# ZEOWINE for the fertilization of a young vineyard-Final version

# ZEOWINE per la concimazione di impianto in viticoltura – versione finale

Deliverable Action C1

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# Summary



# 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.

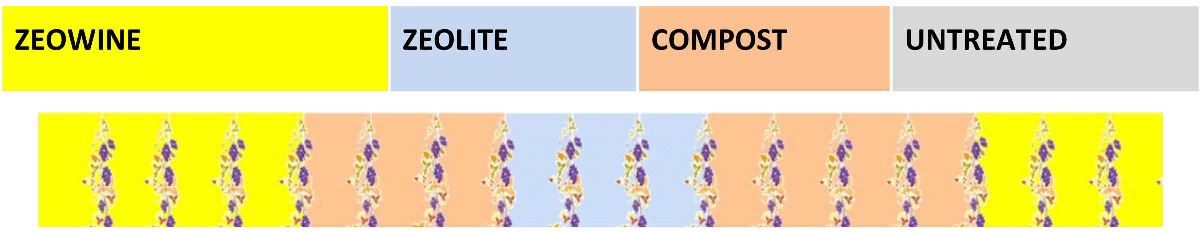



**Figure 1. Experimental layout**

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**), after the second grape harvest (18 months from treatments application, 21 October 2020, **T2**) and after the third grape harvest (30 months from treatments application, 5 October 2021, **T3**) 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. 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

### 4.2.1 - Gas exchange measurements

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 a few seconds the instrument measures the differential concentrations of $CO_2$ 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.

| | | |
|---|---|---|
|  |  |  |
| Fig. 3 – Treatments randomization | Fig. 4– Gas exchange readings | Fig. 5 – Gas exchange readings |

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 $m^{-2}s^{-1}$); stomatal conductance, the degree of stomatal opening which is directly related to the transpiration rates (gs, mmol $m^{-2}s^{-1}$) 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.

### 4.2.2 - Water stress measurements

Vines water stress: was detected with a Scholander's pressure chamber (model 600, PMS Instrument Co., Albany, OR, USA), 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 ($\Psi w$), 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. Measurements were conducted between 12 noon and 1:00 p.m., from flowering to the ripening phase, in the median portion of a primary shoot. Measurements were taken once a day on the following dates: flowering, fruit set, pre-veraison, veraison, mid maturation, full maturation.

Fig. 6 – Performing water stress readings

Fig. 7 – Scholander pressure chamber

Fig. 8 – Leaf preparation

### 4.2.3 - Chlorophyll fluorescence

In the hottest and driest period, from pre-veraison to the ripening, using Handy-PEA® tool (Hansatech Instruments, UK), Chlorophyll a fluorescence transient of dark-adapted leaves was recorded with a saturating flash of actinic light at 3000 µmolm/m$^2$s for 1 s. Briefly, the maximum quantum yield of photosystem II (PSII) was calculated as the ratio $Fv/Fm = (Fm - F0)/Fm$ where Fv represents the variable fluorescence and F m represents the maximal fluorescence of dark-adapted (over a 30-min period) leaves. A 502 SPAD device (Konica Minolta Inc., Japan) was used to measure chlorophyll content in leaves.

## 4.3 - Grape maturity

Technological maturity analyses: consist in determining the sugar content (° Brix), total acidity (g/L 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 g/L 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.


Fig. 9– Sample collection


Fig. 10 – Yield weight


Fig. 11 – Grapes and leaves harvest

Phenolic maturity analyses: the total and extractable polyphenol and anthocyanin values (mg $L^{-1}$) of harvested grapes were obtained through the Glories Method; it consists of 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.

Fig. 12 – Harvest panoramic


Fig. 13 – Sugar and phenolic analyses

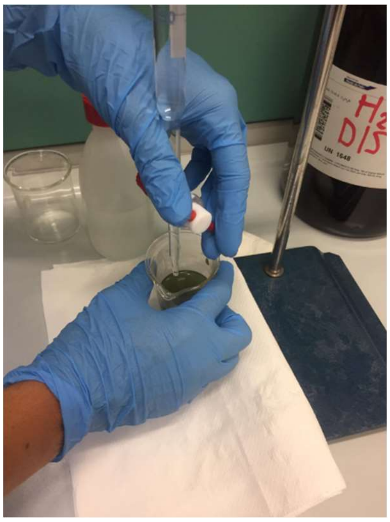


Fig. 14 – Acidity analysis

### 4.5 - Statistical analysis

Data from each season 2019, 2020, and 2021 were separately analyzed by means of one-way ANOVA with soil treatments as the main factor ($P \leqslant 0.05$). In addition, mean values were separated by Fisher's least significant difference (LSD). P value adjustment was performed with the Holm method ($P \leqslant 0.05$). All statistical analyses were performed using R and RStudio (Boston, MA, USA).

# 5 Results

## 5.1 Soil

### 5.1.1 Soil Chemical parameters

The young vineyard soil showed alkaline pH, especially at T3 sampling time (Figure 15) and was not saline (electrical conductivity lower than 0,2 dS $m^{-1}$, Figure 16). CEC values increased with ZEOWINE and commercial compost addition.

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 each sampling time (Figure 18).

The content of TP and TK were higher in the all the treatments, especially in the zeolite and zeowine treated soils in comparison with the control soils (Figure 8 and 10).

The application of zeowine significantly decreased the exchangeable K over time with respect to control soil (Figure 20 and Figure 22), thus indicating the increase in retention of these element.

Similarly, a reduction of available Cu was observed in zeolite and ZEOWINE treatments, at T2 and T3 sampling times, with respect to control soil and to the soil treated with commercial compost.

**Figure 15.** pH at T0, T1, T2 and T3


**Figure 16.** Electrical Conductivity at T0, T1, T2 and T3

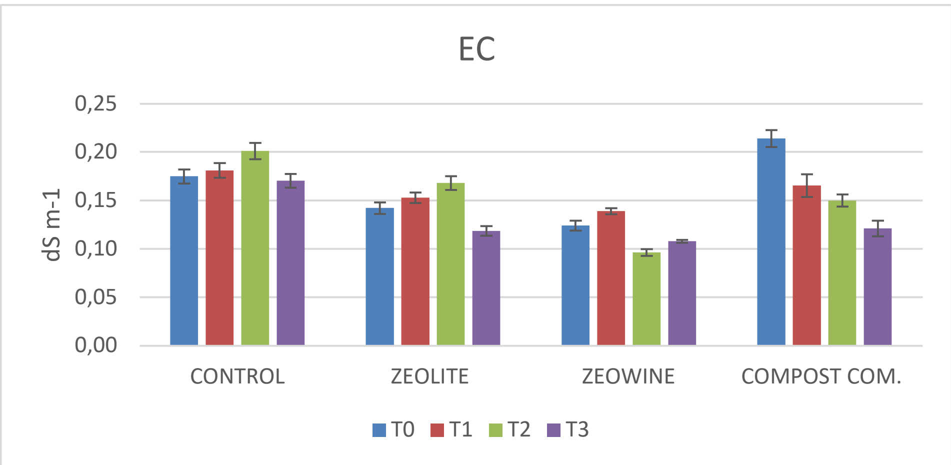


**Figure 17.** Cation Exchange Capacity at T0, T1, T2 and T3

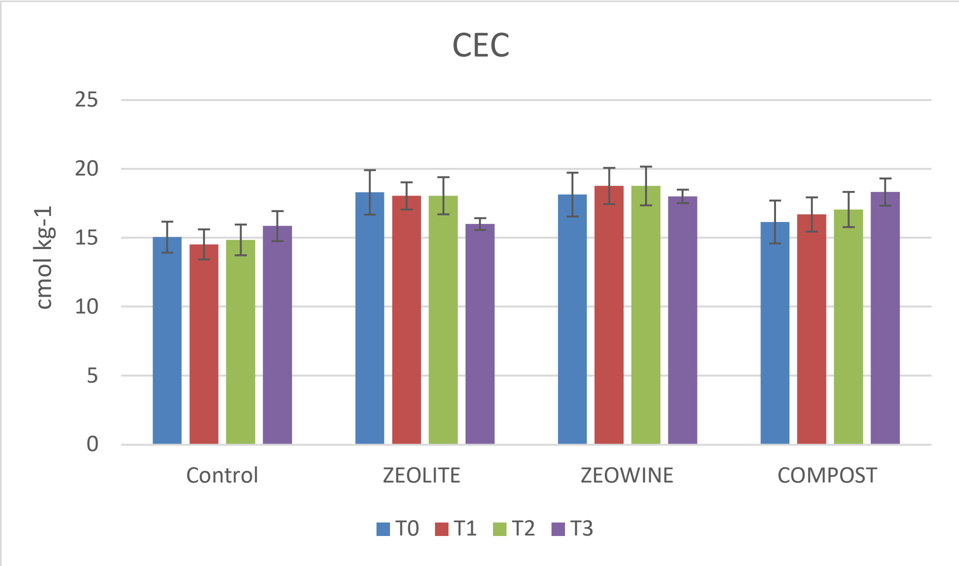



**Figure 18.** Total Organic Matter at T0, T1, T2 and T3

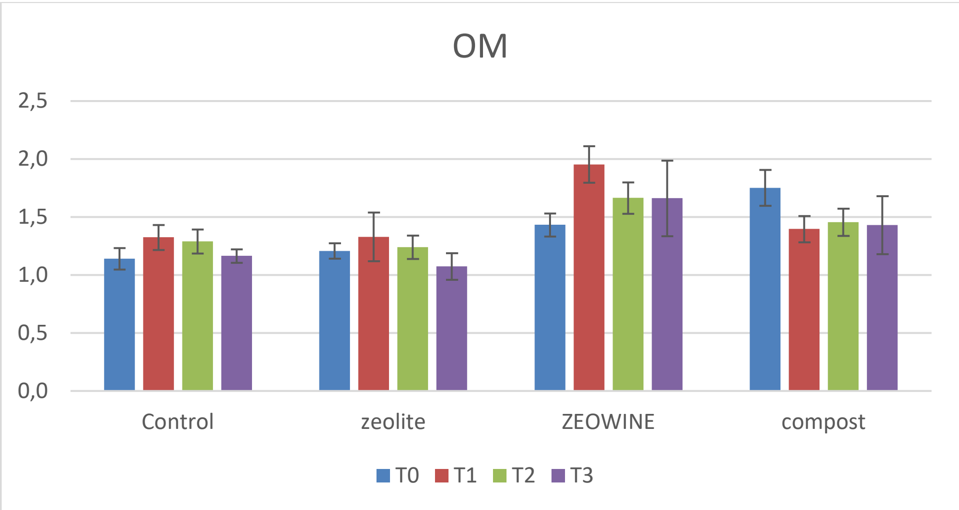



**Figure 19.** Total Nitrogen at T0, T1, T2 and T3

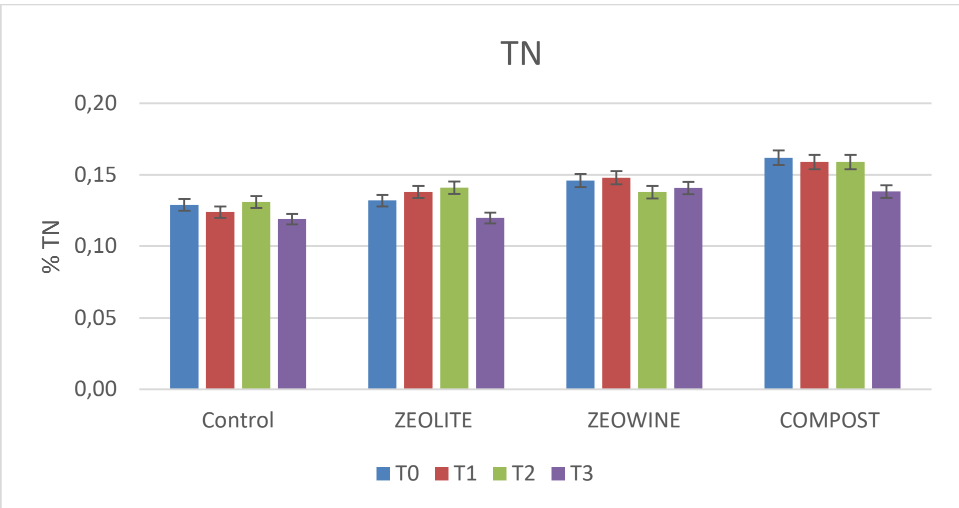


Figure 20. Total phosphorus at T0, T1, T2 and T3

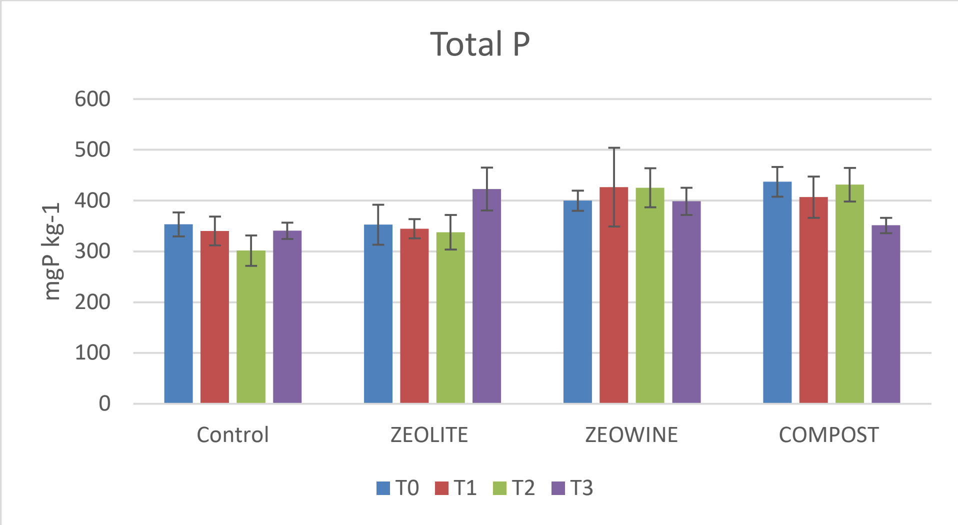



**Figure 21.** Exchangeable phosphorus at T0, T1, T2 and T3

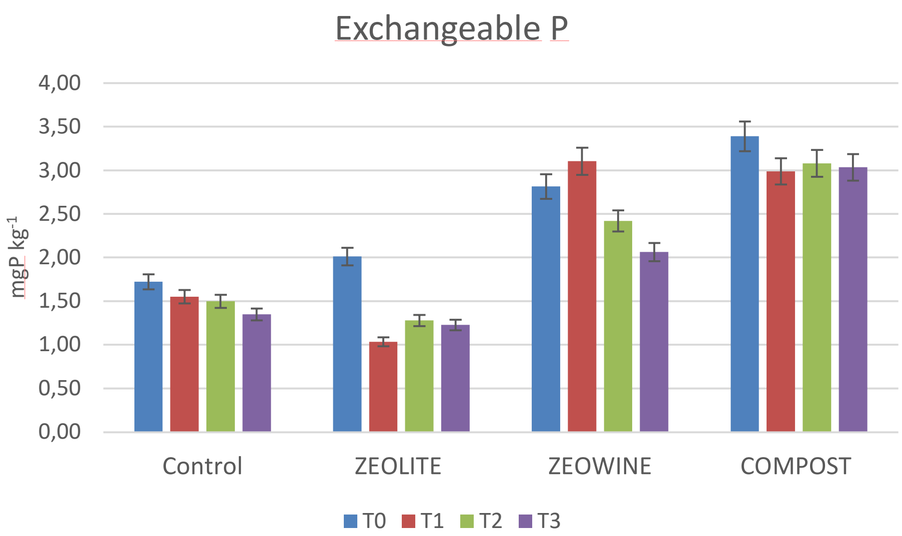



**Figure 22.** Total potassium at T0, T1 and T2

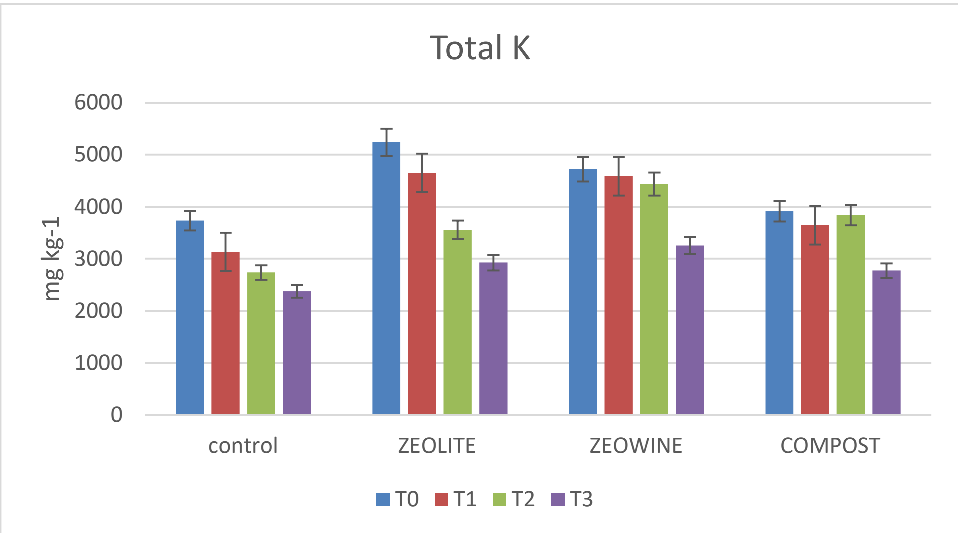


**Figure 23.** Exchangeable potassium at T0, T1, T2 and T3

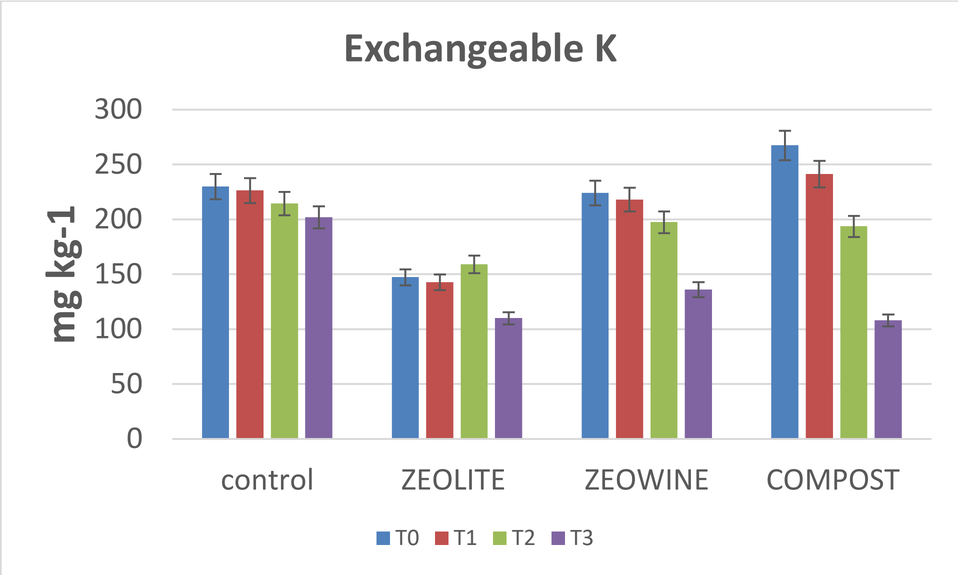



**Figure 24.** Total copper at T0, T1, T2 and T3

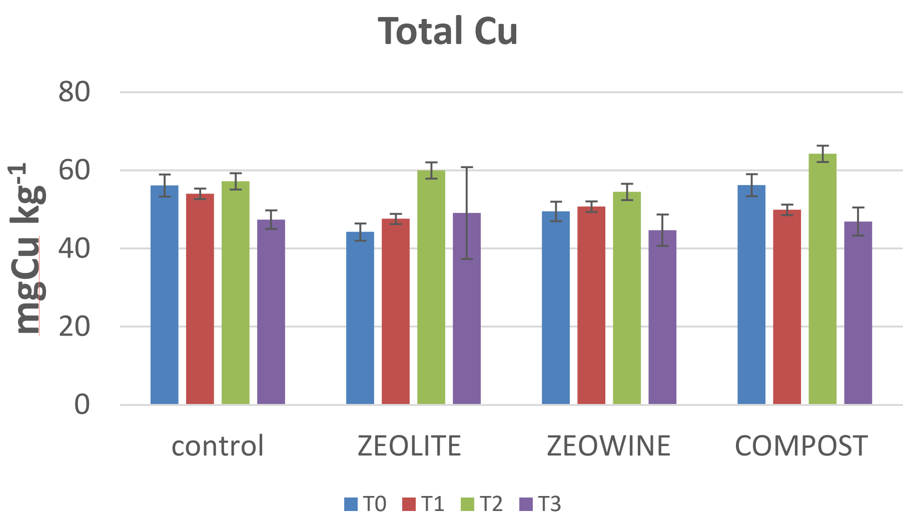



**Figure 25.** Available Cu at T0, T1, T2 and T3

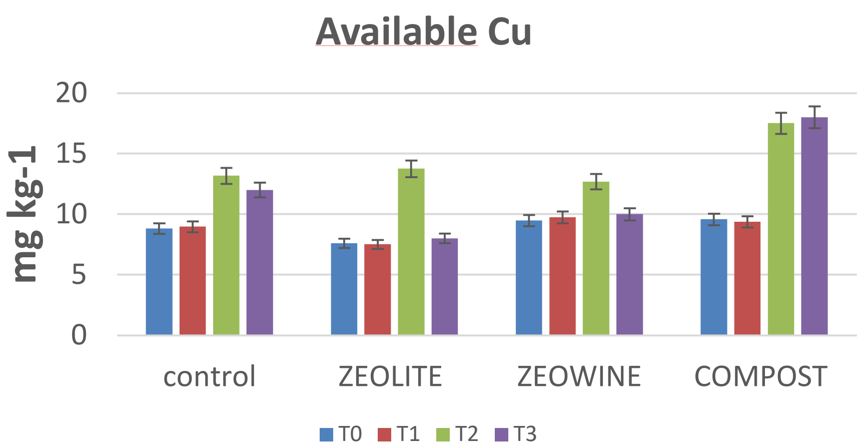
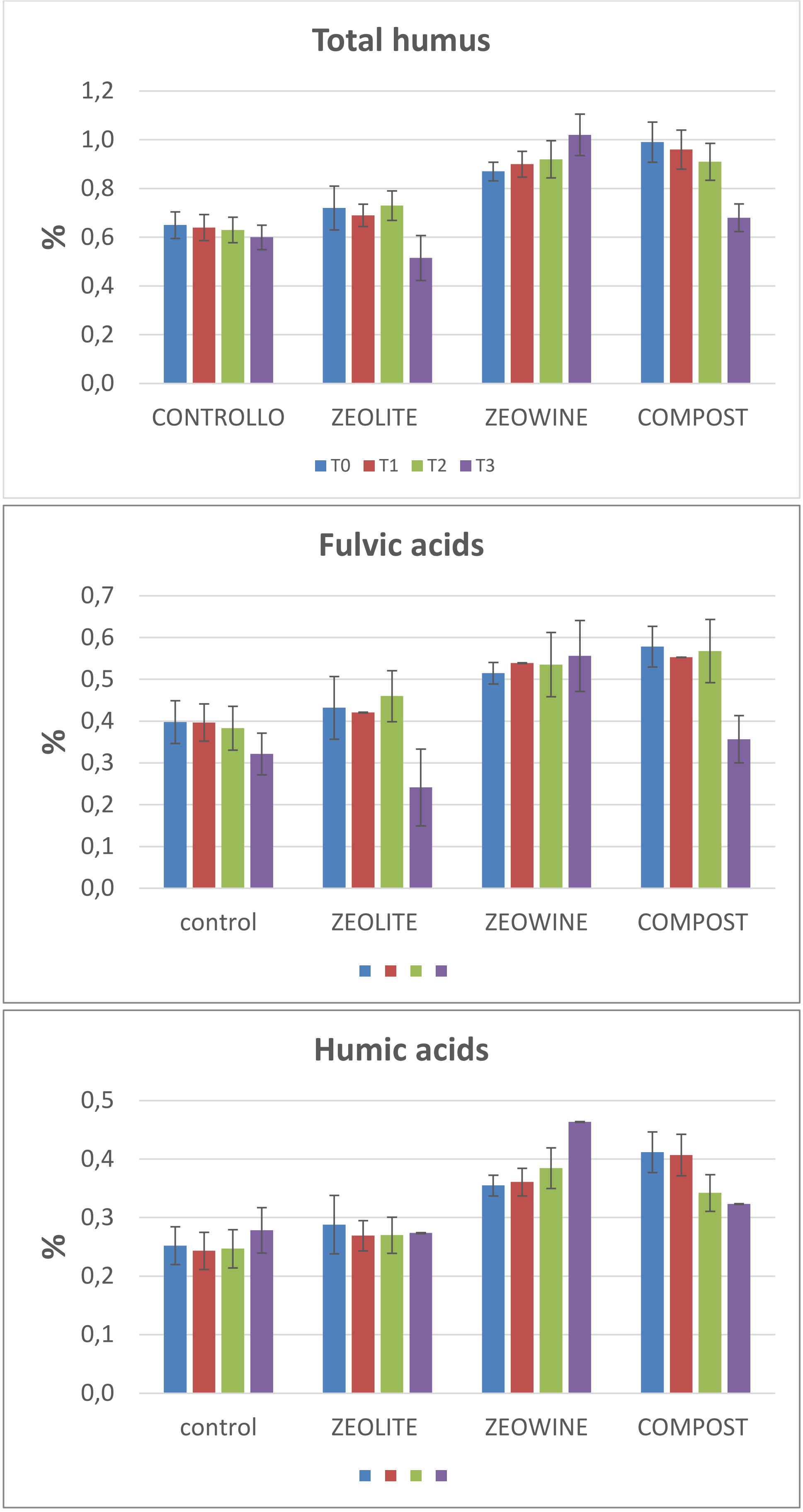


**Figure 26.** Total Humus and fulvic and humic acids at T0, T1, T2 and T3



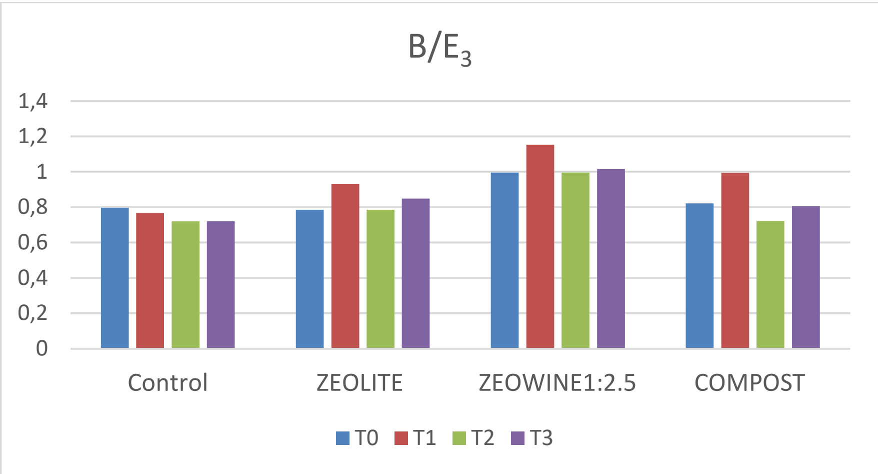
### 5.1.2 Py-GC

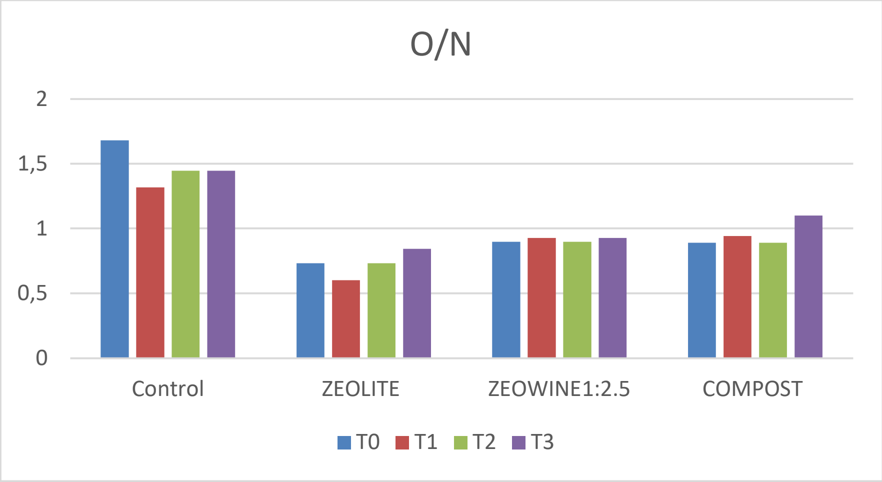
The chemical–structural composition of organic matter was characterized using pyrolysis–gas chromatography. Seven pyrolytic fragments were considered: acetonitrile (E1), acetic acid (K), benzene (B), pyrrole (O), toluene (E3), furfural (N) and phenol (Y). The ratios between the relative abundances of 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 zeolite, 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 at T2 and T3 sampling times with respect to the other treatments. 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 27.** Py-GC at T0, T1, T2 and T3 sampling time, O/N mineralization index, B/E3 humification index.




O/N
2
1,5
1
0,5
0
Control
ZEOLITE
ZEOWINE1:2.5
COMPOST
T0
T1
T2
T3

### 5.1.3 Soil physical parameters

The values of Idric Retense Capacity, measured as available water (% v/v) between -10cm and -100cm, were about 13,7 % in new vineyard untreated control soil. With the zeowine treatment an increase in Idric Retense Capacity higher than 1% was measured at each sampling times (T0, T1, T2 and T3).

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 28.** Idric Retense Capacity at T0, T1, T2 and T3

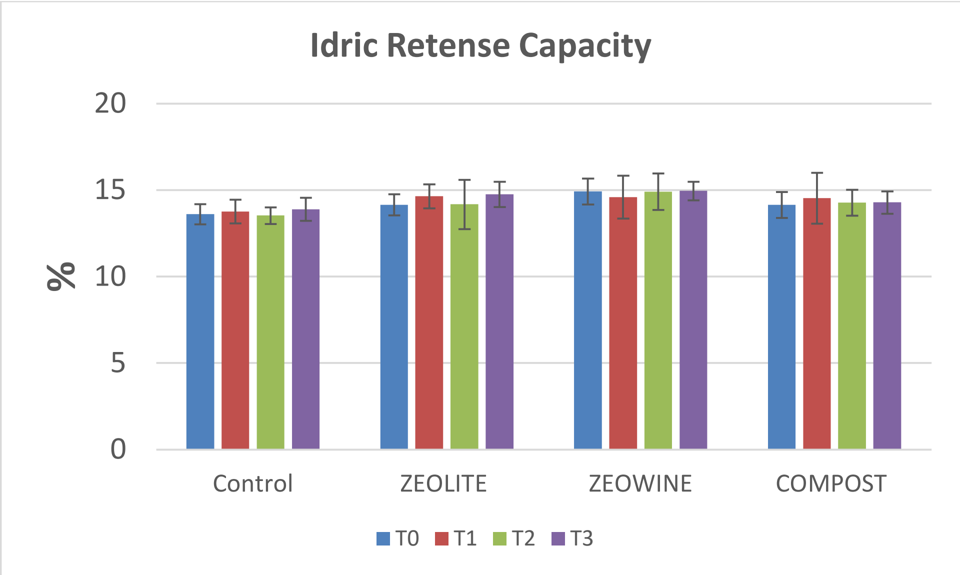
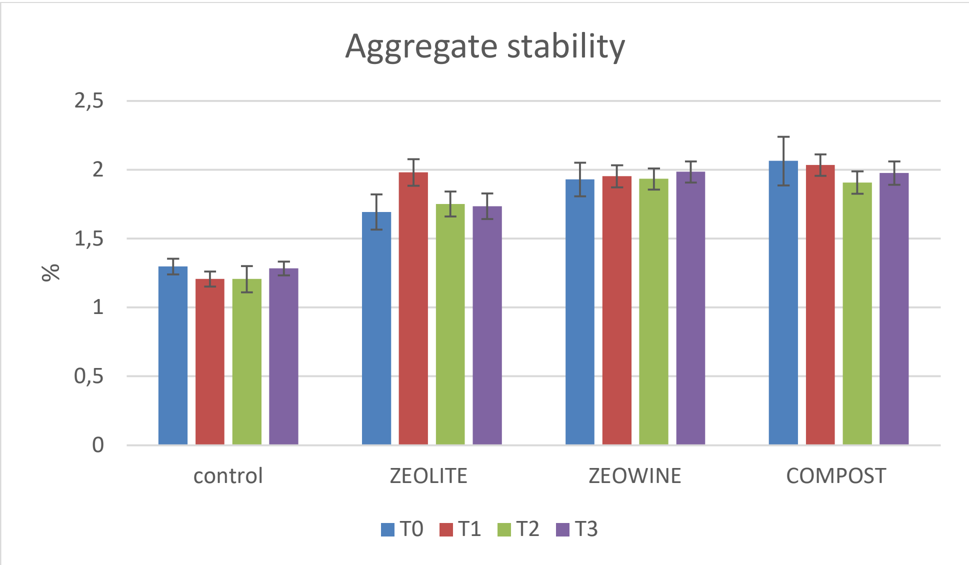



**Figure 29.** Aggregate stability at T0, T1, T2 and T3

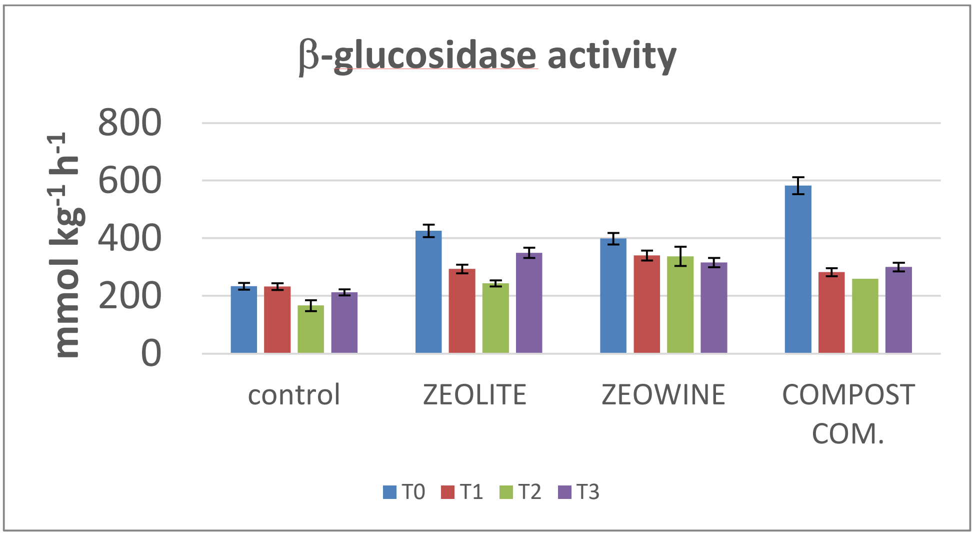


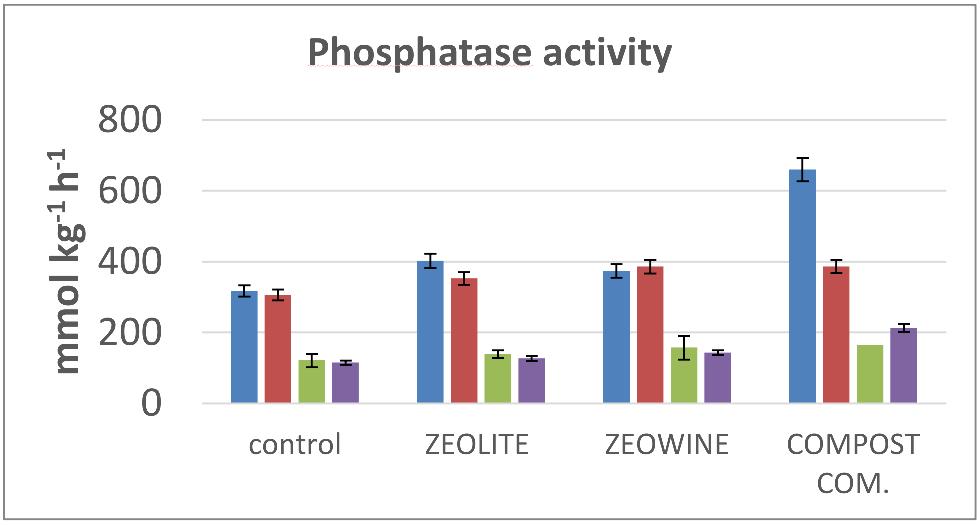
## 5.1.4 Soil Biological and Biochemical parameters

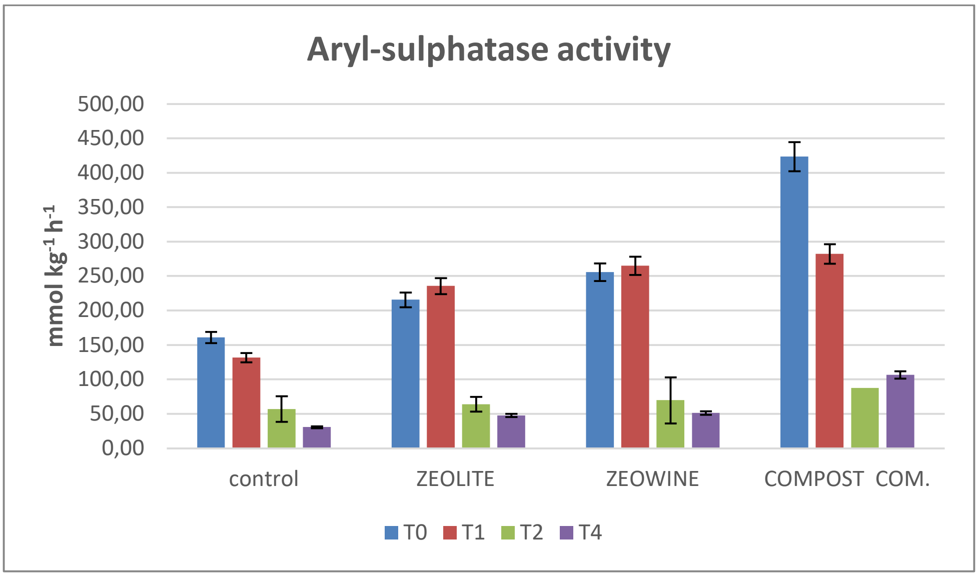
### *5.1.4.1 Soil enzyme activities*

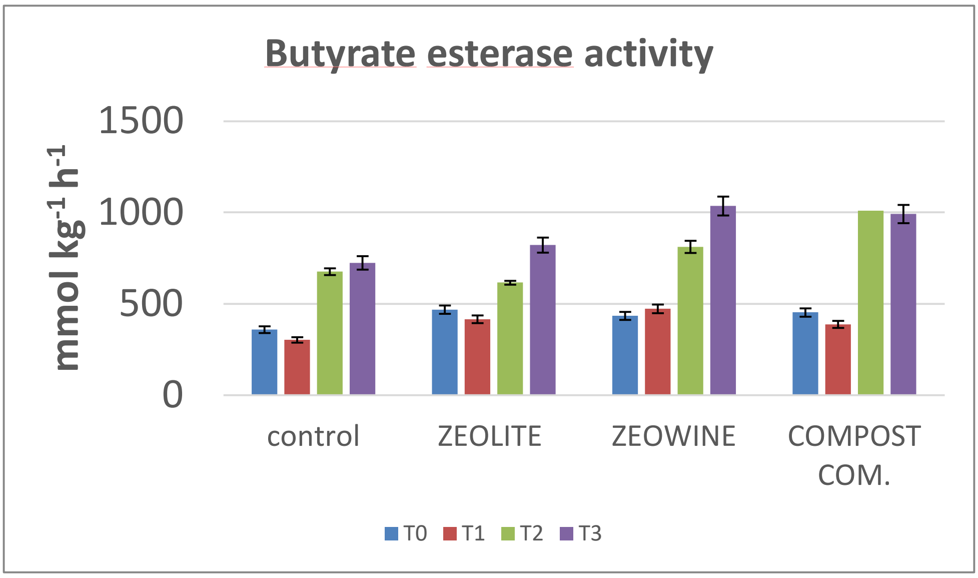
Microbial activity can be used more effectively than chemical-physical parameters as an indicator of variations in soil quality since it responds more rapidly and with a greater degree of sensitivity to such changes. Since the content and the activity of soil microbial biomass are closely related to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

**Figure 30.** Enzyme activities at T0, T1, T2 and T3.




Aryl-sulphatase activity
mmol kg-1 h-1
500,00
450,00
400,00
350,00
300,00
250,00
200,00
150,00
100,00
50,00
0,00
control
ZEOLITE
ZEOWINE
COMPOST COM.
T0
T1
T2
T4


Butyrate esterase activity
mmol kg-1 h-1
1500
1000
500
0
control
ZEOLITE
ZEOWINE
COMPOST COM.
T0
T1
T2
T3

### *5.1.4.2 Soil microarthropod community structure analysis*

*See Annex 1*

## 5.2 Plants

### 5.2.1 - Gas exchange

To focus on climate change, the main objectives of soil management are to maintain an environment that favors the development of the vegetative apparatus, the accumulation of organic matter, the absorption of water and the use of nutrients; management that causes drop-in soil skills to reduce these aspects. However, proper soil management can be expected to restore ecosystem functions that have been degraded. This project highlights the importance of soil management in the Mediterranean area through the application of a new zeolitic-based product against the problems of climate change. Chlorophyll a fluorescence (Fv/Fm, an indicator of photo-oxidative stress) reported differences especially on the hottest days. The photo-oxidative shock inhibited photosystem II (PSII) efficiency, as suggested by the reduction of Fv/Fm ratios. In the Compost treatment, the extreme temperature-induced photo-oxidative stress as could be derived from increased expression of reactive oxygen species (ROS) scavengers and an increased pool size of the xanthophyll cycle pigments. Consequently, in Compost treatment, a robust inhibitory effect on photosynthetic capacity and net $CO_2$ assimilation (Pn) was reported during that period, as a typical impact of PSII deficiency. Gaseous exchanges were affected in zeolite-treated vines; Zeowine showed higher rates of photosynthesis vs. Compost treatment. The photosynthesis trend during the seasons of both treatments reflected that temperature directly influenced the photosynthesis rate by stimulating the activity of photosynthetic enzymes and the electron transport chain (ETC). At low temperatures, the Pn rate increased proportionally with the temperature until it reached an optimum. The higher-summer temperatures reduced Compost photosynthesis. In addition, an increase in the air temperature for the Compost treatment indirectly led to increased leaf temperature, which could stimulate water loss by transpiration and elevate vapour pressure deficit (VPD). Probably the effect on leaf temperature was mediated by water availability, as it was observed from stem water potential data. As with barley and corn seedlings, the application of zeolite to the vineyard soil was found to increase photosynthetic activity. The WUE of zeolite-treated plants was usually higher to compost vines, suggesting that zeolites did increase water consumption with increasing $CO_2$ fixation. Due to zeolitic ability to retain water, plants treated with clinoptilolite (Zeowine and Zeolite) showed significantly lower leaf temperatures in both seasons than zeolite-free composting plants. The lower transpiration rates of compost-treated plants may explain the higher leaf-air temperature that was observed. The synergy of compost and zeolite positively affected water stress; due to the zeolitic skill of adsorption and release of water, the Zeowine application showed less negative water potential values during the most syccitous period in all years (2019, 2020, and 2021). Zeowine increased the water-holding capacity of the soil and improved soil quality in the root zone.

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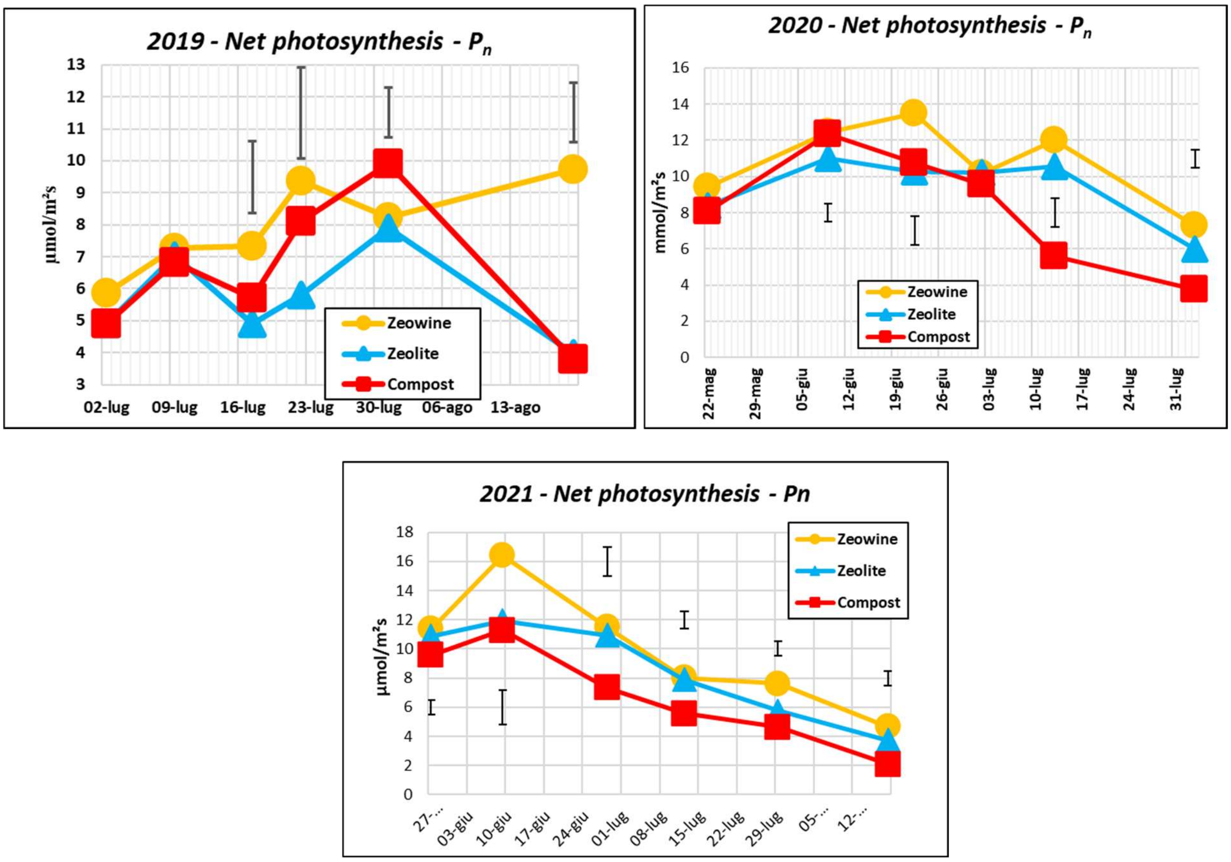


Figure 31 - Net photosynthesis 2019-2020-2021. Vertical bars represent LSD 95%

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Figure 32 - Transpiration rate 2019-2020-2021. Vertical bars represent LSD 95%

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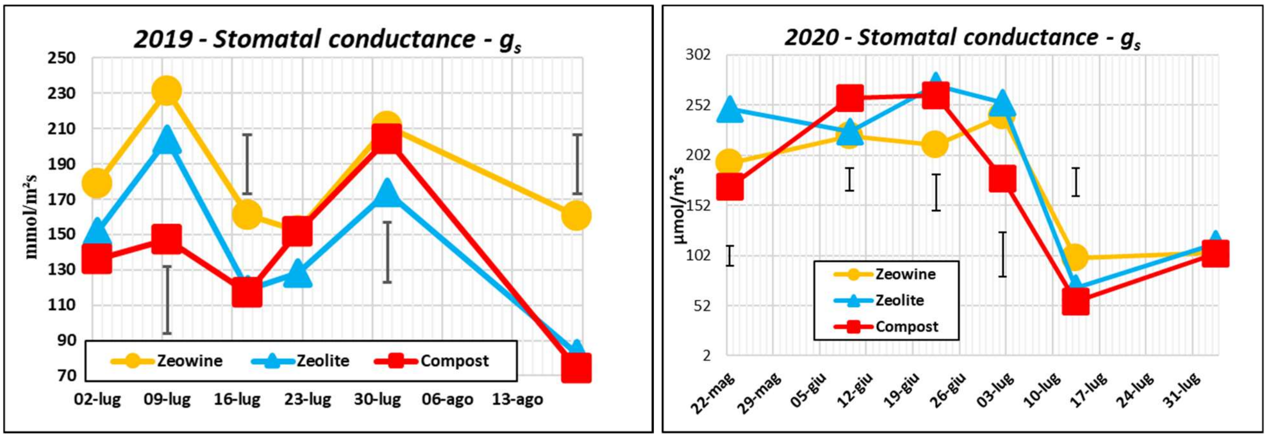

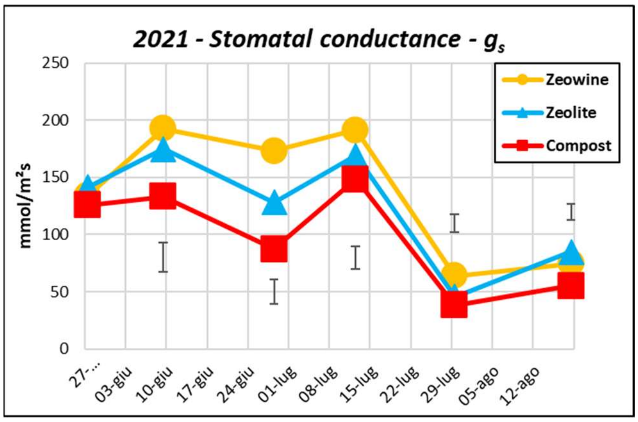


Figure 33 - Stomatal conductance 2019-2020-2021. Vertical bars represent LSD 95%

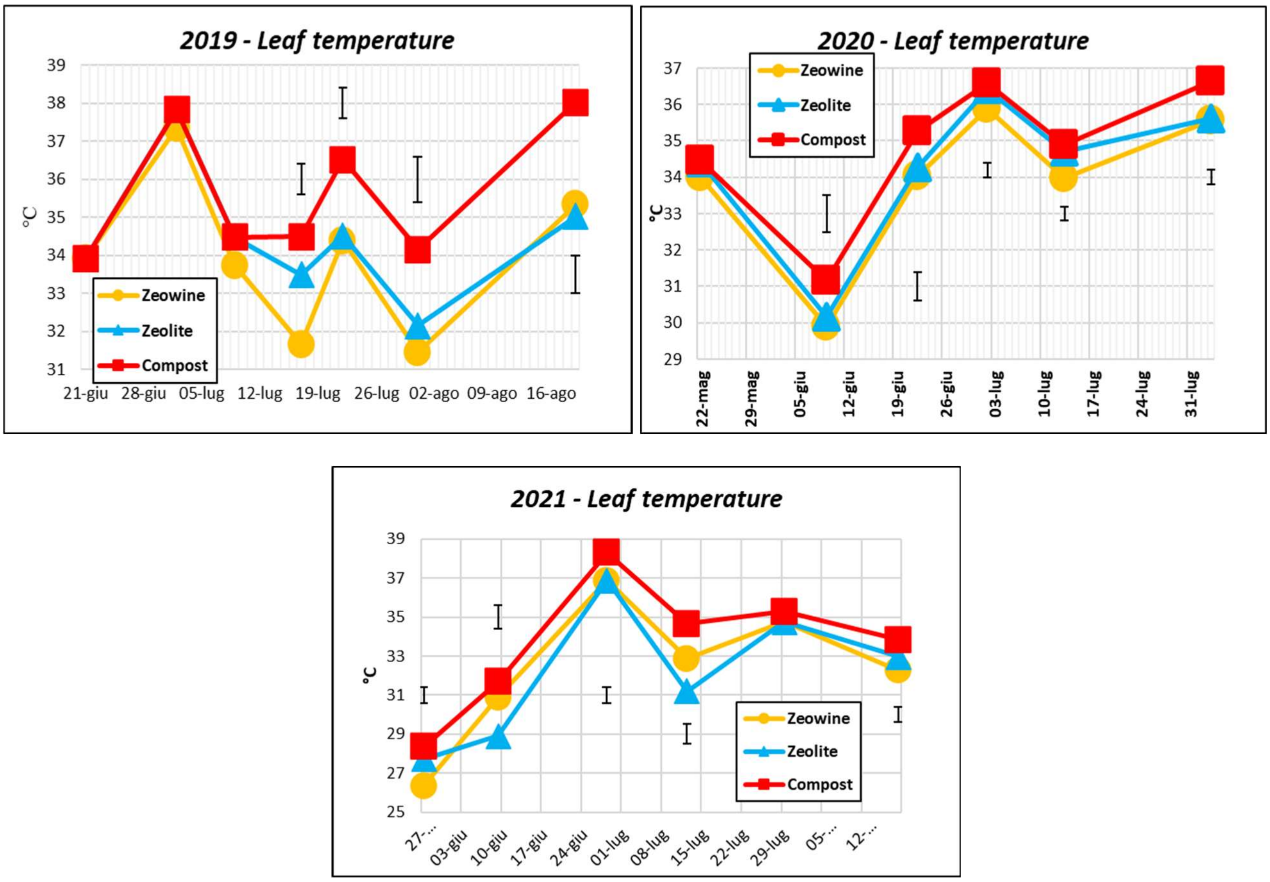


Figure 34 - Leaf temperature 2019-2020-2021. Vertical bars represent LSD 95%

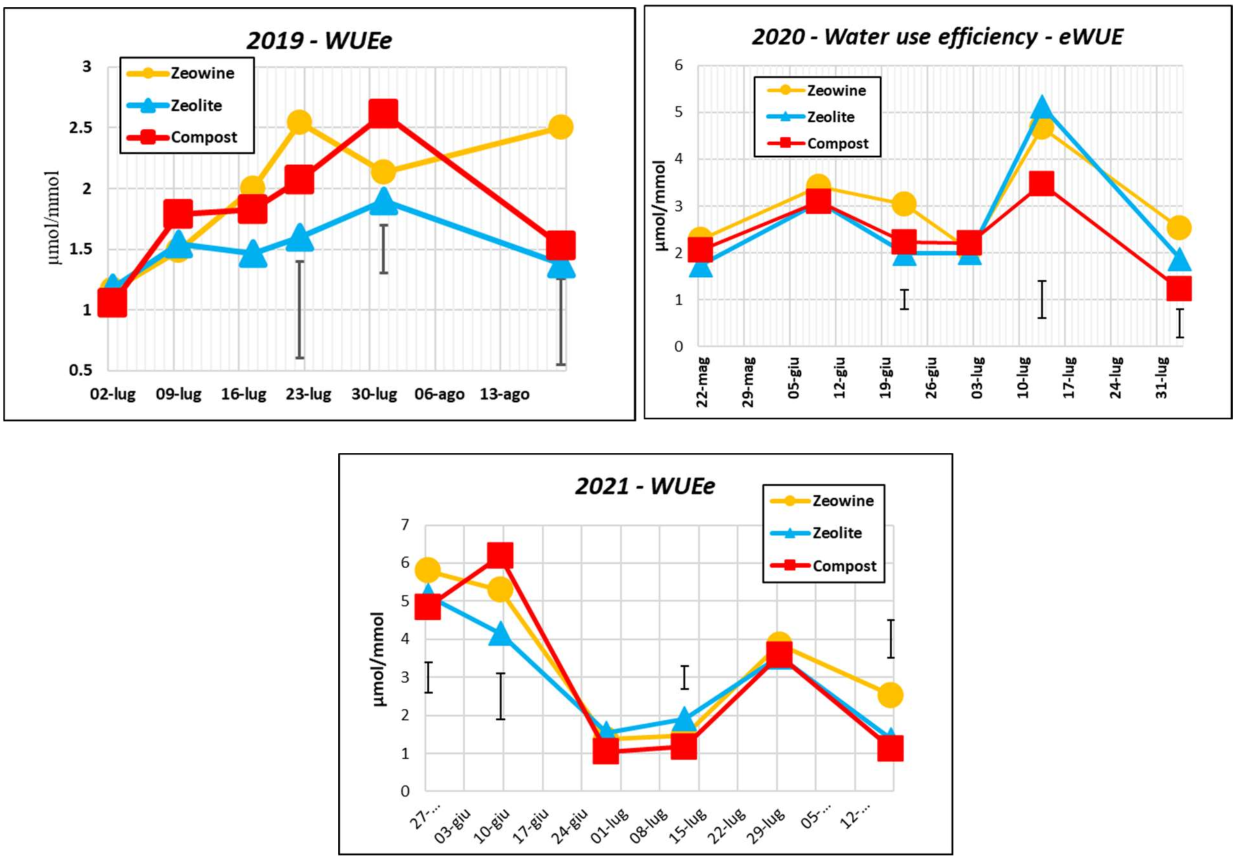


Figure 35 - Water use efficiency 2019-2020-2021. Vertical bars represent LSD 95%

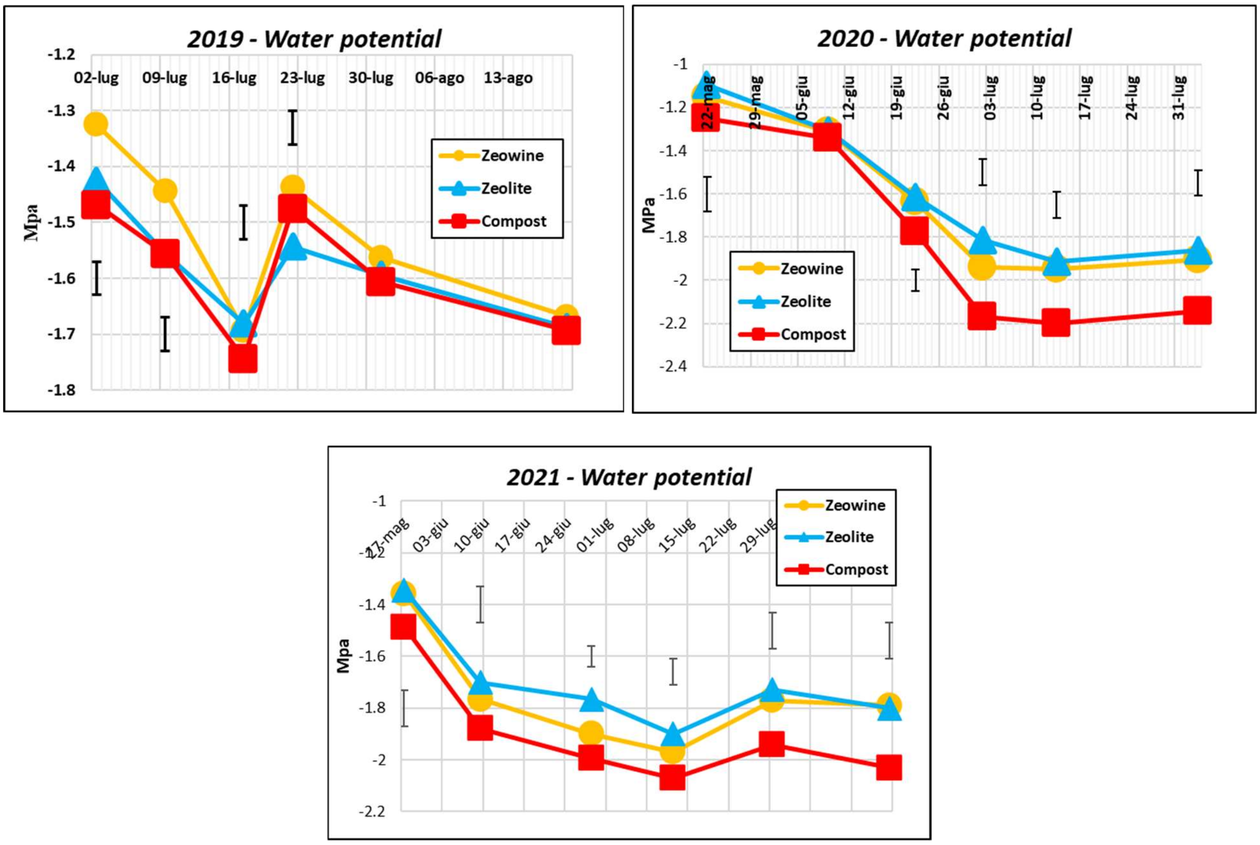


Figure 36- Water potential 2019-2020-2021. Vertical bars represent LSD 95%

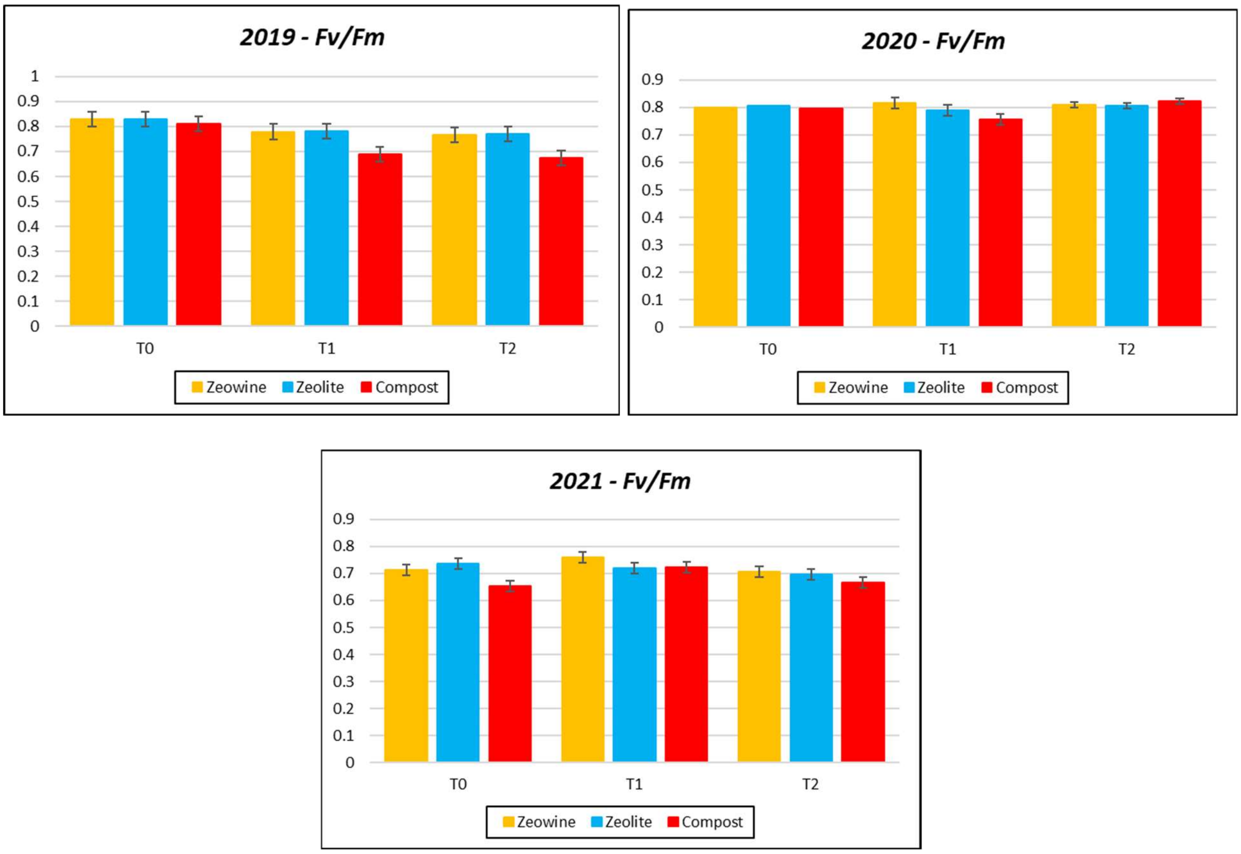


Figure 37- Chlorophyll a fluorescence (Fv/Fm) 2019-2020-2021. Vertical bars represent LSD 95%

## 5.3 - Grape quality and quantity

During the 2020 season, significant differences at full veraison, mid-maturation and harvest were noted in sugar content, while during the 2021 season, significant differences at mid-, full-maturation and harvest were noted in sugar content: Zeowine had the highest values than Compost treatment. At the time of harvest, Zeowine increased the sugar content of 5.50% in 2020 and 2.00% in 2021. The higher sugar content of Zeowine-treated plants was due to their higher rate of photosynthesis. A close link between photosynthesis (Rubisco activity) and vine carbohydrate metabolism was found and it was observed that the photosynthesis rate (Pn) was directly related to the rate of the sugar metabolic process. Moreover, we hypothesize that this correlation was due to the young age of the plants; in fact, as there were few stored carbohydrates, berry sugar accumulation was more sensitive to photosynthesis. In addition, these effects have been linked to the reduction of the leaf temperature, in this case, due to zeolite ability to reflect infrared radiation. However, it cannot be excluded the zeolite capacity to absorb carbon dioxide, determining its increase near the stomata and net photosynthesis increase. This aspect deserves more and deeper investigation.

A 23% (2020) and 31% (2021) increase in the weight of the harvest berry for zeowine treatment was also observed; the ability of zeolite to improve the radical water microclimate led to more hydrated and larger berries.

Regarding phenolic maturity, the greatest differences were found in the composition of extractable and total anthocyanins. At full maturation and harvest, Zeowine berries showed significantly higher extractable and total anthocyanin content compared to Compost berries (2020). The lowest values in total anthocyanins were recorded for the Compost treatment at the three different stages during the seasons. At mid-, full-maturation and harvest Zeowine berries showed significantly higher extractable and total anthocyanin content compared to Compost berries (2021). The higher Brix degree of Zeowine treatment may explain the increased accumulation of anthocyanins in the berries (sugar/anthocyanin relationship). In fact, it was demonstrated that differences in the anthocyanin extractability were highly influenced by the ripeness degree and also, by the soluble solids contents. During the 2020 season, no differences in total polyphenols at harvest were found. At full maturation, no differences in extractable polyphenols were found, while at harvest Compost berries showed significantly higher extractable polyphenol content compared to the Zeowine application. During the 2021 season, significant differences in polyphenols at harvest were found; Zeowine berries showed significantly higher extractable and total polyphenol content compared to the Compost application. Increases in total anthocyanins, but also in total polyphenols and colour intensity, were recorded in wine obtained from vines treated with zeolite leaf applications. Again, the leaf temperature reduction effect may have been decisive, for these results, because linked to higher biosynthesis of phenolic compounds. Considering the promising results obtained by zeolite leaf applications, and in this project, by zeolite soil applications, their synergic use may be desirable, with a view to environmentally friendly crop management.

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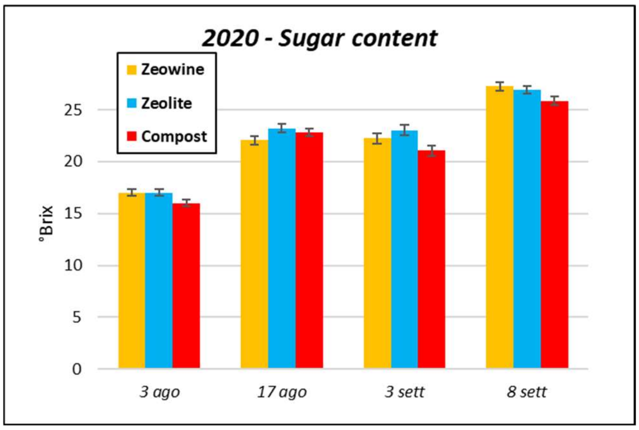


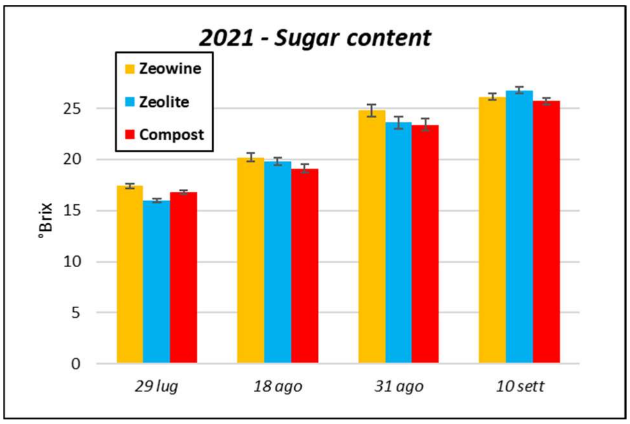



Figure 38 - Sugar content 2020-2021. Vertical bars represent LSD 95%

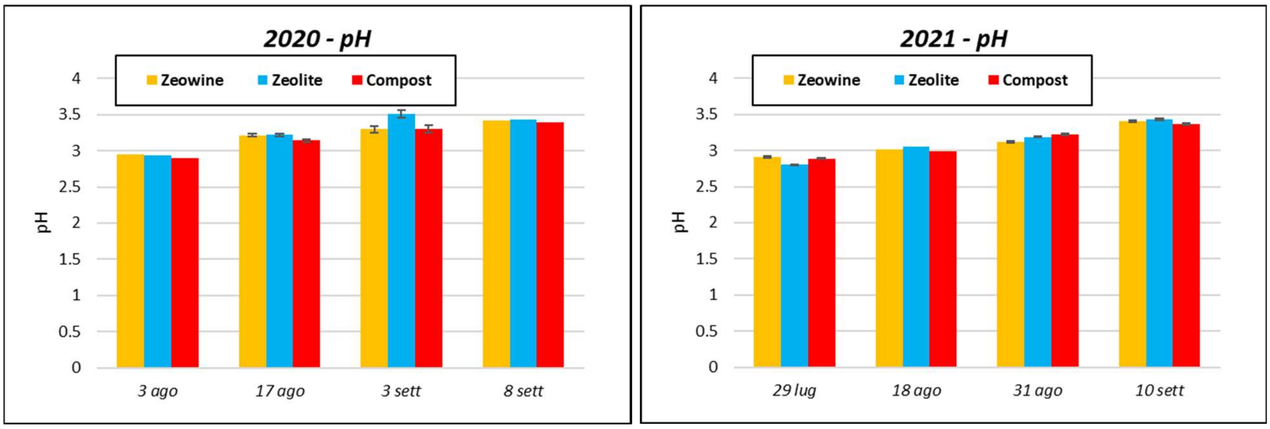



Figure 39 - pH 2020-2021. Vertical bars represent LSD 95%

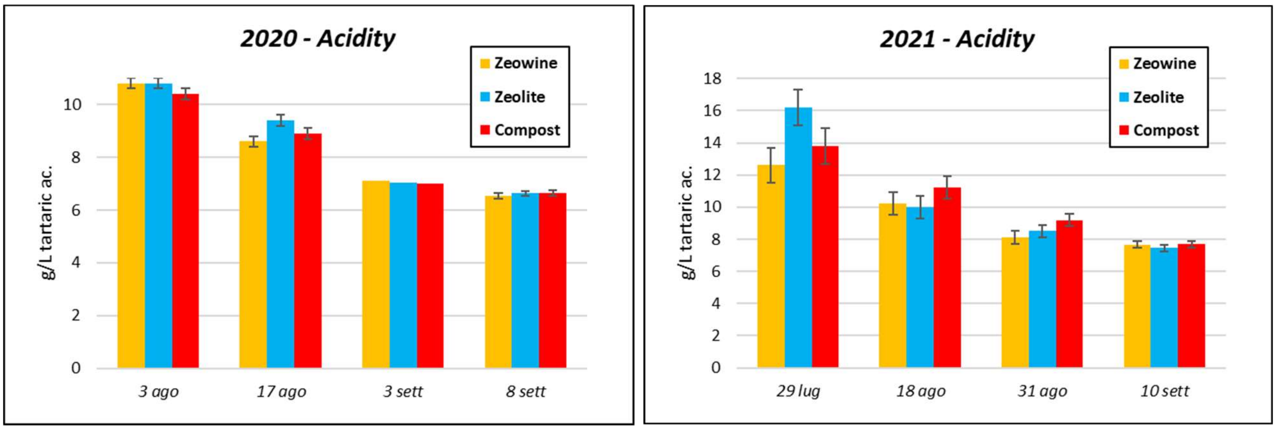



Figure 40 - Acidity 2020-2021. Vertical bars represent LSD 95%

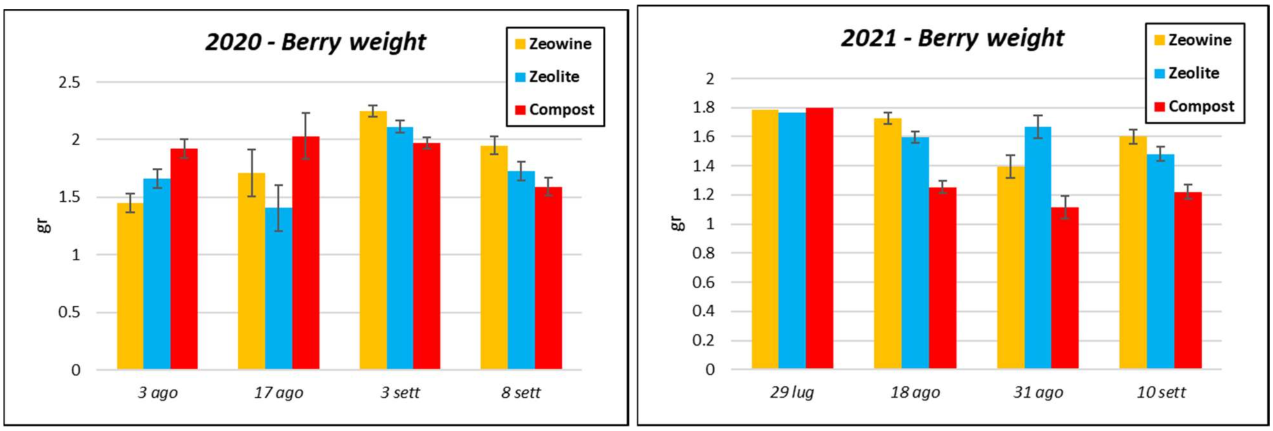
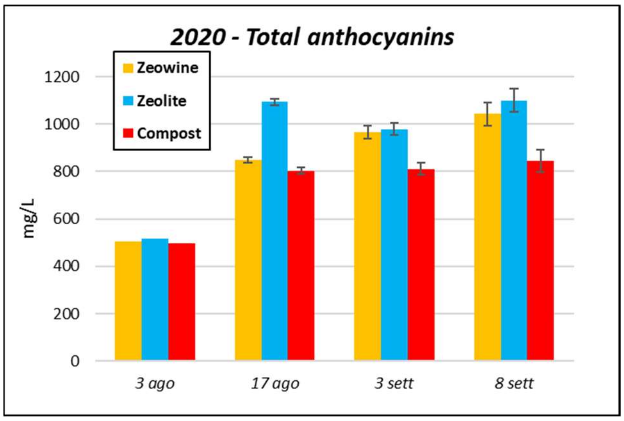



Figure 41 - Berry weight 2020-2021. Vertical bars represent LSD 95%



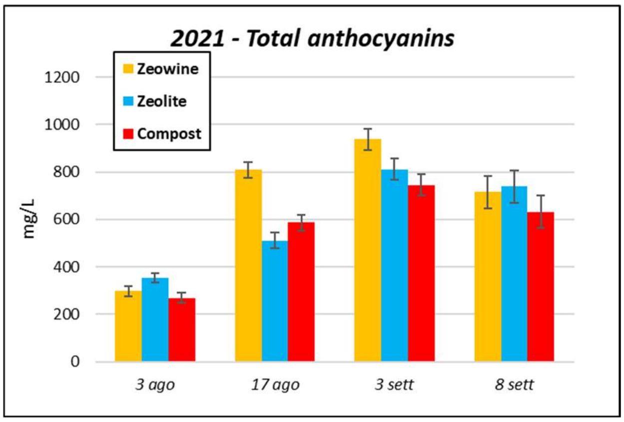
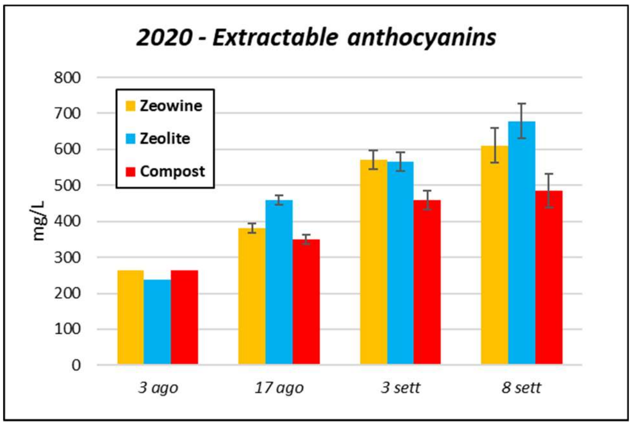



Figure 42 - Total anthocyanins 2020-2021. Vertical bars represent LSD 95%



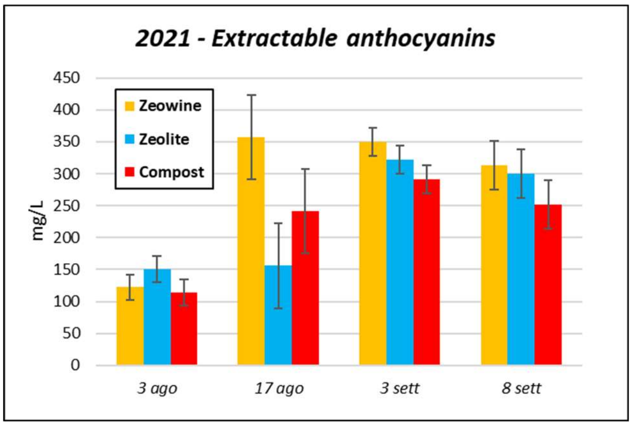
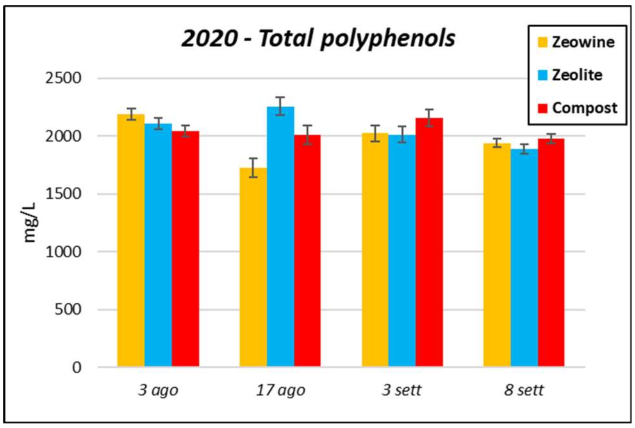



Figure 43 - Extractable anthocyanins 2020-2021. Vertical bars represent LSD 95%



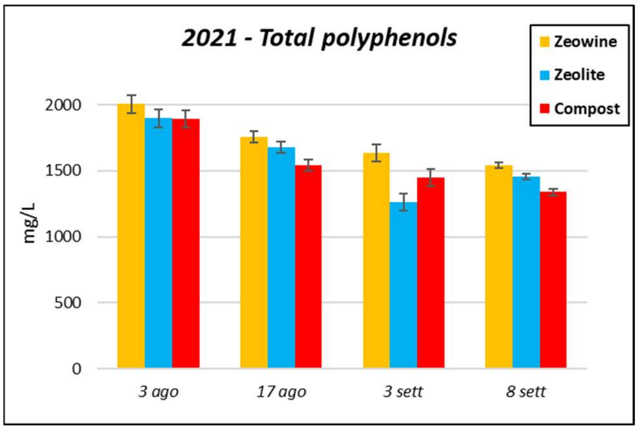



Figure 44 - Total polyphenols 2020-2021. Vertical bars represent LSD 95%

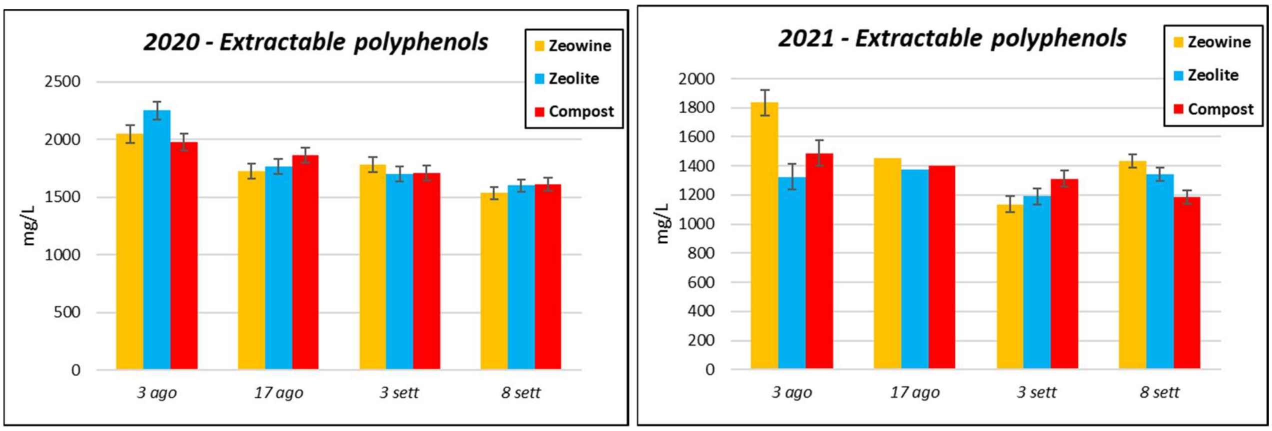



Figure 45 - Extractable polyphenols 2020-2021. Vertical bars represent LSD 95%

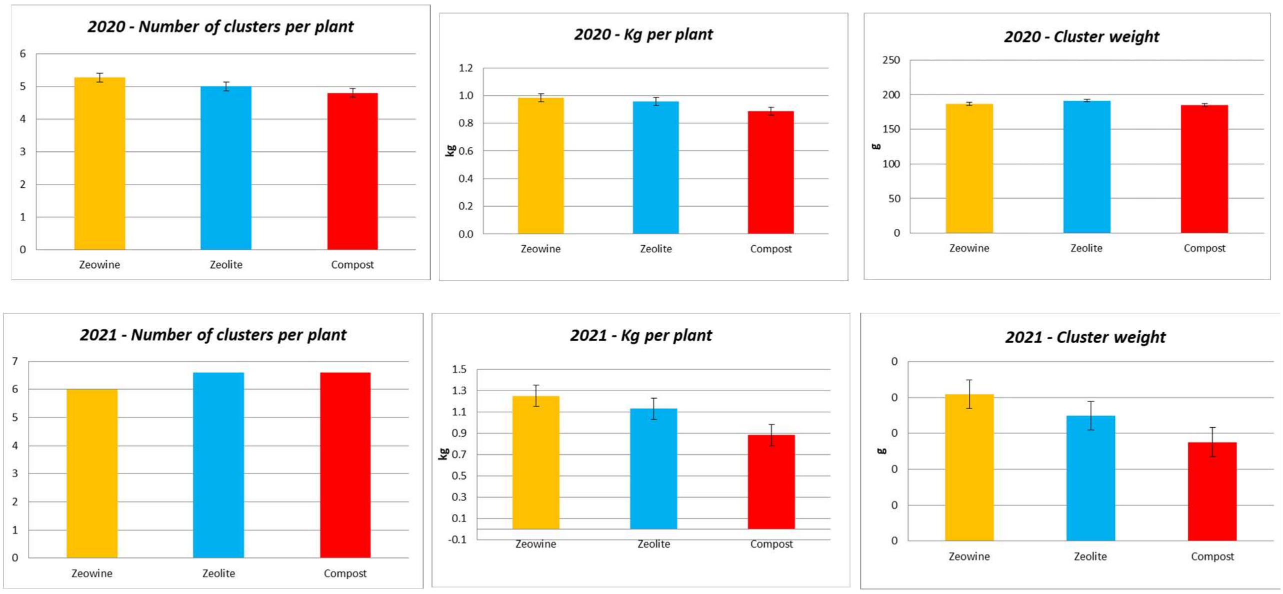



Figure 46 - Productive parameters 2020-2021. Vertical bars represent LSD 95%

It could be concluded that the deleterious effects of global warming, in a new grapevine plant, can be reduced with Zeowine soil improver. The zeolite skill to hold water and exchange nutrients, gave the vines the strength to improve their performance and carry out better production than the compost treatment. The features of zeolite combined with compost could be one of the best solutions to make a stand against drought problems. Therefore, it is in this scenario that the application of Zeowine in the vineyard to the soil can be a valid tool to mitigate the effects of climate change.

# References

Ramesh, K., & Reddy, D.D. (2011). Zeolites and their potential uses in agriculture. Advances in Agronomy, 113, 219-241.

Macci C., Doni S., Bondi G., Davini D., Masciandaro G. & Pistoia A. (2012a) Effects of wild boar (Sus scrofa) grazing on soil properties in Mediterranean environment. Catena, 98, 79-86.

Marx, M.C.; Wood, M.; Jarvis, S.C. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol. Biochem. 2001, 33, 1633–1640.

Sumner M.E. & Miller W.P. (1996) Cation exchange capacity and exchange coefficients. D.L. Sparks (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods, Soil Science Society of America Book Series, Soil Science Society of America Inc., Madison, 1201-1230 pp.

Vepsäläinen, M.; Kukkonen, S.; Vestberg, M.; Sirviö, H.; Niemi, R.M. Application of soil enzyme activity test kit in a field experiment. Soil Biol. Biochem. 2001, 33, 1665–1672.

Yeomans J. C. and J. M. Bremner, "A rapid and precise method for routine determination of organic carbon in soil," Communications in Soil Science and Plant Analysis, vol. 19, no. 13, pp. 1467–1476, 1988.