ZEOWINE for the fertilization of a young vineyard-first version

ZEOWINE per la concimazione di impianto in viticoltura – prima versione

Deliverable Action C1

31/12/2019

Summary

1 Introduction 3

2 ZEOWINE preparation 3

3 ZEOWINE application 4

4 Results 5

4.1 Soil 5

4.1.1 Soil sampling 5

4.1.2 Methods 5

4.1.3 Soil microarthropod community structure analysis 5

4.1.4 Results 6

4.2 Plant 15

4.2.1 Materials and Methods 15

4.2.2 Results 16

The project is co-funded with the contribution of the European Commission, under the LIFE Programme – Environment & Resources efficiency

The information and views set out in this deliverable are those of the author(s) and do not necessarily reflect the official opinion of the European Union. Neither the European Union institutions and bodies nor any person acting on their behalf may be held responsible for the use which may be made of the information contained therein.

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 preparation

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.

Figure 1: zeolite (clinoptilolite) and winery waste piles set up

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 in triplicate, 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.

1 ZEOLITE : 2,5 WASTES	ZEOLITE	COMPOST	UNTREATED

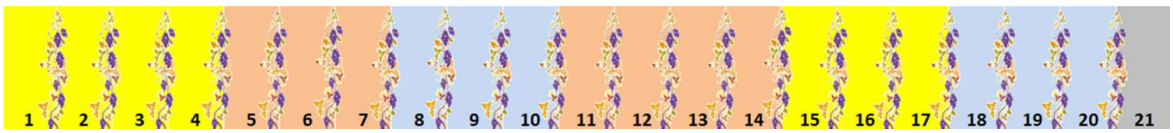


Figure 2. Experimental layout

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 Results

4.1 Soil

4.1.1 Soil sampling

Immediately after treatments (Spring 2019) and after the grape harvest (six months from treatments application, September 2019), 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 Methods

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).
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.

4.1.3 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 3 - Microarthropods extraction by modified Berlese-Tullgren funnels.

4.1.4 Results

The young vineyard soil showed moderately alkaline pH (Figure 4) and was not saline (electrical conductivity, Figure 5). CEC values increased with ZEOWINE 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 soils at T0 sampling time. However, at T1 (six months later), the OM content was higher with respect to the control soils only in the zeowine 1:2,5 treated soils (Figure 7).
The contents of TP and TK were slightly higher in the zeowine-treated soils in comparison with the control soils (Figure 8 and Figure 10). In particular, significantly higher values of TK were detected in the soils treated with zeowine.
However, the application of zeowine significantly decreased the available P and K at T1 sampling time with respect to control soil (Figure 9 and Figure 11), thus indicating the increase in retention of these elements.
Similarly, a lower available Cu was observed in both ZEOWINE treatments, at both sampling times, with respect to control soil and to the soil treated with commercial compost.

Figure 4. pH at the initial sampling time (T0) and after six months (T1)

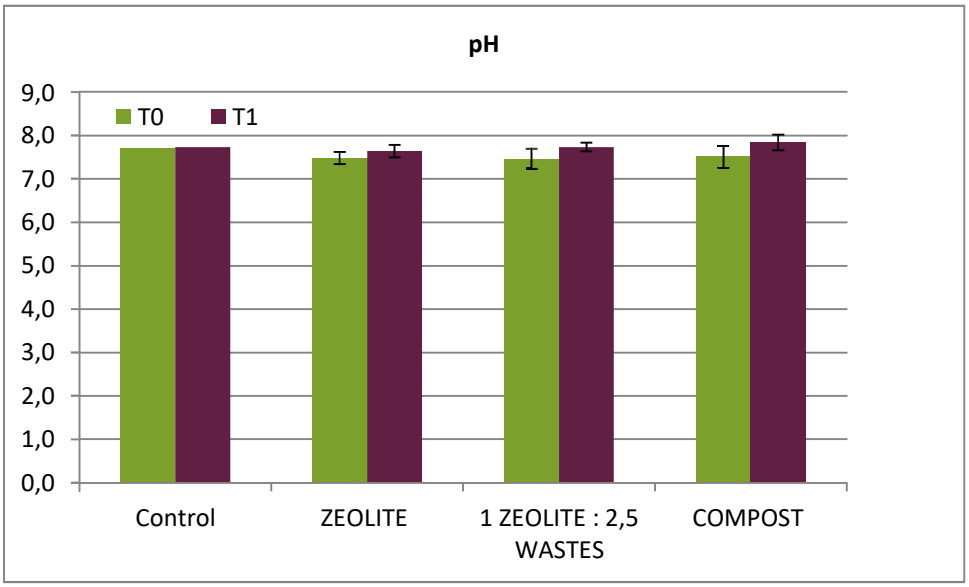


Figure 5. Electrical Conductivity at the initial sampling time (T0) and after six months (T1)

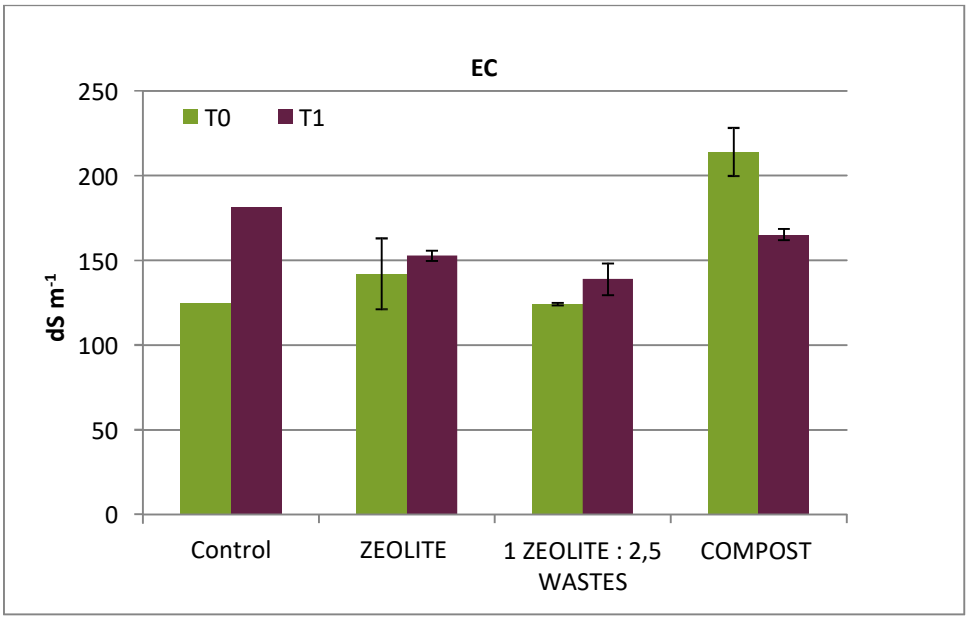


Figure 6. Cation Exchange Capacity at the initial sampling time (T0) and after six months (T1)

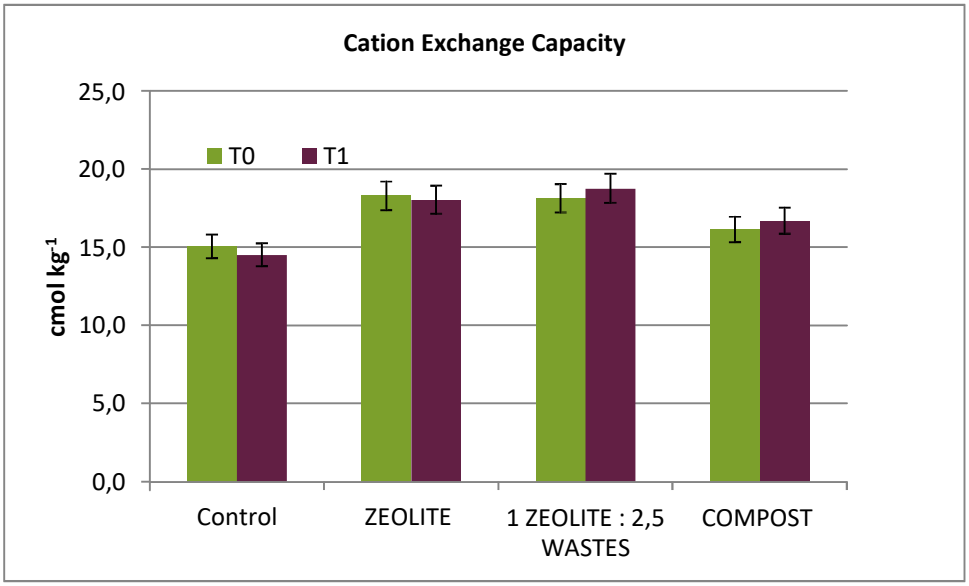


Figure 7. Total Organic Matter at the initial sampling time (T0) and after six months (T1)

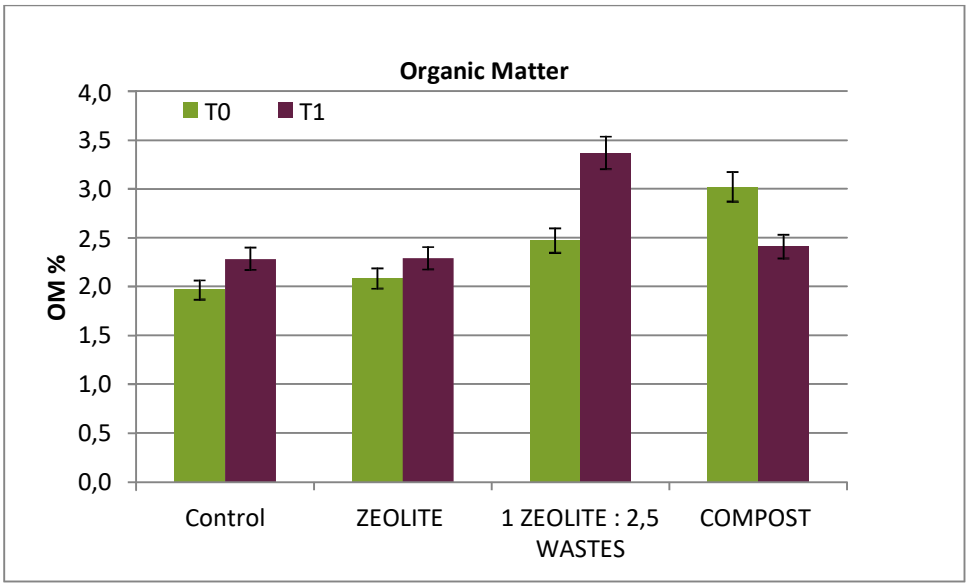


Figure 8 Total phosphorus at the initial sampling time (T0) and after six months (T1)

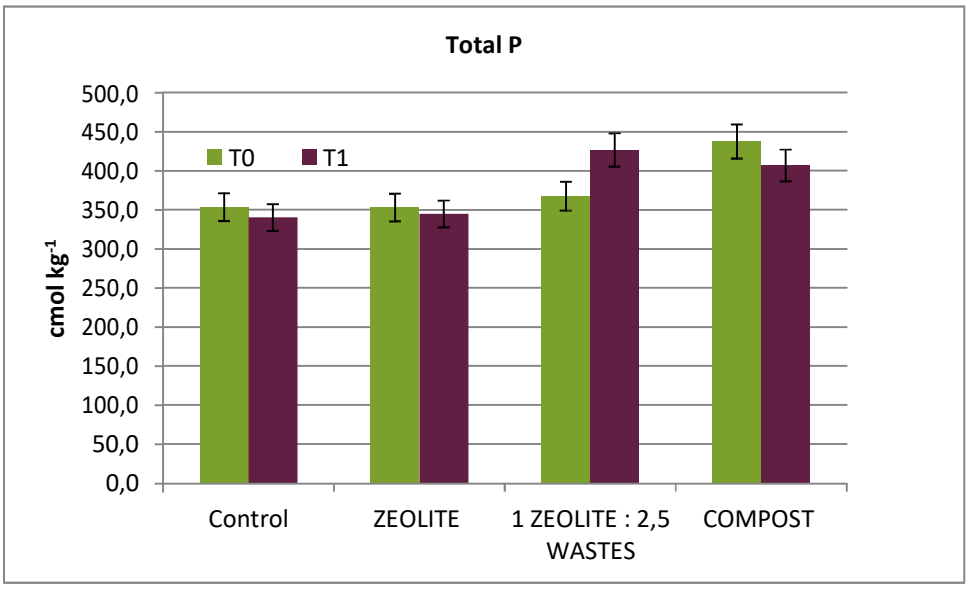


Figure 9 Available phosphorus at the initial sampling time (T0) and after six months (T1)

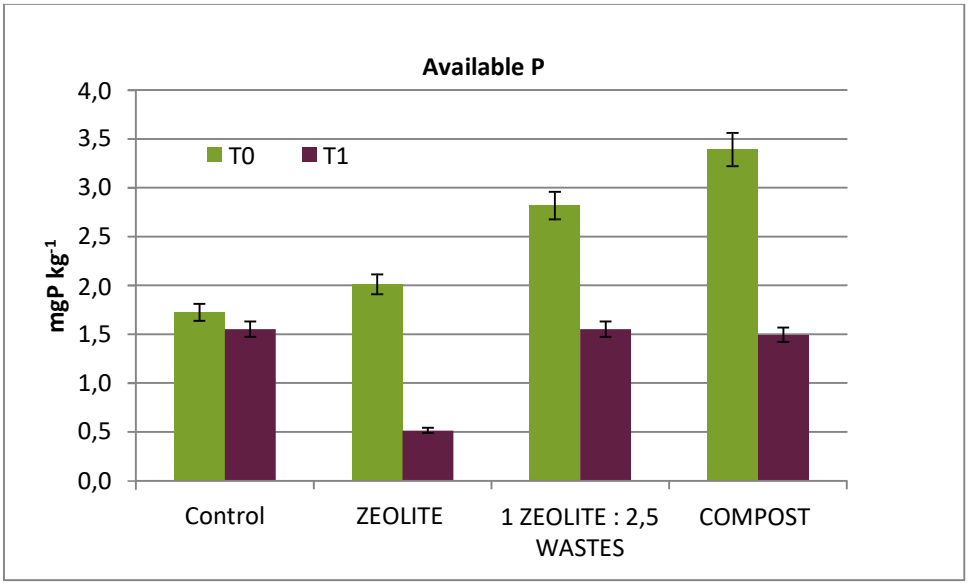


Figure 10 Total potassium at the initial sampling time (T0) and after six months (T1)

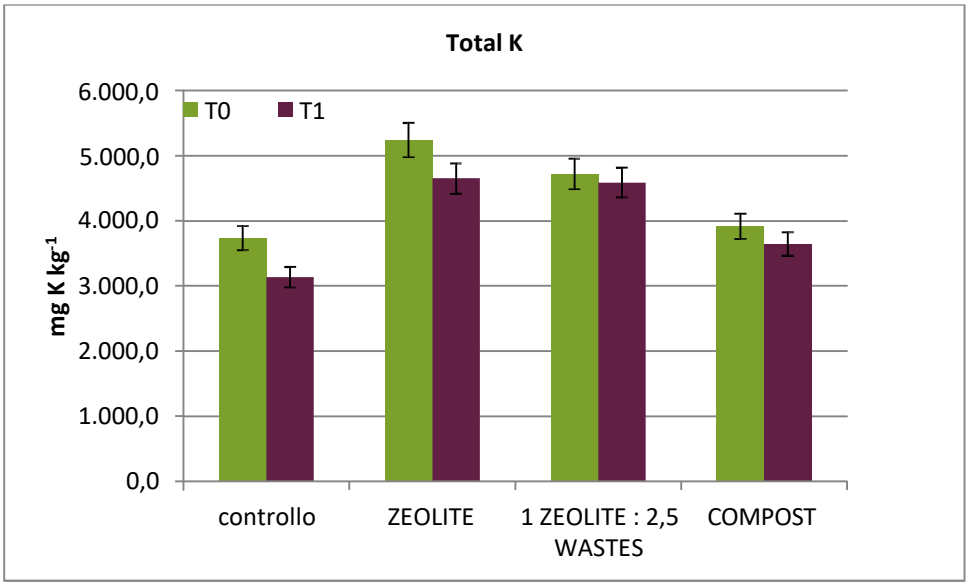


Figure 11 Exchangeable potassium at the initial sampling time (T0) and after six months (T1)

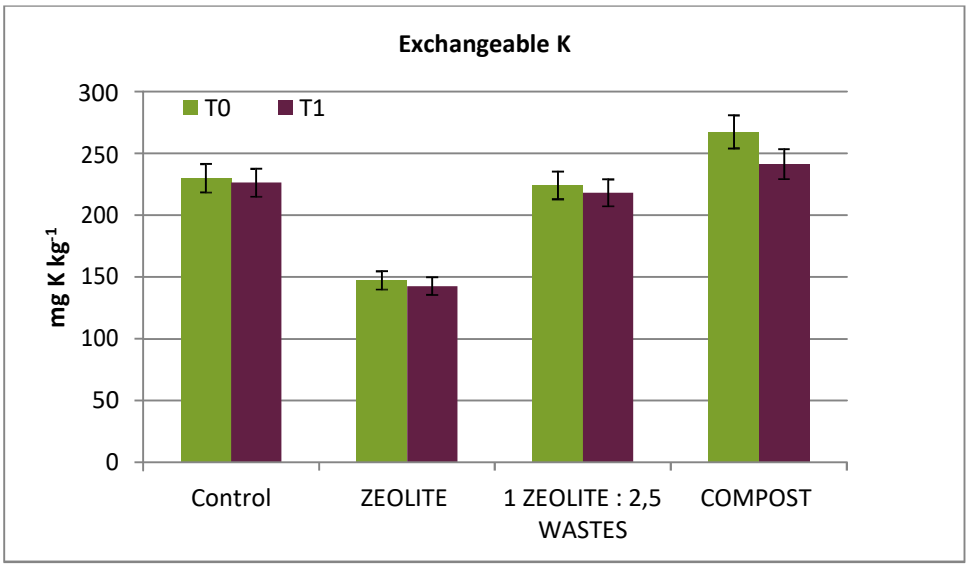


Figure 12. Total copper at the initial sampling time (T0) and after six months (T1)

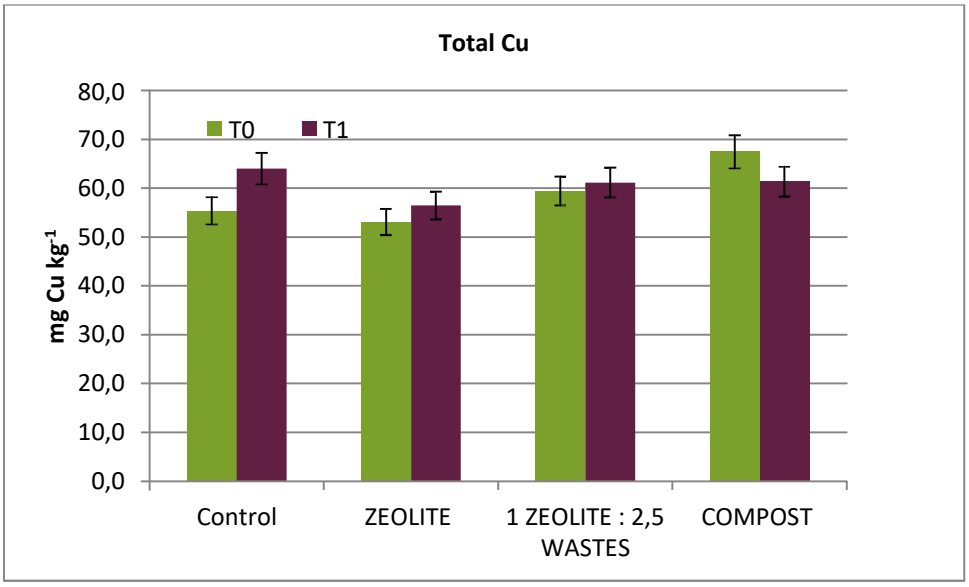
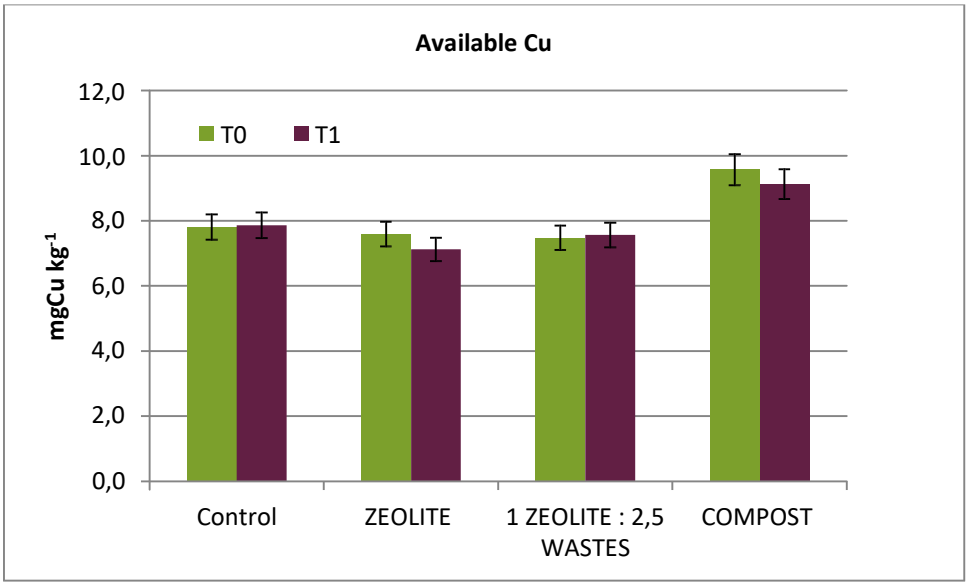


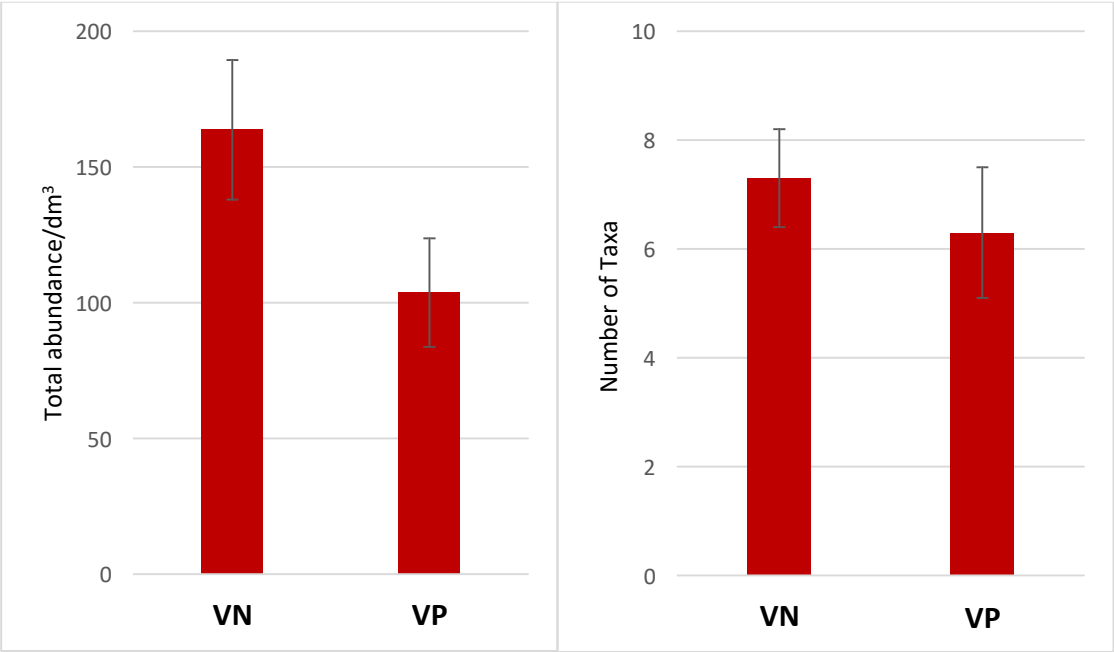
Figure 13 Available Cu at the initial sampling time (T0) and after six months (T1)



Soil microarthropod community structure analysis

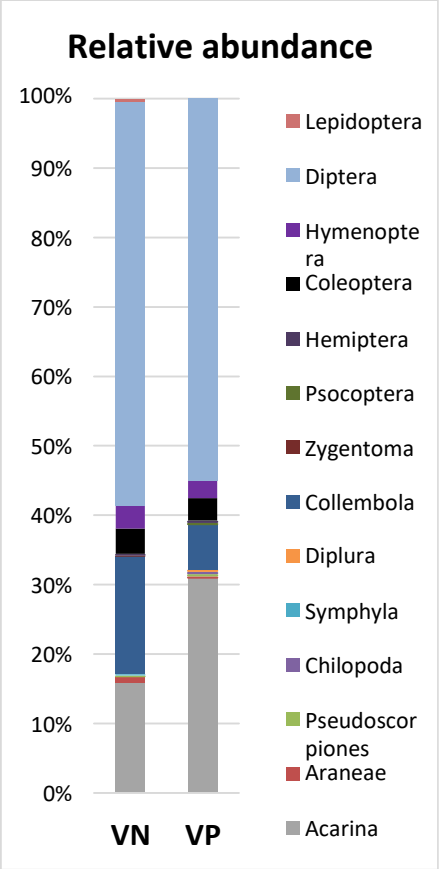
The total abundance and richness were higher in the newly planted vineyard than in the vineyard in production (Figure 14).

Figure 14. Total abundance and richness in 2018, before treatments. VN, newly planted vineyard; VP, vineyard in production. Standard errors are reported.



Fourteen different taxa of microarthropods were identified in the two vineyards. In general, only mites, springtails and Diptera were prominent, while the other taxa showed a very low abundance Figure 15). However, no differences were found between the two vineyards.

Figure 15. Relative abundance of soil microarthropod taxa per vineyard. VN, newly planted vineyard; VP, vineyard in production.



Concerning mites, Oribatids represent the main taxa involved in organic decomposition and were found only in VN. The springtail assemblage was arranged in four taxonomic groups and in three ecological classes, surface-living (epi-edaphic), intermediate (hemi-edaphic) and deep soil-living (eu-edaphic). In both vineyards, springtails were mostly represented by hemi-edaphic forms belonging to Entomobryomorpha groups.

Figure 16. Springtail distribution based on the taxonomic groups Symphyplena, Neelinae, Entomobryomorpha e Poduromorpha.

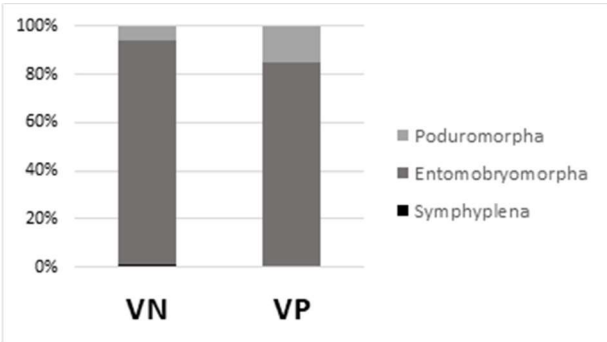
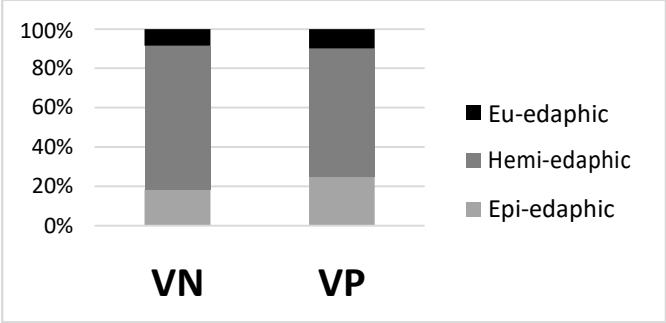
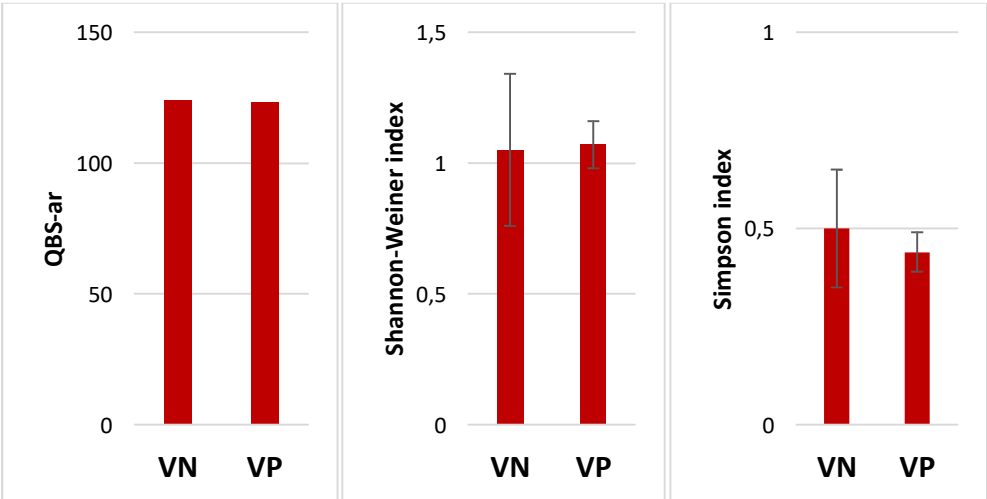


Figure 17. Springtail distribution based on arranged in epi-edaphic, hemi-edaphic and eu-edaphic groups.



In general, the selected indices evidenced a good soil quality. In fact, QBS-ar showed the following values: 124 and 123 for VN and VP, respectively. However, the biodiversity showed low values for Shannon-Weiner index and high values for Simpson index due to the dominance of some taxa (Figure 18). No differences were found between the two vineyards for any indices.

Figure 18. Microarthropod indices before treatments. VN, newly planted vineyard; VP, vineyard in production. Standard errors are reported.



4.2 Plant

4.2.1 Materials and Methods

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-2s-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 (Ψmd), 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.

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.

4.2.2 Results

Figure 19.

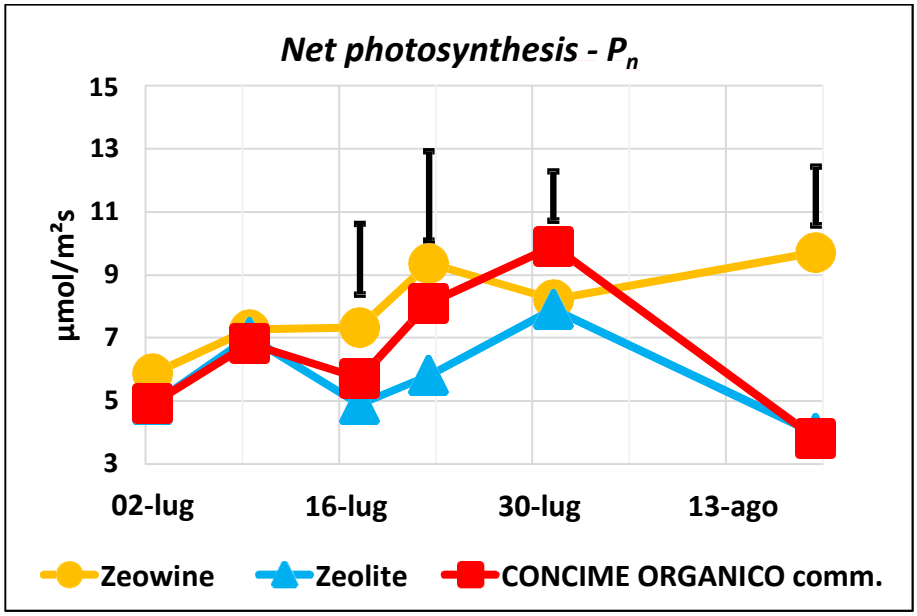


Figure 20.

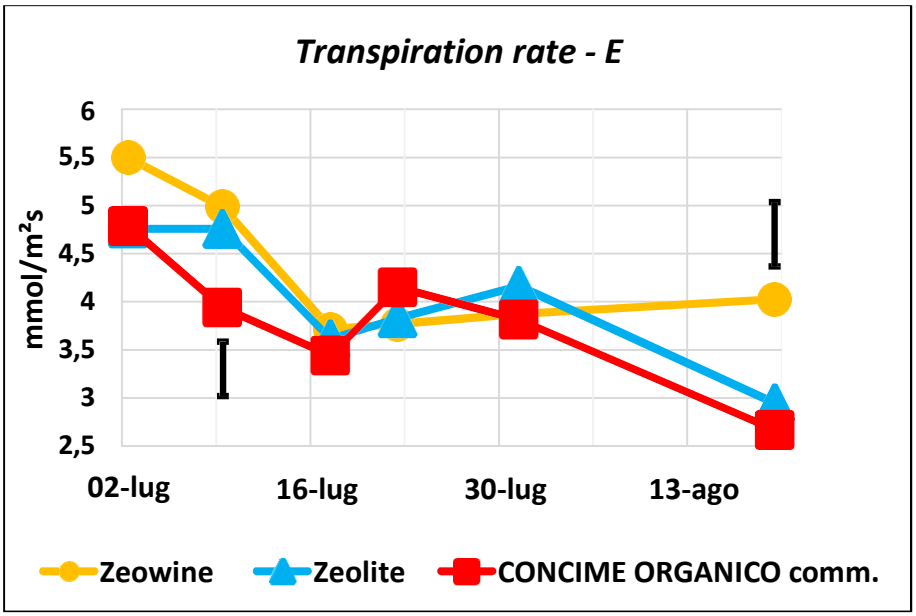


Figure 21.

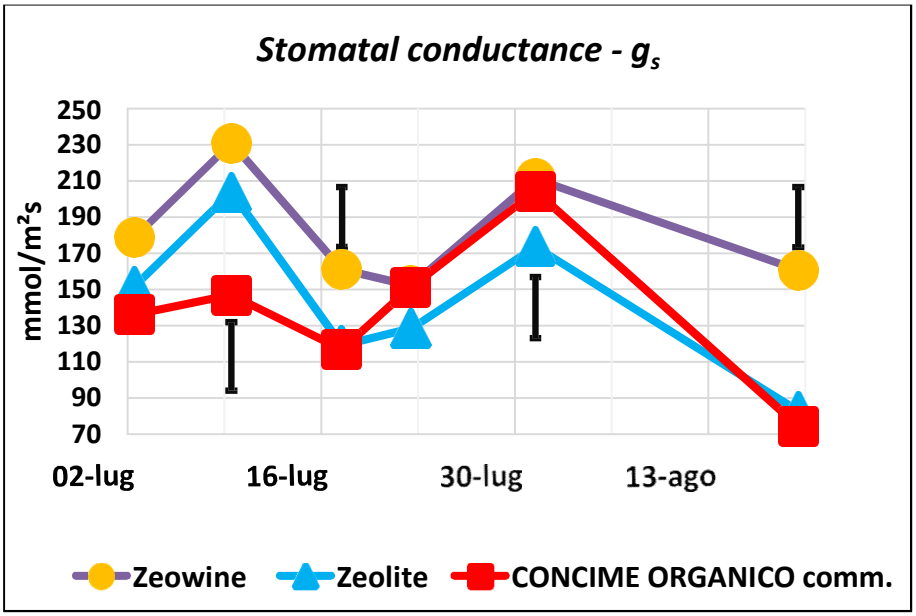
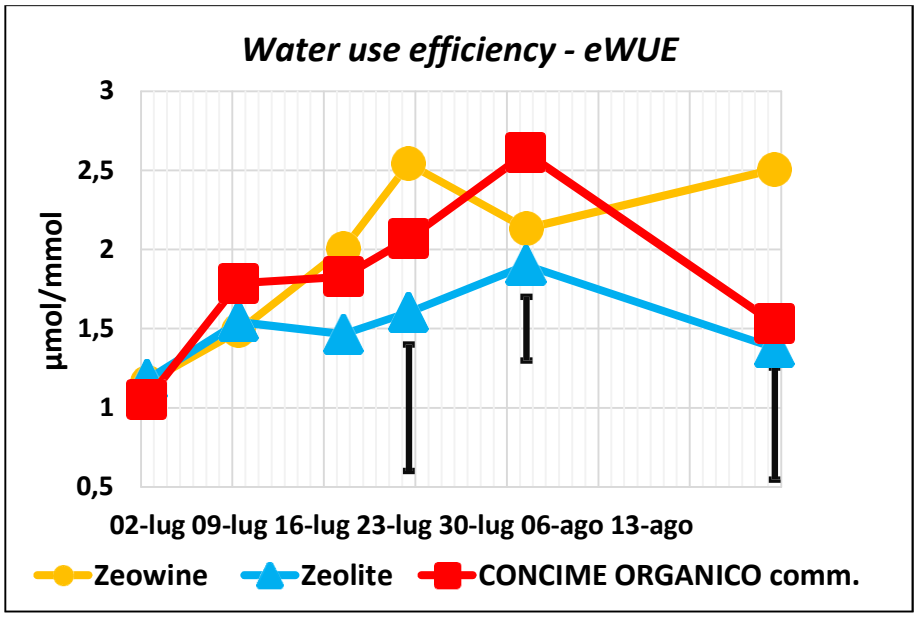
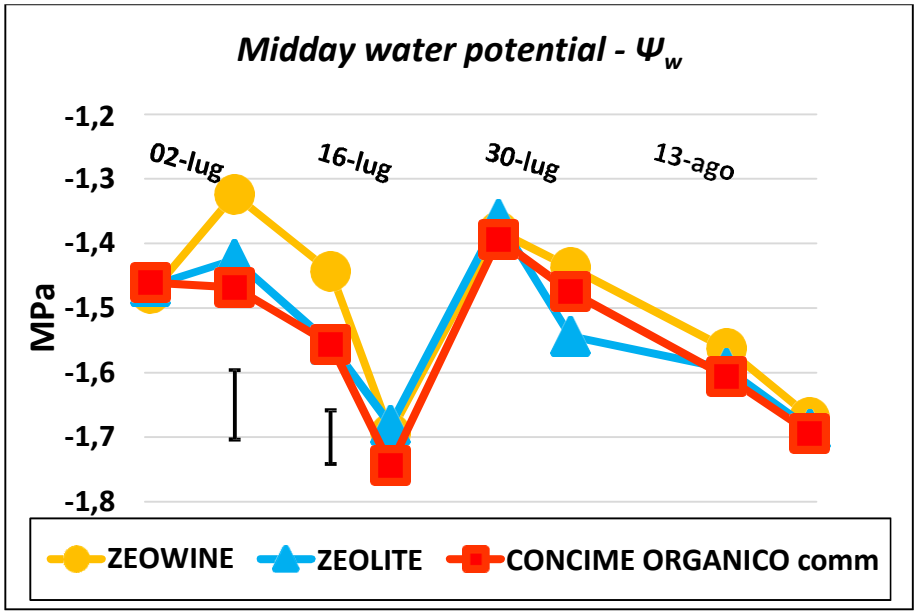


Figure 22.



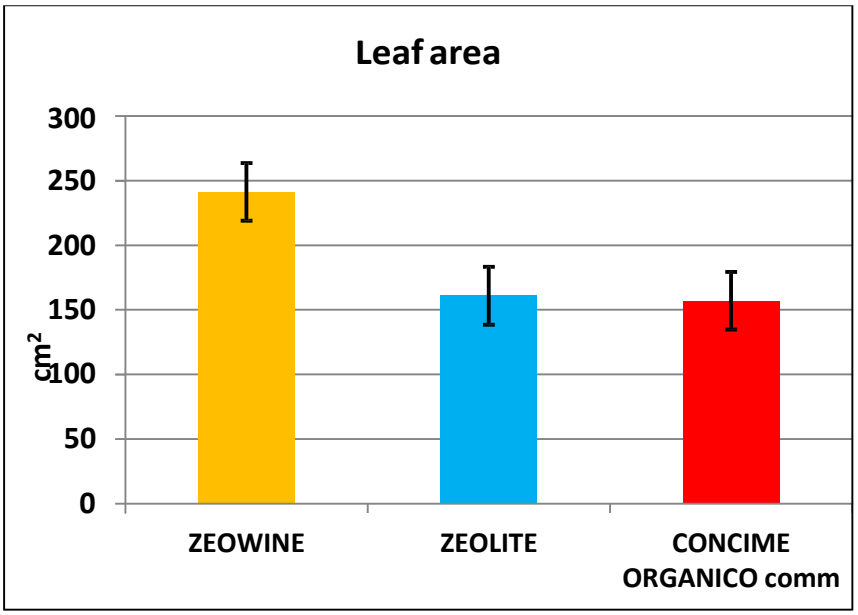
In general, good values of gas exchanges for all theses. Zeolite shows lower photosynthesis and stomatal conductance values. Better gas exchanges for Zeowine, also until late vegetative season (more open stomata, so higher photosynthesis) As in the production vineyard, good values of WUE for all theses.

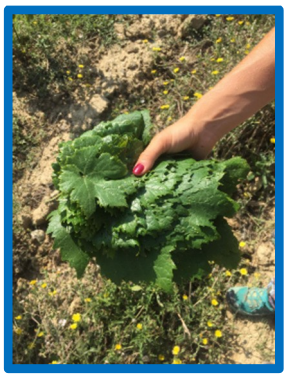
Figure 23.



The midday water potential values are indicative of moderate / severe stress. Less negative values (less stress) for Zeowine thesis on the first dates. This differences disappear in the second part of the season.

Figure 24.





Statistical differences between the theses emerge from the surveys of the leaf area. Greater leaf area was detected for Zeowine.

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.