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Effectiveness of composts from decentralised composting scenarios to promote degraded soil restoration and *R. officinalis* drought resilience

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A R T I C L E I N F O

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A B S T R A C T

Soil degradation and water scarcity affect crop productivity and ecosystem resilience. Biotechnological strategies based on organic waste valorisation offer promising tools for restoring soil fertility and increasing stress tolerance. This study evaluated the effectiveness of four composts produced under different decentralized composting schemes: community (CA), decentralized urban (SO), small-scale agrocomposting from poultry manure (UP), and medium-scale agrocomposting from olive pomace and pig manure (TO), in improving a degraded soil and enhancing rosemary tolerance to drought. Soil microcosms were set up with a degraded soil and amended with different composts. Half of the microcosms were subjected to well-watered conditions and half to water-stress (75% and 20% of the soil water holding capacity, respectively). Composts promptly increased microbial abundance and dehydrogenase activity. With the TO compost plant grew better than other conditions, presumably due to its phosphorus and labile compound enrichment. Under drought stress, CA and SO composts mitigated biomass losses, demonstrating a protective role against drought-induced stress. Soil amended with pig and poultry-derived composts (TO and UP) introduced antibiotic resistance genes and, in the case of UP, the soil water extracts had significant ecotoxicological effects on both *Daphnia magna* and *Lepidium sativum*. In contrast, the CA compost - from organic fraction of municipal solid waste, yard trimmings and donkey manure - displayed the best overall performance, enhancing plant tolerance and soil microbial functionality without ecotoxicological effects. These findings highlight the biotechnological potential of community-scale composting as a circular and effective approach for degraded soil recovery and crop management under water-limited conditions.

1. Introduction

Soils are increasingly exposed to diverse degradation processes that threaten their quality, fertility and ecosystem functions. These include reduced organic matter content, local and diffuse contamination, erosion, sealing, compaction, biodiversity loss, salinization, flooding and landslides [1]. Droughts, heavy precipitation and heat waves, are becoming more frequent and intense across the Mediterranean area, resulting in longer periods during which crops are exposed to prolonged freshwater scarcity [2] and this exacerbates environmental stress for plants and soil biota [3].

Conventional agriculture is the main responsible of soil degradation because it involves an intense use of machinery and chemicals (e.g. fertilizers and pesticides) which negatively affect its quality and ecosystem capability to provide several regulating services [4]. Agriculture and horticulture are heavily dependent on external inputs of mineral nutrients in the form of synthetic fertilizers, derived either from mined resources or, in the case of nitrogen, industrially fixed from atmospheric N. The use of synthetic fertilizers increased by 200–300% between 1970 and 2010 [5,6] and FAO has predicted that worldwide use of synthetic N fertilisers is expected to increase 50% from the 2012 level by 2050 [7]. The inappropriate and excessive use of fertilisers has also contributed to soil acidification, biodiversity loss and nutrient runoff, leading to water body eutrophication and increased greenhouse gas

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emissions [8,9].

Composting can be considered an effective technology for addressing soil degradation and recovering ecological functions [10,11]. Composting is a natural, aerobic biotransformation process in which complex organic matter is broken down by microbial communities into simpler and more stable substances, resulting in a reduction in waste volume and formation of compost, a highly stable, humus like material rich in nutrients [12-14]. This process occurs in several stages under variable temperatures but is typically divided into two main phases performed by active mesophilic (< 45 °C) and thermophilic (> 45 °C) microorganisms [15]. The heat generated in active compost also inactivates some microbial pathogens. When available carbon and other nutrients are depleted, microbial activity decreases and decomposition slows down, at which point the stabilisation phase is reached and mineralisation and humification occur (maturity phase), [16]. Indeed, composts typically contain essential nutrients, humic substances, beneficial microbes and improve key soil properties such as structure, porosity, water-holding capacity and carbon storage [17]. Moreover, compost application can reduce nutrient and contaminant leaching and soil erosion. Overall compost characteristics has the potential to enhance plant growth [12] and tolerance to stress, including drought, as found for carob (*C. siliqua* L.) by Boutasknit et al. [3].

The composition and quality of composts can vary considerably depending on the feedstock origin and processing conditions, influencing their agronomic effectiveness and environmental safety. The nature of organic material – if derived from agro-industrial residues, livestock waste or municipal organic fractions – has been demonstrated to strongly influence nutrient profiles, contaminant load and microbial diversity of a final compost product [18]. However, composts may also carry unwanted contaminants, such as heavy metals, pharmaceuticals and antibiotic resistance genes (ARGs) (e.g. from manure), [19,20] and microplastics (e.g. from municipal solid waste), [21,22]. Some composts rich in organic matter and nutrients confer a high agronomic potential, although they may show side effects linked to contaminant (e.g. heavy metals in manure) or phytotoxic compound (e.g. phenolic acids in pomace) presence [23,24]. Although composting substantially reduces pathogenic microorganisms, the efficiency of different composting strategies in removing microbial contaminants and possible antibiotic-resistant bacteria needs to be investigated before compost applications [25]. Recently works, have reported that ARG spread in soils is not solely determined by external inputs such as manure amendments, but it can also be influenced by extreme environmental conditions, which can affect microbial community structure and functioning [26-28]. In this context, drought represents a major abiotic stress capable of apply strong selective pressure not only on plants, but also on plant-microbiome interactions [28]. On the other hand, olive pomace is characterized by high acidity, salinity and phenolic compound presence, which can cause phytotoxic effect [29] and impose additional selective pressures on soil microorganisms. Nevertheless, the composting process stabilizes its organic fraction [30] and reduces phytotoxicity [29], thereby enhancing its suitability as a sustainable soil amendment.

In the framework of the European Green Deal, which encourages an efficient use of resources, reduction in pollution and waste production, a localised valorisation of municipal and agro-zootechnical waste through its transformation into a compost to be used as fertilizers, fully meets the circular economy principles. In this sense, decentralised composting models (community composting, agrocomposting or composting in decentralised small-scale plants) have emerged as new composting scenarios mainly based on a small-scale and localised management, very close or even in the place where the residual streams have been produced [31]. These scenarios reduce transport expenses and environmental repercussions associated with the traditional centralised management facilities [32].

In this context, this study aimed at evaluating the effectiveness of four composts from different decentralised composting scenarios, in restoring a degraded soil and improving the growth and drought tolerance of *Rosmarinus officinalis* L. in a greenhouse microcosm experiment. The research focused on (i) assessing possible improvements in plant biomass (ii) evaluating microbial community abundance (DAPI counts) and activity (dehydrogenase activity); (ii) determining if compost application can introduce antibiotic resistance genes (qPCR) in the amended soil; and (iii) evaluating possible ecotoxicity of the various compost-amended soils through standard ecotoxicological bioassays. The biotests included the *Aliivibrio fischeri* bioluminescence inhibition test, *Lepidium sativum* germination test and *Daphnia magna* immobilization test.

2. Materials and methods

2.1. Sampling site

Soil samples were collected from the surface layer (0–20 cm) of a former agricultural area in Central Italy (Monterotondo, near Rome) that had been left uncultivated for at least ten years. Prior to abandonment, this area hosted only a few olive trees, distributed very sparsely. The clay-loam soil (36.1% sand, 33.4% clay, 30.5% silt, [33] had 0.82% organic carbon, low concentrations of total nitrogen (1.26 g kg^{-1} dry soil), pH of 8.37, ammonium (0.002 g kg^{-1} dry soil) and nitrate (0.015 g kg^{-1} dry soil), (Table S1). The soil was manually homogenized, with plant roots and any stones removed, and dried at room temperature before its use.

2.2. Composts used for the experiment

A total of four composts, obtained from a variety of decentralised composting scenarios, were used to amend the soil (Table 1). The four composts were:

CA: compost from a community composting model produced in a composting area belonging to a municipality located in the Valencian Community (Spain).

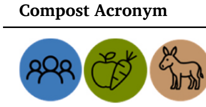
SO: compost from a decentralised urban composting model produced at a small-scale in Catalonia (Spain).

UP: compost obtained from a small-scale agrocomposting (composting capacity: approximately 20–200 t waste $year^{-1}$) produced using a mixture of chicken manure and barn waste in a small composting module located in Navarre (Spain).

TO: compost produced by a medium-scale agrocomposting (composting capacity: approximately 1000–2000 t waste $year^{-1}$) consisting mainly of a mixture of olive pomace (oil mill waste), pruning and pig manure. It was obtained from an installation located in the Valencian Community (Spain).

Both CA and SO composts were obtained mainly from an organic fraction of municipal solid waste, collected separately, and then joined with pruning waste, at a ratio of 1:1 (v:v). In the case of the CA compost,

Table 1
Acronyms and main sources of the four compost used for the microcosm experiment.

Compost Acronym	Composting scenario	% Raw Materials
	Community composting	45% organic fraction of municipal solid waste + 45% yard trimmings + 10% donkey manure
	Decentralized urban composting	50% organic fraction of municipal solid waste + 50% yard trimmings
	Small Agrocomposting	100% Poultry manure
	Medium Agrocomposting	30% Olive pomace + 20% yard trimmings + 50% pig manure

the initial composting mixture contained also 10% (fresh weight) of donkey manure.

The main characteristics of the four composts are reported in Supplementary Material (Table S2). The primary agronomic characteristics of the composts are described in [31]. All the composts showed suitable characteristics for their use as organic amendments. Thus, in accordance with the standardized Dewar test for the evaluation of compost self-heating [34] and the germination index for evaluation the compost phytotoxicity [35], all composts were stable and indicated absence of phytotoxicity. In addition, the values of macro and micronutrients were also found to be within the normal ranges for this type of composts and the concentrations of heavy metals fulfilled the criteria established in the European legislation for their agronomic use [32].

2.3. Microcosm set-up

Microcosms consisting of 1 L capacity plant pots were set up for the experiment. Twenty-four microcosms (3 replicates for each condition) were filled with 0.7 kg of the soil and amended with an aliquot of one of the four compost (CA, SO, UP and TO), to achieve a standardised dose of 210 kg N ha $year^{-1}$. Moreover, in addition to the compost-amended conditions, six un-amended microcosms were performed and used as controls. A *Rosmarinus officinalis* cutting was transplanted in each microcosm. The pots were randomly distributed throughout the greenhouse and maintained under natural temperature and light conditions for five weeks, until the start of the stress treatment. Moreover, three replicate microcosms consisting of unamended soil, without the rosemary plant (NO_PLANT) were set up to assess possible differences between bulk and rhizosphere soil microbiome.

All microcosms were kept at 75% of their maximum water holding capacity (WHC) and under the same natural temperature and light conditions for five weeks. Then half of the compost-amended soil pots were subjected to a drought stress for some days in order to reach a water content corresponding to 20% of the WHC (0 day). The amount of water from each pot was monitored daily by weighing them. Two samplings were performed, one at the start of the “drought stress condition” (0 day) and the second one at 29 days (end).

The experimental conditions are summarized as it follows:

1.	Control soil + Plant: CON
2.	Soil + compost (CA) + Plant: CA_M
3.	Soil + compost (SO) + Plant: SO
4.	Soil + compost (UP) + Plant: UP_M
5.	Soil + compost (TO) + Plant: TO_MP
6.	Control soil + Plant under drought stress (20% of WHC): CON stress
7.	Soil + compost (CA) + Plant under drought stress (20% of WHC): CA_M stress
8.	Soil + compost (SO) + Plant under drought stress (20% of WHC): SO stress
9.	Soil + compost (UP) + Plant under drought stress (20% of WHC): UP_M stress
10.	Soil + compost (TO) + Plant under drought stress (20% of WHC): TO_MP stress
11.	Control soil without rosemary plant: NO PLANT

2.4. Plant growth analyses

The rosemary plants were sampled at the end of the drought experiment at 29 days. They were carefully removed from their pots and washed with distilled water. After determining their fresh weight, the plants were dried in an oven at 60°C for 72 h, after which their dry weight was determined (g).

2.5. Total microbial abundance and dehydrogenase activity

The total microbial abundance (No. cells g $soil^{-1}$) was determined using the direct epifluorescence count method under an epifluorescence microscope (Leica Digital Microscope 4000 B, Leica Microsystems GmbH, Wetzlar, Germany) and using DAPI (4’,6-Diamidino-2-phenylindole dihydrochloride) dye as the fluorescent DNA intercalant, as described in Barra Caracciolo et al. [13].

Dehydrogenase activity reflects the overall microbial respiration rate and therefore biological oxidation of organic matter [36,37]. The method is based on an extraction and subsequent colorimetric determination of the colour intensity of the 2,3,5-triphenyl formazan (TPF) produced from the TTC reduction [37]. The colour intensity was measured with a Thermo Multiskan FC Microplate Photometer (Thermo Fisher Scientific; Waltham, MA, USA). Dehydrogenase was expressed as μg TPF g $soil^{-1}$.

All the microbiological analyses were performed in at least three replicates for each experimental condition and sampling.

2.6. Microbial DNA extraction and quantification of antibiotic resistance genes (ARGs) in soil

Total DNA was extracted using the DNeasy PowerSoil kit (Qiagen, United States) and then used for

quantitative PCR (qPCR) analyses. The qPCR was performed with the CFX96 real-time PCR detection System (Bio-Rad, USA). The SYBR Green detection was used to quantify two sulfamethoxazole-resistance genes (*sul1* and *sul2*), one tetracycline efflux pump gene (*tetA*), the class 1 integron-integrase gene (*intI1*) and a macrolide ARG (*mphE*). These genes belong to classes of antibiotics widely used for treating human and veterinary infections and for this reason they are among the most abundant in agricultural soils [38-41], as observed in previous studies [42,43].

The primer list used and information on the qPCR analysis are reported in Supplementary materials (Table S4).

The quantitative PCR data were expressed as the ratio of ARG or *intI1* gene copy number per 16S copy number to evaluate the relative proportion of each target gene in the bacterial community.

2.7. Ecotoxicity bioassays

The ecotoxicity tests of the compost-amended soil were performed following procedures which simulate a leaching test. In particular, the protocols described in UNI EN 14735:2005 and UNI EN 12457–2:2004 were used to produce soil water extract (elutriates), at a liquid/solid ratio of 1:10, with distilled water acting as the extracting agent. Aliquots of each soil water extract (ca. 50 g soil: 500 mL) were shaken for 24 h at 20 °C in the dark. After the leaching period, each suspension was left for 15 min to allow the solid particles to settle, then each supernatant was centrifuged for 15 min at 9000 rpm. The elutriate samples were then filtered through 0.45 μm cellulose acetate filters (Whatman) and used for ecotoxicological analyses.

The possible ecotoxicity of the amended soil samples, was evaluated by testing soil aqueous elutriates using three different model organisms: the *Aliivibrio fischeri* luminescent bacterium, the *Lepidium sativum* dicotyledonous plant [44] and the *Daphnia magna* crustacean (ISO 6341:2013). All tests were performed in at least three replicates. Positive and negative controls were performed for all three bioassays in accordance with their specific guidelines.

The untreated soil elutriates were used as test controls in order to obtain the net toxicity of the treatments. Data are reported as the percentage effect net of any possible intrinsic toxicity in the soils, as measured in the untreated soil.

The acute toxicity test with *Aliivibrio fischeri* (ISO 11348–3: 2019) is based on the inhibition of the marine bacterium bioluminescence when exposed to a toxic substance. The test was performed using a Microtox® analyser (Model 500, Microbics Corporation, USA) in accordance with the manufacturer's instructions. The light output of the test organism was measured after three exposure times (5, 15 and 30 min) and compared with a toxic-free control to give the inhibition percentage, calculated using the Microtox calculation software (Microtox Omni® software V 4.2). The difference in light output between a sample and a control is ascribable to the effect of the sample on bacteria. According to the ISO protocol, the pH value of each elutriate was measured and corrected (to a range of 6.0–8.0) using a 0.1 M HCl solution before carrying out the tests [45]. Furthermore, the salinity of each sample was adjusted to 2% with an osmotic adjustment solution. The results reported are those obtained after a 30-minute exposure of bacteria to elutriates. A significant inhibition effect is indicated by a value higher than 20% compared to a non-toxic control consisting of a bacterial suspension in distilled water containing NaCl (2%).

The acute toxicity test with the *Daphnia magna* crustacean was carried out in accordance with OECD Test No. 202 (OECD, 2004b) and ISO 6341:2013. The test was performed in the dark at 20 °C for 48 h. For each condition twenty daphnid neonates (five per well) and 10 mL of each elutriate (per well) were used. At the end of the test, the number of immobilised organisms was counted related to the number of actively swimming organisms in each well. The results were expressed as a percentage. Negative and positive controls were carried out alongside the test: a negative control with standard ISO freshwater and a positive control with a solution of 10 mg L^{-1} $ZnSO_4$.

Finally, the acute phytotoxicity test with *Lepidium sativum* L. (cress) was performed in accordance with APAT [44], assessing the toxic effect on reproductive (germination rate) and vegetative (root elongation) endpoints. The test was conducted in triplicate in Petri dishes, using a paper disk, 5 mL of water extract and 10 *L. sativum* seeds. The control test was performed using 5 mL ultrapure water instead of the water extract. The dishes were sealed and placed in a dark growth chamber at 25 °C. After 72 h, the number of germinated seeds and root elongation were assessed.

The Germination Index (GI) was then calculated for each plate according to [44] as follows:

GI = number of germinated seeds × mean root length,

Subsequently it was expressed as percentage per treatment comparing it with the control [46,47]:

GI % = (GI average treated / GI average control) * 100

2.8. Statistical analyses

All the results are reported as average values ± standard errors of at least three replicates. All the statistical analyses were performed using R software (4.3.1version https://www.r-project.org). The microbiological, chemical-physical and plant biomass results were subjected to the Shapiro–Wilk test to verify their normal distribution. Where the data were normally distributed, analysis of variance (ANOVA) was performed using the *aov* function in the R statistical software package. Where significant differences emerged, Tukey (*Tukey's Honestly Significant Difference*, HSD) test was performed using the *TukeyHSD* function. For data that did not meet the normality assumption, the non-parametric Kruskal–Wallis test was applied using *kruskal.test* function. In all cases, a p-value threshold of 0.05 was considered significant.

The Principal Component Analysis (PCA) of the overall data was run to graphically synthesize the complexity of the dataset at 29 days by considering plant biomass (fresh and dry), dehydrogenase activity, microbial abundance, pH, soil humidity, *sul1*, *sul2, tetA*, *intI1* and the sum of all these genes, organic carbon, total kjeldhal nitrogen and electrical conductivity. The PCA was performed, using the packages "*FactoMineR*" and "*factoextra*".

3. Results and discussion

3.1. Plant biomass

At 29 days, the dry biomass (g), (Fig. 1) in the control condition (CON) was significantly higher ($p < 0.05$) than that of the rosemary plant under drought stress (CON stress), showing an expected effect of water deficit on plant growth. Although adding composts increased organic carbon content in all amended soils (Table S3), this did not promote plant growth in all conditions. This can depend on compost quality and possible presence of unwanted compounds from different

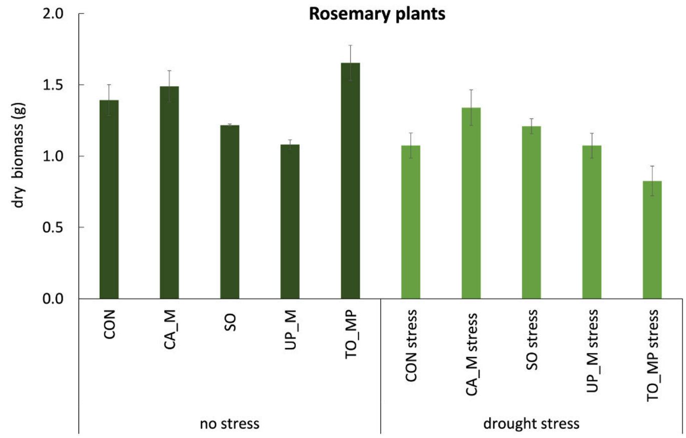


Fig. 1. Plant total dry biomass (g) at 29 days in the watered conditions (left side) and drought conditions (right side). The vertical bars are the standard errors.

feedstocks used (e.g. yard trimmings, manure and organic fraction of municipal solid waste) and environmental conditions during a composting process. Literature data report that in some composts an excess of labile organic compounds such short-chain organic acids (e.g. acetic and propionic and free ammonia) can hamper seedlings and inhibit germination [48], or that heavy metals [24] and other unwanted compounds including emerging contaminants, such as antibiotics [49] can affect plant development. Thus, the TO_MP condition, with the compost containing olive pomace plant residues and pig manure promoted the highest biomass value ($p < 0.05$). This result can be ascribed to the significantly higher phosphorus content (TO: 23.7 ± 1.4 g kg^{-1}; CA: 7.5 ± 0.09 g kg^{-1}; SO: 9.5 ± 0.09 g kg^{-1} and UP: 11.7 ± 0.2 g kg^{-1}) and higher amount of labile carbon in the TO than the other composts. On the contrary, the TO_MP stress condition was the worst for plant growth ($p < 0.05$), and this may be attributable to the lower levels of recalcitrant compounds such as humic acid compared to the other composts (Table S2). Indeed, it has been demonstrated that humic substances can assist plants in their ability to withstand abiotic stresses [50,51]. Moreover, it cannot be excluded that rosemary plants in co-presence of the drought stress were not able to counteract phytotoxic compounds (e.g. phenolics and tannins) occurring in pomace, [52,53].

The rosemary plants in the other amended conditions under drought stress (CA_M stress, SO stress and UP_M stress) were not affected in terms of biomass, demonstrating the effectiveness of the other composts tested in limiting water loss. Indeed, it is well-known that composts increase water retention in soil [3].

3.2. Soil microbial abundance and dehydrogenase activity

Adding compost increased microbial abundance promptly (Fig. 2, 0 d). Microbial abundance (No. cells g^{-1} soil) increased over time (29 days) with significantly ($p < 0.05$) higher values in the planted (CON, CA_M, SO, UP_M, TO_MP and CON stress, CA_M stress, SO stress, UP_M stress and TO_MP stress) than in the un-planted (NO PLANT) conditions. The development of plant roots presumably stimulated microbial growth and this phenomenon commonly refers to a rhizosphere effect [42,43,54,55] However, in water drought (Fig. 2, right side), lower microbial abundance values were observed compared to no stress conditions (Fig. 2, central part), with significantly lower values ($p < 0.05$) in CON stress and UP_M stress. The latter was one of the conditions with the lowest rosemary biomass, suggesting that compost composed exclusively of poultry manure is not a suitable organic fertilizer. In fact, poultry manure resulted rich in soluble salts (see Table S2 and S3), leading to the highest EC value. Moreover, we cannot exclude that in the poultry manure there were toxic substances (e.g. emerging

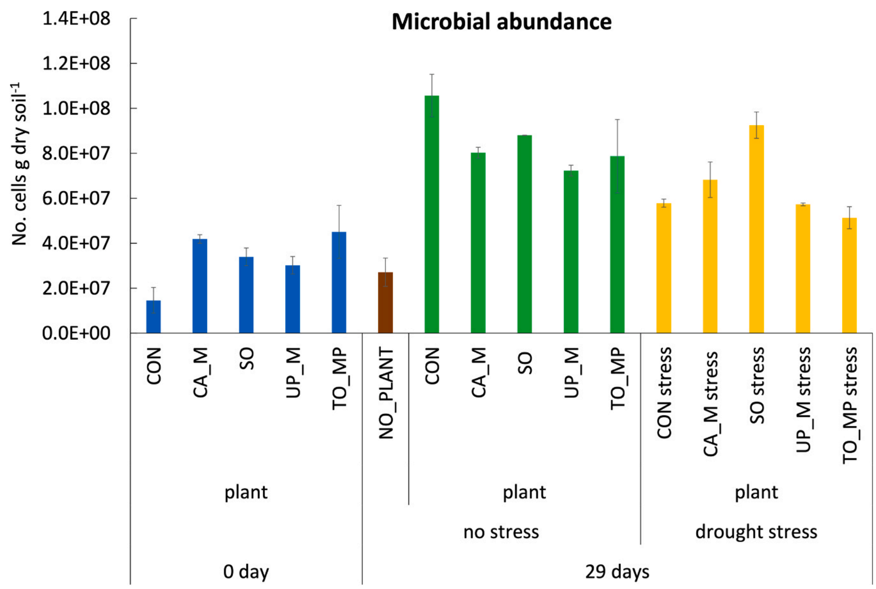


Fig. 2. Microbial abundance (No. cells g soil^{-1}) in the various experimental conditions at 0 day and 29 days. Rosemary plants were always present, except in NO PLANT. The vertical bars are the standard errors.

contaminants such as antibiotics), [20], which in conditions of drought stress exerted their negative effects.

The microbial activity, measured as dehydrogenase (μg TPF g dry soil^{-1}), maintained with similar values between 0 and 29 days in the no stress conditions, except for a decrease in UP_M and an increase in TO_MP (condition with the highest plant biomass), (Fig. 3). Overall, drought stress affected negatively ($p < 0.05$) microbial activity (except for SO stress) with the lowest values in UP_M stress. Microbial activity is known to be inhibited in a dry substrate [56]. In line with the microbial abundance, all conditions at 29 days showed significantly higher dehydrogenase activity values ($p < 0.05$) than the NO PLANT (Fig. 3), confirming the positive rhizosphere effect on the microbial community.

3.3. Quantification of antibiotic resistance genes (ARGs) in soil

The ARGs analysed were searched for considering the most commonly antibiotic found in agricultural soils, such as sulphonamides (*sul1*, *sul2*), macrolides (*mphE*) and tetracyclines (*tetA*), [57-59]. Moreover, the *intI1* gene, considered a proxy for antibiotic resistance [60], was also quantified.

The amount of ARGs in CON was comparable to that found in other degraded soils, with *sul2* the most abundant gene [43]. Because most European soils have been used in the recent past for agricultural purposes and the phenomenon of antibiotic resistance is a natural response of microorganisms to biotic (e.g. competition) and abiotic (e.g. natural toxic substances) stress it is unlikely to detect "zero antibiotic resistance" in a soil [57]. However, the relative gene abundances (No. gene copy 16S^{-1}) found in UP_M and TO_MP were higher ($p < 0.05$) than in the other conditions (Fig. 4, 0 day). In particular, the highest values were in the soil amended with the compost containing pig manure, pomace and trimmings (TO_MP: *sul1* +*sul2* +*intI1* +*tetA*+*mphe* = 5.53 E-03 copies 16S^{-1}) and in that amended with compost containing

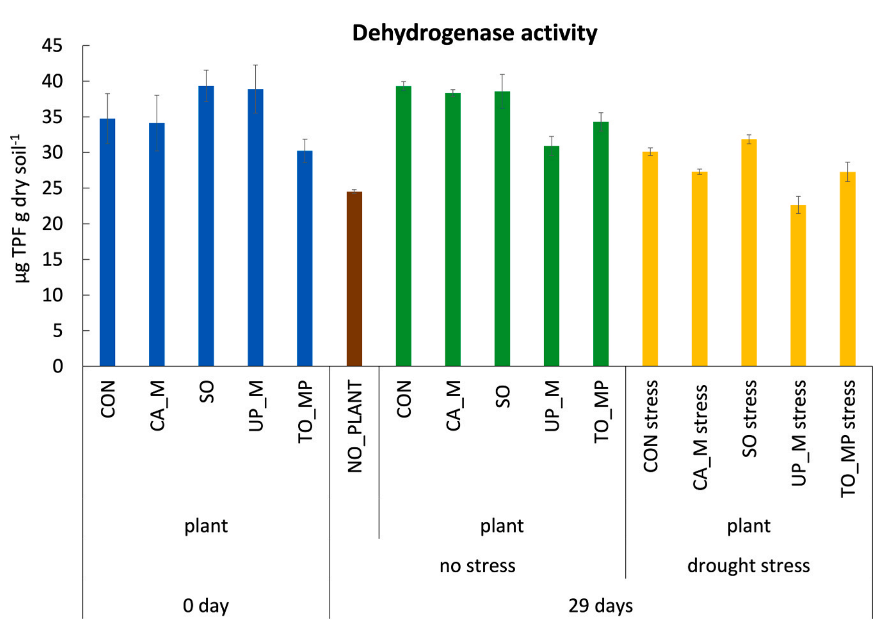


Fig. 3. Dehydrogenase activity (μg TPF g^{-1} dry soil h^{-1}) in the various experimental conditions at 0 day and 29 days. Rosemary plants were always present, except in NO PLANT. The vertical bars are the standard errors.

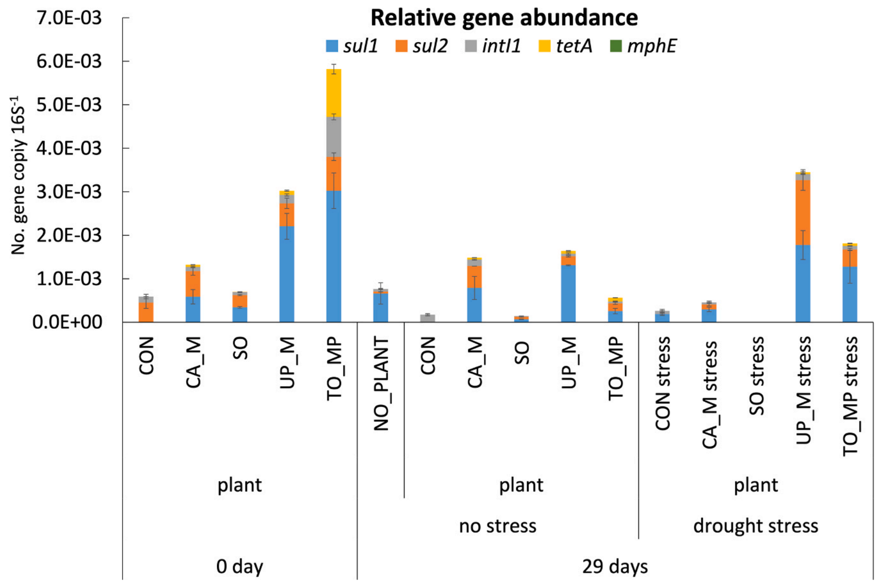


Fig. 4. Relative gene abundances (No. gene copy $16S^{-1}$) in the various experimental conditions at 0 day and 29 days. Rosemary plants were always present, except in NO PLANT. The vertical bars are the standard errors.

exclusively poultry manure (UP_M: *sul1* +*sul2* +*intI1* +*tetA* +*mphe* = 3.03 E-03 copies $16S^{-1}$). At 29 days, a general decrease in the relative gene abundance was observed in all conditions. This over time decreasing trend following organic amendment application has been found in other works [42].

The compost containing pig manure, pomace and trimmings (TO_MP) promoted the best rosemary growth (Fig. 1) and because ARGs decreased significantly at 29 days, this condition was confirmed to be the best option in absence of a drought stress.

On the other hand, the drought condition influenced ARG persistence over time. In fact, in the UP_M stress, the *sul2* gene increased ($p < 0.05$) at 29 days compared to the initial value (0 day). Moreover, in the TO_MP stress a lower ARG decrease ($p < 0.05$) compared to TO_MP was observed for both the *sul1* and *sul2* genes (Fig. 4, on the right side). These results confirm that composts containing pig or poultry manure may introduce ARGs in agricultural soils [20] and that drought conditions may act as a selective pressure which favor antibiotic resistant microorganisms or enhance gene transfer mechanisms [61,62]. On the other hand, sulphonamide resistance genes are highly frequently present in pig manure [63] and in bacteria species from pig farms [64].

It has been recently reported that antibiotic resistance mechanisms can confer tolerance to abiotic stresses, including water stress [26,27,65]. Moreover, other two recent works [42,66] have shown how extreme temperatures acted as a co-selection mechanism maintaining *sul1* and increasing *sul2* genes as a response to this stress. In any case, the effects of key abiotic factors (e.g. drought condition and changes in temperatures) on ARG spread in soils have not been deepened so far.

Interestingly, the condition with the compost containing donkey manure (CA_M stress) showed to be the best not only for facing plant drought stress and stimulating plant growth (Fig. 1), but also because it did not promote ARG spread neither in "no stress" nor in "drought stress" conditions. Indeed, donkeys are generally not raised in intensive animal farming and for this antibiotic use can be limited.

In any case, comparing the overall ARGs found in both pig and poultry composts, their values were significantly lower than those in pig and poultry manure reported in other works [19,20,67]. Further studies aimed at evaluating the antibiotic resistance phenomenon under environmental stress linked to increasing temperatures and drought are desirable.

3.4. Ecotoxicity bioassays

Three test organisms (*Aliivibrio fischeri*, *Lepidium sativum* and *Daphnia magna*) were used for their known sensitivity to various contaminants [68,69]. Table 2 reports the results of the tests performed on soil water extracts from the different experimental treatments. This approach was used to assess possible presence of toxic unknown compounds in the various composts [23]. The *Aliivibrio fischeri* bacterium showed no inhibition effects in any condition, with values always below the 20% toxicity threshold, indicating absence of acute toxicity. The Germination index (IG%) of *L. sativum* seeds was below (< 80%) the toxicity threshold value in the case of SO, UP_M and TO_MP conditions. This bioassay relies on an early developmental endpoint which is very sensitive (precautionary screening) and may not reflect effects on plant growth in a real soil. However, it cannot be excluded that a compost produced by urban waste or manure can contain substances (e.g. contaminants, salts) which can influence negatively root elongation in a water solution [48,70,71].

Finally, *D. magna* was highly negatively affected (immobilization > 90%) in the case of the UP compost suggesting the presence of toxic compounds in the poultry manure.

3.5. Principal component analysis (PCA)

At 29 days, the PCA of the non-stress condition (Fig. 5a) explains 71.97% of the total variance. PCA clearly discriminates samplings depending on the experimental condition. Firstly, the NO PLANT differs markedly from the other ones. In fact, this condition is negatively correlated with the dehydrogenase activity (DHA), microbial abundance, organic carbon and total nitrogen (NTK), confirming the positive interactions established between plant roots and its microbiome (rhizosphere effect), [42,43,54,55]. Among conditions containing manure TO_MP and CA_M are positively associated with microbial parameters and plant growth supporting how above mentioned on the positive effect of these composts. PCA also highlights how poultry manure (UP_M) is strongly correlated with ARGs.

At 29 days, the PCA of the drought stress condition (Fig. 5b) explains 67.29% of the total variance. The experimental conditions are distinguishable in three different groups: 1. CA_M stress and SO stress which show positive correlations with plant growth and microbial activity; 2. TO_MP stress and UP_M stress associated with *sul1*, *sul2*, *tetA*, *intI1* genes; and 3. CON stress inversely correlated to microbial abundance

Table 2
Acute effects of water extracts from each experimental condition assessed with: *Aliivibrio fischeri* (% bioluminescence inhibition at 30 min); *Lepidium sativum* (% Germination index at 72 h); *Daphnia magna* (% Immobilization at 48 h).

Test organism:			*Aliivibrio fischeri*	*Lepidium sativum*		*Daphnia magna*
			Bioluminescence inhibition (%)	Germination index (%)		Immobilization (%)
Soil water extract	**0 day**	**CON**	14.95 ± 0.94	89.26 ± 1.14	0.00 ± 0.00	
		CA_M	0.00 ± 0.00	86.50 ± 9.26	0.00 ± 0.00	
		SO	5.00 ± 0.49	53.04 ± 3.84	13.33 ± 0.20	
		UP_M	6.04 ± 0.51	48.46 ± 3.03	93.33 ± 0.14	
		TO_MP	0.00 ± 0.00	46.89 ± 1.14	60.00 ± 0.28	

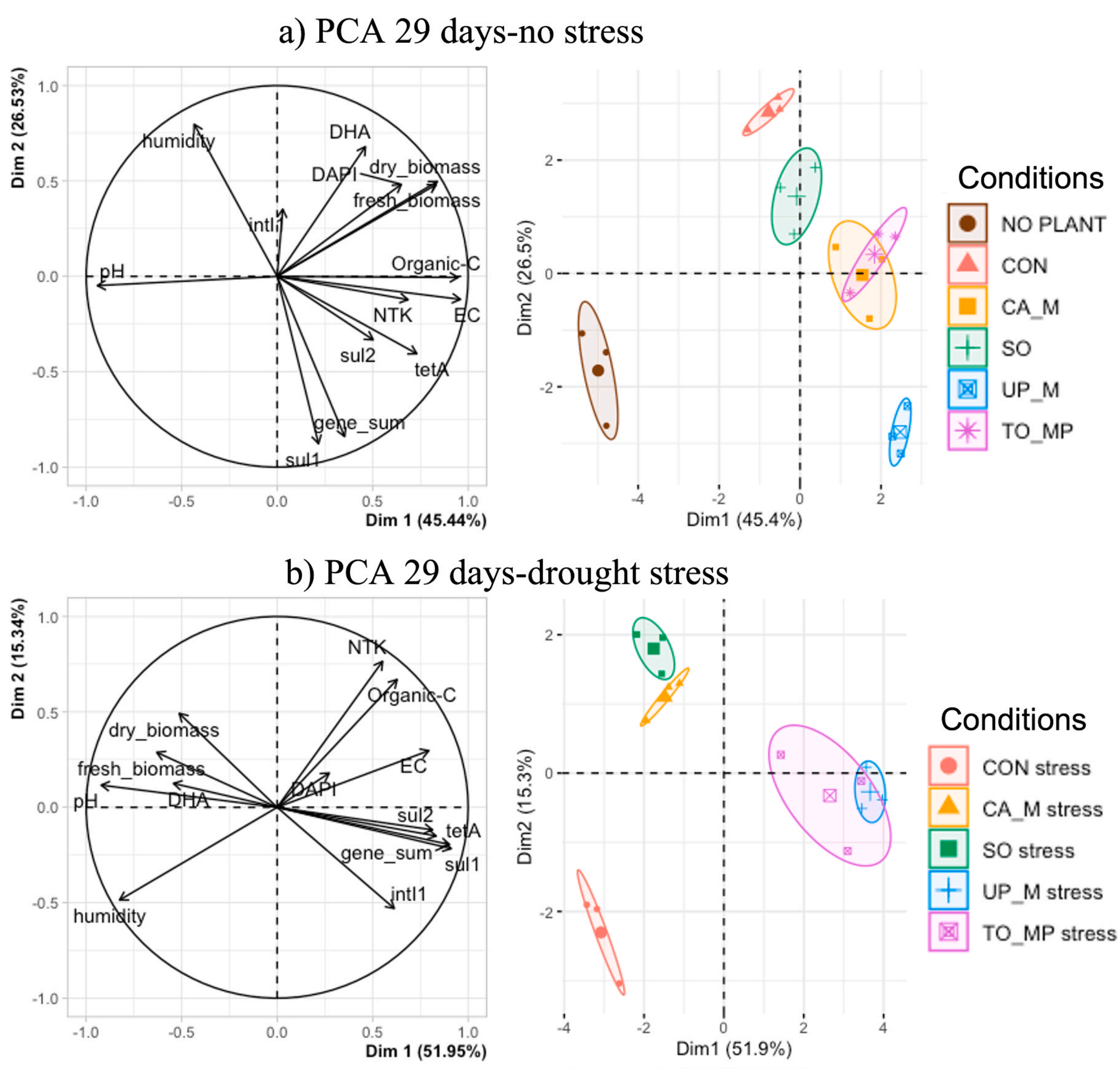


Fig. 5. Principal component analysis (PCA) at 29 days **a)** no stress condition; **b)** drought stress. The PCA was obtained from 14 parameters: plant biomass (fresh and dry), dehydrogenase activity (DHA), microbial abundance (DAPI), pH, soil humidity, *sul1*, *sul2*, *tetA, intI1,* sum of genes (*sul1*+ *sul2* + *tetA*+ *intI1*), organic carbon, electrical conductivity (EC) and total Kjeldhal nitrogen (NTK).

(DAPI), organic C and NTK. Overall, this analysis confirms how the quality of a compost can be evaluated, not only for its agronomic value, but also considering the microbial community associated to rhizosphere and possible introduction of emerging contaminants such as ARGs.

The length of each vector represents the contribution of the corresponding variable on the principal component (Dim1 and Dim2), while the direction of each vector indicates the correlation between the variable and the principal components. Data points are grouped into coloured ellipses representing a 95% confidence interval, showing samples' segregation by different condition.

4. Conclusions

The rosemary plant was confirmed to be an adaptable species to a degraded soil, with low organic matter content and water stress conditions. The positive plant-microbiome interactions established in rosemary rhizosphere promoted microbial abundance and activity over time. Although all organic amendments increased overall soil organic carbon content, only some composts, depending on their composition, promoted plant growth and differently if in presence (CA_M stress) or absence (TO_MP) of the drought stress. In general, compost containing in their composition manure (TO and CA), having a higher amount of available nutrients for plants, had the best performance for its growth. Manure can sometimes introduce ARGs in soil, however this phenomenon was negligible in the case of donkey (CA_M stress) and limited to an initial ARG load in the case of pig (TO_MP). In fact, ARGs decreased at 29 days with values comparable to the CON ones.

Differently, the compost composed exclusively of poultry manure (UP) was not only unsuitable for plant growth, owing to its high salinity and presence of toxic compounds, which hindered also microbial growth, but also introduced ARGs which increased at 29 days under drought stress.

Overall results suggest that it is crucial to consider the characteristics of both a compost and a soil before selecting fertilisation practices. Depending on the type of soil degradation (e.g. water scarcity, compaction, salinity, low organic matter content or high pH), it is necessary to apply on a soil an organic amendment with different characteristics and origin. Composting can be a useful and virtuous process for producing a good organic amendment, however it is very important to test its final quality, through an ecological approach, before its application on a soil.

CRediT authorship contribution statement

De Carolis Chiara: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Barra Caracciolo Anna:** Conceptualization, Validation, Formal analysis, , Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Álvarez-Alonso Cristina:** Methodology, Formal analysis, Investigation, Writing – original draft. **Bustamante Maria Ángeles:** Conceptualization, Validation, Investigation, Funding acquisition, Writing – original draft, Writing – review & editing. **Grenni Paola**: Investigation, Resources, Writing – review & editing, Visualization. **Rolando Ludovica**: Methodology, Investigation. **Narciso Alessandra:** Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft. **Nogués Isabel:** Conceptualization, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbt.2026.02.007.

Data availability

Data will be made available on request.

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