with hexamminecobalt(III) chloride and measuring the excess of Co^{3+} , and Ca^{2+} , Mg^{2+} , K^{+} and Na^{+} in the extracts by inductively coupled plasma–optical emission spectrometry (ICP-OES, Ametek, Germany) [26]. The carbonate content was quantified by volumetric method through the reaction of soil sample with 6 M HCl [27]. The particle-size distribution was measured by pipette method [28]. The amount of total organic carbon (OC) and nitrogen (TN) was determined by dry combustion (EA 1110, Thermo Fisher, Waltham, MA, USA) after removing inorganic carbon by HCl. The amount of stable carbon isotope (^{13}C) was determined by an isotope ratio mass spectrometer (IRMS Delta C or DELTA+XL, Thermo Finnigan MAT, Bremen, Germany), and it was then expressed as $\delta^{13}\text{C}$ (‰) with respect to the V-PDB universal reference standard for ^{13}C . Total P (TP) was determined after treating samples with suprapure HCl and HNO_3 mixture (3:1 v/v) in microwave oven (Milestone 1200, Milestone Inc., Sorisole, Bergamo, Italy) [29]. The available P (POlsen) was extracted using 0.5 M NaHCO_3 at pH 8.5 [30]. The amount of TP and POlsen was then measured by ICP-OES (Ametek, Germany).

2.3.2. Soil Biochemical Parameters

The C and N of microbial biomass (C_{mic} and N_{mic} , respectively) were determined as the difference between C and N extracted with 0.5 M K_2SO_4 (1:4 soil:solution ratio) from chloroform-fumigated and not-fumigated soil samples [31]. For C_{mic} and N_{mic} , a correction factor of 0.45 and 0.54, respectively, has been then applied, as proposed by Vance et al. [31]. The C and N extracted from not-fumigated soil samples represented the labile pool of C and N ($\text{K}_2\text{SO}_4\text{-C}$ and $\text{K}_2\text{SO}_4\text{-N}$).

The microbial respiration was determined placing the soil samples in closed glass jars, incubating them at 25 °C in the dark at field capacity and trapping the evolved CO_2 by 0.5 N NaOH. The C- CO_2 evolved after 1, 2, 4, 7, 10, 14, 17, 21, 28 days was determined by 0.05 N HCl titration of the excess of NaOH [32]. From data respiration, the following indicators have been calculated: basal respiration (BR), as the hourly respiration rate; CO_2

cumulative (C_{cum}), as the cumulated CO_2 evolved during the incubation period; metabolic quotient (qCO_2), as the hourly CO_2 evolved per unit of microbial biomass, and used to evaluate the effects of external disturbances [33]; the mineralization quotient (qM), as the ratio between the C_{cum} and the OC, indicating the efficiency of micro-flora in metabolizing soil OC [34]. The microbial quotient ($qMIC$) was also calculated, as the $C_{mic}:OC$ ratio and it represents the percentage of microbial C with respect to the total organic C [34].

Additionally, a simplified biological fertility index (BFIs), was calculated. This type of index was used to discriminate soil biological fertility status under different agrosystems [35–37].

2.3.3. Macronutrient Content in Rhizosphere and Tomato

The concentration of P, K, S, Na, Ca, and Mg in rhizospheric soil was detected by ICP-OES (Ametek, Germany) after treating samples with suprapure HCl and HNO_3 mixture (3:1 v/v) in microwave oven (Milestone, 1200) [29].

The macronutrients concentration was also analyzed in plant tissues and fruits by ICP-OES, after digesting of finely ground sample in microwave oven with suprapure HNO_3 and 30% H_2O_2 mixture (4:1.5 v/v).

2.4. Data Treatment

Differences in the physicochemical and biochemical soil properties between sites have been verified by Kruskal–Wallis test using R software 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). We chose to use a non-parametric test because in our dataset some deviations from normality occurred, as well as the residues of the different groups had different ranges of variation (heteroskedastic), as verified by aov R implementation. Therefore, the key assumptions for ANOVA test calculation (i.e., normality and homoskedasticity of the subgroups) were not respected. The p-value greater than 0.05 indicates that there was no difference in ranks between the different groups and the significant codes.

In order to obtain the BFIs, the correlation coefficients were calculated among the soil parameters included in the biological fertility index (BFI) proposed by Benedetti and Mocali [38] to identify and remove the redundant variables. Furthermore, the BFIs calculation was based on the sum of the scores given by non-correlated parameters (Table S2 of the Supplementary Materials).

3. Results

3.1. Soil Physicochemical Properties

The investigated agricultural sites showed a different particle-size distribution; in particular, we found a silty loam texture in MEZ, clay/silty clay in MO and sand/sandy loam in RA (data not shown).

Tables 1 and 2 report the values of soil physicochemical characteristics in the three different sites. Generally, no differences were detected in the physicochemical soil properties between top- and subsoil (0–20 and 20–40 cm, respectively), while significant differences were evident among the pedoclimatic areas.

The pH ranged from neutral (MEZ) to subalkaline (RA, MO), while the EC varied from moderate saline (MEZ) to no saline soils (RA and MO) (Table 1). MEZ and RA soils were poor in carbonates ($<10\text{ g kg}^{-1}$), while a slight increase in MO soils was evident, where the $CaCO_3$ content reached 22 and 21 g kg^{-1} , respectively (Table 1). Both for top- and subsoils, the CEC values trend was $MEZ > MO > RA$ (Table 1). Calcium was, in general, the most represented cation on the exchange complex and its concentrations showed the highest and the lowest values in MEZ and RA, respectively. The content of exchangeable Mg and K was similar in MEZ and MO and higher than in RA. Besides calcium, MEZ

soils showed the highest concentrations of exchangeable Na, while the lowest one was detected in MO.

Table 1. Mean and standard error (SE) values of soil pH, electrical conductivity (EC), CaCO₃ content, cation exchange capacity (CEC), contents of exchangeable Ca, Mg, K and Na in 0–20 and 20–40 cm soil layers of processing tomato fields located in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy. Different letters mean significant differences (Kruskal–Wallis test, $p < 0.05$) among sites within 0–20 and 20–40 cm soil layers.

Soil Depth		MO		MEZ		RA	
		Mean	SE	Mean	SE	Mean	SE
0–20 cm	pH	8.3 ^a	0.1	7.1 ^c	0.2	7.7 ^b	0.1
	EC	690 ^b	158	1465 ^a	221	975 ^b	72
	CaCO ₃	21.1 ^a	1	1.4 ^c	0.2	7.1 ^b	0.8
	CEC	21.5 ^b	1.9	40.2 ^a	5.3	5.5 ^c	2.6
	Ca	17.1 ^b	0.5	32.9 ^a	0.9	3.5 ^c	1.4
	Mg	2.3 ^a	0.1	3.5 ^a	0.1	0.2 ^b	<0.1
	K	1.4 ^a	0.1	0.4 ^a	0.1	0.1 ^b	<0.1
	Na	0.3 ^c	0.05	2.5 ^a	0.3	1.4 ^b	0.2
20–40 cm	pH	8.4 ^a	0.1	6.8 ^c	0.3	7.8 ^b	0.1
	EC	847 ^b	216	2774 ^a	598	931 ^b	99
	CaCO ₃	22.1 ^a	0.7	1.5 ^c	0.2	7.7 ^b	0.9
	CEC	20.8 ^b	2.1	39.8 ^a	4.7	8.5 ^c	3.5
	Ca	17.4 ^b	0.5	32.4 ^a	2.9	3.1 ^c	1.4
	Mg	2.4 ^a	0.1	1.6 ^a	0.7	0.2 ^b	0.1
	K	0.4 ^a	0.1	0.5 ^a	0.1	0.1 ^b	<0.1
	Na	0.3 ^c	0.1	4.5 ^a	0.8	1.2 ^b	0.2

As expected, due to ancient land reclamation, peaty MEZ soils were enriched in OC (99.1 and 96.5 g kg⁻¹, respectively, in top- and subsoil) (Table 2). In MO site, soil showed significantly higher OC content (14.4 g kg⁻¹) than RA (7.5 g kg⁻¹). Similarly, total N showed the highest concentrations in MEZ while the lowest ones in RA. The OC:TN ratio showed the highest values in MEZ, while no differences occurred between RA and MO (Table 2). The isotopic C signature showed high values in RA, and low ones in MEZ.

Due to the high variability, few differences occurred for total P and the P Olsen contents. In fact, TP showed some differences only in topsoil where MO had a higher TP content than MEZ and RA. For P Olsen, the topsoil showed higher values in MO than in MEZ, while the subsoil showed the lowest values in MEZ (Table 2).

Table 2. Mean and standard error (SE) values of soil organic C (OC) and total N contents, C:N ratio, $\delta^{13}\text{C}$, total and available P contents (TP and POlsen, respectively), labile organic C and N contents ($\text{K}_2\text{SO}_4\text{-C}$ and $\text{K}_2\text{SO}_4\text{-N}$, respectively), basal and cumulative respiration (BR and Ccum, respectively), microbial biomass C and N contents (Cmic and Nmic, respectively) in 0–20 and 20–40 cm soil layers of processing tomato fields located in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy. Different letters mean significant differences (Kruskal–Wallis test, $p < 0.05$) among sites within 0–20 and 20–40 cm soil layers.

Soil Depth			MO		MEZ		RA	
			Mean	SE	Mean	SE	Mean	SE
0–20 cm	OC	g kg^{-1}	14.4 ^b	0.5	99.1 ^a	10.2	7.5 ^c	0.6
	TN	g kg^{-1}	2.1 ^b	0.1	6.1 ^a	0.6	1.1 ^c	0.1
	OC:TN		6.8 ^b	0.5	16.2 ^a	0.2	6.8 ^b	0.1
	$\delta^{13}\text{C}$	‰	−26.2 ^b	0.1	−26.9 ^c	0.1	−25.7 ^a	0.2
	Ptot	g kg^{-1}	1.2 ^a	0.2	0.8 ^b	0.1	0.7 ^b	0.1
	P Olsen	mg kg^{-1}	4.6 ^a	1.6	2.7 ^b	0.2	2.9 ^{ab}	0.9
	$\text{K}_2\text{SO}_4\text{-C}$	mg kg^{-1}	92 ^b	3	378 ^a	30	70 ^c	4
	$\text{K}_2\text{SO}_4\text{-N}$	mg kg^{-1}	28 ^b	5	67 ^a	7	18 ^b	1
	BR	$\text{mg C-CO}_2 \text{ kg}^{-1}$	15 ^b	2	120 ^a	5	13 ^b	2
	Ccum	$\text{mg C-CO}_2 \text{ kg}^{-1}$	688 ^b	57	2,567 ^a	125	125 ^b	49
	Cmic	mg kg^{-1}	438 ^a	18	143 ^b	35	121 ^b	19
	Nmic	mg kg^{-1}	21	2	25	5	15	3
20–40 cm	OC	g kg^{-1}	14.1 ^b	0.7	95.6 ^a	15.8	7.9 ^c	0.6
	NT	g kg^{-1}	2.1 ^b	0.1	6.5 ^a	0.6	1.1 ^c	0.1
	OC:TN		6.7 ^b	0.5	14.7 ^a	0.2	7.2 ^b	0.1
	$\delta^{13}\text{C}$	‰	−26.5 ^{ab}	0.6	−26.8 ^b	0.3	−25.7 ^a	0.2
	Ptot	g kg^{-1}	1.1	0.1	0.8	0	0.7	0.1
	P Olsen	mg kg^{-1}	3.8 ^a	1.3	1.5 ^b	0.3	4.3 ^a	0.1
	$\text{K}_2\text{SO}_4\text{-C}$	mg kg^{-1}	96 ^b	5	405 ^a	35	80 ^c	6
	$\text{K}_2\text{SO}_4\text{-N}$	mg kg^{-1}	23 ^b	2	60 ^a	5	31 ^b	6
	BR	$\text{mg C-CO}_2 \text{ kg}^{-1}$	12 ^b	3	58 ^a	7	11 ^b	3
	Ccum	$\text{mg C-CO}_2 \text{ kg}^{-1}$	443 ^b	75	2,843 ^a	188	143 ^b	88
	Cmic	mg kg^{-1}	208	17	146	31	132	20
	Nmic	mg kg^{-1}	17	2	25	6	15	3

3.2. Soil Biochemical Properties

Soil biochemical properties are shown in Table 2. Similar to physicochemical data, in general no significant differences in the microbial parameters between the soil depths were found, while several differences among the three pedoclimatic areas were observed. As expected, MEZ showed the highest values of C and N labile pools ($\text{K}_2\text{SO}_4\text{-C}$ and $\text{K}_2\text{SO}_4\text{-N}$). Furthermore, higher concentrations of $\text{K}_2\text{SO}_4\text{-C}$ were found in MO than in RA. The MEZ soils had the highest C-CO₂ emissions, both as basal and cumulative soil respiration, while no differences occurred between MO and RA. The microbial C content trend was MO > MEZ = RA in topsoils, while the microbial N values were similar among sites, both in top- and subsoils.

The qMIC trend was MO > RA > MEZ in the topsoil, while in the subsoil MEZ showed the lowest qMIC values and no differences occurred between MO and RA (Figure 2). For both soil layers, the qCO₂ showed the highest and lowest values in MEZ and MO, respectively (Figure 2). The qM of the topsoil showed the highest values in MO and the lowest ones in RA. In subsoil, instead, MEZ had a higher qM compared to MO and RA (Figure 2).

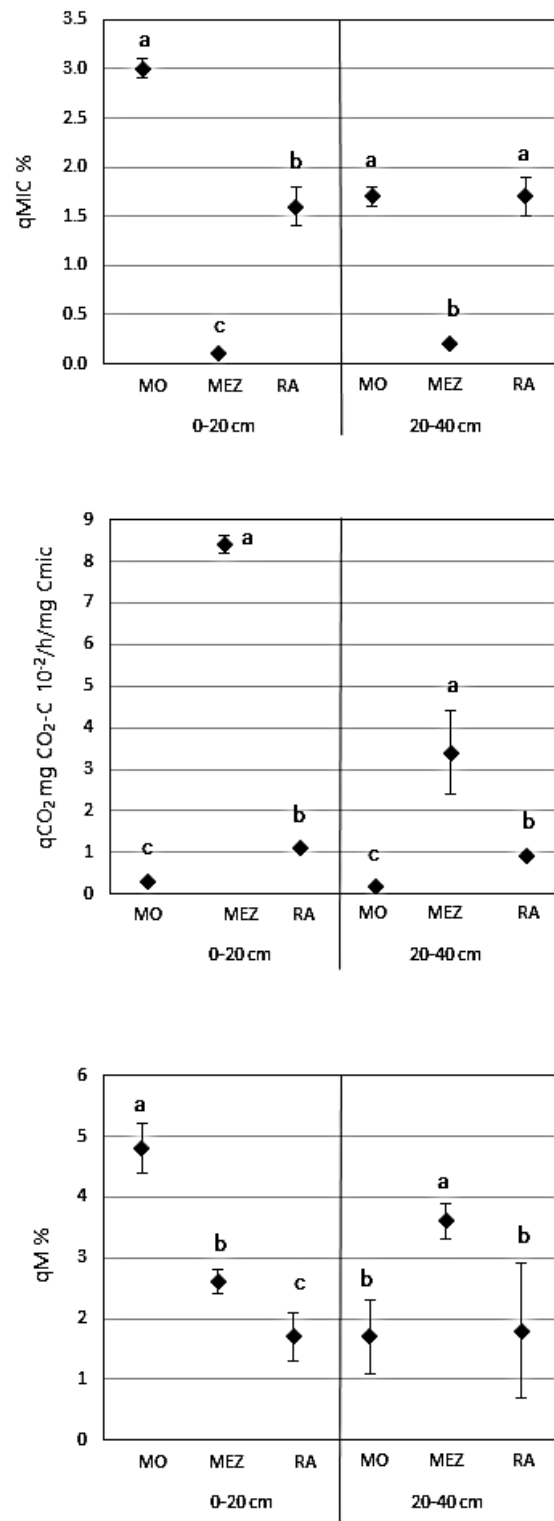


Figure 2. Microbial (qMIC), mineralization (qM) and metabolic (qCO₂) quotients and in 0–20 and 20–40 cm soil layers of processing tomato fields located in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy. Error bars represent standard error of the mean. Different letters mean significant differences (Kruskal–Wallis test, $p < 0.05$) among sites within 0–20 and 20–40 cm soil layers.

Checking the correlations among the values of indicators used for BFI calculation (Table 3), we found a strong positive correlation between OC amount and the CO₂ evolved during the 28-day incubation time ($r = 0.62$ and 0.63 for BR and Ccum, respectively) as well as between BR and Ccum ($r = 0.99$).

Table 3. Correlation matrix among the indicators used for biological fertility index (BFI) calculation in the three investigated sites. In bold, significant ($p < 0.05$) correlations are reported.

	OC	BR	Cum	Cmic	qCO ₂	qM
OC	1					
BR	0.62	1				
Ccum	0.63	0.99	1			
Cmic	0.19	0.06	0.06	1		
qCO ₂	-0.29	-0.09	-0.09	-0.63	1	
qM	-0.69	-0.32	-0.32	-0.32	0.68	1

OC: organic C; BR: basal respiration; Ccum: cumulative respiration; Cmic: microbial C; qCO₂: metabolic quotient; qM: mineralization quotient.

Therefore, the BFIs took into account the following indicators: OC and microbial C amount, mineralization quotient and metabolic quotient. In the BFI formulation, the range of soil fertility classes is (i) BFIs = 4 stressed soils with very low fertility; (ii) $4 < \text{BFIs} \leq 8$ pre-stress soils; (iii) $8 < \text{BFIs} \leq 12$ soils with intermediate fertility; (iv) $12 < \text{BFIs} \leq 16$ good fertility soils; (v) $16 < \text{BFIs} \leq 20$ soils with very high fertility. Thus, the values of BFIs, calculated for investigated soils, in both 0–20 and 20–40 cm soil depth were MO = 15 corresponding to IV fertility class (good), MEZ = 11 in III fertility class (intermediate) and RA = 6 in II fertility class (pre-stress) (Table 4).

Table 4. The simplified biological fertility index (BFIs) scores and corresponding fertility classes calculated in 0–20 and 20–40 cm soil layers of processing tomato fields located in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy.

Scheme	Site	BFIs Score	BFIs Class
0–20 cm	MO	15	IV (good)
	RA	6	II (pre-stress)
	MEZ	11	III (intermediate)
20–40 cm	MO	15	IV (good)
	RA	6	II (pre-stress)
	MEZ	11	III (intermediate)

3.3. Nutrient Content into Rhizosphere and Tomato Organs

Figure 3 shows the nutrient content in rhizosphere and in different organs of tomato plants. With the exception of Mg, significant differences in nutrient content in the rhizospheric soil were detected among sites (Table S3 of the Supplementary Materials); in particular, MO soils had the highest content in Ca, K, Na and P while MEZ soils had the highest content in S. The lowest concentrations of Ca and Na were found in the rhizosphere of MEZ and RA, respectively. In the tomato organs, instead, very few differences were found and only S seemed to maintain the detected difference among rhizospheric soils, being the highest in the young leaves and stem of plants grown in MEZ soils (Figure 3 and Table S3 of the Supplementary Materials).

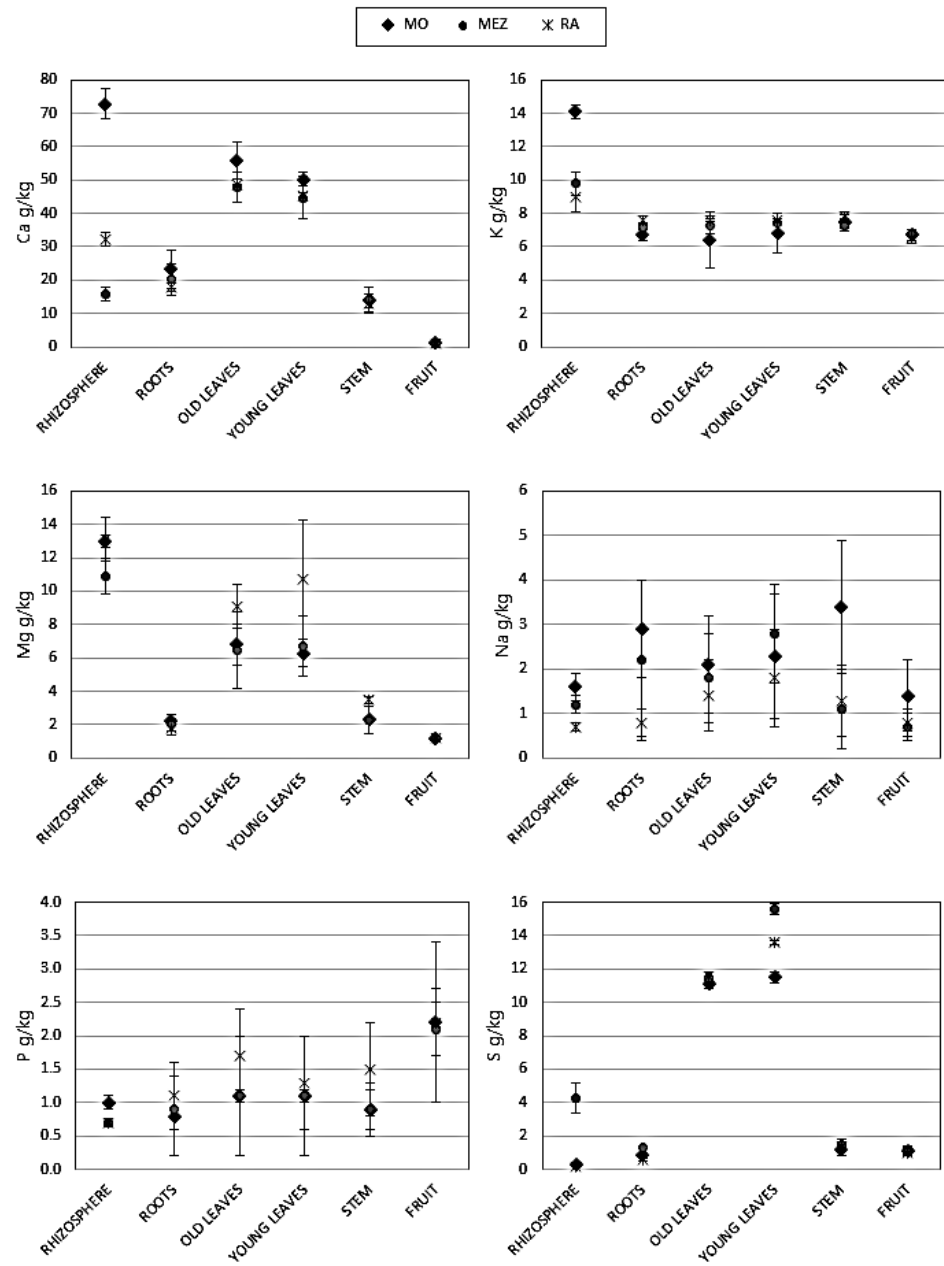


Figure 3. Ca, K, Mg, Na, P and S concentrations in rhizosphere soil, root, old and young leaves, stem and fruit of processing tomato plants cultivated in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy. Error bars represent standard error of the mean.

4. Discussion

4.1. Processing Tomato Cultivation Causes Higher Stress Conditions in Soils of MEZ and RA Study Sites Than in Those of MO Study Site

The highest OC content and the most negative $\delta^{13}\text{C}$ value (ca. -27‰) in MEZ soils was in agreement with the presence of ancient reclaimed peats. Indeed, in peatlands, accumulation of OC occurs and the $\delta^{13}\text{C}$ values of the accumulated plant debris are preserved due to anoxic conditions that limit the organic matter degradation in the peat and, therefore, no isotopic C fractionation occurs [39]. The highest OC content resulted in a higher CEC, which is an important soil feature in cropland ecosystems because it allows retention of the nutrients added through fertilization.

It is noteworthy to mention that, despite MEZ soils showing the highest concentrations of soluble C and N, which are the most easily available forms for microorganisms [40] promoting soil microbial biomass growth [41], the C_{mic} content was low. Moreover, MEZ soils had the lowest q_{MIC} and the highest q_{CO_2} . While the highest labile organic matter concentration can be assigned to the highest OC content [42], the low C_{mic} concentrations and the high q_{CO_2} should be attributed to the high OC:TN ratio of the soil organic matter. In fact, the high OC:TN ratio observed in MEZ soils, suggesting a low organic matter quality [43], likely prevented the microbial biomass growth [44]. However, in MEZ, we cannot exclude the possible depressing effect of salinity on microbial community [45]. The more stressful conditions occurring in MEZ study sites were, however, confirmed by the high value of q_{CO_2} . In fact, the q_{CO_2} depicts the energetic efficiency of the microbial community [34]; therefore, a high q_{CO_2} indicates a high energy demand for microbial biomass maintenance [46]. The lowest q_{MIC} in MEZ soils would further suggest a scarce efficiency of microbes to convert the soil organic carbon into microbial biomass [47]. Additionally, in MEZ soils, the q_{MIC} value was greatly lower than 2, which is considered a critical stress threshold for soils with neutral pH [34]. These findings supported the stressful condition for microbial biomass growth in MEZ soils.

In contrast to MEZ soils, RA and MO had different C use efficiency and the microbial respiration. Soils of RA study sites, having the coarser texture, showed the lowest OC and $\text{K}_2\text{SO}_4\text{-C}$ contents which might explain the low values of microbial respiration and C_{mic} . However, RA soils had the highest $\delta^{13}\text{C}$ values which would suggest how the small microbial population harboring in RA soils possess a high metabolism. This was also showed by the quite high q_{MIC} , which resulted in an accelerated soil organic matter decomposition. MO soils are characterized by a scarce OC content and high incorporation of soil organic C in the microbial biomass, as highlighted by the generally higher values of q_{MIC} and q_{M} . Furthermore, MO soils showed the lowest q_{CO_2} values suggesting relatively low stress levels [48], lower also than in RA.

The low soil organic matter quality of the MEZ soils and the imbalance between the current use and properties of these soils should contribute to reduction in the C use efficiency and growth of microbial population with respect to RA and MO soils. We cannot in fact exclude the effect of diverse fertilization strategies on the greater C use efficiency and the low microbial respiration occurring in MO and RA soils compared to MEZ ones. In fact, both in MO and RA, a higher amount of N fertilizers was added compared to MEZ which likely had a positive effect on soil microbial respiration [49,50]. Additionally, the contrasting soil texture between MO and RA soils might, at least partially, explain the lower microbial stress condition in MO than in RA—it being well known that finer soil texture promotes the microbial biomass growth [51] in contrast to coarser ones [52].

Overall, our findings illustrated that, in MEZ soils, despite the high concentration of organic C also in labile form, the microbial community does not immobilize that C into the microbial biomass, but it is lost through CO_2 emission (i.e., high basal and cumulative respiration values) and/or leached. On the contrary, MO soils, showed a less stressed microbial community characterized by a more efficient use of C resources. RA soils, instead, although showing similar microbial features as the MO ones, cannot be considered a

healthy system. Indeed, taking into account the $qCO_2:OC$ ratio, a qualitative index proposed by Dilly [53], both RA and MEZ soils had values greater than 400, which testifies an inefficient ecophysiological energy state of the system [53].

The better soil biological and biochemical quality of MO study sites is further confirmed by the BFI value which classifies it as a soil with a “good” biological fertility. Conversely, RA soils converged in a class characterized by “pre-stress” conditions, while MEZ soils were featured by an “intermediate” biological fertility. It is interesting to observe that the differentiation occurring for BFIs between MEZ and MO is missing for BFI. In particular, according to BFI, both MEZ and MO soils converged in “good” class, suggesting an overestimation of the index likely due to the presence of redundant and strongly correlated variables in its calculation. The proposed BFIs well describe the interaction between microbial communities and their activity even in soils where the high organic matter can mask some processes. Furthermore, the indications recorded by BFIs were coherent to the suggestions given by the $qCO_2:OC$ ratio proposed by Dilly [53].

4.2. Crop Quality

Despite the different microbial replies to the diverse soil types, very few differences of nutrient concentrations in tomato plant organs were detected. The general lack of differences of nutrient concentrations in plant organs can be attributed to the balanced use of chemical fertilizers applied by the farmers. Specifically, the amount of nutrients added to the soils was adjusted on the base of nutrient contents already present in soil with the aim to satisfy the plant nutritional requirements. However, noteworthy were the higher yield and brix grades of processing tomatoes grown on MO soils compared to MEZ and RA (Table S1 of the Supplementary Materials). Due to the role of soil nutrients on tomato yield [54,55], the highest yield of processing tomatoes grown on MO could be attributed to the highest concentrations of Ca, K and P in the rhizosphere of tomatoes located in MO. Instead, the lower yield of processing tomatoes cultivated in MEZ could be due to the higher salinity [56]. However, the differences of yield and brix grades might also be assigned to the diversity of the soil biological and biochemical fertility, which is a key component for crop production and quality [57,58]. Hence, it appears that the critical soil conditions detected by the BFIs relating to inefficient C use by soil microbial communities can lead to a worsening trend of crop production. Overall, in our opinion the data indicated a good suitability of MO area to the studied farming system, but an environmental risk related to soil degradation exists in the MEZ and RA sites.

5. Conclusions

Soil is an essential part of the CZ and is deeply influenced by anthropogenic actions, such as agricultural activities. Therefore, there is a need for measurable proxies that indicate the health status of agricultural soils. In this light, our study provided evidence about the usefulness of the biochemical properties to evaluate the stressful conditions of soils. Specifically, low values of $qMIC$ and $Cmic$ and high values of qCO_2 indicate soils where the microbial community has a low fitness to incorporate carbon in their own structures and, therefore, a scarce growth of microbial biomass. In this sense, MEZ and RA sites showed less biologically active soils. Furthermore, our study highlighted how the BFIs, where the highly correlated parameters were removed, could be an effective indicator of soil stress conditions.

The better yield and brix grades of processing tomatoes observed in those soils with higher values of BFIs, $qMIC$ and $Cmic$ and lower values of qCO_2 (MO sites) confirmed our second hypothesis about the influence of soil biological fertility on crop yield and quality.

Finally, the present paper shows that agroecosystems, i.e., important CZ components, have to be investigated with suitable biogeochemical indexes that help to understand the soil functioning, giving indication for a sustainable agricultural management. Indeed, a soil characterized by scarce biological fertility could indicate the occurrence of degradation processes.

Supplementary Materials: The following are available online at www.mdpi.com/2075-163X/11/2/219/s1, Table S1: Amount of nitrogen (N), phosphorus (P₂O₅) and potassium (K₂O) applied by fertigation to soil, the mean yield and brix degree of tomatoes of processing tomato fields located in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy, Table S2: The scores of indicators for BFI calculation, Table S3: Results of Kruskal–Wallis test on the nutrients content in rhizosphere (Rhizo) and tomato organs (Roots, Old and Young leaves, Stem, Fruit) in processing tomato fields located in Modena (MO), Ferrara (MEZ) and Ravenna (RA) provinces, Emilia Romagna Region, Italy.

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