ZEOWINE for the fertilization of a young vineyard- second version

ZEOWINE per la concimazione di impianto in viticoltura – seconda versione

Deliverable Action C1

31/12/2020

Sommario

1 Introduction

Due to the increasing pressure imposed to agricultural soils and to their consequent reduction in fertility, the development of management strategies able to increase the productivity and quality of soils has become a common priority.

In particular, Mediterranean vineyards are exposed to severe risk of soil quality decline due to erosion, loss of organic matter, contamination and compaction. In intensive viticulture, the continuous working practices using heavy machinery and inappropriate tillage, for eliminating competition between vines and other plants for water and nutrients, are responsible for increasing soil erosion rates, and CO_2 emissions.

LIFE ZEOWINE is a demonstration project which aims to improve the protection and management of the soil, the well-being of the vine and the quality of grapes and wine, thorough the soil

application of ZEOWINE compost, an innovative product deriving from the composting of wastes from the wine production chain and zeolite.

Starting from the results of previous experimentations, which aimed to evaluate the effectiveness of the application of zeolite and compost in a separate way in other productive chains, we proceed with the intention of applying, for the first time, either in a **new vineyard plant** and in **productive vineyards**, the ZEOWINE product, with effect in terms of performance in soil management and in soil and plant biodiversity.

The synergy of the positive effects of ZEOWINE on the soil and on the plant will be demonstrated by the improvement of nutritional and water efficiency, the reduction of the need for fertilizer supply, the closure of the production cycle of the waste material from the supply chain and the improvement of the quality of the wines produced.

2 ZEOWINE production

During the first year of the project, the waste material came from the wine supply chain of the 2018 harvest was mixed with the natural zeolite, clinoptilolite 80% with granulometry 0.2-2.5 mm, to form five different composting piles characterized as follows:

- One control pile: 9 tons of pomace and stems (control);
- Three piles ZEOWINE 1:2.5 w/w: 2.5 tons of zeolite + 6.5 tons of pomace and stems;
- One pile ZEOWINE 1:10 w/w: 1 ton of zeolite + 9 tons of pomace and stems.

The composting piles were periodically turned (at least once a month) with a scraper to promote aeration. In order to maintain a moisture of about 50% an irrigation by sprinklers on the top of each pile as needed was performed. Temperature and humidity were recorded every day until the end of the termophilic phase, successively every week. Three composite samples for each pile were collected at the start of composting, at the end of termophilic phase and at the end of composting process.

The samples were air-dried and sieved (2 mm) and stored at room temperature until physical, chemical, biochemical, toxicological and hydrological analyses.

3 ZEOWINE application

In the spring 2019, the obtained ZEOWINE composts (ZEOWINE 1:2.5) have been applied to the new vineyard (cultivar Sanforte). The demonstration site selected for the experimentation is located in

the San Miniato area (Pisa, Tuscany) in Central Italy. The climate is typically Mediterranean, semiarid, with a mean annual precipitation of 859 mm and a mean annual temperature of 14.3°C. Soil classification was Calcixerept (Soil Survey Staff, 2014) with a sandy clay loam texture (51.1% sand, 28.3% clay and 20.6% silt) (USDA classification), an organic matter (OM) content of 1.8% (±0.2), a high level of carbonate (bivalve shells were very common) and a slightly alkaline pH.

In this new vineyard, **Sanforte cultivar**, the vine spacing is 2 m between rows x 0.8 m between plants.

In this site the following treatments (**in triplicate**) have been applied:

- **Commercial compost** (compost) (20 t/ha)
- **Zeolite** (10 t/ha)
- **ZEOWINE 1:2.5** (30t/ha)
- **Control soil** (untreated)

The vineyard was divided into 9 sub-plots (0,15 ha, 8 m x 20 m), where compost, ZEOWINE 1:2.5 and zeolite were applied, and mixed by plowing to a depth of 30 cm. So as to isolate soil variability, the sub-plots were chosen and judged to be independent true replicates. An additional untreated sub-plot was chosen as control soil.

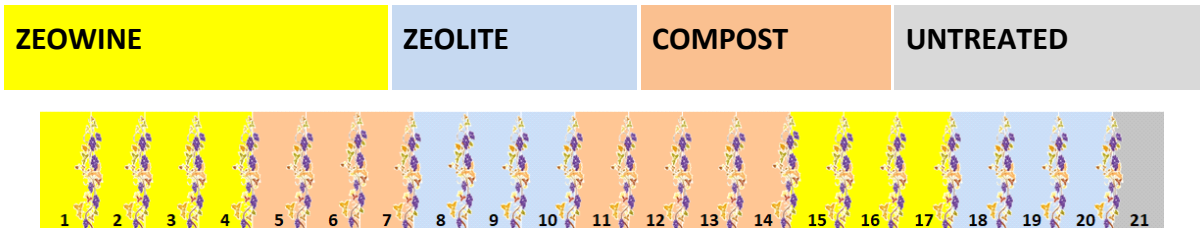


Figure 1. Experimental layout

For the ZEOWINE and the commercial compost distribution, a spandicompost was used; this to ensure the simple and uniform spreading of amendments over the entire soil surface.

4 Materials and Methods

4.1 Soil

4.1.1 Soil sampling

Immediately after treatments (Spring 2019, 5 July **T0**), after the grape harvest (six months from treatments application, 14 November 2019, **T1**) and after the second grape harvest (18 months

from treatments application, 21 October 2020, **T2**) three composite soil samples were taken from each of the three sub-plots per each treatment, between rows at the 0–30 cm layer.

4.1.2 Soil chemical parameters

Electrical conductivity (EC) and pH were measured in a 1/10 (w/v) aqueous solution using selective electrodes. Total organic carbon (TOC) and Total Nitrogen (TN) content were measured with LECO, U.S.A. RC-412 Multiphase Carbon and FP-528 Protein/Nitrogen Determinators, respectively.

Total phosphorus (TP) and potassium (TK) were extracted with nitric-perchloric acid digestion (HNO_3:$HClO_4$, 5:2) in microwave. Total phosphorus (TP) was measured using the method reported by Murphy and Riley (1962). Available potassium (Kav) was extracted with ammonium acetate (Helmke and Sparks, 1996). Total (TK) and available potassium (Kav) were determined by ICP-OES (Varian AX Liberty). N–NH_4 and N–NO_3 were determined in 1:10 (w/v) KCl extracts 0.5 M; N–NH_4 was detected with ion selective electrode (Seven- Multi, Mettler Toledo) and N–NO_3 was detected by Norman et al. method (1985). Sodium pyrophosphate (0.1M, pH 11) at 60 °C for 24h under shaking at 200 oscillation min−1 was used to extract Total Humic Carbon (THC). The THC extract was separated into humic (HA) and fulvic (FA) acids by addition of H_2SO_4; the extract was kept overnight at 4 °C, and then the flocculent (HA) and the supernatant (FA) were centrifuged. THC and FA were determined by the Yeomans and Bremner (1988) method, while HA were obtained by subtracting FA from THC. Cation-Exchange Capacity (CEC) of the soils was determined by Sumner and Miller (1996) method, using barium chloride (pH 8.1). Pyrolysis-gas chromatography (Py–GC) was used to evaluate soil organic matter quality. It is based on a rapid decomposition of organic matter under a controlled high flash of temperature, in an inert atmosphere of gaseous N2 carrier. The obtained pyrolytic fragments were separated and quantified by using the gas chromatographic technique (CDS Pyroprobe 190 coupled to a Carlo Erba 600 GC) (Macci et al., 2012a).

4.1.3 Soil physical parameters

Bulk Density (BD), Particle Density (PD), Total Pore Space (TPS), Air Content (AC), Water-holding capacity (WC), Easily Available Water (EAW), and Water Buffer Capacity (WBC), were determined following sand-box method.

Aggregate stability refers to the ability of soil aggregates to resist disruption when outside forces (usually associated with water) are applied. The procedure involves repeated agitation of the aggregates in distilled water.

4.1.4 Soil biological parameters

4.1.4.1 Enzyme activities

Total β-glucosidase, phosphatase, arylsulphatase and butyrate esterase activities were tested by the method of Marx et al. (2001) and Vepsäläinen et al. (2001) using the 4-Methylumbelliferyl β-glucosidase, 4-Methylumbelliferyl phosphate, 4-Methylumbelliferyl sulphate and 4-Methylumbelliferyl butyrate, respectively, as substrates. Fluorescence (excitation 360 nm; emission 450 nm) of the product 4-Methylumbelliferone was measured with an automated fluorimetric plate-reader (Infinite® F200PRO Tecan) after 0, 30, 60, 120, 180 min of incubation at 30 °C.

4.1.4.2 Soil microarthropod community structure analysis

The analysis of soil microarthopod community structure was carried out at the CREA of Florence. The sampling was carried out in spring 2019 and involved a total of 27 plots identified in the farm between the vineyard in production and the newly planted vineyard (three points of each treatment). In each point a soil sample, of approximately 2 Kg, was collected from each point by means of a special corer devoted to the mesofauna sampling (a 10 cm cube). Soil samples were placed in a plastic bag and stored at 4°C until arrival to the laboratory. A sub-sample of about 50 g was prepared from each soil sample and stored at -20°C for the molecular analysis. Microarthropods were extracted from the soil samples using modified Berlese-Tullgren funnels following the Standard methodology (Parisi et al., 2005) and observed at the stereomicroscope (Fig. 3). The edaphic microarthropods community was characterized using: i) individual abundance; ii) richness determined by counting the number of taxa; iii) biodiversity indices, Shannon-Weiner index and Simpson index; (4) QBS-ar index according to Parisi et al., (2005). This index is based on the life-form approach and its values are the summa of EMI (Eco-Morphological Index) scores, ranging between 1 and 20 for each organism depending on its adaptation to the edaphic habitat.

Fig 2 - Microarthropods extraction by modified Berlese-Tullgren funnels.

4.2 Plants

Leaf gas exchanges: were detected with a portable infrared gas analyzer (CIRAS 3, PP Systems Herts). The detection is based on the absorption of infrared radiation by water and carbon dioxide, with a maximum peak at 426 ± 0.15nm. The main components of the instrument are: an air supply unit with a known concentration of carbon dioxide and water vapor; an infrared analyzer; a cuvette clamp, called also assimilation chamber, in which the leaf portion is enclosed. In few seconds the instrument measures the differential concentrations of CO2 and water vapor between the sample of the air entering the assimilation chamber, at known concentrations, and the sample of the air leaving the assimilation chamber, influenced by the leaf gas exchanges. The CIRAS 3 allows to detect several parameters, among which we can find: transpiration rate (E, mmol m-2 s-1); net photosynthesis or assimilation per unit of surface (Pn, µmol m-2s-1); stomatal conductance, the degree of stomatal opening which is directly related to the transpiration rates (gs, mmol m-2s-1) and is determined by measuring the resistances of the stomatal rim and of the boundary layer at the diffusion of water vapor on the tissues of the leaf surface; extrinsic Water use efficiency (eWUE, µmol mmol-1) or the ratio between net photosynthesis and transpiration rate. The measurements with CIRAS were carried out on 25 leaves / thesis to monitor the physiological status of the vines.

Vines water stress: was detected with a Scholander's pressure chamber, which determines the negative hydrostatic pressure of the xylem, which is believed to be very close to that of the entire plant. The water potential is measured by applying increasing pressure of gas on a cut leaf which is placed inside the pressure chamber; the petiole faces outwards, passed through a hole equipped with a gasket, present in the chamber lid. When the leaf is cut, the petiole appears dry because the water present in the capillaries is recalled to the xylem by osmosis of the surrounding cells. The

pressure applied into the chamber to get water out from the petiole is equivalent, with a negative sign, to the value of the water potential of the leaf. The water potential of the tissue under examination is closely linked to the water condition of the plant and, therefore, is possible to assume the measured water potential of the leaf as the potential for the whole plant. During the day, the water potential can vary based on the variation of the evapotranspiration demand of the environment and the water reserves of the soil. The potential was measured at noon (Ψw), after leaving the leaves in the dark in the absence of perspiration for an hour to balance the water potential of the leaf with that of the entire branch.

Technological maturity analyses: consist in determining the sugar content (° Brix), total acidity (gL-1 of tartaric acid), pH of the must and berry weight (g). The procedure carried out for the quantification of the sugar is as follows: the first step consists in mechanically extracting the must from the grapes by squeezing the berries of the various theses, collected in bags. The sugar content was determined with the use of a field refractometer, which is a metal tube inside which there is a prism where few drops of must are placed. The instrument measures the refraction angle, through a graduated scale, which undergoes a beam of light which is intercepted by the instrument and passes through the liquid to be analyzed. The data provided is expressed in °Brix since the concentration of soluble solids in a liquid is directly proportional to the refractive index of the liquid itself. The sugar content is the average result obtained from 7 repetitions of each thesis. Total acidity is the sum of the titratable acids bringing the must or wine to pH 7, by adding an alkaline solution of known strength. To determine the total acidity, 7.5 ml of must was taken, to which 3 drops of the blue bromothymol indicator were added. The base used for titration is 0.1 N sodium hydroxide contained in a 50 ml burette equipped with a tap. The neutral pH is reached when the solution reaches a green color, due to the blue of bromothymol. The total acidity value, expressed in gL-1 of tartaric acid, corresponds to the quantity of sodium hydroxide used for the titration. For the determination of the pH of the must was used a pH-meter formed by a display and a glass electrode, which must be calibrated before analysis with two solutions at known pH. The must obtained from the various samples is put into beakers, in which the pH meter electrode is immersed and after a few seconds it is possible to view the value on the display. Between one measurement and another, the electrode was cleaned by immersing it in a beaker with distilled water. Another data analyzed was the weight of a single berry, obtained from a sample of 100 berries that were weighed. The weight obtained was then divided by 100 and the average weight of one berry was obtained. The

sugar content, the total acidity and pH in the graphs are the average results obtained from 7 repetitions (samples of berries) for each thesis.

Productivity: at harvest, the yield per vine (kg) was determined. The number of bunches per plant was also counted, consequently it was possible to mathematically calculate the average weight of the bunch (g). The yield was obtained from the average weight of 20 vines per repetition of each thesis.

Phenolic maturity analyses: the total and extractable polyphenol and anthocyanin values (mgL-1) of harvested grapes were obtained through the Glories Method; it consist in two extractions from berry skins, one in soft conditions that simulate the winemaking process, the other in strong acid conditions capable of completely eliminating the diffusion barriers and leading to a total extraction of the secondary compounds. The two solutions obtained are aqueous, one at pH 1 (HCl N / 10) and the other at pH 3.2. Furthermore, in order to have a greater extraction efficiency, it is recommended to break the berries as well as the 1:1 dilution of the obtained pulp. The contemporary breakage of the grape seeds induces the partial extraction of the tannins which is important for defining the characteristics of the grapes.

Statistical analysis: the collected data were entered on the Microsoft Excel calculation program and subsequently subjected to the analysis of variance using the statistical program SPSS Data Editor, with separation of the averages through the DMS test at 95% of probability. This program allows to formulate a linear factorial model by inserting both the parameter whose dependence wants to be demonstrated (Dependent variables) and the fixed parameters (Feed Factors). In graphs, bars indicate the statistical difference. Where the values differ more than the width of the bars it is to be considered that values are different, therefore statistically significant.

5 Results

5.1 Soil

5.1.1 Soil Chemical parameters

The young vineyard soil showed moderately alkaline pH (Figure 3) and was not saline (electrical conductivity lower than 0,2 dS m^{-1}, Figure 4). CEC values increased with ZEOWINE addition (Figure 5).

Total organic matter of the soil samples treated with compost, both compost alone and zeolite-based compost (zeowine), increased with respect to the control and zeolite treated soils at T0

sampling time. However, at T1 sampling time, the OM content was higher with respect to the control soils only in the zeowine 1:2,5 treated soils (Figure 6).

The content of TP was higher in the zeowine and commercial compost treated soils in comparison with the control and zeolite treated soils (Figure 8). However, significantly higher values of TK were detected in the soils treated with zeolite and zeowine.

The application of zeowine significantly decreased the available P and K at T1 and T2 sampling times with respect to control soil (Figure 9 and Figure 11), thus indicating the increase in retention of these elements.

Similarly, a slightly reduction available Cu was observed in ZEOWINE treatment, at T2 sampling time, with respect to control soil and to the soil treated with commercial compost.

Figure 3. pH at T0, T1 and T2

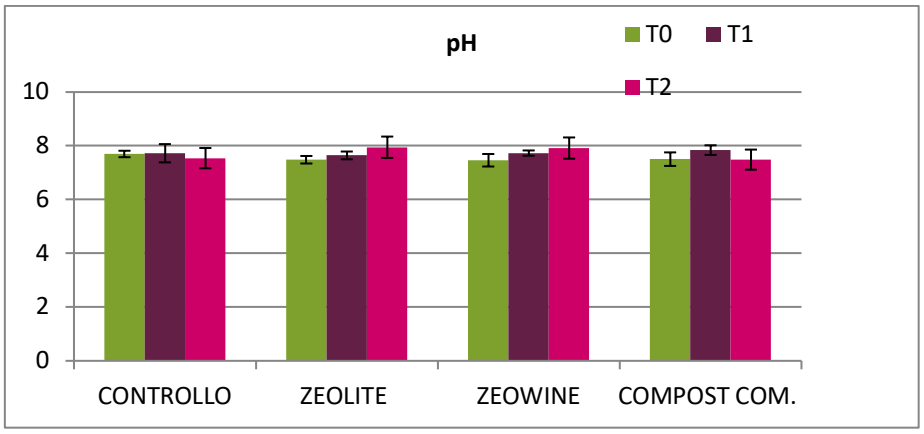


Figure 4. Electrical Conductivity at T0, T1 and T2

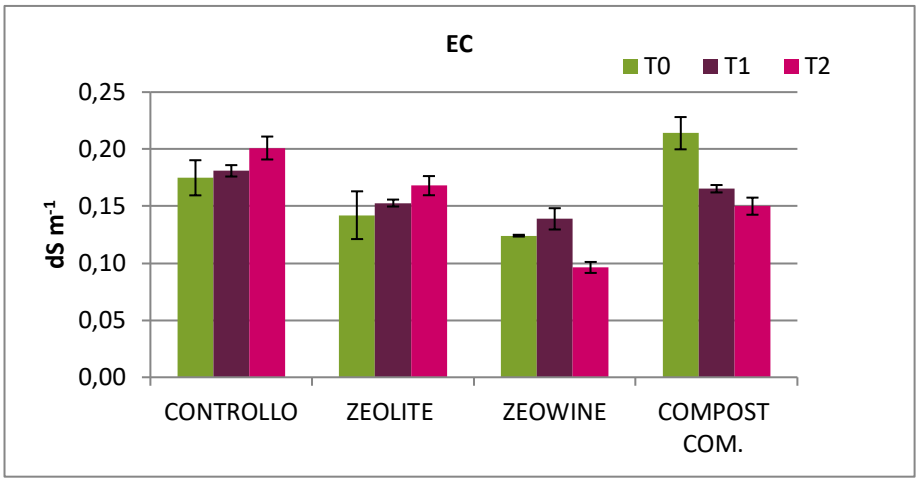


Figure 5. Cation Exchange Capacity at T0, T1 and T2

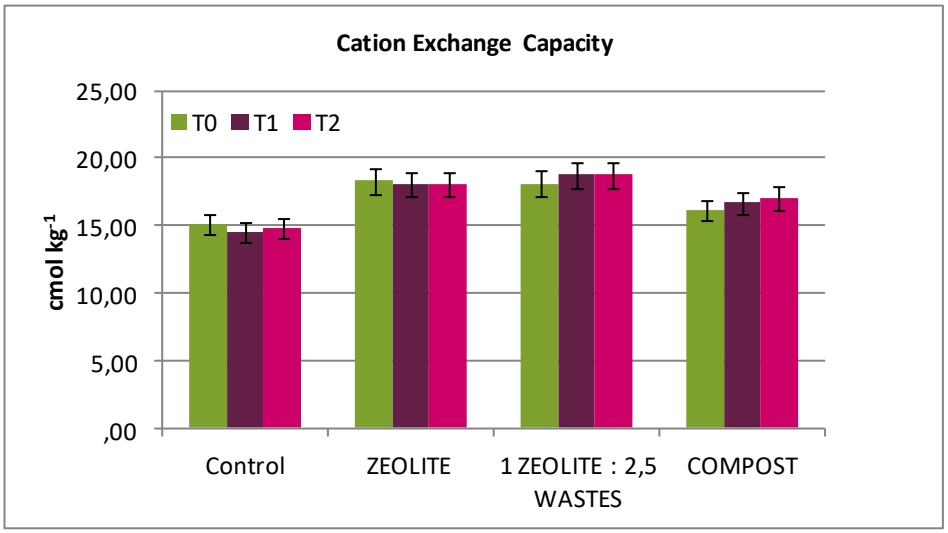


Figure 6. Total Organic Matter at T0, T1 and T2

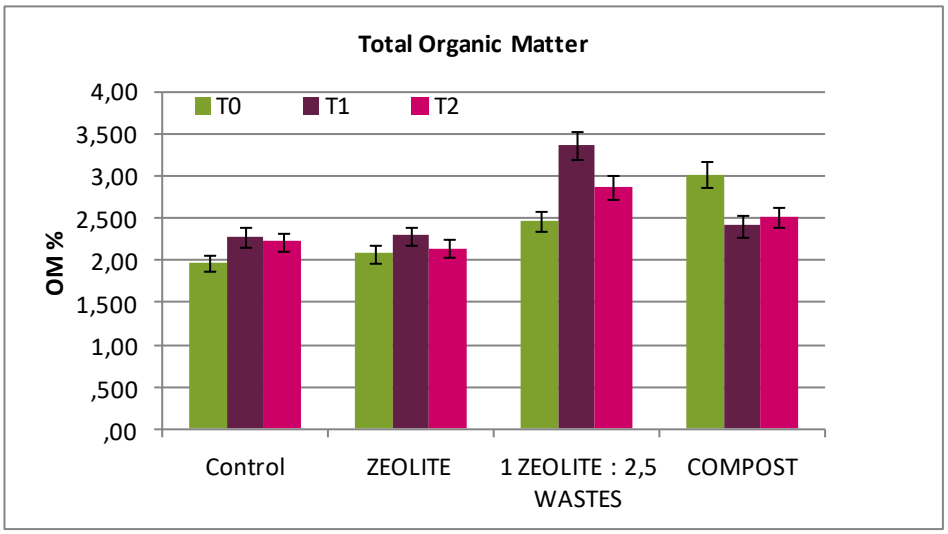


Figure 7. Total Nitrogen at T0, T1 and T2

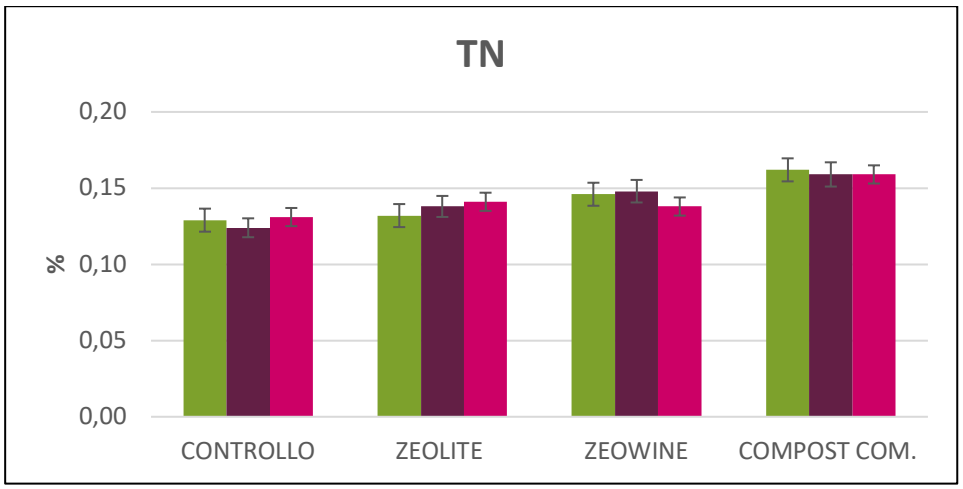


Figure 8. Total phosphorus at T0, T1 and T2

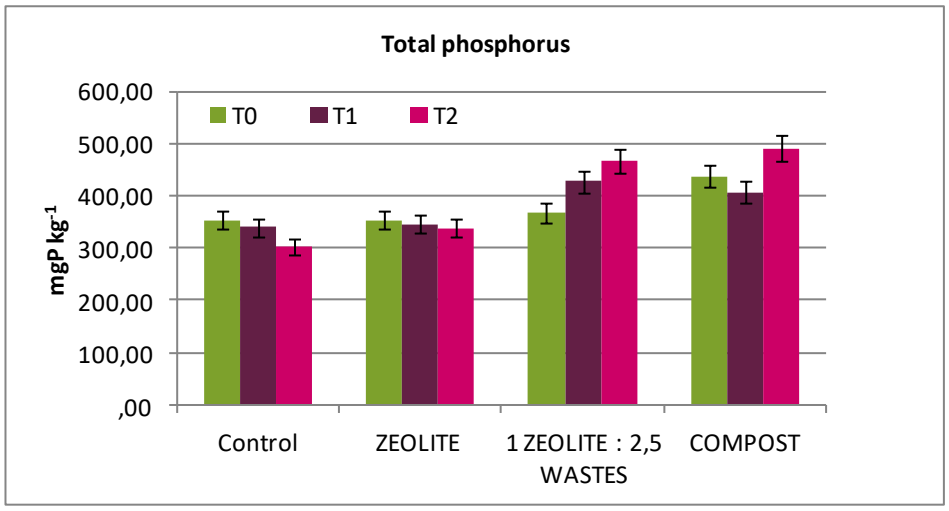


Figure 9. Exchangeable phosphorus at T0, T1 and T2

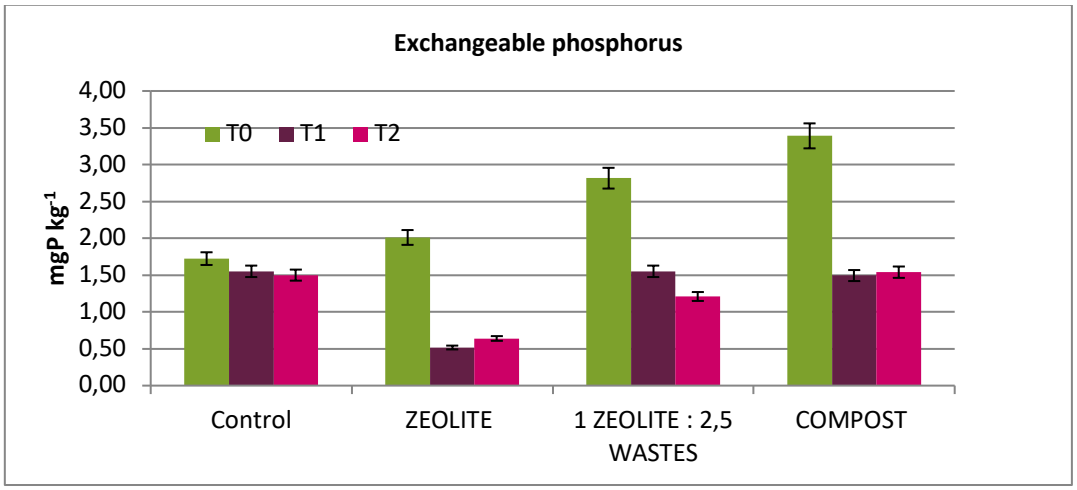


Figure 10 Total potassium at T0, T1 and T2

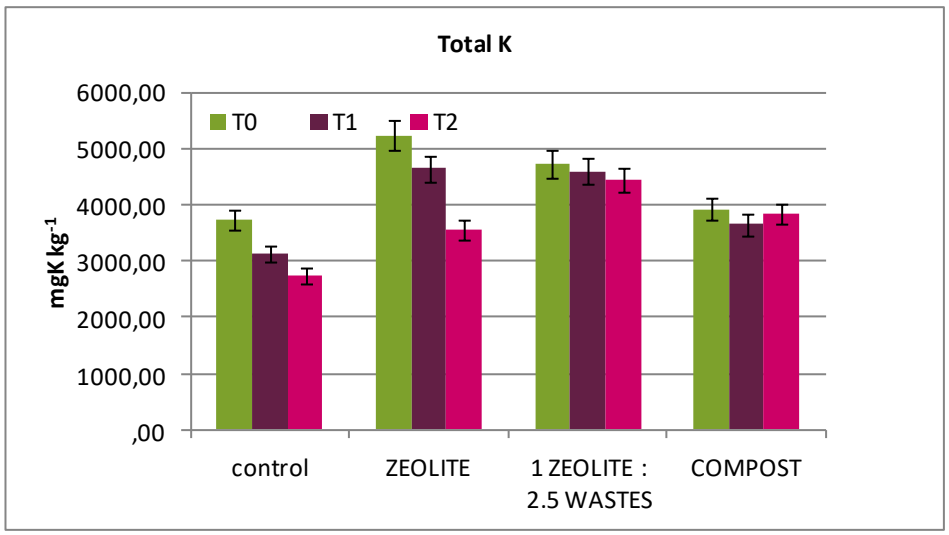


Figure 11 Exchangeable potassium at T0, T1 and T2

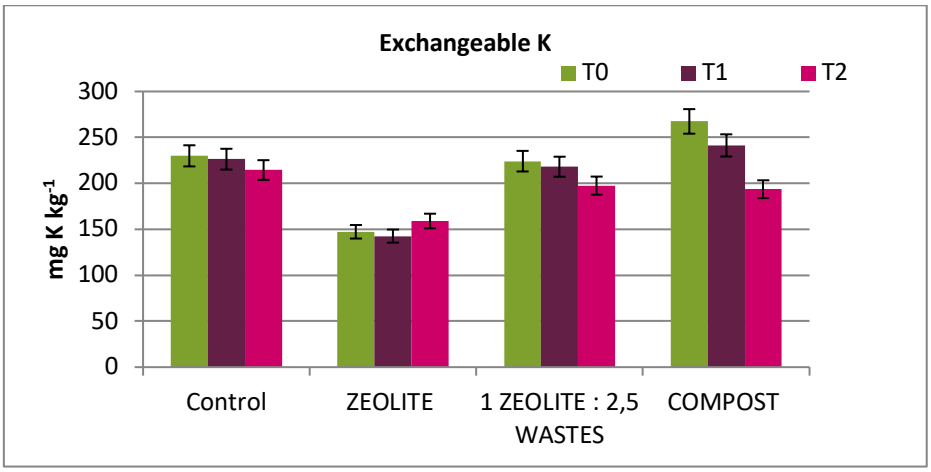


Figure 12. Total copper at T0, T1 and T2

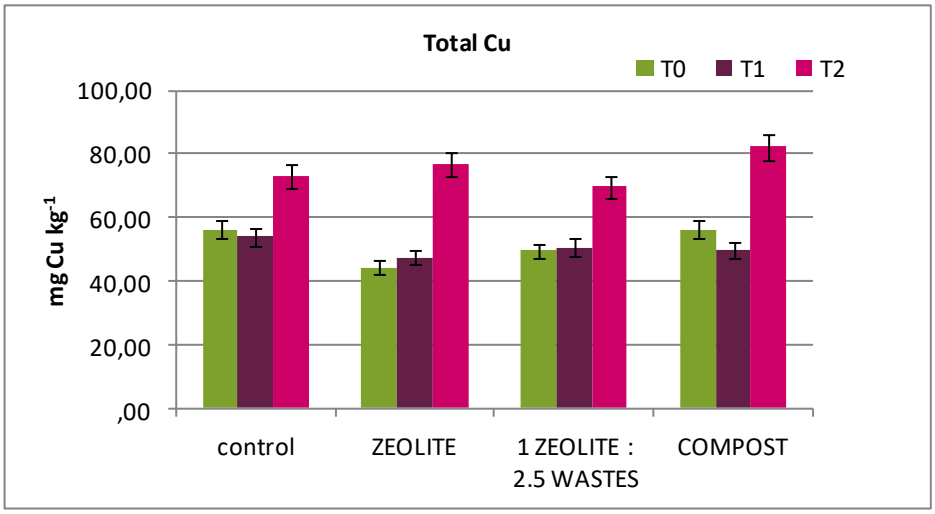


Figure 13 Available Cu at T0, T1 and T2

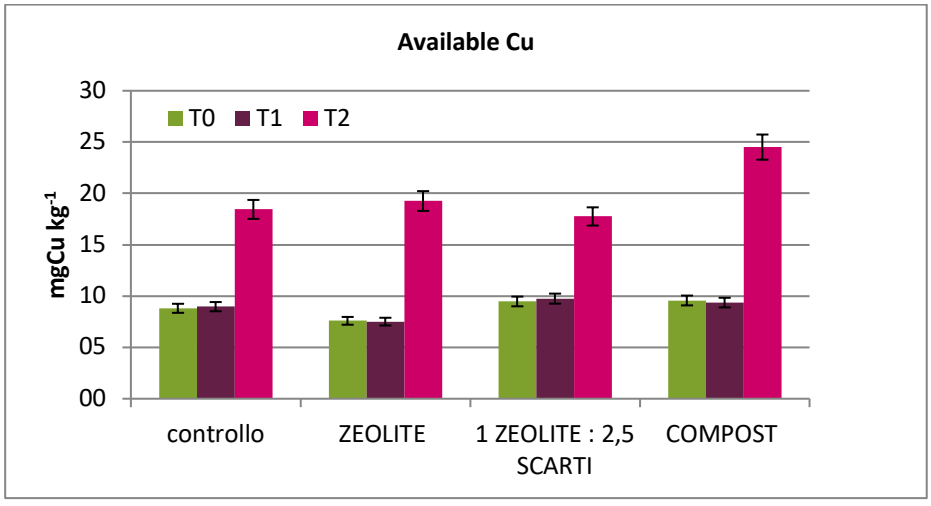
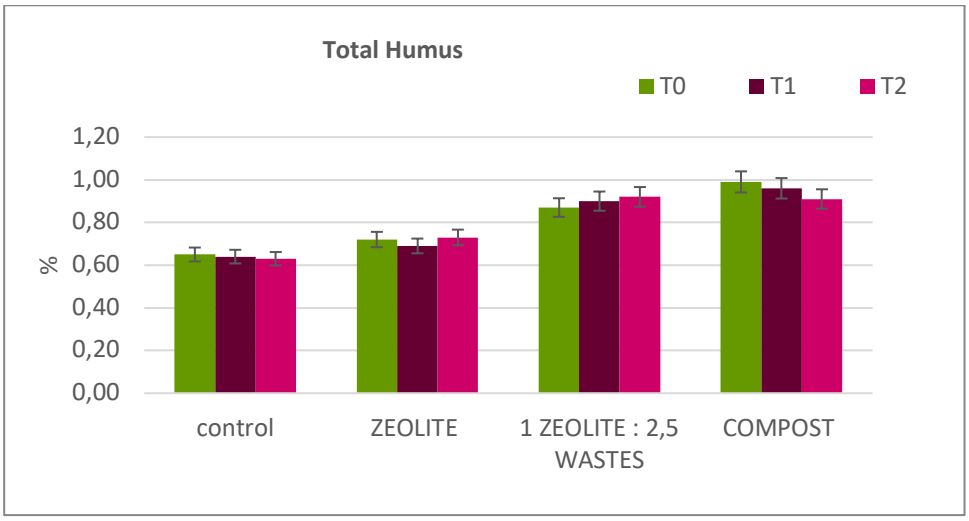


Figure 14. Total Humus at T0, T1 and T2



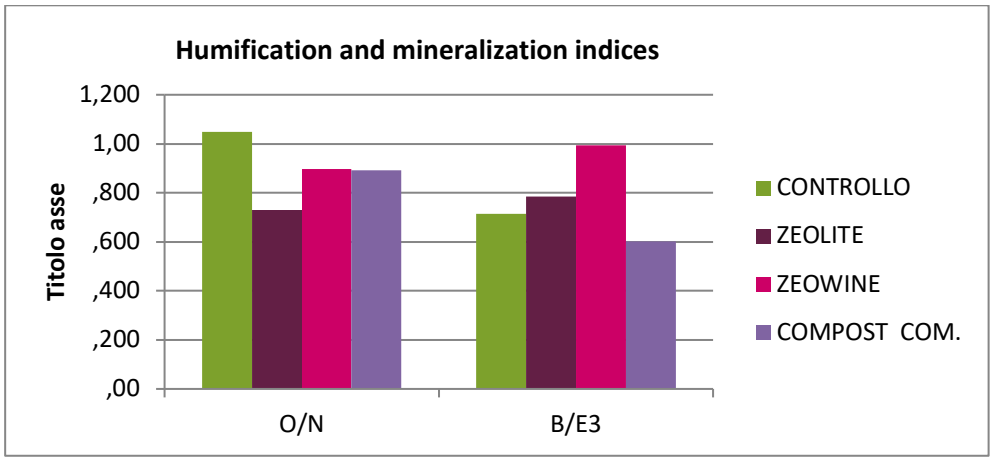
5.1.2 Py-GC

The chemical–structural composition of organic matter was characterized using pyrolysis–gas chromatography. Seven pyrolytic fragments were considered: acetonitrile (E1), acetic acid (K), benzene (B), pyrrole (O), toluene (E3), furfural (N) and phenol (Y). The ratios between the relative abundances of some of the peaks were calculated as indicators of the extent to which mineralization and humification processes take place in soil. In particular, the ratio between pyrrole and furfural (O/N) expresses the mineralization of fresh organic matter. However, the ratio between benzene and toluene (B/E3) expresses the humification of organic matter (Gispert et al., 2018).

In zeowine and commercial compost soil treatments, lower values of the O/N pyrrole/furfural) index compared to the control soil were observed.

The humification index B/E3 was higher in ZEOWINE-treated soil. This result can be attributed to the increase in condensed aromatic structures (benzene) and the decrease in less-condensed humic substances producing E3 (toluene) in ZEOWINE-treated soil in comparison with the control, zeolite and commercial compost treated soils.

Figure 15 Py-GC at T1 sampling time, O/N mineralization index, B/E3 humification index.



5.1.3 Soil physical parameters

The values of Idric Retense Capacity, measured as available water (% v/v) between -10cm and -100cm, were about 13,5 % in new vineyard untreated control soil. With the zeowine treatment an increase in Idric Retense Capacity of about 1% was measured at the three different sampling times (T0, T1, and T2).

The results showed a larger aggregate stability in the zeowine treated soil with respect to control soil, which may indicate a better conservation of the soil architectural frame.

Figure 16. Idric Retense Capacity at T0, T1 and T2

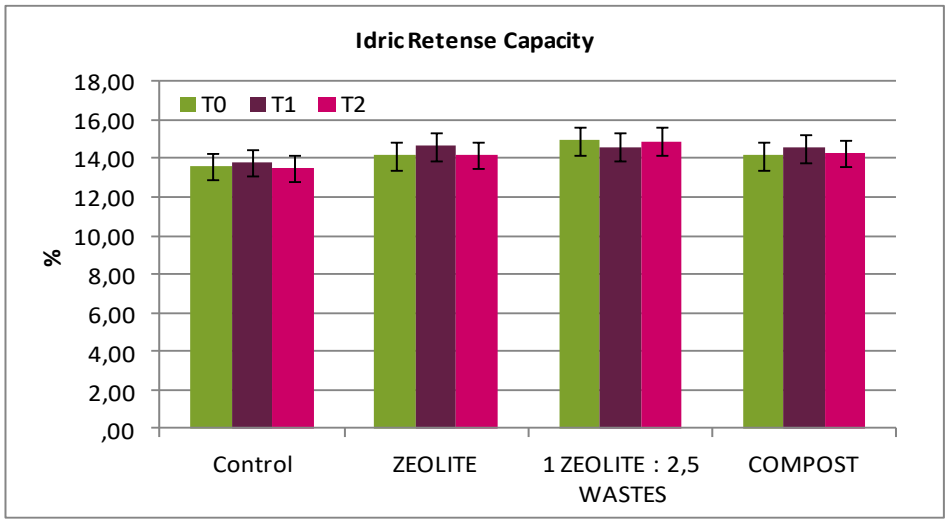
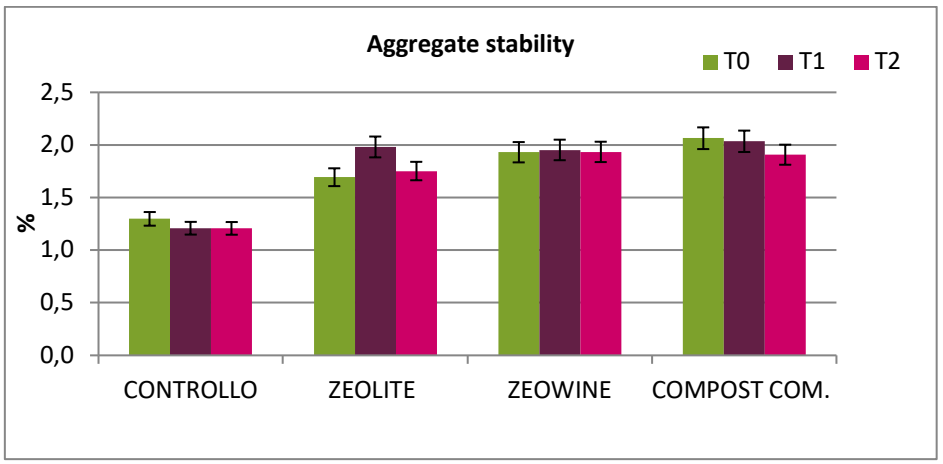


Figure 17. Aggregate stability at T0, T1 and T2

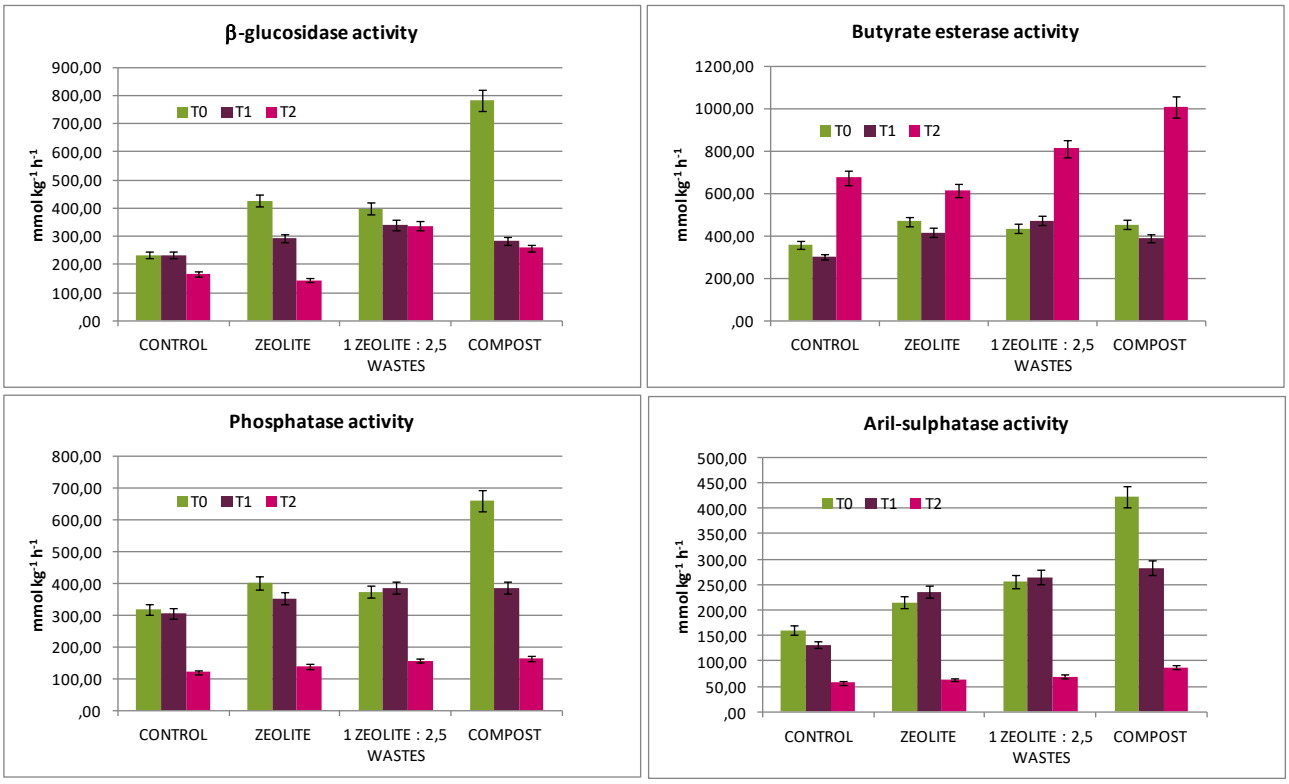


5.1.4 Soil Biological and Biochemical parameters

5.1.4.1 Soil enzyme activities

Microbial activity can be used more effectively than chemical-physical parameters as an indicator of variations in soil quality since it responds more rapidly and with a greater degree of sensitivity to such changes. Since the content and the activity of soil microbial biomass are closely related to the organic matter input, as expected, zeowine and commercial compost addition improved the microbiological and biochemical conditions of the new vineyard soils.

Figure 18. Enzyme activities at T0, T1 and T2.



5.1.4.2 Soil microarthropod community structure analysis

Fig. 19 – Total abundance in 2019, after the first year of treatments. Standard errors are reported.

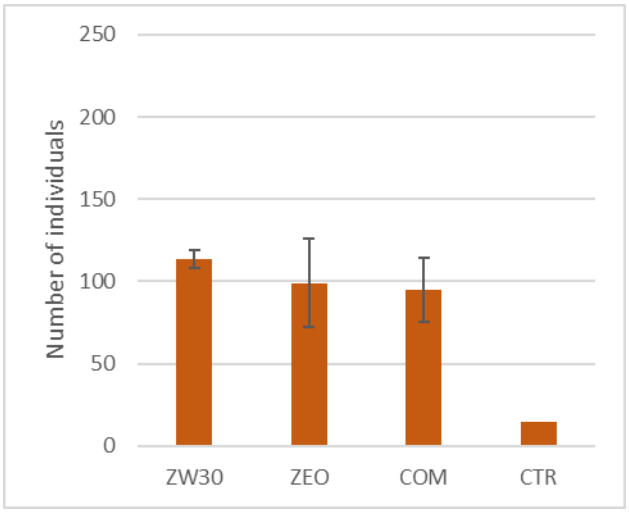
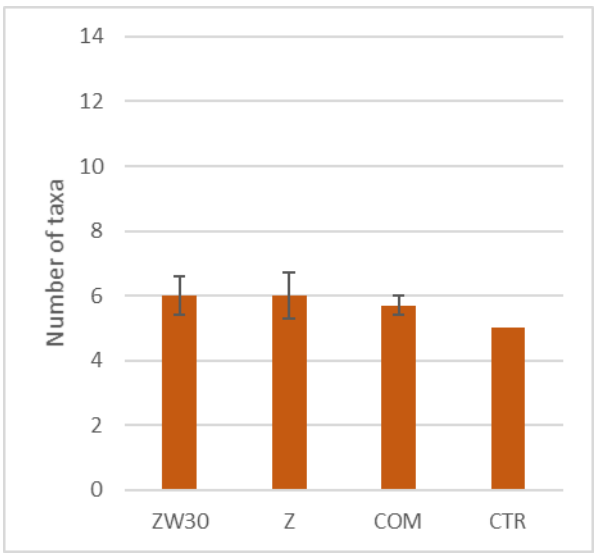
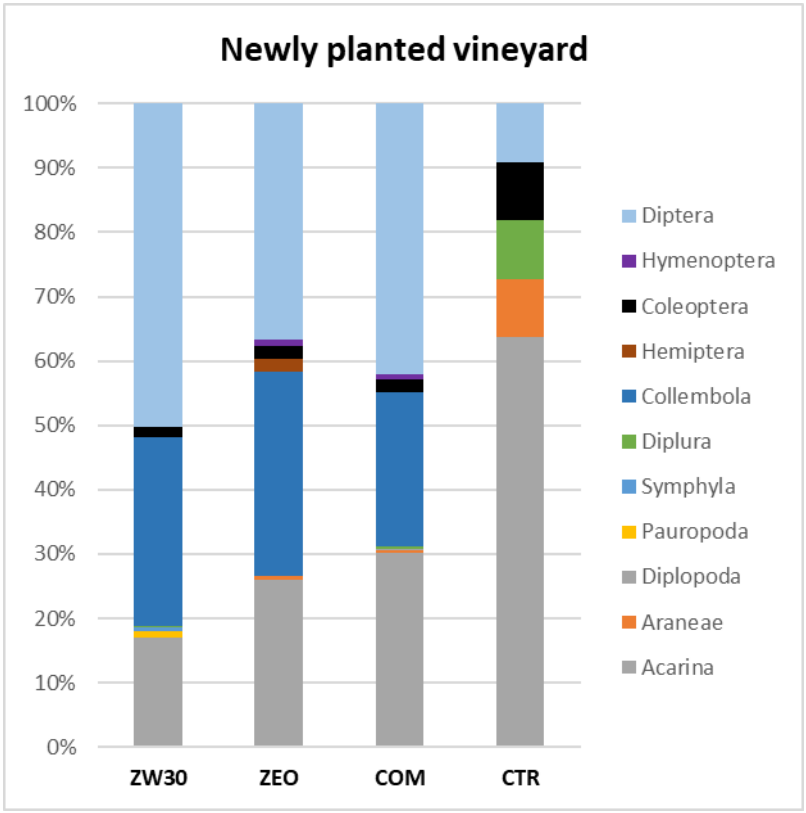


Fig. 20 – Number of taxa in 2019, after the first year of treatments. Standard errors are reported.



In newly planted vineyard, only eleven taxa were found. All treatments showed an increase in Diptera than Control, at the same time, mites and Coleoptera decreased.

Fig. 21 - Relative abundance of soil microarthropod taxa per vineyard.


Newly planted vineyard
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
ZW30
ZEO
COM
CTR
Diptera
Hymenoptera
Coleoptera
Hemiptera
Collembola
Diplura
Symphyla
Pauropoda
Diplopoda
Araneae
Acarina

Soil microarthropod indicators

No differences were recorded among different managements in the new vineyard for Shannon-Weiner and Simpson indices (Fig. 22 and 23)

Fig. 22– Shannon-Weiner index in 2019, after the first year of treatments. Standard errors are reported.

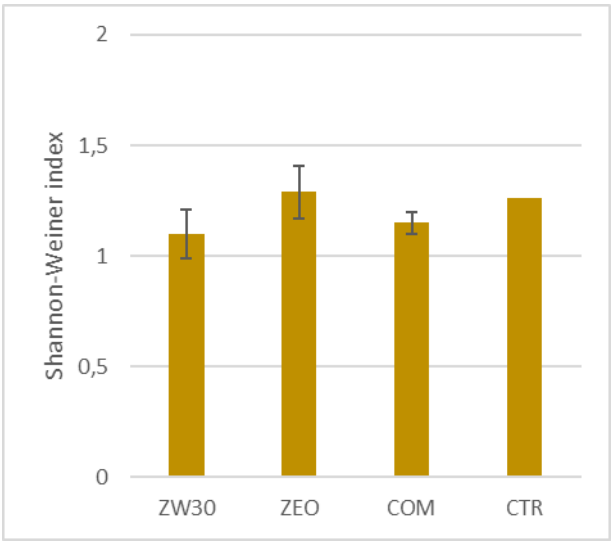
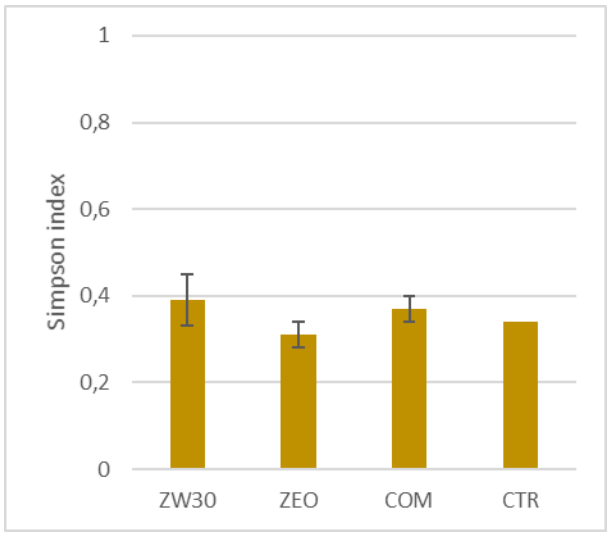
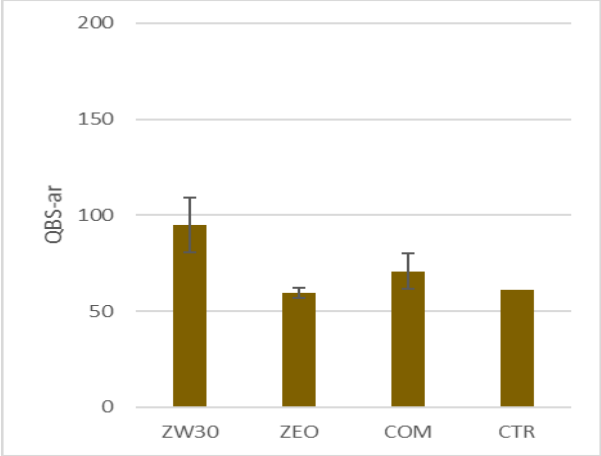


Fig. 23 – Simpson index in 2019, after the first year of treatments. Standard errors are reported.



Instead, QBS-ar evidenced significant differences among treatments in new vineyard. In particular, zeowine treatment showed the highest value. In general, the good soil quality is reached when the QBS-ar value is proximality close to 100 in agricultural soils (Parisi et al., 2005). In this case, only zeowine treatment reached this threshold.

Fig. 24 QBS-ar in 2019, after the first year of treatments. Standard errors are reported.

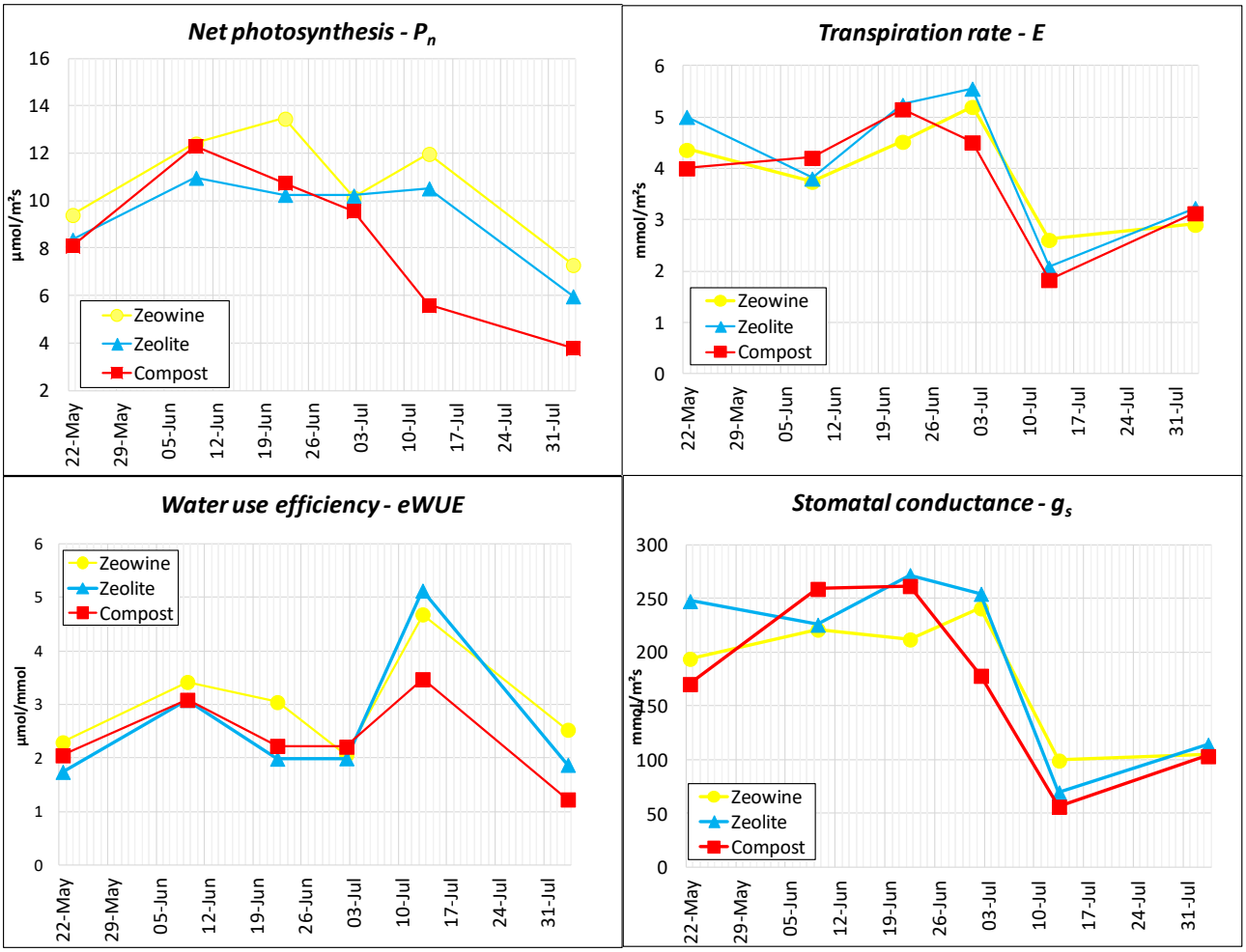


5.2 Plants

The results of 2019 season, concerning the young vineyard at CMM estate, have been reported in the deliverable "ZEOWINE for the fertilization of a young vineyard-first version" prepared on December 2019.

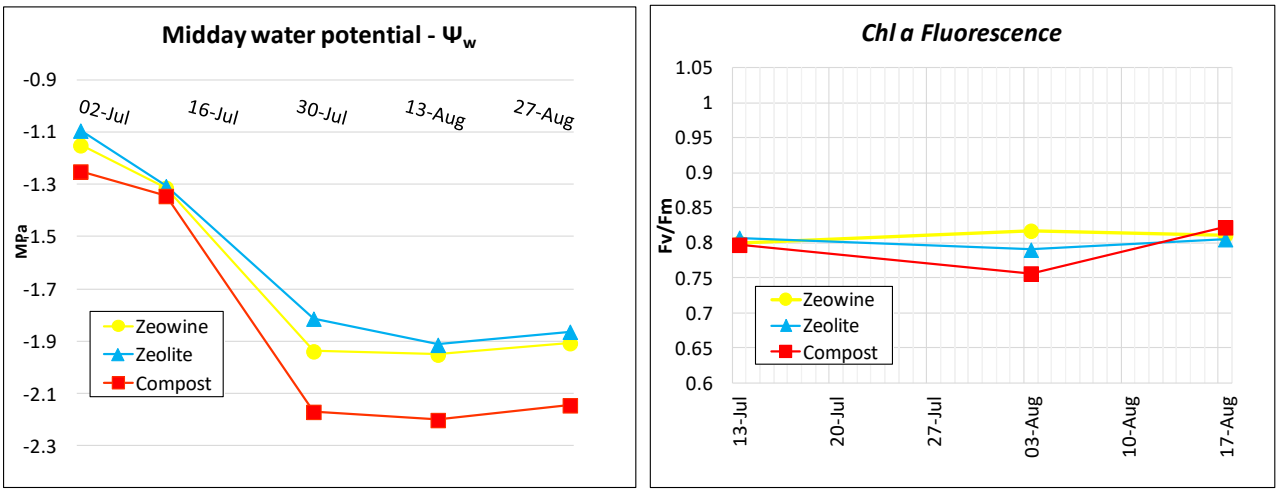
As regards 2020 season, the data already processed are presented below.

Figure 25 Leaf gas exchanges of the young vineyard at CMM estate.



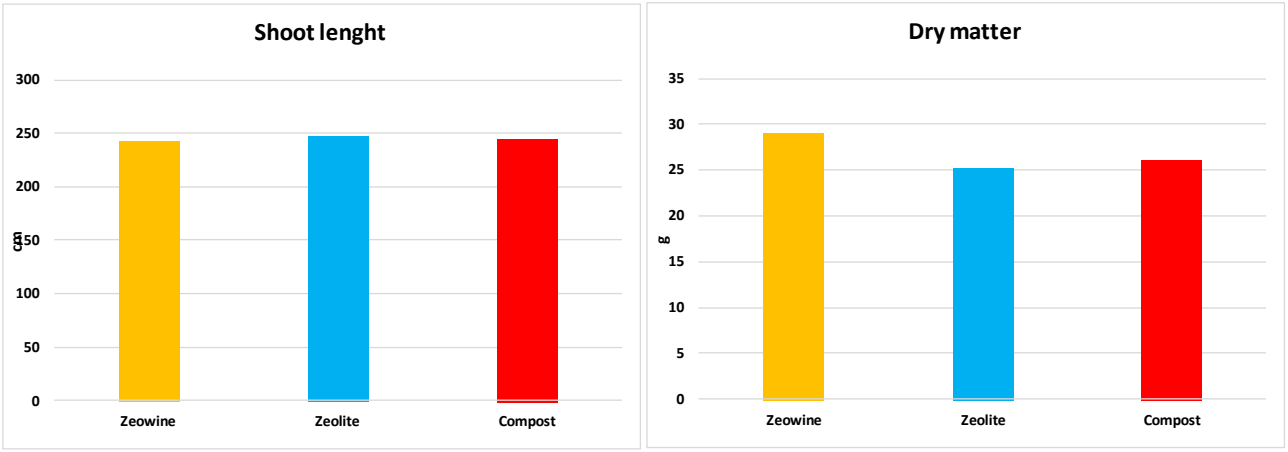
In the young vineyard, leaf gas exchanges followed the same trend as in the productive vineyards, despite less marked differences between the treatments.

Figure 26. Midday water potential and chlorophyll a (Chl a) fluorescence of the young vineyard at CMM estate.



Compost showed a higher water stress, while the values of chlorophyll fluorescence indicated that photosystem II was not damaged in any treatment.

Figure 27. Shoot length and dry matter of the young vineyard at CMM estate.



As regard the shoot length at the end of the season, no differences were recorded among treatments. Dry matter accumulated at the end of the season was slightly higher in Zeowine than the other two treatments.

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