Deliverable action B4: Soil quality indices

Introduction: soil quality definition

During the last decades, the Scientific Community is increasingly drawing attention on soil. Soil is not yet considered simply a sort of substrate for fibre and food production, but a fundamental component of the biosphere involved in the maintenance of environmental quality at local, regional and global scale. In this context, the necessity of defining criteria for (i) determining the health of soils and (ii) developing indexes for soil quality comparisons in time and space is becoming one of the main topics of soil research. In order to reach these objectives, it necessary to develop a flexible procedure for indexing able to be easily modified according to the different soil types (Andrews et al., 2002). Moreover, it should be able to classify the different categories of dynamic quality, evaluate their evolution in time and finally use results to quantify long term effects of different soil use (Andrews et al., 2002).

Soil quality can be defined in terms of agricultural productivity, environmental quality or land use applications; further definitions include “fitness for use” and “the capacity of a soil to function”. Thus, soil quality is the ability of a soil to perform the functions necessary for its intended use.

Soil functions include:

- Sustaining biological diversity, activity, and productivity
- Regulating water and solute flow
- Filtering, buffering, degrading organic and inorganic materials
- Storing and cycling nutrients and carbon
- Providing physical stability and support

Since the soil functions are very difficult to be measured, the soil quality approach considers soil parameters as indicators of the function analysed
In particular, dynamic soil properties, which are soil characteristics susceptible to significant changes in a single year or growing season, are considered (Fig. 1).
In order to assess the soil dynamic properties, biological, physical and chemical soil parameters are considered as indicator and measured.
An indicator to be effective in the evaluation of soil quality, must meet the following requirements (Doran and Parkin, 1996; Doran and Zeiss, 2000):

1. Sensitivity to changes in soil management. "An indicator should be sensitive enough to reflect the influence of different types of management and climate changes on long-term soil quality (Doran and Parkin, 1996). Soil organisms (biological indicators), in particular, have this requirement, being able to respond significantly to human activity (Pankhurst et al., 1997; Wolters and Schafer, 1994).
2. To be well correlated with the positive functions of soil.
3. Be helpful to explain ecosystem processes. Indicators should explain why the soil "work" or "will not work" as expected by providing guidance on how to act if necessary to return to the original situation.

4. Be understandable and useful to decision makers.
5. Be easy and cheap to measure. The indicators of the quality of the soil should be accessible both in economic terms (Pankhurst et al., 1997 Ndiaye et al., 2000) that the time required for their determination, and being of easy execution (Dick et al., 1996).

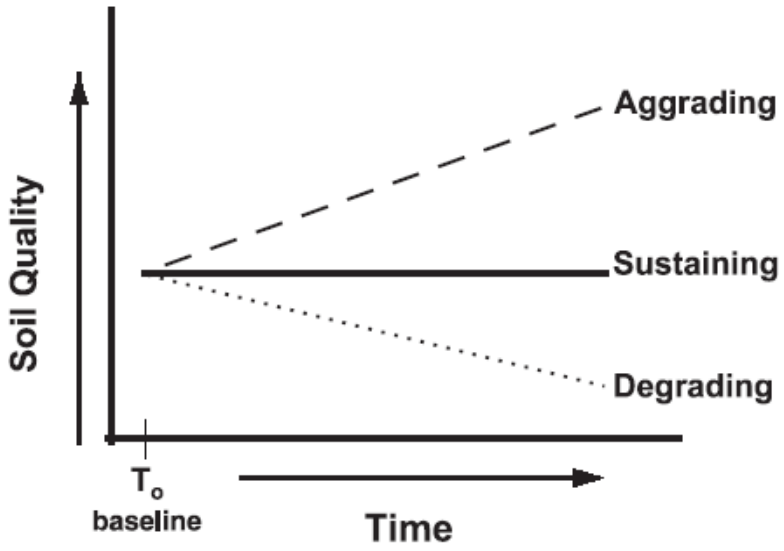


Fig. 1 Possible evolution of soil quality

A common strategy for evaluating soil quality consists in selecting a minimum data set of soil quality indicators (MDS) and condensing it in a synthetic index (IQ).
In detail, the indexing of the dynamic quality of the soil takes place through three steps:
• selection of appropriate indicators to represent the soil functions that you want to monitor;
• transformation of the indicator value in a nnormalisedscore;
• Integration of the data of the indicators nnormalisedto obtain an index value of soil quality (IQ).

Working methodology for LIFE DOP Project

During LIFE DOP the following working steps were implemented to assess the changes in soil quality caused by different management practices (Dynamic or management dependent features).

Soil sampling

Representative samples of each experimental plot were collected according to standard procedure.

Soil samples were collected from a depth of 40 cm by randomised sampling at the beginning of the trial (before treatment, time 0) and at the end of the experimental plan (time f).

Definition of Synthetic Soil Quality Index (IQ)

The following biological, physical and chemical indicators selected basing on expert opinions as significant soil functions were measured and employed to construct the Minimum Data Set in order to describe soil quality.

In particular, the following parameters will be measured:

- Organic Carbon
- Organic Matter
- Total Nitrogen
- pH
- C/N ratio
- Texture
- CEC (Cation Exchange Capacity)
- Available Phosphorus
- Soil respiration
- WHC (Water Holding Capacity)
- PLFA (Phospholipid Fatty Acid)

To define if and how the use of the tested ffertilisersis able to affect soil quality, previous parameters will be used to calculate the quality indexes (IQ).
Soil parameters were be grouped in soil functions (i.e. nutritional, biological etc..). Afterwards, the kind of variation ("*more is better","less is better","optimum"*) and the relative theoric range of variation was identified depending on for each indicator of the MDS.

Aim

The analytical investigations reported in this deliverable were carried out in order to aanalysethe preliminary and final conditions ("zero time" and "final time") of the soils considered in the experiments by the LIFEDOP project, before the application of the various management practices compared. The results will form part of the dataset used by the Ricicla group in evaluating the soil quality. These analyses were repeated at the end of the experimentation period (2021) to allow a comparison of the results, and by indexing the dynamic quality of the soil, to evaluate which practices will be more sustainable to improve soil quality.
For this purpose, two techniques were applied, such as the analysis of soil respiration and the analysis of PLFA (phospholipid fatty acids), in order to determine the extent of the microflora present in the sampled soils, from a qualitative-quantitative point of view. The analysis of respiration, through the measurement of the CO_2 produced by the samples, made it possible to quantify the extent of the soil biome from a quantitative point of view. The analysis of PLFA,

on the other hand, made it possible to study the quantity and composition of the microbial population. PLFA (phospholipid fatty acids) are important components of the cell membrane and are widely used as chemotaxonomic markers in soil microbiology studies (Zelles, 1999).

Material and methods

Demofarms

The soil samples analysed came from two experimental fields located in the Mantua area and belonging to two farms growing cereal crops. Field Z is located at the Bertoletta farm (Via Birla, 46020 Pegognaga, Mantua - N 44.975480; E 10.90209499) consisted in a 10-hectare plot cultivated with maize (Zea Mais) during the experimentation. Field G is located at the Corte Zamiola farm (Via Circonvallazione Est, 43, 42045 Luzzara RE - 44 ° 57'17.44''N; 10 ° 41'49.63''E) and consists of two adjacent plots of 2.38 and 2.45 hectares also in this case cultivated with corn (Fig. 2).

Fig. 2 Field Z (left) and field G (right)

Field Z was subjected to 5 different management practices corresponding to 4 experimental theses plus the control; for field G, 2 experimental theses plus the control were applied. The control, in both cases, consists of the standard management practices commonly adopted in the territory. Each thesis was replicated three times according to an experimental design in randomised blocks, for a total of 15 samples for the Z field and 9 samples for the field.
All the soil samples were collected with random sampling at a depth of 40 cm.
The innovative practices researched are for z thesis:

- replacement of mineral fertilisation with digestate and application of the digestate by injection or drop distribution;
- minimum tillage and instead of conventional tillage;

The innovative practices researched for G thesis are:

- Use of solid digestate in place of chemical fertilization on presowing
- use of cover crop.

Field Z

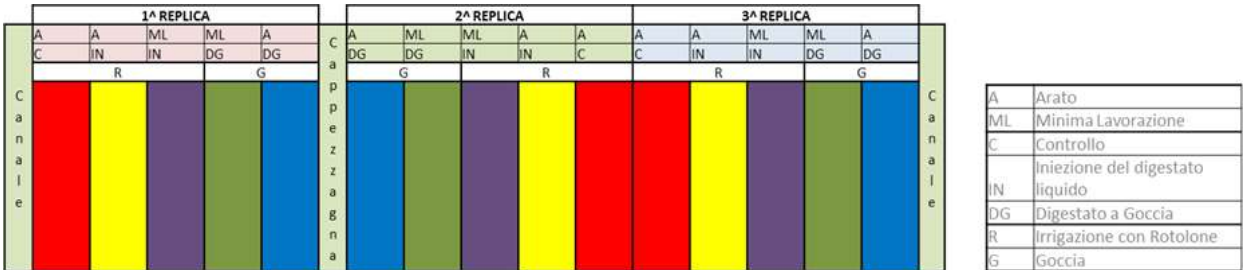


Fig. 3 Experimental design Field Z

The control thesis of the Z field, corresponding to the samples Z1, Z10 and Z11, consists of a conventional tillage of the fields by plowing, fertilisation with slurry during autumn and addition of 170 units of urea in the spring.

The second thesis, corresponding to samples Z2, Z9 and Z12, consists of plowing, injection of liquid digestate in pre-sowing (94 nitrogen units) and liquid digestate distribution in emergency with 234 units of nitrogen.
The third thesis, corresponding to samples Z3, Z8 and Z13, consists in the conservative tillage (strip tillage) and fertilisation techniques similar to thesis 2.
Thesis 4, corresponding to samples Z5, Z6 and Z15, consists application of liquid digestate distributed in different moments by fertirrigation for a total of 246 nitrogen units and conventional plowing.
Finally, thesis 5, corresponding to samples Z4, Z7 and Z14, consists of minimal tillage and fertilisation similar to that reported for thesis 4.

Field G

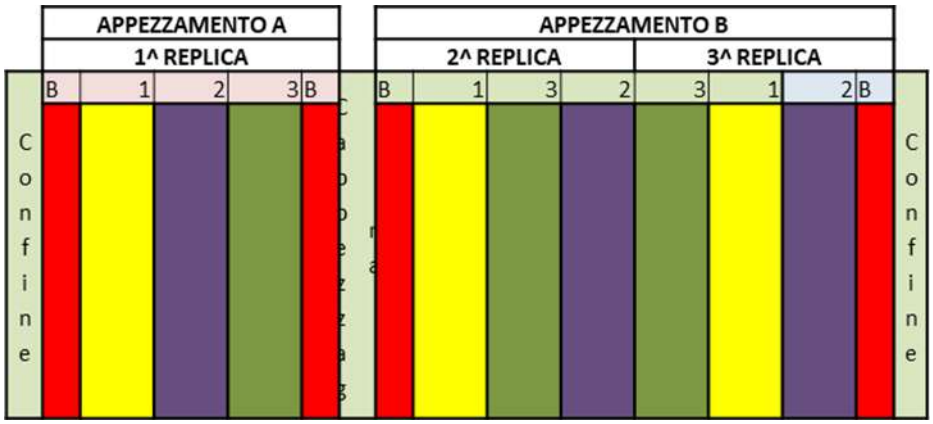


Fig. 4 Experimental design Field G

The control thesis of field G (1G), corresponding to the samples GZ1, GZ4 and GZ8, consists in fertilisation with 281 units per hectare of synthetic nitrogen (urea). The 2G thesis, corresponding to samples GZ2, GZ6 and GZ9, consists of a fertilisation management with 76 units / ha of nitrogen in the form of solid fraction of digestate (50 m^3, 0.51% N, efficiency 30%) in pre-plowing, to which 175 units / ha of synthetic nitrogen (urea) in rising were added.

The 3G thesis, corresponding to the GZ3, GZ5 and GZ7 samples, consists of fertilisation with solid fraction of digestate in autumn (quantity and characteristics of the digestate equal to those reported for the 2G thesis), 175 units / ha of urea and sowing of cover crops (weed of grasses) concluded in spring with roller crimper and followed by sod sowing.

Determination of soil respiration

The method (titrimetic method, ISO 16072:2002) involves the use of hermetically sealed jars in which a weighed quantity of the soil to be tested and a "control" is introduced. The analyses are carried out in duplicate. In detail, the following operations were performed:
- Weigh 20 g of sieved and mortared soil in the jars and add water in order to bring them to the maximum water capacity, taking care that there is no free water that could cause anoxic conditions.
- Fill the beakers with 20 ml of sodium hydroxide (NaOH 0.1 N) and place them inside the airtight jar with the soil.
- Close everything tightly and let respiration take place.
- Titrate with hydrochloric acid (HCl 0.1 N) the "not consumed" soda (frequency of 3-4 days in the initial period, weekly in the central period and biweekly towards the end of the analysis) using a solution of barium chloride dihydrate (0.5M $BaCl_2$) and phenolphthalein (0.5 g phenolphthalein, 50 ml ethanol, make up to 100 ml with distilled water) as an indicator. Titration of the NaOH with HCl reveals the exact amount of NaOH that has remained in the beaker, that is, the amount that has not reacted with the CO_2 produced by microbial respiration. It follows that if a soil breathes a lot, it produces a lot of carbon dioxide which, reacting with the NaOH to form Na2CO2, will decrease its quantity, so that the titration hydrochloric acid used for this soil will be less than that used for a soil that breathe much less. This analysis was conducted for 59 providing the data underlying the construction of the respiration curves. The respiration curve shows the temporal trend of soil respiration.
In particular, using the following formula, the CO_2 production of the samples used can be calculated.

$$RCO_2 = \frac{2,2\,(V\,m\,b - V\,m\,p)}{Pd\,w}$$

in which:
- RCO_2 is the CO_2 production rate of a unit of dry soil (mg CO2 · g-1 dry soil)
- 2.2 is the factor (1 ml of 0.1 N HCl corresponds to 2.2 mg of CO2) (mg ml-1)
- Vmb is the average volume of HCl consumed in the control, in ml
- Vmp is the average volume of HCl consumed in the sample considered, in ml
- Pdw is the dry weight of the sample considered, in g

The respiratory dynamics in these standardised conditions is usually characterised by a high initial respiration rate that gradually decreases due to the exhaustion of the nutritional resources readily available for the decomposing microorganisms, tending to an ideal plateau in which, all accessible organic matrices are exhausted. The respirometric curve therefore

appears as a function characterised by a higher initial inclination which, progressively in different times and dynamics depending on the soil, tends to decrease until it settles on minimum values.

Phospholipid fatty acid determination

The phospholipid fatty acid (PLFA) were determined by using the methodology proposed by Buyer and Sasser (2012). To examine the soil communities in term of bacteria and fungi, the following attribution was applied.

Nomenclatura IUPAC	Formula	Marker	Referenze
Decanoic acid, methyl ester	C11:0	General bacteria	Fernandes et al., 2013
Dodecanoic acid, methyl ester	C12:0	General bacteria	Chen et al., 2016
Tridecanoic acid, methyl ester	C13:0	General bacteria	Chen et al., 2016
Methyl 2 hydroxydecanoate	2OH C10:0	Gram Negative	Willers et al., 2015
Methyl 13 methyltetradecanoate	iC15:0	Gram Positivo	Gharaibeh e Voorhens, 1996; Ibekwe e Kennedy, 1999; Kaur et al., 2005; Quideau et al., 2016
Methyl 12 methyltetradecanoate	aC15:0	Gram Positivo	Gharaibeh e Voorhens, 1996; Ibekwe e Kennedy, 1999; Kaur et al., 2005; Quideau, et al. 2016
Methyl 3 hydroxytetradecanoate	3OH C14:0	Gram Negative	Willers et al., 2015
Methyl tetradecanoate	C14:0	General bacteria	Zelles, 1997; Kaye, 2005; Treonis et al., 2004
Methyl myristoleate	C14:1	Gram Negative	Zelles, 1999
Pentadecanoic acid, 14 methyl,	iC16:0	Gram Positivo	Kaye et al., 2005; Chen et al., 2016; Quideau et al., 2016;
Pentadecanoic acid, methyl ester	C15:0	Gram Positivo	Gharaibeh e Voorhens, 1996, Zelles, 1997
Hexadecanoic acid, methyl ester	C16:0	Gram Negative	Gharaibeh e Voorhens, 1996; Zelles, 1997; Treonis et al., 2004
9 Hexadecenoic acid, methyl ester, (Z)	$C16:1^{9}$	Gram Negative	Gharaibeh e Voorhens, 1996; Zelles, 1997 e 1999
Methyl 15 methylhexadecanoate	iC17:0	Gram Positivo	Gharaibeh e Voorhens, 1996; Kaye et al., 2005; Chen et al., 2016; Quideau et al., 2016
cis 9,10 Methylenehexanedecanoate	C17:0Δ	Gram Negative	Guckert et al., 1985; Gharaibeh e Voorhens, 1996; Ibekwe e Kennedy, 1999; Kaur et al., 2005
Heptadecanoic acid, methyl ester	C17:0	General bacteria	Kaye et al., 2005; Zelles, 1997
Octadecanoic acid, methyl ester	C18:0	Gram Positivo	Gharaibeh e Voorhens, 1996; Zelles, 1997; Treonis et al., 2004
Methyl elaidate trans	C18:ω9	Fungi	Kaye et al., 2005; Quideau et al., 2016; Treonis et al., 2004
Methyl oleate cis	C18:1	Gram Negative	Gharaibeh e Voorhens, 1996, Zelles, 1997
9,12 Octadecadienoic acid (Z,Z) methyl	C18:2 ω9 12	Fungi	Hedrick et al., 2007
9,12,15 Octadecatrienoic acid,	18:3 ω3	Fungi	Zelles, 1999; Buyer e Sasser, 2012
cis 9,10 Methylenoctadecanoate	c19:0Δ	Gram Negative	Guckert et al., 1985; Gharaibeh e Voorhens, 1996; Zelles, 1999, Quideau et al., 2016
Eicosanoic acid, methyl ester	C20:0	Gram Negative	Li et al., 2009
Methyl cis 11 eicosenoate	C20:1	Gram Negative	Zelles, 1997
Docosanoic acid, methyl ester	C22:0	Gram Negative	Li et al., 2009
Nonadecanoic acid, methyl ester	C19:0	Gram Negative	Zelles, 1999; Buyer e Sasser, 2012

Results and Discussion

Soils characteristics: evolution before and after the treatments

The main chemical-physical characteristics of the soils considered in this report were:
Water Holding Capacity (WHC) texture, pH, CEC Cation Exchange Capacity), TOC (Total Organic Carbon), Ntot (total nitrogen), C / N ratio, Pass (available phosphorus).The results of the chemical-physical analyses of the samples follow, divided by field of origin, and the statistical processing to which they were subjected in order to verify their homogeneity at zero time and any differences at the final time.

Water Holding Capacity

Water Holding Capacity (WHC) for the soils (time zero and final time) are reported in Table 0. This parameter does not show any variation over time and treatment, probably because it is affected by the texture (clay content increases WHC) and soil organic matter content, parameters that rarely change in short term experiments, except little increase in some soils of this experiment for organic matter content.

Table 0. Soil Water Holding Capacity

Z thesis average values		
Campione (Tesi)	**WHC (time zero)**	**WHC (final time)**
	(g 100 g^{-1} DM)	
1Z (testimone)	37.2±1.5	39.9±1.7
2Z	35.3±3.2	37.8±3.5
3Z	39.1±2.7	41.7±3.0
4Z	36.6±0.9	39.3±1.1
5Z	34.9±3.4	37.2±3.8
G thesis average values		
G0 (testimone)	47.2±2.2	48.9±1.7
G1	45.8±1.8	43.4±2.3
G3	48.1±4.2	47.2±1.6

Texture

Field Z

The samples were divided into texture classes using the USDA (United State Department of Agriculture) classification method. The soils of field Z were mainly placed in the USDA "basically clayey" class, presenting a medium-high clay content that varies from a minimum value of 24% to a maximum value of 40% of the total dry matter (DM), with an average of 32

± 6%. This analysis was not repeated at the end because this parameter is very stable and it is believed that it cannot be changed in a few years as a result of different agronomic practices.

Chemical characterization

The chemical characteristics of the field Z at time zero are reported in Table 1 and, summarised on the basis of the treatment, in Table 2.
Data reported in table 1 show that the pH of the soils presents a low variability, ranging from soils with an acid reaction (min value 5.8) to soils with a slightly acid reaction (max value 6.6). The average value of 6.21 reflects a greater positioning of the samples in the range of light acidity (6.1-6.7 in which 13 samples out of 15 are placed) rather than in the narrow acidity (5.4-6.0, 2 samples out of 15). The Corg ranges from values of 1.1 to 2.1 (% of dry matter) with an average of 1.56, corresponding to an average content of 2.7% of organic matter on the total dry matter. This datum, if compared to the texture of the medium clayey soils, corresponds to a normal value. The total nitrogen ranges from low values (min value 0.09%) to high values (max value 0.22%) with an average value of 0.17% corresponding to a medium value. The C / N ratio varies from a minimum value of 7.4 to a maximum of 14.4. Although the average of 9.1 corresponds to a balanced value (values between 9 and 12 are considered balanced) only three of fifteen samples are actually in this range (Z2, Z3 and Z7). Two samples (Z8 and Z9) have higher values while the remaining 10 samples have a C / N ratio lower than 9, highlighting the tendency of the sampled soils to present a rapid mineralisation dynamic. This fact could contribute to rapid organic matter degradation with negative repercussions on fertility of soils.

Table 1 Chemical characteristics of the field **Z (time zero)**

CAMPO Z						
Campione (Tesi)	pH	CSC (cmol+/kgss)	Ntot (%ss)	Corg (%ss)	C/N	Pass (mg(kg)
Z1 (0)	6,3	12	0,22	1,9	8,6	140
Z10 (0)	6,2	9,8	0,22	1,9	8,6	46
Z11 (0)	6,2	14	0,21	1,6	7,6	48
Z2 (2)	6,1	16	0,22	2,1	9,5	91
Z9 (2)	6,2	17	0,09	1,3	14,4	25
Z12 (2)	6,6	15	0,17	1,4	8,2	21
Z3 (3)	6,1	17	0,19	1,8	9,5	38
Z8 (3)	6,1	14	0,1	1,2	12,0	19
Z13 (3)	6	26	0,18	1,5	8,3	38
Z5 (4)	6,3	19	0,19	1,6	8,4	51
Z6 (4)	6,2	11	0,16	1,4	8,8	40
Z15 (4)	6,5	10	0,19	1,5	7,9	42
Z4 (5)	6,4	12	0,21	1,8	8,6	45
Z8 (5)	5,8	25	0,12	1,1	9,2	30
Z14 (5)	6,2	17	0,19	1,4	7,4	31

The CSC of the soils has a high variability ranging from a minimum of 9.8 to a maximum of 25 (cmol + / kg ss). Excluding the minimum value (Z10) and a few others just above sufficiency (a CEC <10 cmol / kg is considered low), most of the samples have a medium-high cation exchange capacity, a value in accordance with the average clayey texture of the samples. As for the other nutritional elements, all soils have a very high supply of potassium (minimum value: 730 mg K / kg ss), according to the texture, while the amount of assimilable phosphorus has a high variability for which it is more appropriate to focus on the individual cases. In fact, if two thirds of the samples (10 out of 15) have normal-high values of assimilable phosphorus (i.e.> 37 mg Pass / kg ss), in as many as 5 out of 15 samples the Pass values were found to be low enough to potentially constitute a limiting factor for soil fertility.

The structural data were grouped according to the plots constituting the theses and the mean and standard deviation of each group were calculated (Table 2).

Table 2. Chemical characteristics of the field Z (time zero): average values

CAMPO Z valori medi parcelle						
TESI	Ph	csc	Ntot	Corg	C/N	Pass
1Z (testimone)	6,2 ± 0,06	11,9 ± 2,1	0,22 ± 0,01	1,8 ± 0,17	8,3 ± 0,6	78 ± 53
2Z	6,3 ± 0,26	16 ± 1	0,16 ± 0,07	1,6 ± 0,44	10,74 ± 3,3	45 ± 39
3Z	6,1 ± 0,06	19 ± 6,2	0,16 ± 0,05	1,5 ± 0,3	9,94 ± 1,9	31 ± 10
4Z	6,3 ± 0,15	13,3 ± 5	0,18 ± 0,02	1,5 ± 0,1	8,4 ± 0,4	44 ± 6
5Z	6,1 ± 0,31	18 ± 6,5	0,17 ± 0,05	1,43 ± 0,35	8,4 ± 0,9	35 ± 8
Media Campo Z	**6,2 ± 0,2**	**15,6 ± 4,9**	**0,17 ± 0,04**	**1,56 ± 0,28**	**9,1 ± 1,8**	**47 ± 30**

The data was subjected to analysis of variance (ANOVA) in order to evaluate its homogeneity. The results of the test confirmed, for all variables, the hypothesis that the slight variability of the parcels is not due to actual structural differences but is rather the result of natural samples variability.

The chemical characteristics of the field Z at final time are reported in Table 3 and, summarised on the basis of the treatment, in Table 4.

Table 3 Chemical characteristics of the field Z (final time)

CAMPO Z						
Campione (Tesi)	pH	CSC (cmol+/kgss)	Ntot (%ss)	Corg (%ss)	C/N	Pass (mg/kgss)
Z1 (0)	6,5	15	0,21	1,8	8,6	86
Z10 (0)	6,2	10	0,23	1,8	7,8	45
Z11 (0)	6,3	12	0,20	1,6	8,0	39
Z2 (2)	6,7	16	0,22	1,7	7,7	45
Z9 (2)	6,1	21	0,22	1,9	8,6	51
Z12 (2)	5,9	15	0,19	1,5	7,9	43
Z3 (3)	6,7	18	0,24	1,8	7,5	42
Z8 (3)	6,2	14	0,22	1,7	7,7	29
Z13 (3)	6,5	19	0,23	1,9	8,3	50
Z5 (4)	6,2	19	0,15	1,3	8,7	47
Z6 (4)	6,6	15	0,20	1,8	9,0	41
Z15 (4)	5,8	16	0,13	1,6	12,3	46
Z4 (5)	6,1	19	0,18	1,9	10,6	33
Z8 (5)	6,3	16	0,20	1,3	6,5	42
Z14 (5)	6,2	19	0,14	1,1	7,9	55

To better assess if and to what extent the different fertilisation treatments determined substantial effects data reported in Table 4, as average values, and compared with Table 2 can help us. In general, the different fertilisation techniques don't affect soil characteristics, probably because only long-term experiments could be able to show this, anyway, as expected, a tendency for the soil subjected to conservative management (minimal tillage), fertilised by injection of digestate show a tendency in the increasing both of total nitrogen and organic carbon. This because as known, minimum tillage is a typical soil conservation technique and organic nitrogen and carbon, subjected to a more rapid turnover, are soil parameter able to show rapid changes also in relatively short time.

Table 4. Chemical characteristics of the field Z (final time): average values

CAMPO Z valori medi parcelle						
Campione (Tesi)	pH	CSC (cmol+/kgss)	Ntot (%ss)	Corg (%ss)	C/N	Pass (mg/kgss)
1Z (testimone)	6,3±0,2	12±2	0,21±0,02	1,7±0,1	8,1±0,4	57±26
2Z	6,2±0,4	17±3	0,21±0,01	1,7±0,2	8,1±0,5	46±4
3Z	6,5±0,3	17±3	0,23±0,01	1,8±0,1	7,8±0,4	40±11
4Z	6,2±0,4	17±2	0,16±0,04	1,6±0,3	10,0±2,0	45±3
5Z	6,2±0,1	18±2	0,17±0,03	1,4±0,4	8,3±2,1	43±11

Field G

The soils of field G, according to the USDA classification method, are placed in the USDA "loamy" textural class which identifies soils characterised by a balanced distribution between sand, silt and clay.

The chemical characteristics of the field G at time zero are reported in Table 5 and, summarised on the basis of the treatment, in Table 6.

Table 5 Chemical characteristics of the field Z (time zero)

CAMPO G						
CAMPIONE (TESI)	Ph	CSC (cmol/kg)	Ntot (%ss)	Corg (%ss)	C/N	Pass (mg(kg)
GZ1 (0)	6,2	20	0,15	1,3	8,7	29
GZ4 (0)	6,2	18	0,17	1,4	8,2	20
GZ8 (0)	6,1	25	0,16	1,5	9,4	21
GZ2 (2)	6,1	25	0,16	1,4	8,8	38
GZ6 (2)	6,2	22	0,17	1,5	8,8	30
GZ9 (2)	6,6	18	0,13	1,2	9,2	25
GZ3 (3)	6,3	23	0,17	1,3	7,6	20
GZ5 (3)	6,6	24	0,24	1,7	7,1	24
GZ7 (3)	6,1	23	0,18	1,5	8,3	27

Data reported in Table 5 show that the reaction of the samples of the G field have a variability lower than the samples of the Z field, and all the samples are "slight acid" (pH = 6.1-7). The Corg content also has a lower variability with values ranging from 1.3% to 1.7 % dm; the average of 1.42% (equivalent to 2.45% in SO) reveals an average endowment high if related to the loose texture of these lands. The total nitrogen supply presents average values (we consider average values between 1.0 and 2.0 g / kg) in all the samples except the GZ5 sample characterised by a high supply (2.4 g / kg). The C / N ratio presents a low variability with values between 7.1 and 9.4 with an average of 8.4. Only two samples (GZ8 and GZ9) have a value between 9 and 12, considered balanced. The others have values <9. Therefore, what has already been stated for the soils of field Z in the relevant section remains valid. The CEC of soils has medium-high values ranging from 18 to 25 cmol + / kg dm with an average value of 22. As regards the other nutritional elements, all soils have a very high supply of potassium (minimum value: 870 mg K / kg DM) while the supply of available phosphorus tends to be low: only one sample (GZ2) exceeds the value that is conventionally considered sufficient (37 mg / kg DM) while the remaining 8 samples presented low values (minimum value of 20 mg / kg).

The structural data were grouped according to the plots constituting the future theses and the mean and standard deviation of each group were calculated (Table 6).

Table 6. Chemical characteristics of the field Z (time zero): average values

CAMPO G						
TESI	Ph	CSC (cmol/kg)	Ntot (%ss)	Corg (%ss)	C/N	Pass (mg(kg)
G0 (testimone)	6,2 ± 0,06	21 ± 3,6	0,16 ± 0,01	1,40 ± 0,1	8,7 ± 0,6	23 ± 5
G1	6,3 ± 0,26	21 ± 3,5	0,15 ± 0,02	1,36 ± 0,15	8,9 ± 0,2	31 ± 6
G3	6,3 ± 0,25	23 ± 0,6	0,19 ± 0,04	1,50 ± 0,20	7,7 ± 0,6	23 ± 3
Media campo G	6,2 ± 0,20	22 ± 2,7	0,17 ± 0,03	1,42 ± 0,15	8,5 ± 0,7	26 ± 6

Also in this case the data were subjected to analysis of variance (ANOVA) in order to evaluate its homogeneity. The results of the test confirmed, for all variables, the hypothesis that the slight variability of the parcels is not due to actual structural differences but is rather the result of natural sample variability.

Soil characteristics at the end of the experiments are reported and summarised in Table 7 and 8.

Table 7 Chemical characteristics of the field G (final time)

CAMPO G						
Campione (Tesi)	pH	CSC (cmol+/kgss)	Ntot (%ss)	Corg (%ss)	C/N	Pass (mg/kgss)
GZ1 (0)	6,1	21	0,12	1,2	9,2	27
GZ4 (0)	6,2	23	0,18	1,3	7,2	25
GZ8 (0)	6,5	17	0,13	1,2	9,2	19
GZ2 (2)	6,3	21	0,16	1,3	8,1	31
GZ6 (2)	6,2	23	0,19	1,5	7,9	28
GZ9 (2)	6,3	23	0,18	1,5	8,3	25
GZ3 (3)	6,5	22	0,20	1,6	8,0	25
GZ5 (3)	6,1	25	0,25	1,6	6,4	36
GZ7 (3)	6,1	24	0,24	1,8	7,5	31

Comparing data reported in Table 6 (time zero) and Table 8 (final time) it is possible to find some differences, also in this case, relative to soil organic matter. In particular, treatment G3 (fertilisation with solid fraction of digestate in autumn, urea and sowing of cover crops) seems to determine an increase of total nitrogen and organic carbon as consequence of the presence of the cover crops. At the same, time, however, the C to N ratio for this treatment decrease from 8.5 to 7.3. This means, in the short time, an improvement of soil organic matter that is, at the same time, more easily degradable. In this sense, only in the long term will be possible assess if the soil organic matter increase can be considered only "apparent". Finally, the increase of available phosphorous does not seem to be statistically significant.

Table 8. Chemical characteristics of the field Z (final time): average values

CAMPO G valori medi parcelle						
Campione (Tesi)	**pH**	**CSC (cmol+/kgss)**	**Ntot (%ss)**	**Corg (%ss)**	**C/N**	**Pass (mg/kgss)**
G0 (testimone)	6,3±0,2	20±3	0,14±0,03	1,23±0,06	8,5±1,1	24±4
G1	6,3±0,1	22±1	0,18±0,01	1,43±0,12	8,1±0,2	28±3
G3	6,2±0,2	24±1	0,23±0,03	1,67±0,11	7,3±0,8	31±6

Microbial community composition

Field Z

The PLFAs at T=0 Y had very different values. This result can be due to the difficult to represent microorganism composition in a full scale field but also to the heterogeneity of the soil considered in the filed Z.
Nevertheless, the absence of difference suggested that the field had a homogenous condition as baseline for the successive treatment. The data were more coherent in term of composition since few constant PLFA were the main contributors to the total amount. All these PLFA came by microbial community that was prevalent respect to the fungi in agreement with literature (Zhang et al, 2012).
The predominance of bacteria is proper of agricultural soil for the higher C turnover, physical perturbation and not-stable growing condition.

After three year of treatment the PLFA had similar value with that of T=0 Y. Moreover, again very high difference were found thus no significant changes are detectable. In any case was confirmed a commune evolution for the soil probably attributable to the external condition (i.e. soil moisture, climate, etc…) rather than the soil management system.

Table 9a. PLFAs of the field Z (time zero)

.

TIME=0

PLFA	T 2Z			T3Z			T5Z			T4Z			T1Z (control)		
	Z2	Z9	Z12	Z3	Z8	Z13	Z4	Z7	Z14	Z5	Z6	Z15	Z1	Z10	Z11
	µg/g dry matter														
C12:0	1.4	0	0	0,27	0.52	0.06	0	0.22	0.15	0.51	0.16	0.08	1.9	0	0
C13:0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20H C10:0	5.1	4.4	11.2	1.11	2.2	0.4	1.4	1.5	2.8	2.6	3.1	0.6	1.8	2	4.8
iC15:0	6.8	2.7	2	0.8	1.6	0.9	0.2	1.8	1.6	1.4	0.7	0.3	36.3	0.2	0.2
aC15:0	4.2	0.4	2.4	0.59	0.7	0.5	0.06	1.5	1.1	0.5	0.3	0.1	21.4	0.2	0.2
3OH C14:0	0	3.1	0	0	0	0	0	0	0	0.07	0	0	0	0.05	0
C14:1	25.3	0.5	5.5	3.9	1.9	0.5	5.6	7.7	8.8	2.2	3.4	1.7	45.9	2.1	1.7
iC16:0	3.7	0.2	0	0.5	0.9	0.8	0.02	0.8	0.9	0.7	0.2	0.3	28	0.01	0
C15:0	2.3	3	0	0.4	0.6	0.5	1.9	0.2	0.2	0.8	0.1	0.2	6.1	0.03	0
C16:19	15.1	0.6	3.93	1.2	4.9	4.4	5.3	2.3	19.2	9.9	2	7.1	28.5	2.6	5.9
iC17:0	4.5	0.8	1.2	0.5	1.7	0.6	2.5	8.3	0.8	0.8	0.3	12.7	12.7	0.3	0.5
C17:0Δ	1.8	0.1	1.6	2.4	0.8	0.4	0	1.9	1.6	1.2	0.5	0.4	1.4	0.4	0.7
C17:0	0.6	27.5	0	0.3	0.4	0.1	1.77	0.04	0.06	0.4	0.02	0.1	3.6	0.02	0
C18:1	46.6	49.6	15.7	17.2	19.5	11	21.7	13.4	44.8	36.75	10.8	10.9	69	23.7	41.8
C18:2w912	18.8	8	17.3	22.7	19.8	12.9	3.1	6	87.9	58.35	11.6	15.8	46.7	47.6	89.9
C18:3 w3	12.8	0.5	0	4.08	3.9	2.4	4	0.7	14.1	10.17	1.5	2.2	3.6	8.5	14
C19:0Δ	11.2	0.9	1.9	1.6	0.4	2.8	0.04	1.8	0.6	18.8	0.5	0.9	24.5	0.8	0.6
C20:0	4.5	0.08	1.5	0.6	2	1.3	2.8	1.6	2.17	1.6	1.1	0.4	10	0.4	0.4
C20:1	0.4	1.7	4.3	0.2	0.3	0.1	0	0.8	0.4	0.14	0.4	0.6	0.7	0.1	0.4
C22:0	6	1.7	4.5	1.7	3	1.8	0.7	2.7	2.4	1.7	1.9	0.4	13.9	0.09	0
C19:0	2.5	1.7	0	1.1	0.8	0.4	0.2	0	2.3	1	0.3	0.6	0.7	2	3.4
SUM	173.6	107.48	73.03	61.15	65.92	41.86	51.29	53.26	191.88	149.59	38.88	55.38	356.7	91.1	164.5
MEAN± SD	118±51			56.3±13			98.8±81			81.3±60			204.1±137		

Table 9b. PLFAs of the field Z (final time)

TIME=3 y

PLFA	T 2Z			T3Z			T5Z			T4Z			T1Z (control)		
	Z2	Z9	Z12	Z3	Z8	Z13	Z4	Z7	Z14	Z5	Z6	Z15	Z1	Z10	Z11
	µg/g dry matter														
C12:0	0.7	0.0	0.0	0.3	0.5	0.1	0.0	0.1	0.1	0.3	0.2	0.1	1.8	0.0	0.0
C13:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20H C10:0	2.4	7.5	10.1	1.0	1.6	0.7	1.0	1.4	2.6	1.6	5.4	0.6	3.2	2.0	9.3
iC15:0	2.7	3.2	1.1	0.8	0.8	1.8	0.1	1.2	1.5	2.8	0.9	0.3	53.4	0.1	0.2
aC15:0	2.7	0.6	2.7	0.4	0.5	1.0	0.0	1.2	0.9	0.3	0.5	0.2	42.0	0.2	0.4
3OH C14:0	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0
C14:1	12.6	1.2	7.2	3.9	2.3	0.9	6.9	10.7	7.2	1.4	8.3	2.1	46.5	3.0	1.5
iC16:0	1.7	0.2	0.0	0.7	0.7	1.3	0.0	0.6	0.4	1.3	0.2	0.3	30.1	0.0	0.0
C15:0	1.2	5.3	0.0	0.3	0.4	1.0	1.2	0.2	0.5	0.9	0.2	0.2	11.1	0.0	0.0
C16:19	10.9	0.6	3.4	1.3	4.2	8.6	4.6	1.1	25.2	6.4	2.2	9.3	55.0	2.3	5.5
iC17:0	2.5	1.4	1.3	0.3	1.1	1.1	1.7	10.8	0.8	0.5	0.6	22.8	23.7	0.2	0.5
C17:0Δ	0.7	0.1	1.0	2.6	0.5	0.8	0.0	2.3	2.0	2.2	0.7	0.6	2.4	0.3	0.5
C17:0	0.2	64.1	0.0	0.4	0.5	0.2	3.5	0.0	0.1	0.3	0.0	0.2	3.8	0.0	0.0
C18:1	22.0	89.0	14.8	14.3	14.3	25.9	42.3	11.9	55.3	65.3	19.9	19.6	82.1	17.9	82.0
C18:2w9 12	6.3	14.3	11.4	20.2	10.9	24.0	5.7	6.1	63.7	58.3	21.2	16.0	33.9	39.2	175.4
C18:3 w3	5.6	0.8	0.0	4.1	3.0	3.8	7.9	0.5	15.2	4.8	2.6	2.4	3.9	10.5	25.8
C19:0Δ	5.3	1.6	1.8	1.1	0.3	5.4	0.1	1.7	0.5	20.6	0.9	1.6	21.3	0.6	1.2
C20:0	3.0	0.2	2.1	0.6	2.4	1.5	6.6	1.6	2.3	0.9	2.1	0.8	10.6	0.2	0.8
C20:1	0.1	3.7	3.1	0.2	0.2	0.2	0.0	1.1	0.3	0.2	0.9	1.1	0.6	0.1	0.9
C22:0	3.0	2.9	4.2	2.4	3.7	2.8	1.1	1.8	4.7	1.3	3.3	0.7	27.1	0.1	0.0
C19:0	0.8	2.4	0.0	0.7	0.2	1.0	0.4	0.0	2.0	1.6	0.4	0.6	0.6	1.2	5.0
SUM	84.4	204.5	64.4	55.6	48.2	82.2	83.1	54.3	185.4	171.0	70.5	79.4	453.0	78.1	309.1
MEAN± SD	118 ±76			62±18			107.6±69			107±56			280±189		

Field G

At the start of the project the PLFA of Filed G was completely different than that of GZ. Again. a wide heterogeneity was found between the different part of the field thus no significant difference was found. The predominance of bacteria was found in the Field G too. The increase of PLFAs amount found at the end of the project did not due to the different treatment but rather to other chemical-physical parameters that affected similarly the community.

Table 10a. PLFAs of the field GZ (time zero)

TIME=0 Y

PLFA	2G			3G			T1Z (control)		
	GZ1	GZ4	GZ8	GZ2	GZ6	GZ9	GZ3	GZ5	GZ27
	µg/g dry matter								
C12:0	0.8	0.5	0.8	0.3	0.9	1.2	1.6	0.6	0.3
C13:0	0	0	0	0.07	0	0	0	0	0
20H C10:0	81.8	23.2	62.1	37.6	53.7	116.2	70.7	57.5	1.4
iC15:0	117	75.2	104.6	41.2	120	158.4	181	78.2	2.5
aC15:0	3.5	30.3	53.7	17.3	65	82.3	99.3	32.8	1.6
3OH C14:0	4	0	0	0	0	0	0	0	0
C14:1	89.3	0.06	3	1.9	1.5	19.9	7.7	0.2	1.3
iC16:0	10.5	1.1	1.2	0.5	1.5	1.6	2.2	1.04	1.5
C15:0	101	3	3.5	1.6	4.4	6	6.5	3.7	0.8
C16:19	129	0.6	2	6.5	0.6	4.4	2.4	2.8	0.3
iC17:0	6.2	109	94.42	1073	121	207	217	101	1.3
C17:0Δ	0.4	0.2	0.2	0.2	0.2	0.1	0.1	0.2	1.2
C17:0	3.5	3.7	3.4	0.2	2.2	6.1	7.2	3.9	0.1
C18:1	331	26	704	148	529	656	24.4	435	2.8
C18:2w9 12	3.5	0.5	0.1	0.8	0.5	0.2	2.5	0.3	0.2
C18:3 w3	4	3.9	0.3	0.5	0.9	0.1	0.5	0.2	
C19:0Δ	89.3	44	2.9	24	37.7	29.1	40.3	39.6	0.1
C20:0	10.5	11.4	11.7	7.2	8.2	6.4	9.8	6.3	22.8
C20:1	102	546	104	572	1616	200	477	1432	0.41
C22:0	129	120	140	45	180	200	261	162	0.2
C19:0	6.2	2.3	21.1	0	17.7	9.1	33.3	4.2	5.2
SUM	1222.5	107.48	73.03	61.15	65.92	41.86	356.7	91.1	164.5
MEAN± SD		468±653			56.31±13			204±137	

Table 10b. PLFAs of the field GZ (final time)

TIME=3 Y

PLFA	2G				3G			T1Z (control)	
	GZ1	GZ4	GZ8	GZ2	GZ6	GZ9	GZ3	GZ5	GZ27
	µg/g dry matter								
C12:0	1.7	0.8	1.8	0.5	1.8	2.5	0.6	2.5	1.3
C13:0	0	0	0	0.1	0	0	0	0	0
20H C10:0	155.4	43.8	103.1	69.7	99	220.8	2.6	133.6	95.5
iC15:0	187.2	100	197.7	53.7	237.6	253.4	5	240.7	147.8
aC15:0	9.1	53	71.4	29.7	133.8	214	3.3	173.8	43.6
3OH C14:0	8	0	0	0	0	0	0	0	0
C14:1	177.7	0.2	5.6	4.9	2.8	39.6	2.4	20.3	0.4
iC16:0	19.7	1.3	3.2	0.6	2.4	3	2.4	2.7	2.7
C15:0	204	5.9	4.3	3.1	8.6	12.1	1.6	12.9	4.5
C16:19	374.1	0.7	4	7.6	1.2	12.8	0.6	2.9	5.5
iC17:0	13.8	218	113.3	2103.9	223	459.5	2.4	434	121.2
C17:0Δ	0.6	0.3	0.4	0.3	0.4	0.2	2.4	0.2	0.4
C17:0	5.8	9.6	7.5	0.5	4.5	10.1	0.2	18.7	8.7
C18:1	625.6	52	1091.2	290.2	1244.7	1239.8	6.6	48.8	674.3
C18:2w9 12	4.7	1	0.2	1.6	0.9	0.3	0.4	5	0.5
C18:3 w3	7	7.3	0.6	0.9	1.4	0.2	0	0.9	0.4
C19:0Δ	167.9	88.9	3.9	47.5	73.2	54.7	0.2	81.4	52.7
C20:0	27.7	23.9	20.5	14.8	9.6	16.9	26.8	20.6	11
C20:1	124.4	1310.4	195.5	1345.9	3168.6	244	0.8	1144.8	2692.2
C22:0	255.4	228	369.6	83.8	282.4	396	0.3	495.9	427.7
C19:0	7.4	3.7	25.7	0	45.1	10.9	13.3	53.3	5.1
SUM	2377.3	2149	2219.4	4059.4	5541.1	3190.8	71.7	2892.9	4295.4
MEAN± SD	2248±117				4263±1188			2420±2151	

Microbial community: respirometric activity

Respiration measurements are useful for better assessing: the effects of soil disturbance,. microbial biomass, microfauna activity (Anderson and Ineson, 1982) and the mineralisation rate of organic matter. Among the various methodologies available, the titrimetric method that measures the evolution of CO2 (ISO, 2002) was used in this study.
The results of the respirometric analyses are reported below, divided by field of origin of the samples, expressed both on the dry weight of the soil and for gram of organic matter. Exposing the data obtained is followed by a brief discussion of the results in the light of the statistical

processing with which they were analysed The results were subjected to the analysis of variance test (ANOVA) and, crossed with the data of the soil analyses, to a correlation analysis in order to verify the possible presence of correlations between the characteristics of the soils and their respirometric levels.

Field Z

The cumulative respiration values (calculated by monitoring the production of CO_2) measured in the 59 days of observation in the soils of field Z are shown in Figure 5 and reported in Table 9. The values shown correspond to the average of the two replicates that were carried out in the laboratory for each sample.

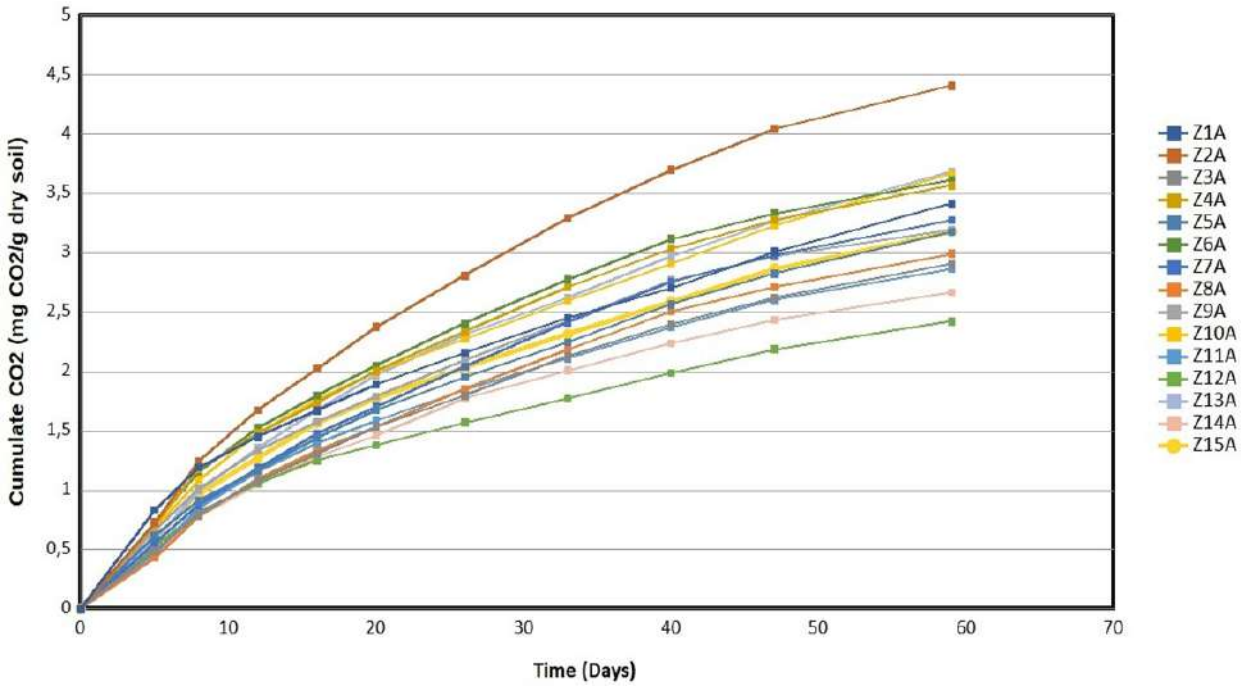


Fig. 5 Cumulative values of soil sample respiration: Field Z (time zero)

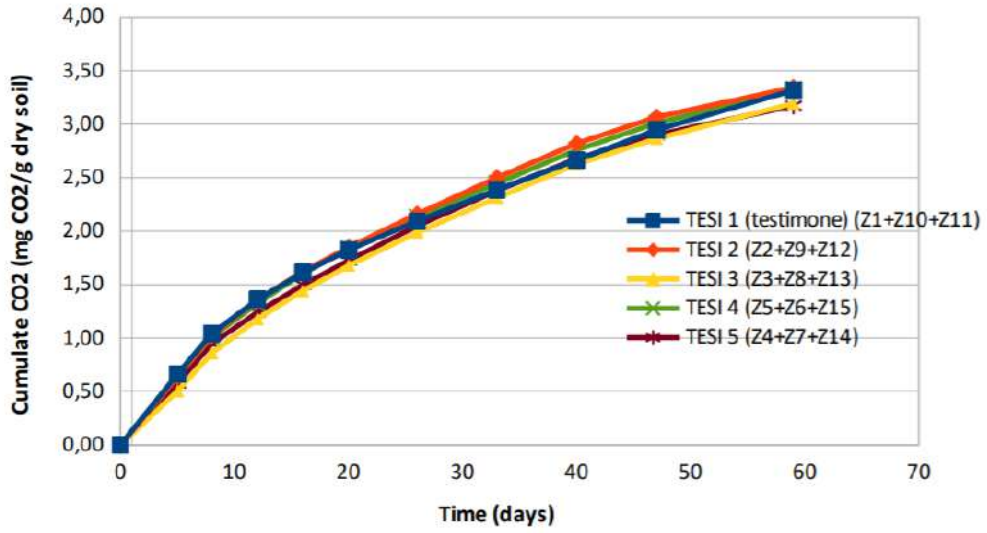
The mean respiration rate of the Z-field samples was 3.26 mg CO2 / g soil, 1.32 mgCO2 / g higher than the G-field mean. The respiration of the soils of the Z field showed a greater variability than the samples of the G field, with values ranging from 2.42 to 4.40 mg CO2 / g. This greater variability is however due to an extreme value (Z2), excluding which the variability becomes slightly lower than that of the G field. These results also show good homogeneity to be used to compare different management practices.

Table 11. Cumulative CO_2 production value (data expressed in mgCO2 / g) at the end of 59 days. Field Z. Average of the two replicates.

Campione	CO2 (mgCO2/g dry soil)
Z1A	3,41 ± 0,51
Z2A	4,40 ± 0,51
Z3A	2,90 ± 0,17
Z4A	3,56 ± 0,41
Z5A	3,17 ± 0,59
Z6A	3,61 ± 0,15
Z7A	3,27 ± 0,66
Z8A	2,99 ± 0,45
Z9A	3,19 ± 0,67
Z10A	3,66 ± 0,82
Z11A	2,86 ± 0,15
Z12A	2,42 ± 0,37
Z13A	3,68 ± 0,88
Z14A	2,66 ± 0,26
Z15A	3,18 ± 0,32

Sample Z2 is the one that produced the most carbon dioxide (4.40 mgCO2 / g soil), a result perfectly in line with the high organic carbon content (2.1%). The minimum value is that corresponding to the sample Z12 (2.42 mgCO2 / g soil). All the other samples were fairly homogeneously distributed between 2.7 and 3.7 mgCO2 / g soil.

Also for respiration the results were grouped and averaged by type of thesis (Fig. 6).



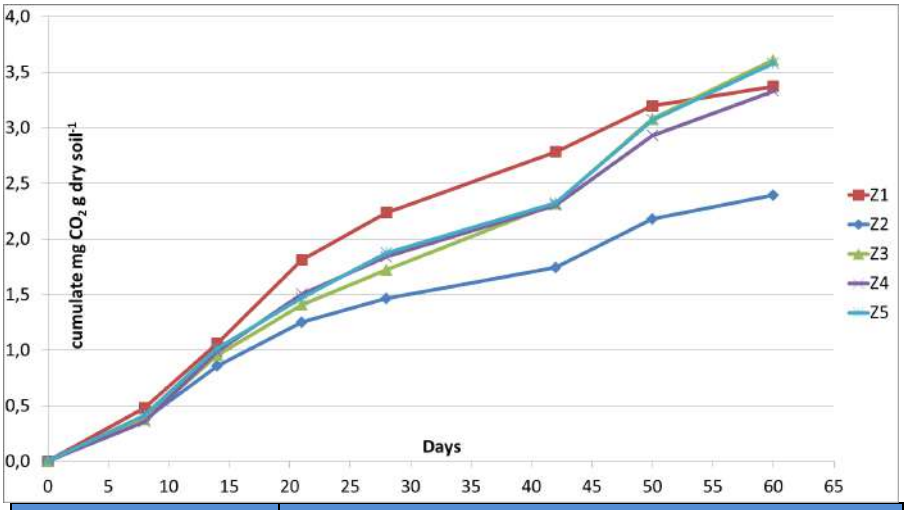
Tesi	Media cumulata mgCO2/g dry soil (t=59 days)
TESI 1 (testimone) (Z1+Z10+Z11)	3,32 ± 0,57
TESI 2 (Z2+Z9+Z12)	3,34 ± 1,03
TESI 3 (Z3+Z8+Z13)	3,19 ± 0,59
TESI 4 (Z5+Z6+Z15)	3,32 ± 0,38
TESI 5 (Z4+Z7+Z14)	3,17 ± 0,55
Media generale campo Z	3,27 ± 0,62

Fig. 6. Averages of the theses, cumulative values of respiration, Z field (**time zero)**

The averages of the theses showed, as can be seen very well in Fig. 6, a very low variability. The mean respiration rate of the Z field was 3.27 ± 0.62 mgCO2 / g dry soil. The analysis of variance (ANOVA) showed that there are no significant differences between the respirations of the various theses and the variability is only random (sign = 0.98).

The correlation analysis between respiration values and the chemical-physical characteristics of the soil showed that there are moderate positive correlations between the amount of CO_2 produced and the organic carbon content (TOC; r= 0.415). The correlation with the quantity of SO is probably significant as SO acts as an energetic and nutritional substrate for the microbial population; it therefore appears justified that as the SO content of the samples increases, their level of respiration increases. The analysis also showed a strong correlation (r = 0,84) between the total nitrogen content (Ntot) and the organic carbon (TOC); this correlation is perfectly in line with what is reported in the literature (Cheng et al., 2016).
Soil respiration has been determined at the end of the experiment to assess the effects of the different management techniques (Fig. 7) on the quantity and potential degradability of soil organic matter. Figure 7 reports the average results for the 5 theses. Differently from what is expected, the control (Z1) shows the highest rate of respiration. Thesis Z3, Z4, Z5 seem to be very similar and the treatment that shows lower respiration rate (Z5) is relative to the soil managed by fertilisation of liquid digestate and minimum tillage.

Considering all the data, it is therefore not possible to define which of the treatments may have most influenced the heterotrophic biological activity of the soil.



Tesi	Media cumulate mgCO2/g dr soil (t=59 days)
1Z (testimone)	3,37±0,13
2Z	2,39±0,39
3Z	3,61±0,51
4Z	3,33±0,44
5Z	3,58±0,15

Fig. 7. Averages of the theses, cumulative values of respiration, Z field **(final time)**

Field G

The cumulative respiration values (calculated by monitoring the production of CO_2) measured in the 59 days of observation in the soils of field G are shown in Figure 8 and reported in Table 10. The values shown correspond to the average of the two replicates that were carried out in the laboratory for each sample.

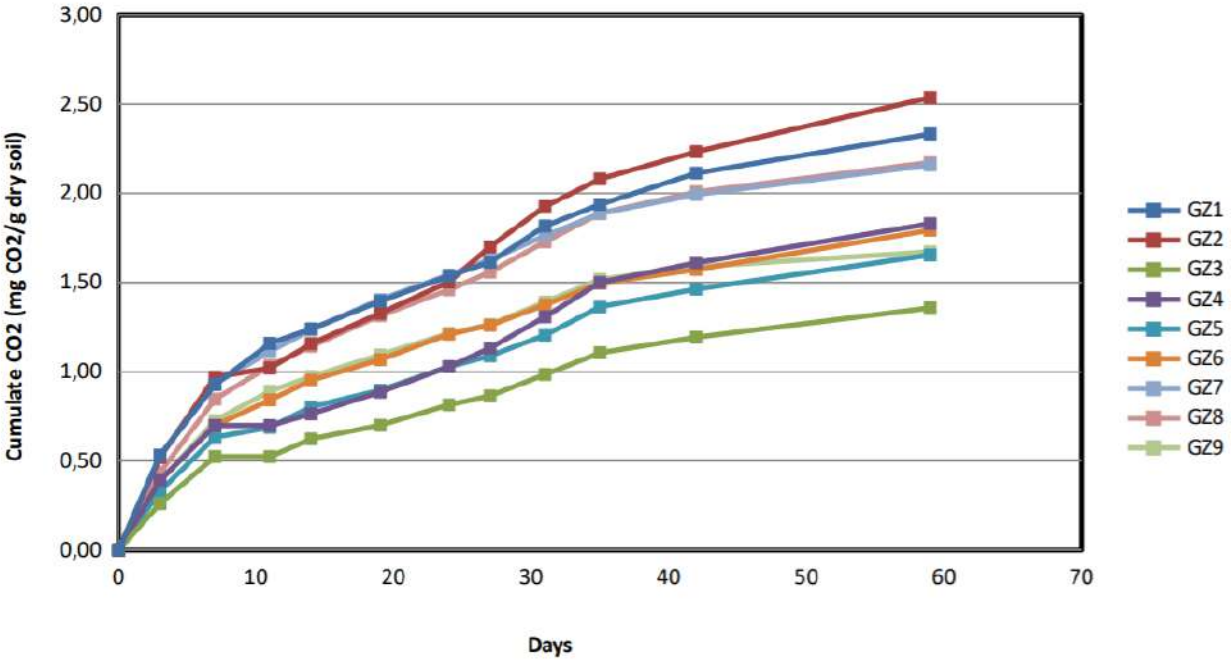


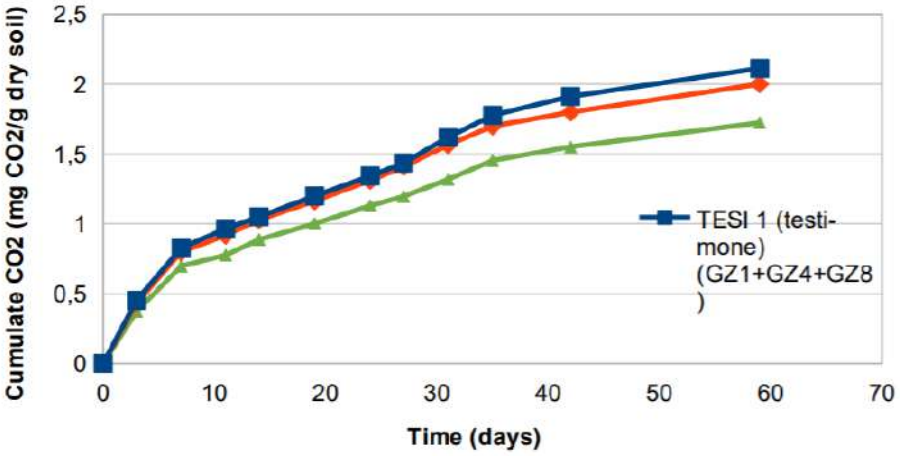
Fig. 8 Cumulative values of soil sample respiration: Field G

The production of CO2 in the 59 monitoring days showed a rather low variability. This result is in line with the homogeneous management that the fields have undergone so far and the slight differences may be due to the natural spatial variability of the characteristics of the soils, highlighted in the above analyses. This rather low variability is envisaged as a good starting point for the evaluation of the different agronomic management. The GZ2 sample produced the most carbon dioxide (2.53 mgCO2 / g soil) followed by GZ1, GZ8 and GZ7 (which produced 2.33, 2.17 and 2.15 mgCO2 / g soil of CO2, respectively). The sample that gave the lowest result was GZ3 with a value of 1.36 mgCO2 / g soil. The remaining 4 samples (GZ4, GZ6, GZ9 and GZ5) were placed in a range of values between 1.83 and 1.66 mgCO2 / g soil.

Table 12. Cumulative CO_2 production value (data expressed in mgCO2 / g) at the end of 59 days. Field G. Average of the two replicates.

sample	CO2 (mgCO2/g dry soil)
GZ1	2,33 ± 0,09
GZ2	2,53 ± 0,36
GZ3	1,35 ± 0,13
GZ4	1,83 ± 0,24
GZ5	1,65 ± 0,09
GZ6	1,79 ± 0,07
GZ7	2,15 ± 0,04
GZ8	2,17 ± 0,14
GZ9	1,67 ± 0,15

The respiration results were grouped and averaged by type of thesis (Fig. 9).



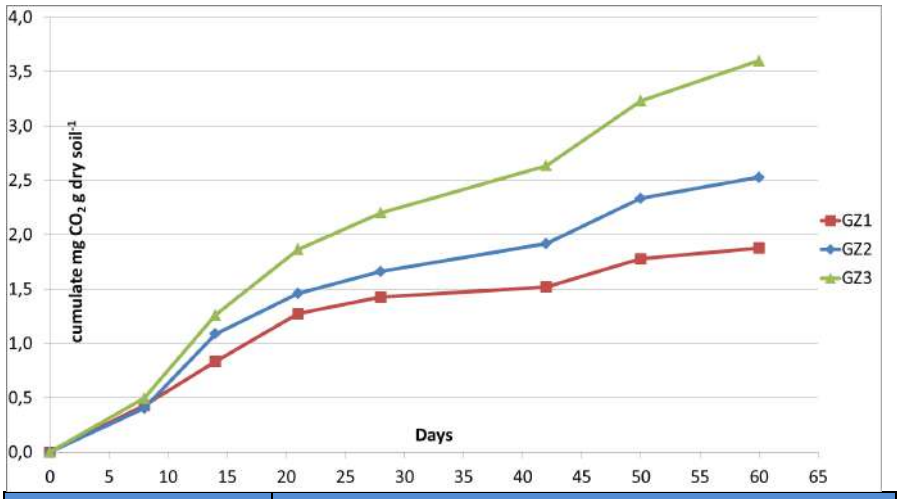
Tesi	Media cumulata mgCO2/g dry soil (t=59 days)
TESI 1 (testimone) (GZ1+GZ4+GZ8)	2,11 ± 0,26
TESI 2 (GZ2+GZ6+GZ9)	2 ± 0 ,47
TESI 3 (GZ3+GZ5+GZ7)	1,72 ± 0,4
Media generale campo G	1,95 ± 0,38

Fig. 9. Averages of the theses, cumulative values of respiration, G field, (time zero)

The results of the averages of the theses have, for obvious reasons, shown even less variability. The mean respiration rate of the G field was 1.95 ± 0.38 mgCO2 / g dry soil. The analysis of variance (ANOVA) showed that there are no significant differences between the respirations of the various theses and this variability is only random (sign = 0.2).

The correlation analysis between the respiration values and the chemical-physical characteristics of the soil showed strong correlation (r = 0.87) between the total nitrogen content (Ntot) and organic carbon (TOC); this correlation is perfectly in line with what is reported in the literature (Cheng et al., 2016).

At the end of the experiment the respiration rate of the samples GZ were measured and the results are reported in Figure 10. In this case, except for the last measurement, as expected, the control shows the lowest respiration. The other two treatments, corresponding to ZG2 (fertilisation by solid fraction of digestate and mineral fertiliser and GZ3 (fertilisation by solid fraction of digestate and mineral fertiliser plus cover crop), **show higher respiration rate for the soil subjected to the presence of the cover crops.** This data agrees with the increase of total nitrogen and organic carbon detected in the soil (Table 6 vs Table7) as probably consequence of the presence of the cover crops.
Considering all the data, it is therefore possible to assess that the presence of the cover crops is able to influence the heterotrophic biological activity of the soil more than the solid fraction of digestate alone.



Tesi	Media cumulate mgCO2/g dr soil (t=59 days)
GZ1 (testimone)	1,87±0,21
GZ2	2,52±0,13
GZ3	3,60±0,26

Fig. 10. Averages of the theses, cumulative values of respiration, G field **(final time)**

Soil Quality index consideration

In order to evaluate the improvement of the soil quality the most commune parameters have been proposed and successively measured. However, starting from the theoretical approach, the application of soil indexing procedure required to be contextualised respect to the experimental condition and the crop management system adopted.

In order to evaluate the effect of the treatment for each parameter, different trends and references value were attempt for each parameter, taking into consideration the treatment carried out

Table 13 trend and reference value for the parameters selected.

		Theoretical range	Significance respect to the treatment carried out
Organic Carbon	*more is better*	*Value at t=0, increase statistically significant*	+ amending can improve the C content
Organic Matter	*more is better*	*Value at t=0, increase statistically significant*	+ amending can improve the C content
Total Nitrogen	*more is better*	*Value at t=0, increase statistically significant*	+ amending can improve the C content
pH	*optimum*		fertilisation and amending did not affect significantly the pH value. optimum value can be considered that typical of the soil
C/N ratio	*optimum*		Value equilibrated 8-12. For lower values losses of nitrogen, for highest values nitrogen immobilisation Fresh organic matter is able to lower C to N ratio
Texture	*optimum*		fertilisation and amending did not affect the texture. optimum value can be considered a loamy soil.
CEC (Cation Exchange Capacity)	*more is better*	*Value at t=0, increase statistically significant*	Amending with high concentration of mature organic matter can increase CEC
Available Phosphorus	*more is better*	*Value at t=0, increase statistically significant*	Fertilisation are able to influence pH (optimum for available P is 6.5-6.8) and / or directly added P to soil
WHC (Water Holding Capacity)	*more is better*	*Value at t=0, increase statistically significant*	organic matter contributes to increase WHC by the presence of negative charges (COOH and OH functional group dissociation)
Soil respiration	*more is better*	*Value at t=0, increase statistically significant*	Microbial parameters are directly influenced by the C addition, N improvement and presence and strip tillage
PLFA (Phospholipid Fatty Acid)	*more is better*	*Value at t=0, increase statistically significant*	

Basing on Table 13 we can conclude that some parameters were not adequate to monitor the soil quality changes occurred during the project but, probably these parameters are useful in long term experiments (more than 10 years). In some case the chosen parameters are not sensible to treatments (i.e texture, pH change) in other case (i.e microbial parameters) their variation is influenced by several components, often not caused by the treatment adopted
Moreover, soil heterogeneity affected the results from a numerical point of view.
For this reason, it was not useful to proceed with a global numerical standardization of the measured parameters (MDS) and calculation of the index, but on the other hand, taking into consideration the available parameters more suitable to describe the effect of the different treatments done in the short term (i.e. organic Carbon total Nitrogen) it is possible to conclude that:

- after three years of treatment the PLFA did not show difference between treatments;
- the presence of the cover crops is able to influence the heterotrophic biological activity of the soil more than the solid fraction of digestate alone;
- fertilisation with solid fraction of digestate in autumn, urea and sowing of cover crops seems to determine an increase of total nitrogen and organic carbon as consequence of the presence of the cover crops.
- the soil subjected to conservative management (minimal tillage), fertilised by injection of digestate in pre-sowing and in emergency show increasing both of total nitrogen and organic carbon;
- organic fertilisation by digestate is able to improve soil fertility when, conservative technique (minimum tillage) is adopted.
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