Nature-based solutions using organic amendments for biorestoration of alkaline spoil material

Ludovica Rolando [a], Anna Barra Caracciolo [a,*], Gian Luigi Garbini [a,b], Andrea Visca [a], Livia Mariani [a], Antonio Finizio [c], Valentina Mazzurco-Miritana [d], Isabel Nogues [d], Paola Grenni [a,e]

[a] Water Research Institute-National Research Council (IRSA-CNR), Montelibretti 00010, Rome, Italy
[b] Department of Ecological and Biological Science (DEB-Tuscia University), 01100 Viterbo, Italy
[c] Department of Earth and Environmental Sciences, University of Milano Bicocca, 20126 Milan, Italy
[d] Research Institute on Terrestrial Ecosystems-National Research Council (IRET-CNR), Montelibretti 00010, Rome, Italy
[e] NBFC, National Biodiversity Future Center, Piazza Marina, 61, Palermo 90133, Italy

ARTICLE INFO

ABSTRACT

There are several tunnelling projects underway in Europe and worldwide to facilitate connections between distant areas or ones with geographical constraints (e.g. mountains). As a result, the tunnel industry has been producing huge amounts of excavated material (spoil material: SM), which can be re-used for different purposes (including green areas). Adding lime can be a practical procedure at a construction site for the stabilization of SM and makes it possible to manage it better at its final destination site. This work reports a nature-based solution (NBS) for decreasing the highly basic pH (12.53 ± 0.01) of the SM after adding lime, using three different organic fertilizers which are by-products of local olive oil (pomace), compost and digestate production. For this purpose, microcosm experiments were performed with SM amended with compost, pomace or digestate. Half of the microcosms were also seeded with *Medicago sativa* to evaluate its growth in terms of biomass. The changes in pH and in the structure and functional potential of the microbial community were evaluated in the amended SM at the start and end (4 months) of the experiment. Aerial and root plant biomass and concentrations of leaf chlorophyll and total phenolic compounds were also measured. The microbial biodiversity was evaluated using amplicon sequencing (16S rRNA gene) and a predictive functional analysis was performed by processing sequencing data with the PICRUSt2 tool.

Adding the amendments to SM significantly decreased the pH values by 4 units and introduced organic carbon, nitrogen, and active microbial populations. Although pomace promoted the lowest pH decrease (7.98 ± 0.18) and the highest microbial abundance and activity, it did not favour the highest plant growth. Adding compost and digestate to SM can be considered the best NBS for decreasing pH and supporting plant development. *M. sativa* did not have any direct effect on lowering pH, demonstrating in this study the key role of microorganisms in responding efficiently to this environmental stress and mitigating it. In accordance with these results, predicted bacterial genes coding for membrane proteins (*nuo*, *mrp*) and for indoleacetic acid metabolism (*ami*, *nthA*, *ipdC*) involved in pH regulation were identified among the microbial populations.

1. Introduction

Anthropogenic activities (e.g. infrastructure construction, buildings, landfills, quarries) have been continuously impacting natural ecosystems through their input of chemicals, alteration of landscapes and contribution to consumption and degradation of soil (Amato et al., 2017). Several degradation processes threaten soils, such as soil erosion, soil consumption, soil salinity/alkalinity, landslides or forest fires, often due to incorrect relationships between humans and soil. Soil is a limited resource, especially in developed countries, such as Italy, and its consumption needs to be controlled (Dazzi and Lo Papa, 2013). Soil consumption and its associated vegetation cover removal for urban sprawl and implementation of large infrastructure projects and landfills, significantly interfere with the key regulating, supporting, provisioning and cultural services provided by ecosystems (Saccá et al., 2017). The

* Corresponding author.
E-mail address: barracaracciolo@irsa.cnr.it (A. Barra Caracciolo).

Abbreviations

ASV	amplicon sequencing variants
NBS	nature-based solution
OA	organic amendment
SM	spoil material
SS	Surface soil
Comp	SS + SM + compost
Dig	SS + SM + digestate
Pom	SS + SM + pomace
Comp_P	SS + SM + compost + *M. sativa*
Dig_P	SS + SM + digestate + *M. sativa*
Pom_P	SS + SM + pomace + *M. sativa*
Control	SS + SM
Control_P	SS + SM + *M. sativa*
Plant	SS + *M. sativa*

highest proportions of artificial cover in EU Countries were found in Malta (32.9 %), Belgium (13.4 %), the Netherlands (12.2) and Luxembourg (11.9 %); Italy, Germany, Denmark and the UK have similar values (about 4 %), and these percentages have been increasing (Orgiazzi et al., 2018). The EU soil strategy for 2030 set out concrete measures for protecting and restoring soils and ensuring their sustainable use, including re-use, to be achieved by 2050 (EC, 2006). In this context, the current challenge is to combine technological progress and environmental protection. In the last few years, the underground excavation industry has been growing with a view to both decreasing motor vehicle traffic in large cities and favouring easier and faster interconnections between distant areas or ones with geographical constraints, such as mountains. The implementation of major engineering works such as highways and high-speed railways in mountain regions, like in Italy, requires tunnelling excavation (Barra Caracciolo et al., 2019). In this context, millions of cubic meters of excavated material (spoil material: SM) are produced and large areas (in terms of hectares) of countryside or natural soil cover are used for the temporary deposit of the SM and for various construction site needs (e.g. bungalows for work camp buildings and areas for machinery parking). SM consists of rocks and altered portions of rocks which can have a significant proportion of fine components (Bilotta et al., 2022). At the EU level, specific guidelines for excavated material management are reported in the Waste Framework Directive 2008/98/EC. The legal framework describes the technical characteristics required for SM re-use, considering both environmental protection and the costs of treatment or use as a virgin raw material. In accordance with circular economy objectives, excavated material recycling makes it possible to recover scrap material and to prevent waste production, avoiding the use of landfills for its disposal. Storing SM in deposit areas for several days before its final use is a common practice for ensuring soil drying and biodegradation of the chemicals used for soil conditioning during tunnelling (Barra Caracciolo et al., 2021, 2019; Grenni et al., 2018; Mariani et al., 2020). SM is used as a by-product in compliance with the above-mentioned EU Directive and national legislation and site-specific protocols (Grenni et al., 2019; Mariani et al., 2022, 2020), which require analysis of some organic and inorganic contaminants (Italian DM 161/2012, DPR 120/2017). Once the SM suitability is confirmed from an environmental point of view, the addition of lime (1–6 % in weight) can be a feasible method for chemically stabilizing excavated materials (particularly for spoil materials rich in clay and silty components). Lime produces short term modifications (flocculation and agglomeration of clay minerals, reducing plasticity and moisture content) and a long-term reaction resulting in stabilization of the material, cementing and increasing its strength (Oggeri et al., 2014). Lime treatment makes possible SM handling for different purposes, including material for filling natural depressions for

landscape restoration (Barra Caracciolo et al., 2021). Although lime facilitates SM management, it unavoidably leads to a significant increase in its pH up to very high values ($>$12), (Barra Caracciolo et al., 2021). It is known that pH is a key factor for soil organisms. Soils with pH values higher than 8.0 are defined as highly alkaline (Lopes et al., 2021). Most terrestrial biotas have pH tolerance ranges around neutral; for example, pH values for plants are generally between slightly acid to neutral (Msimbira and Smith, 2020). Alkaline soil causes toxic effects on plants in terms of cellular osmotic stress, ion toxicity, oxidative stress, and bicarbonate/carbonate (HCO_3^-/CO_3^{2-}) stress, affecting plant development (Cao et al., 2022; Liu et al., 2022). In order to adapt to alkaline stress, plants have evolved multiple strategies, such as accumulation and synthesis of small-molecules (such as proline, soluble proteins, betaine, sugar, polyols and polyamines), and the capacity to exclude excessive ions (e.g., Na^+ and Cl^-) and acidify rhizosphere and scavenging systems (including antioxidant enzymes and antioxidants) (Cao et al., 2022; Fang et al., 2021). However, these strategies vary between species and strongly basic pH values are not tolerated (Msimbira and Smith, 2020).

High alkaline or acidic conditions influence some microbial cellular components which can be hydrolysed or enzymes which can be denatured (Atlas and Bartha, 1998). Microorganisms are a key soil component and responsible for several ecosystem functions, such as degradation of organic matter and nutrient cycling, thus ensuring soil fertility and showing a homeostatic capacity versus environmental changes, including pH variations (Atlas and Bartha, 1998; Saccá et al., 2017). Microbes which exhibit a better growth at pH values $\geq$ 9 are termed alkaliphiles (Jin and Kirk, 2018). However, for survival they need to maintain their cytoplasm at a neutral pH, regulating at the same time the osmotic potential within the cell and obtaining enough nutrients and energy. Indeed, alkaliphilic microorganisms have developed strategies for alkaline pH homeostasis such as: (i) increase in metabolic acid production through amino acid deaminases and sugar fermentation; (ii) increase in ATP synthase, which couples H^+ entry to ATP generation; (iii) changes in cell surface properties and (iv) increase in expression and activity of monovalent cation/proton antiporters. The latter is the main mechanism used for alkaline environments (Padan et al., 2005). Monovalent cation/proton antiporters are ion transporter-related transmembrane proteins which can mediate intracellular ion and pH homeostasis by exchanging monovalent cations (e.g. Na^+, K^+) and H^+ across membranes (Swartz et al., 2005).

The present work deals with a case study of a tunnel excavation in Central Italy where millions of tonnes of excavated material were produced and temporarily stored at the construction site. The majority of the SM was lime-treated and planned to be used for filling the bottom of natural depressions close to the excavation area in order to reshape the landscape. A re-greening project (use of different endemic plants) is planned in this area. In order to prevent the possibility that the lime-treated SM hamper plant growth if roots reached it, the addition of organic amendments (digestate, pomace and compost) was evaluated as a nature-based solution (NBS) for promoting a pH decrease and a valid alternative to the use of chemical treatments (e.g. gypsum, sulphur salts). Although there are several organic amendments which could be useful for decreasing pH, the amendments used in this experiment were chosen because they are by-products of the regional production chains, in order to ensure that the project is more in line with the circular economy.

Microcosm experiments were performed mixing the organic amendments with SM and testing the growth of the *Medicago sativa* plant. The improvement of the SM quality in the different experimental conditions was evaluated in terms of pH, organic carbon, nitrogen content, microbial abundance and activity 4 months after the plant seeding. Moreover, the microbial community composition was identified by sequencing the 16S rRNA gene and the predictive functional composition of the community was also evaluated. Finally, the plant physiology was evaluated in terms of aerial and root biomass and concentrations of leaf chlorophyll and total phenolic compounds.

2. Materials and methods

2.1. Spoil material and surface soil

The SM (about 1.5 million m^2) obtained for the execution of a highway tunnel of 7.5 km length and treated with lime was temporary stored at a construction site located close to Florence (Tuscany, Italy). The construction area close to the tunnel was used not only for base camp needs and as a SM temporarily deposit, but also for the storage of the surface soil previously removed and piled up (piled soil), for its subsequent reuse in the re-greening project. In fact, for the realization of the construction site, the surface soil layer (about 0–30 cm) was removed, piled and accumulated until the end of the overall engineering works (tunnel, service station and area re-greening). The green restoration of the entire site will comprise the filling of natural land depressions with SM as the base and the piled surface soil as the top layer.

2.2. Organic amendments

The compost, digestate and olive pomace used in this study were by-products of regional production chains, with a view to the circular economy and waste valorisation. Compost was obtained from mixed sources, such as the organic fraction of municipal waste (collected separately) through a controlled transformation and stabilization process; it came from the composting plant in Faltona (Florence, IT). Digestate derived from an anaerobic treatment of animal origin waste, waste from agro-industrial activities and from the processing of untreated wood and natural textiles, and matrices intended for a composted green soil improver; it was provided by the "Colline Senesi" biogas plant (Arezzo, IT). Olive pomace was degreased olive flour and was provided by the company Caldini Guido S.r.l. The main chemical and physical characteristics of the organic amendments (OAs) are shown in Tables S2 and S3.

2.3. Microcosm set up

Microcosms were filled with a layer of SM mixed with compost, digestate or pomace at the bottom and a layer of the piled soil. Both the SM and piled surface soil were collected from the construction site. *M. sativa* (cultivar Maraviglia, germination rate 85 %, Sgaravatti), also called alfalfa, was selected for its adaptability and capacity to improve soil fertility (Li et al., 2012). Microcosms, which reproduce natural conditions on a small scale (Matheson, 2008), consisted of 1.4 L capacity plant pots. They were filled with spoil material (SM, 600 g) mixed with one of the OAs on the bottom and covered with 600 g of the surface soil (SS). The OAs were mixed with SM at 5 t/ha for compost (33 g), 3 % for digestate (18 g) and 50 t/ha for pomace (72 g), in accordance with Directive 91/676/EEC and the Italian Legislative Decree 152/06.

Half of all the pots were seeded with alfalfa (10 seeds for each pot). The pots were kept in a growth chamber at 23 °C, with 50 % humidity, 60 % ventilation and 14 h of light and 10 h of dark, and watered with distilled water for about 4 months (121 d).

The overall experimental conditions can be summarized as follows:

1. Surface soil (0–3 cm, SS) + Spoil material (3–6 cm, SM) amended with compost: **Comp**
2. SS + SM amended with digestate: **Dig**
3. SS + SM amended with pomace: **Pom**
4. SS + SM with compost + *M. sativa*: **Comp_P**
5. SS + SM with digestate + *M. sativa*: **Dig_P**
6. SS + SM with pomace + *M. sativa*: **Pom_P**
7. SS + SM: **Control**
8. SS + SM + *M. sativa*: **Control_P**

Finally, some pots were set-up with only surface soil (1200 g) and seeded with *M. sativa* (**Plant**) to compare plant growth and microbial community structure in the absence of the SM. Three replicates were set up for each condition. Two destructive samplings were carried out: an initial one (0 d) and a final one (4 months). At each sampling, aliquots (composite samples of ca. a total of 50 g for each replicate, sampled randomly) of surface soil (0–3 cm) and SM (3–6 cm) were collected from each microcosm for physiochemical and microbiological analyses. After sampling, the aliquots for the physiochemical and microbiological analyses were processed immediately and those for NGS analysis were stored at −20 °C until processing.

2.4. pH, soil water content, organic carbon and nitrogen

The pH was measured in distilled water (1:2.5) and the soil water content by the gravimetric analysis, in accordance with official methods (DM 13/09/1999 and s.m.i.). Aliquots of air-dried soil (15–20 mg each) were used for the total organic carbon (OC) and nitrogen (N) analyses, using an elemental carbon analyser (Carlo Erba NA 1500 series 2 C/H/N/O/S). For the organic carbon analyses, the samples were acidified with 20 μL 5 M ultrapure HCl and kept at 50–60 °C for 30 min in order to remove inorganic carbon. Further details are described in Barra Caracciolo et al. (2020).

2.5. Microbial abundance, and microbial activity

The total microbial abundance (N. cells/g) was measured by the epifluorescence direct count method using DAPI as the DNA fluorescent intercalant. Formaldehyde-fixed soil samples (1 g each) were processed as reported in detail in (Barra Caracciolo et al., 2015; Grenni et al., 2009). The epifluorescence microscope was a Leica DM 4000B (Leica Microsystems, GmbH, Wetzlar, Germany).

The overall microbial activity (μg TPF/g) was measured as dehydrogenase activity, using the colorimetric determination of the 2,3,5-triphenyl formazan (TPF) produced from the reduction of the colourless 2,3,5-triphenyltetrazoliumchloride (TTC) in soil samples (6 g each), as previously reported (Grenni et al., 2012, 2009). The determinations were performed using a Multiskan Sky Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.6. Microbial community structure: DNA extraction and sequencing of 16S rDNA amplicons

The total DNA of the prokaryotic community was extracted from 0.25 g of soil (for each replicate) using the DNeasy PowerSoil kit (QIAGEN GmbH, Hilden, Germany), in accordance with the manufacturer's recommendations. A DNA-free sample was also analysed as the negative control during the whole workflow. The extraction yield and quality of the DNA were assessed using spectrophotometric measurements (Multiskan Sky Microplate Spectrophotometer, Thermo Scientific). DNA extracts were stored at −20 °C until processing. They were used as the template for sequencing the hypervariable V3-V4 region of 16S rRNA with MiSeq Illumina. The 341F (CCTACGGGAGGCAGCAG) and 805R (GACTACHVGGGTATCTAATCC) primers for Prokaryotes were used (Takahashi et al., 2014). The raw sequences were imported and demultiplexed using the QIIME2 next-generation microbiome bioinformatics platform v2019.11 (Bolyen et al., 2019) and denoised with the DADA2 plug-in described by Callahan et al. (2016). The primers were removed using the "trim-left-f" (forward) and "trim-left-r" (reverse) primer DADA2 functions (Mazzurco Miritana et al., 2020). These move the sequences to a specific position. The exact length of the primers was 17 nucleotides for the forward ones and 21 nucleotides for the reverse ones.

The amplicon sequencing variants (ASV) obtained were sorted using the Silva 132 database (https://www.arb-silva.de) for Prokaryotes (Abarenkov et al., 2020) with a naive Bayes classifier trained on the amplified regions with 80 % confidence (Bokulich et al., 2018). The sequencing data of non-amended SM (Control and Control_P) are not

reported owing to the very low amount of DNA obtained from extraction.

The bacterial diversity was analysed using the Evenness and Shannon diversity indices, while the Chao 1 index (Chao et al., 2004) was used as an estimator of potential richness (calculated with Qiime2 using the alpha diversity function).

2.7. Predictive functional analysis of soil Prokaryotes

The PICRUSt2 software tool (https://github.com/picrust/picrust2) made it possible to predict the relative abundance of several genes in the functional profiling of the prokaryotic community, using the 16S rRNA gene amplicon datasets (Douglas et al., 2020). In particular, the ASV table of prokaryotic cells generated by DADA2 was the input. The prediction by KEGG Orthology (Kyoto Encyclopedia of Genes and Genomes Orthologs, https://www.genome.jp/kegg) and of gene functions Enzyme Commission number relative abundances was performed with hidden-state prediction (Louca and Doebeli, 2018) and used to infer metabolic pathway abundances (Ye and Doak, 2009). The data are reported as EC (Enzyme Commission number) relative abundances (%).

2.8. Medicago sativa biomass, foliar chlorophyll and antioxidants contents

At the end of the experiment, all the alfalfa plants were carefully removed from the pots, washed with distilled water to remove any particles attached and weighed (aerial parts + roots) to determine their fresh weight. The plant dry weights were determined by drying them at 60 °C for 72 h in an air-forced oven. The concentration of chlorophyll and total phenolic compounds was estimated from the fresh plant material at the end of the experiment. The extraction was performed from 300 mg of sample leaves with 80 % ethanol for 48 h. Each analysis was performed in duplicate for each extract. Total leaf chlorophyll content was determined spectrophotometrically at 663 and 645 nm and was calculated as described in Zeng et al. (2017).

The amount of total phenolic compounds extracted was determined with the Folin–Ciocalteu reagent as described in Ugulin et al. (2015). Gallic acid was used as the standard and the total phenolic compounds were expressed as mg of gallic acid equivalents per g of fresh weight.

2.9. Statistical analysis

Prior to performing each statistical analysis, the Shapiro-Wilk test of normality was performed in order to select a parametric or a non-parametric test for each data set. The data regarding the microbiological, chemical-physical and plant biomass parameters were compared with each other with ANOVA using the *AOV* function of the statistical program R (ver. 4.3). Where significant differences were found, pairwise comparisons were made to evaluate which conditions were significantly different, using the Tukey's Honestly Significant Difference test with the TukeyHSD function of R. A significance threshold of 0.05 was considered.

The correlation between microbiological data (e.g. abundance vs activity) was performed using Pearson correlation.

The values of the diversity indices, obtained for each individual condition based on the abundance of Amplicon Sequencing Variants (ASVs), were compared for each pair of conditions, using a non-parametric statistical test (*Wilcox test*), executed using the QIIME2 software. The differences among the conditions at class, genera level and ASVs (based on Bray-Curtis distance matrix) were calculated using PERMANOVA test (Analysis of Multivariate Variance with Permutations), carried out using the *adonis2* function of R. The effects of OAs and *M. sativa* on the prokaryotic community were also evaluated in a principal-coordinate analysis (PCoA).

The differences among the relative abundances of the genes revealed by the predictive functional analysis were calculated using ANOVA (*aov*

function of R) and subsequently Tukey's test (*TukeyHSD* function of R) to determine which conditions were significantly different. In any case, a significance threshold of 0.05 was considered. Moreover, the structure of the microbial community (at genus level) was mapped using heatmap modules in the "R" statistical package ("ComplexHeatmap"), The heatmap reports the most abundant prokaryotic genera (top 25 genera, net of "unclassified" ones) detected in the overall samples corresponding to >1 % of total identified genera, and the dataset used for heatmap was normalized by mean and standard deviation (z-score). Differences among conditions were evaluated using the PERMANOVA test.

3. Results and discussion

Preliminary analyses showed that the lime-treated SM had an average pH of 12.53 ± 0.01 and the surface soil of 8.58 ± 0.01, showing how the latter was partially influenced by the lime treatment due to its temporary storage close to the area where the SM was treated with lime.

Initially, there were no microorganisms detected in SM, probably owing to its very high pH (12.53 ± 0.01). Adding OA increased the SM organic carbon and nitrogen contents and added active microbial populations into the SM. There was no immediate pH reduction (Table 1 and Supplementary Materials, Tables S2 and S3) because the three OAs had a neutral or slightly acid pH.

The basic pH (8.58 ± 0.01), low organic carbon (0.85 ± 0.03 %), and low microbial abundance ($7.61 \times 10^6 \pm 8.35 \times 10^2$ cells/g) and activity (19.60 ± 4.54 μg TPF/g) of the surface soil (Table 1) were presumably due to its degradation and deconstruction during piling for ca. 2 years at the construction site and its proximity to the area where the lime treatment took place. Other authors have also reported that piled soils in construction projects are subject to loss of their structure and organic matter due to poor resistance to external erosion agents (Zhang et al., 2021).

At 4 months (Fig. 1), OAs caused an overall significant decrease (p-value < 0.001, Supplementary Materials, Table S6) in pH of SM, with values from 7.98 ± 0.18 to 8.75 ± 0.19 in the plant absence conditions, and with the lowest value in Pom. The latter result was presumably due to organic acid presence in the pomace. The microbial degradation of the organic substance in the amendments presumably promoted the release of various compounds (e.g. NH_4^+, CO_2, organic acids) which led to a pH decrease (Msimbira and Smith, 2020) in the treated SM conditions. A pH decrease was also observed in the not amended conditions (9.24 ± 0.06 in Control and 10.30 ± 0.33 in Control_P), however with values significantly different from the OA conditions. This result suggests a possible pH regulating role of the microorganisms, which presumably migrated from the surface soil to SM.

The plant presence did not favour any additional pH decrease in SM (values from 8.36 ± 0.11 to 8.61 ± 0.07) compared to the conditions in the absence of plants. In fact, any significant further pH reduction was observed with *M. sativa* presence (Fig. 1), suggesting the key role of microorganisms in the pH regulation.

Fig. 2 shows the percentages of organic carbon (A) and total nitrogen (B) measured at 4 months in the SM (3–6 cm) and in the surface soil (0–3 cm) in each single microcosm condition, in the presence (P) and absence of alfalfa. The highest percentages of OC (9.50 ± 0.00 %) and N (0.50 ± 0.00 %) in SM where found in the Pom condition (p-value < 0.01), in line with their initial values (Table 1, SM + Pomace). Interestingly, N % were higher (except for Comp) in the absence of plants, suggesting a probable competition for this limiting factor between the plants and microorganisms.

All the three OAs promoted an overall significant and long-term increase (p-value < 0.001) in microbial abundance and activity in SM (Fig. 3 A and B), with the highest values in the Pom condition ($6.69 \times 10^8 \pm 7.10 \times 10^7$ cells/g and 558.78 ± 30.99 μg TPF/g, respectively). A significant correlation (p-value < 0.01; $R = 0.94$) between microbial abundance and activity was also found after 4 months.

At the end of the experiment, the pH in the surface soil covering the

Table 1

Total microbial abundance, microbial activity, organic carbon (OC %), nitrogen (N %), pH and moisture (% H$_2$O) at day 0 in the surface soil, spoil material (SM) and SM mixed with Compost, Digestate or Pomace; n.d.: not detected.

Day 0	Microbial abundance (N. cells/g)	Microbial activity (μg TPF/g)	OC (%)	N (%)	pH	% H$_2$O
Surface soil	$7.61 \times 10^6 \pm 8.35 \times 10^2$	19.60 ± 4.54	0.85 ± 0.03	0.25 ± 0.03	8.58 ± 0.01	10.2 ± 0.01
SM	n.d.	n.d.	n.d.	n.d.	12.53 ± 0.01	8.7 ± 0.01
SM + compost	$2.13 \times 10^7 \pm 1.10 \times 10^3$	185.38 ± 0.62	3.40 ± 0.25	0.25 ± 0.03	12.25 ± 0.01	9.5 ± 0.01
SM + digestate	$3.29 \times 10^7 \pm 1.43 \times 10^3$	32.24 ± 0.32	2.25 ± 0.03	0.25 ± 0.03	12.50 ± 0.01	9.0 ± 0.01
SM + pomace	$2.90 \times 10^7 \pm 9.06 \times 10^2$	910.06 ± 9.34	6.85 ± 0.41	0.40 ± 0.03	11.58 ± 0.01	6.8 ± 0.01
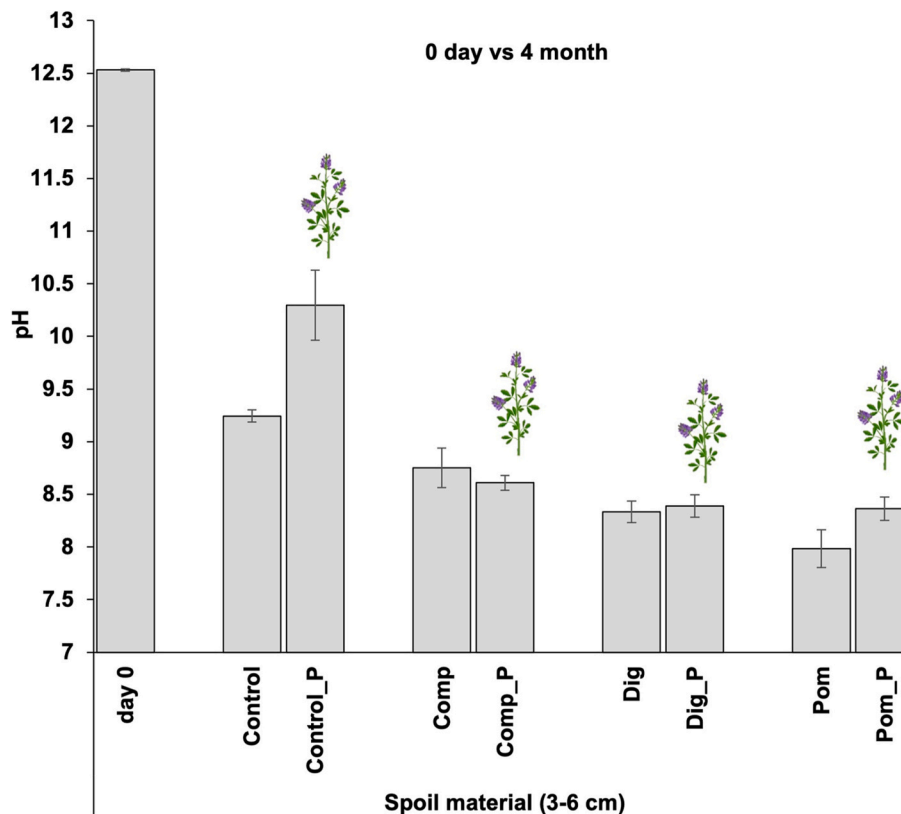
Fig. 1. pH of the spoil material at 0 d and 4 months, under different conditions with the relative standard errors. Comp: compost; Dig: digestate; Pom: pomace, P: plant.

SM decreased only slightly, from an initial value of 8.58 ± 0.01 to average values of 8.36 ± 0.08 and 8.35 ± 0.03 in absence and plant presence, respectively (with no significant differences). Moreover, Organic Carbon (OC%) maintained values substantially similar to the initial ones (OC%: from 0.85 ± 0.03 at 0 d to 1.05 ± 0.12 in the absence and to 0.89 ± 0.06 in the presence of plants at the end of the experiment, reported as mean values), while nitrogen decreased slightly (N%: from 0.30 ± 0.03 at 0 d to 0.28 ± 0.02 in the absence and to 0.22 ± 0.01 in the presence of plants at, reported as mean values).

The physiochemical and microbiological results suggest that the quality of the SM, which was initially a poor substrate, significantly improved, showing pH, nutrients, microbial abundance and activity values also higher than the covering surface soil, which proved the latter was degraded owing to its accumulation at the construction site.

The increase in soil quality following OAs addition has been found in several works (Barra Caracciolo et al., 2015; Garbini et al., 2022; Innangi et al., 2017) although to our knowledge this is the first study on lime-treated spoil material. On the other hand, the plant presence, which generally increases microbial abundance, activity and soil quality, promoting synergic relationships between bacteria and roots in the rhizosphere (Barra Caracciolo and Terenzi, 2021; Bustamante et al., 2020; Di

Lenola et al., 2020), did not in this study favour either a pH decrease or nutrient increase. The latter results can be ascribed to the fact that the SM, because it came from underground, and had been treated with lime, was an inhospitable substrate (pH 12.53 ± 0.01) and was devoid of nutrients (Table 1). Consequently, the microorganisms colonizing it from the OAs, probably outcompeted plants in acquiring nutrients in this pioneer environment. Strong interspecific competitive interactions can occur in degraded soil since both microorganisms and roots require the same limiting nutrients, essential for growth (Kuzyakov and Xu, 2013; Turbé et al., 2010). However, microorganisms were more efficient in a prompt acquisition of nutrients, by degrading organic substances; plants, on the other hand, were able to access nutrients only after they were made available by microbial activity. This phenomenon was particularly evident with pomace where N and OC concentrations were the highest (Fig. 2), but were significantly lower with plants present.

Compared to the initial substrate, the non-amended SM (Control and Control_P) at 4 months improved slightly in terms of OC, N and pH. These results were presumably due, as above mentioned, to soil which mixed with SM during pot watering, leaching both organic carbon and microbial populations. However, the microbial populations, presumably migrated from the covering surface soil owing to the watering, were not
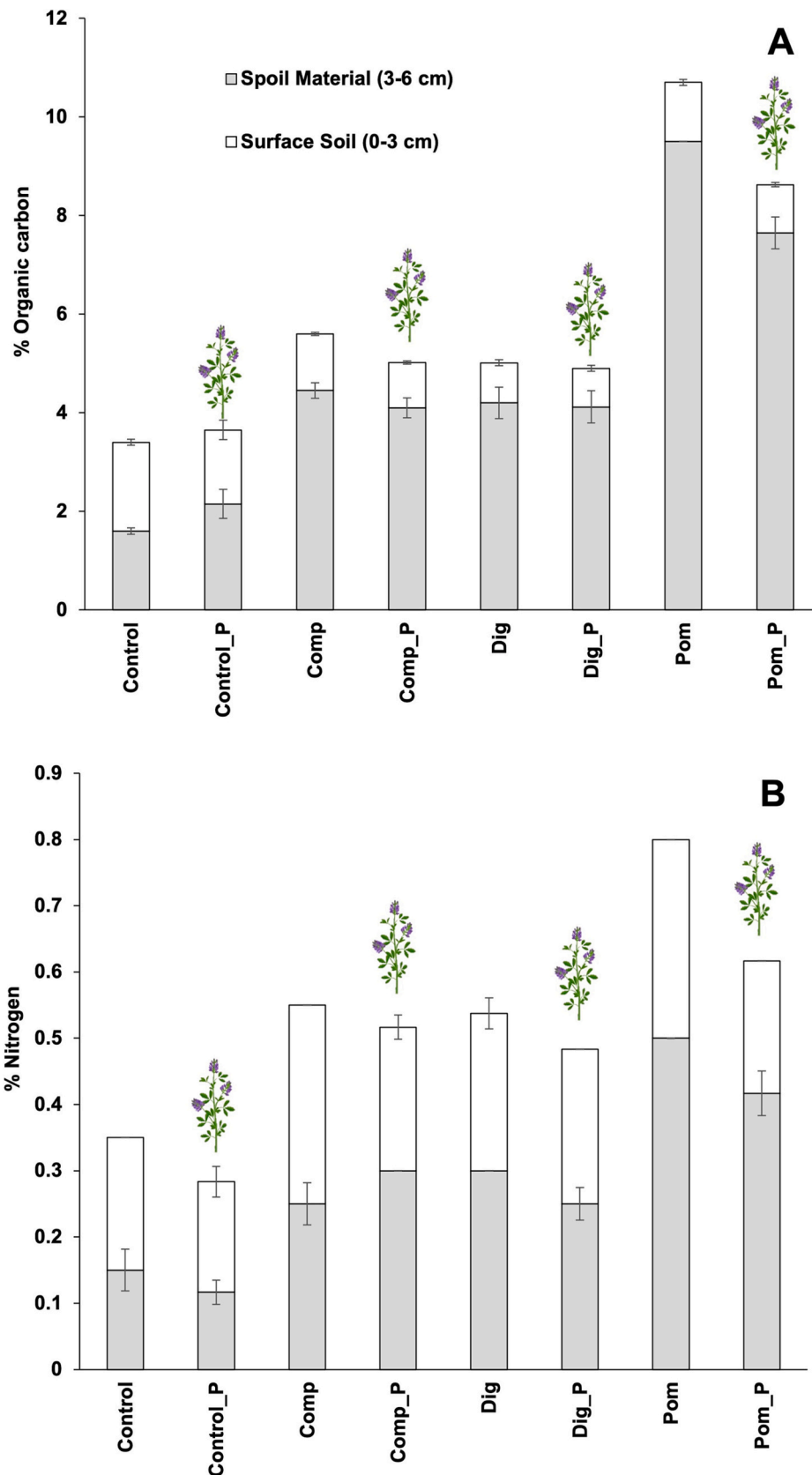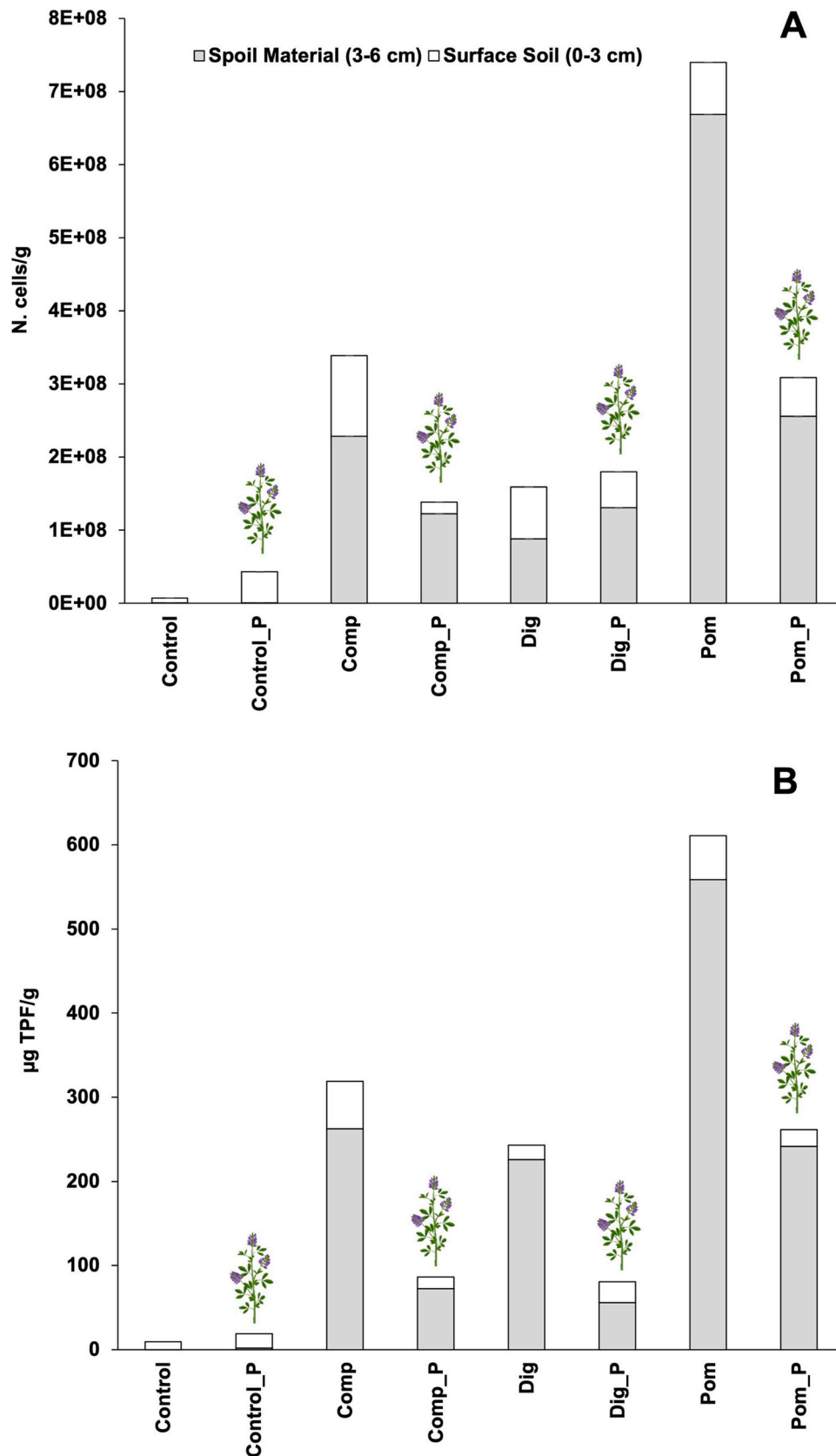
Fig. 2. A) Organic carbon and B) total nitrogen at 4 months, in the different conditions. Comp: compost; Dig: digestate; Pom: pomace; P: plant. The histograms in white represent the surface soil (0–3 cm) and in grey the spoil material (3–6 cm).

Fig. 3. A) Total microbial abundance (N. cells/g) and B) Dehydrogenase microbial activity (µg TPF/g) at 4 months, in the different experimental conditions. Comp: compost; Dig: digestate; Pom: pomace; P: plant. The histograms in white represent the surface soil (0–3 cm) covering the spoil material (3–6 cm), represented by the grey histograms.

very active (Fig. 3 A and B) due to the persistence of the alkaline environment (pH 9–10.30).

Although pomace promoted an overall increase in microbial abundance and activity in the SM, the plant biomass (aerial part and roots, Fig. 4), showed the lowest values (*p*-value < 0.05). This could be ascribable to some compounds in pomace which can be toxic (e.g. phenol acids) for plants, as reported in other works (Muscolo et al., 2019; Paraskeva and Diamadopoulos, 2006; Pinho et al., 2017). In fact, Li et al. (2010) analysed the modes of action of soil phenolic compounds as plant growth inhibitors and found they influenced nutrient uptake, protein synthesis and photosynthesis, among other things. Conversely, the

Comp_P and Dig_P conditions showed the highest biomass values (p-value < 0.05) and roots developed inside the overall SM (Fig. 4).

Finally, the fact that plants grown on the surface soil alone (Plant) had a low biomass was in line with the low soil nutrient content (Table 1 and Fig. 2). Plant growth was found to be in line with leaf chlorophyll content. In fact, there were lower values for Pomace (1.9 µg/g ± 0.2) and Plant (1.7 µg/g ± 0.2) than other conditions (Control_P: 2.6 µg/g ± 0.4; Comp_P: 2.3 µg/g ± 0.4; Dig_P: 2.5 µg/g ± 0.2). Plant biomass and chlorophyll content are parameters that are strictly related, since chlorophyll is a pigment necessary for plant photosynthesis (Croft et al., 2017), an essential metabolic pathway for plants, contributing to their
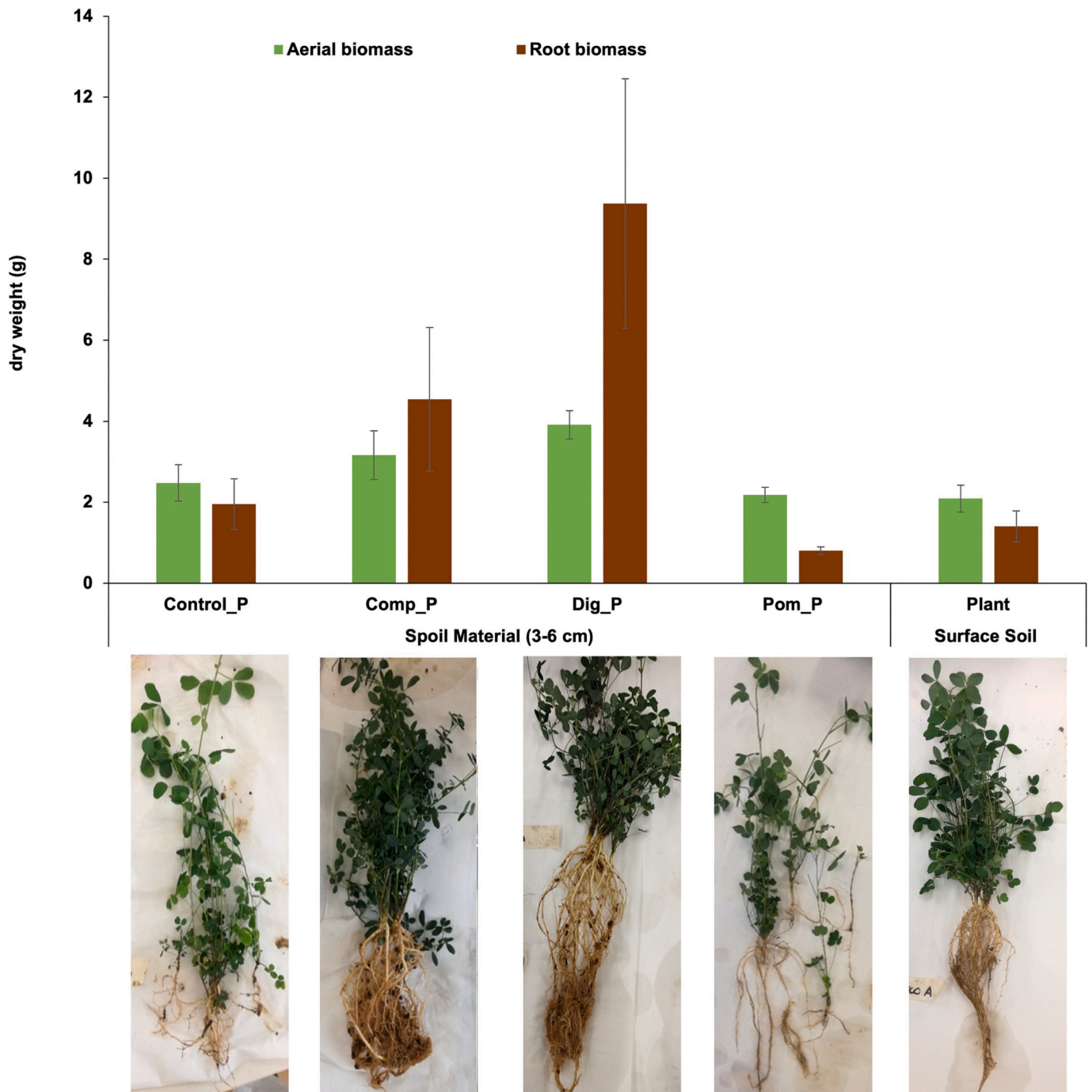

Fig. 4. Aerial and root biomass (dry weight, g) of the plants under different conditions at 4 months.
Control_P: Surface soil + spoil material; Comp_P: Surface soil + spoil material with compost; Dig_P: Surface soil + spoil material with digestate; Pom_P: Surface soil + spoil material with pomace; Plant: pots with only surface soil. The vertical bars are the relative standard errors.

growth and biomass production (Calzadilla et al., 2022). An increase in chlorophyll content is usually correlated with an increase in plant biomass (Fedeli et al., 2023; Liu et al., 2019; Robles-Aguilar et al., 2019). *Chlorophyll content* together with nutrient availability are the most important factors determining plant growth (Kirschbaum, 2011).

The total phenolic compounds in the plants were found to be significantly (*p*-value < 0.05) higher in all conditions with SM (Control_P: 22.0 μg/g ± 0.7; Comp_P: 18.0 μg/g ± 1.3; Dig_P: 20.7 μg/g ± 1.4; Pom_P: 18.8 μg/g ± 1.4) than in the surface soil alone (Plant: 14.7 μg/g ± 0.9). Total phenolic compounds play an important antioxidant role in plant cells and their synthesis is generally activated in response to stress conditions (Šamec et al., 2021). Consequently, plants grown in pots with SM presumably activated a response to reduce oxidative stress caused by growth in an alkaline environment. In this condition plants accumulated and stored phenolics in leaves. Moreover, plant stress may also be due the improper structure of the SM which was probably less suitable than surface soil for plant growth. In fact, the highest phenolic compound amount was found in Control_P. Soil structure has been shown to influence plant phenolic compound production (Alotaibi and Abd-Elgawad, 2023).

The analysis of the microbial community structure at 4 months made it possible to identify the bacterial community involved in improving the SM (Figs. 5 and 6). The SM results are reported with those obtained from microcosms containing exclusively the surface soil at 0 d (Plant day 0) and at 4 months in a plant presence (Plant). The diversity indices (Chao1, Shannon, and Evenness) were calculated using Amplicon Sequencing Variants (ASV), Table 2.

The highest values for microbial biodiversity, which is related to soil quality (Onet et al., 2019; Schloter et al., 2003), were found in the presence of compost and plant (Comp_P: Chao1 = 740.92 ± 21.91, Shannon = 8.37 ± 0.00, Evenness = 0.88 ± 0.04) and the lowest in pomace in the absence of plants (Pom: Chao1 = 328.00 ± 12.79, Shannon = 6.28 ± 0.03, Evenness = 0.75 ± 0.27).

Adding OAs influenced the distribution of the ASV in the different conditions (Fig. 5, Supplementary Materials, Table S11 and PCoA, Supplementary Materials, Fig. S1). The composition of microbial classes (% ASV) found at 4 months in SM under the different conditions were significantly different (PERMANOVA, *p*-value < 0.001, R = 0.68). A general predominance of *Proteobacteria* (*Alpha* and *Gamma*), with a percentage varying from 38 % to 56 %, was observed in all amended conditions, with a significant higher abundance of *Alphaproteobacteria* (14–30 %) in plant presence. The latter group comprise nitrogen-fixing bacteria, such as *Rhizobiaceae*, which are known to colonize root nodules of *M. sativa* (Song et al., 2017).

Interestingly, SM amended with pomace, both in the presence (Pom_P) and absence of plants (Pom), had the lowest biodiversity (Table 2), underlining the selective effect of this amendment. In fact, the microbial community was more active (Fig. 3B) and selected (Fig. 5) compared to the other conditions, with a predominance of *Proteobacteria* (56 and 53 % for Pom and Pom_P, respectively).

In contrast, in the surface soil (Plant day 0 and Plant, Fig. 5), a dominance of *Actinobacteria* was observed (46 % in the absence of plants, 30 % in the presence of plants) and this result confirms that it was not in a good quality state, as this class predominates in stressed and oligotrophic soils (Dube et al., 2019), as in this case (Table 1). It is known that, with accumulation of soil organic matter and nutrients, the abundance of *Actinobacteria* decreases, and that of *Proteobacteria* increases (Zeng et al., 2017). The *Proteobacteria* group includes most bacterial species involved in the main biogeochemical cycles and is typically the most abundant phylum found in a good quality state soil (Barra Caracciolo et al., 2020, 2015).

The most abundant prokaryotic genera (top 25 genera, net of "unclassified" ones) detected in the samples are depicted as a heatmap in Fig. 6. The dataset was normalized using means and standard deviations (z-score), reporting genera from the most abundant (red colour) to the least abundant one (blue colour). It is possible to observe three clusters, the first one consisting of Plant day 0, Dig and Plant; the second one Comp, Comp_P, Dig_P, Pom_P, and the third Pom alone, confirming that the OAs modulated differently the bacterial populations colonizing the SM.

In particular, *Azotobacter* was present only in the Pom condition at 10 %, *Sphingomonas* was significantly different (*p*-value < 0.001) between the conditions containing pomace (Pom and Pom_P) and the others and it was significantly (p-value < 0.001) higher in Pom (42 %) than in Pom_P (19 %). These results are in accordance with the capability of these genera to degrade cellulose and compounds such as phenol acids (Omer and Mohamed, 2012; Tao et al., 2022) and for this reason they were also found in pomace in other works (Milanović et al., 2019; Schmid et al., 2014).

In accordance with the above, bacterial genera, such as the stress-tolerance gram-positive *Bacillus* and *Streptomyces* were the most abundant in the degraded surface soil (Plant day 0 and Plant, Fig. 6), (Pang et al., 2022; Roy et al., 2022).

The above results show the microorganisms' versatility and adaptability to extreme conditions, such as high SM alkalinity. In this case, they presumably enacted adaptive strategies to alleviate pH stress (Santini et al., 2015), using membrane transporters to maintain cytoplasm neutrality (Padan et al., 2005; Swartz et al., 2005). In fact, thanks to the software PICRUSt2, which predicts the potential functional profile of the microbial communities, eight genes encoding membrane proteins involved in cellular pH homeostasis (*ndh*, *nha*, *cha*, *nqr*, *ktr*, *nuo*, *mrp*, *nat*) were identified (Fig. 7).

The genes found ranged from 0.90 % in Pom to 1.20 % in Dig. In all conditions a predominance of the *nuo* operon (p-value < 0.05), responsible for the translocation of protons outside the cell and often associated with the antiporter *mrp*, an active transporter that catalyzes the transport of K$^+$, Na$^+$ or Li$^+$ outside and H$^+$ inside cells (Bongaerts et al., 1995; Swartz et al., 2005), was observed.

In the case of the pots in Plant condition (Fig. 7), the genes identified suggest that soil bacteria also enacted homeostatic mechanisms, and this was confirmed by the decrease in initial pH from 8.57 (Plant day 0) to 8.35 (Plant) measured at 4 months.

Other functional genes involved in organic matter degradation and responsible for the extracellular metabolite release were potentially
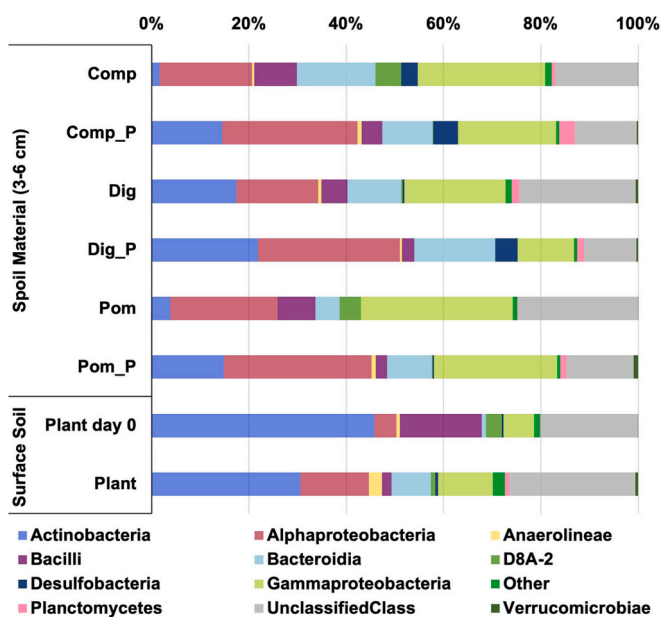

Fig. 5. Relative abundances (%) of bacterial classes detected at 4 months in Spoil material treated with compost (Comp), digestate (Dig) or pomace (Pom) in the absence or presence of *M. sativa* (P); Surface soil at 0 d (Plant day 0) and at 4 months (Plant).
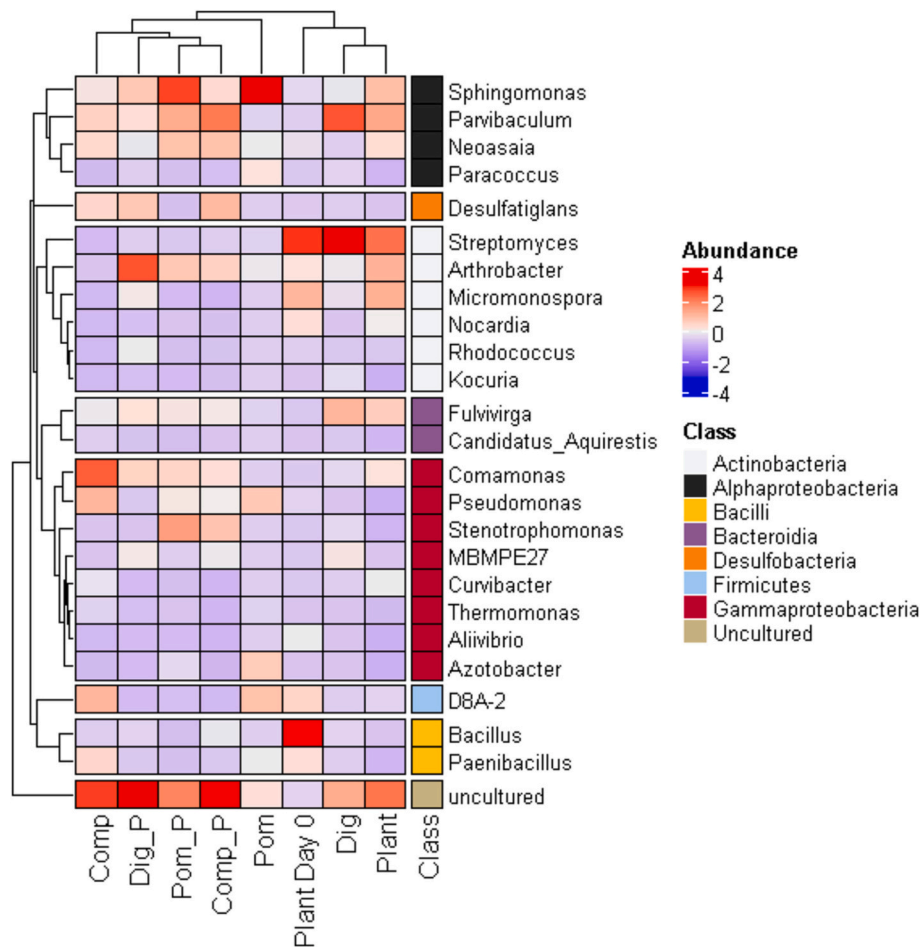
Fig. 6. Heatmap with relative abundance (%) of the main bacterial genera at 4 months in Spoil Material treated with compost (Comp), digestate (Dig) or pomace (Pom) in the absence or presence of *M. sativa* (P); Surface soil at 0 d (Plant Day 0) and at 4 months (Plant).

Table 2
Chao1, Shannon and Evenness indices $\pm$ standard errors calculated at 4 months in Spoil Material treated with compost (Comp), digestate (Dig) or pomace (Pom) in the absence or presence of *M. sativa* (P); Surface Soil at day 0 (Plant day 0) and at 4 months (Plant).

	Condition	Chao1 Index	Shannon Index	Evenness
Spoil material (3–6 cm)	Comp	551.85 ± 57.38	7.43 ± 0.00	0.82 ± 0.16
	Comp_P	740.92 ± 21.91	8.37 ± 0.00	0.88 ± 0.04
	Dig	542.00 ± 10.95	7.64 ± 0.01	0.84 ± 0.06
	Dig_P	508.90 ± 46.49	7.27 ± 0.00	0.81 ± 0.08
	Pom	328.00 ± 12.79	6.28 ± 0.03	0.75 ± 0.27
	Pom_P	599.32 ± 25.29	7.86 ± 0.00	0.85 ± 0.04
Surface soil	Plant day 0	1002.50 ± 231.97	8.84 ± 0.01	0.89 ± 0.22
	Plant	1064.70 ± 31.20	9.43 ± 0.00	0.94 ± 0.05

identified (Fig. 8). For example, indoleacetic acid metabolism genes (*ami*, *nthA*, *ipdC*) were found. In particular, the *ami* gene was the most abundant and with the highest percentages in Pom (p-value < 0.05), where the highest pH decrease (from 12.53 ± 0.01 to pH 7.98 ± 0.18) was observed. The *ami* gene promotes soil acidification regulating other genes encoding for enzymes responsible for the synthesis of carboxylates involved in phosphorus solubilisation and its release in soil (Alemneh et al., 2021). The *ami* gene was also found in the Plant condition and this result can support the hypothesis that soil bacteria metabolism was also active and promoted organic matter re-cycling with a final organic carbon increase.

This experiment confirms the primary role of microbial populations in modifying and colonizing extreme environments thanks to their rapid adaptability and wide metabolic versatility, which allows them to respond efficiently to environmental stress. Once the environment has been made suitable for the growth of other species and in particular plant ones, synergistic relationships can be established between the plant roots and the microorganisms which colonize the same habitat (Kuzyakov and Xu, 2013). In fact, plants can help natural microorganisms in transforming, removing and containing contaminants, improving soil quality (Di Lenola et al., 2020). Moreover, plant-microbe interaction helps plant growth and development under different types of abiotic and biotic stresses (Kumar and Verma, 2018).

Finally, although a specific economic feasibility was not performed, it is clearly recognisable that the use of these OAs in this experiment and, even more, in the large area to be restored, has the double benefit of being in line with circular economy requirements and having economic benefits, since the OAs provided by local production are cheaper than any chemical treatments (e.g. gypsum, sulphur salts) commonly used in agriculture for decreasing soil pH (McKenna et al., 2019).
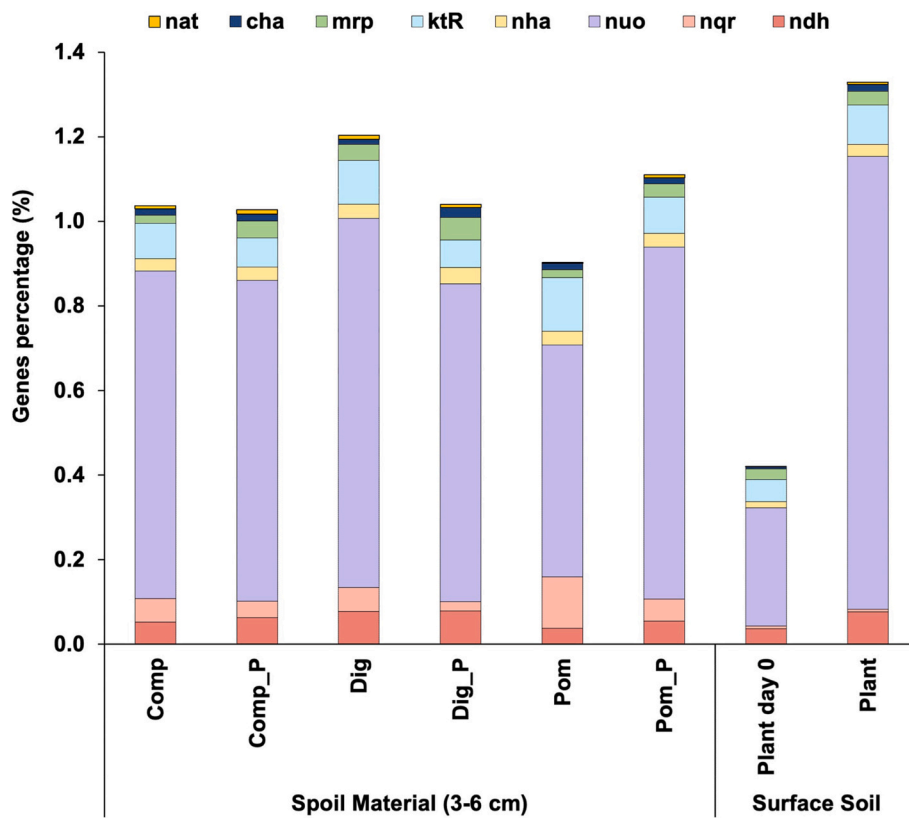
Fig. 7. Predictive functional analysis: relative abundance (%) of functional genes encoding for membrane transporters (*ndh, nha, cha, nqr, ktr, nuo, mrp, nat*) at 4 months in Spoil material treated with compost (Comp), digestate (Dig) or pomace (Pom) in the absence or presence of *M. sativa* (P); Surface soil at 0 d (Plant day 0) and at 4 months (Plant).
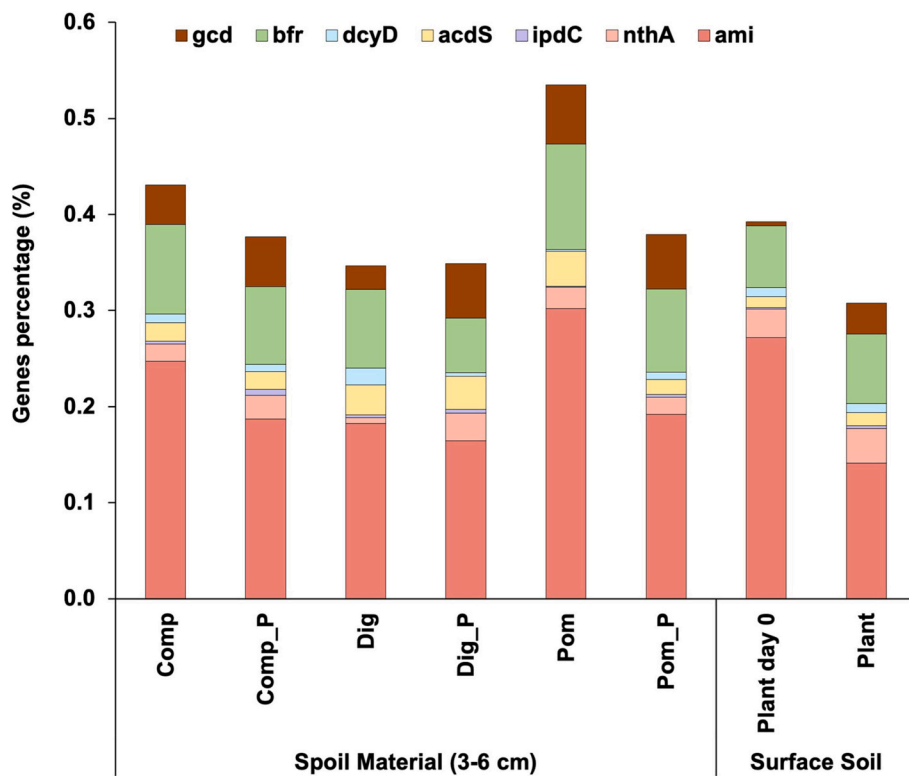

Fig. 8. Predictive functional analysis: relative abundance (%) of some genes responsible for organic matter degradation and release of extracellular metabolites (*ami, nthA, ipdC, acdS, dcyD, bfr*, gcd) at 4 months in Spoil material treated with compost (Comp), digestate (Dig) or pomace (Pom) in the absence or presence of *M. sativa* (P); Surface Soil at 0 d (Plant day 0) and at 4 months (Plant).

4. Conclusions

Adding digestate or compost selected in this study to SM can be considered the best nature-based solution (NBS) as it favoured both a significant pH decrease and the highest *Medicago sativa* growth. The improvement in SM quality found in this microcosm experiment was ascribable to the proliferation of microbial populations from OAs, which were able to initially survive a high pH and low nutrient substrate and then modify it. In fact, at 4 months a significant increase in microbial abundance and activity was observed and a predominance of the *Proteobacteria* (Alpha and Gamma), which include most bacterial species involved in the main biogeochemical cycles, was found. The plant presence neither decreased pH nor increased organic carbon or nitrogen content, showing the pioneer role of bacteria in improving a poor substrate and making it more favourable for plant growth if compared to unamended conditions.

Although additional research is needed to determine the possible long-term effects of organic amendments on soil quality, and to identify expressed genes involved in pH regulation, the results here obtained are encouraging because they show a significant improvement in the substrate within 4 months, applying a green low-cost solution in line with the circular economy. This approach can also be applied to other degraded soils or waste materials, in order to enhance their quality, reduce waste generation and carbon footprint, and preserve natural resources. Finally, this work shows how a NBS can be a valid alternative, in decreasing pH, to chemical treatments (e.g. gypsum, sulphur salts).

The results obtained are being used in a real case study where a green restoration of a construction site area with SM mixed with soil is planned. A field experiment using the compost and digestate as OAs for revegetation and reforestation of this area with different species is in progress.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105070.

References

Abarenkov, K., Zirk, A., Piirmann, Timo, Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2020. UNITE QIIME release for Fungi. UNITE Community [WWW Document]. https://doi.org/10.15156/BIO/786385.

Alemneh, A.A., Cawthray, G.R., Zhou, Y., Ryder, M.H., Denton, M.D., 2021. Ability to produce indole acetic acid is associated with improved phosphate solubilising activity of rhizobacteria. Arch. Microbiol. 203, 3825–3837. https://doi.org/10.1007/s00203-021-02364-w.

Alotaibi, M.O., Abd-Elgawad, M.E., 2023. Soil structure influences proteins, phenols, and flavonoids of varied medicinal plants in Al Jubail, KSA. Saudi J. Biol. Sci. 30, 103567 https://doi.org/10.1016/j.sjbs.2023.103567.

Amato, F., Martellozzo, F., Nolè, G., Murgante, B., 2017. Preserving cultural heritage by supporting landscape planning with quantitative predictions of soil consumption. J. Cult. Herit. 23, 44–54. https://doi.org/10.1016/j.culher.2015.12.009.

Atlas, R.M., Bartha, R., 1998. Microbial Ecology: Fundamentals and Applications, 4th ed. Benjamin/Cummings, Menlo Park, California.

Barra Caracciolo, A., Terenzi, V., 2021. Rhizosphere microbial communities and heavy metals. Microorganisms 9, 1462. https://doi.org/10.3390/microorganisms9071462.

Barra Caracciolo, A., Bustamante, M.A., Nogues, I., Di Lenola, M., Luprano, M.L., Grenni, P., 2015. Changes in microbial community structure and functioning of a semiarid soil due to the use of anaerobic digestate derived composts and rosemary plants. Geoderma 245–246, 89–97. https://doi.org/10.1016/j.geoderma.2015.01.021.

Barra Caracciolo, A., Ademollo, N., Cardoni, M., Di Giulio, A., Grenni, P., Pescatore, T., Rauseo, J., Patrolecco, L., 2019. Assessment of biodegradation of the anionic surfactant sodium lauryl ether sulphate used in two foaming agents for mechanized tunnelling excavation. J. Hazard. Mater. 365, 538–545. https://doi.org/10.1016/j.jhazmat.2018.11.002.

Barra Caracciolo, A., Grenni, P., Garbini, G.L., Rolando, L., Campanale, C., Aimola, G., Fernandez-Lopez, M., Fernandez-Gonzalez, A.J., Villadas, P.J., Ancona, V., 2020. Characterization of the belowground microbial community in a poplar-phytoremediation strategy of a multi-contaminated soil. Front. Microbiol. 11, 2073. https://doi.org/10.3389/fmicb.2020.02073.

Barra Caracciolo, A., Grenni, P., Mariani, L., Rauseo, J., Di Lenola, M., Muzzini, V.G., Donati, E., Lacchetti, I., Gucci, P.M.B., Finizio, A., Beccaloni, E., Patrolecco, L., 2021. Mesocosm experiments at a tunnelling construction site for assessing re-use of spoil material as a by-product. Water 13 (2), 161. https://doi.org/10.3390/w13020161.

Bilotta, E., Casale, R., di Prisco, C.G., Miliziano, S., Peila, D., Pigorini, A., Pizzarotti, E.M., 2022. Handbook on Tunnels and Underground Works: Volume 1: Concept–Basic Principles of Design. CRC Press.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bongaerts, J., Zoske, S., Weidner, U., Linden, G., 1995. Transcriptional regulation of the proton translocating NADH dehydrogenase (nuoA-N) of *Escherichia coli* by electron acceptors, electron donors and gene regulators. Mol. Microbiol. 16, 521–534. https://doi.org/10.1111/j.1365-2958.1995.tb02416.x.

Bustamante, M.Á., Michelozzi, M., Barra Caracciolo, A., Grenni, P., Verbokkem, J., Geerdink, P., Safi, C., Nogues, I., 2020. Effects of soil fertilization on terpenoids and other carbon-based secondary metabolites in *Rosmarinus officinalis* plants: a comparative study. Plants 9, 830. https://doi.org/10.3390/plants9070830.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Calzadilla, P.I., Carvalho, F.E.L., Gomez, R., Lima Neto, M.C., Signorelli, S., 2022. Assessing photosynthesis in plant systems: a cornerstone to aid in the selection of resistant and productive crops. Environ. Exp. Bot. 201, 104950 https://doi.org/10.1016/j.envexpbot.2022.104950.

Cao, Y., Song, H., Zhang, L., 2022. New insight into plant saline-alkali tolerance mechanisms and application to breeding. Int. J. Mol. Sci. 23 (24), 16048. https://doi.org/10.3390/ijms232416048.

Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T.-J., 2004. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecol. Lett. 8, 148–159. https://doi.org/10.1111/j.1461-0248.2004.00707.x.

Croft, H., Chen, J.M., Luo, X., Bartlett, P., Chen, B., Staebler, R.M., 2017. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. Glob. Chang. Biol. 23, 3513–3524. https://doi.org/10.1111/gcb.13599.

Dazzi, C., Lo Papa, G., 2013. In: Costantini, E.A.C., Dazzi, C. (Eds.), Soil Threats-The Soils of Italy. Springer, Netherlands, Dordrecht, pp. 205–245. https://doi.org/10.1007/978-94-007-5642-7_8.

Di Lenola, M., Barra Caracciolo, A., Ancona, V., Laudicina, V.A., Garbini, G.L., Mascolo, G., Grenni, P., 2020. Combined effects of compost and Medicago sativa in recovery a PCB contaminated soil. Water 12 (3), 860. https://doi.org/10.3390/w12030860.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688. https://doi.org/10.1038/s41587-020-0548-6.

Dube, J.P., Valverde, A., Steyn, J.M., Cowan, D.A., van der Waals, J.E., 2019. Differences in bacterial diversity, composition and function due to long-term agriculture in soils in the eastern free state of South Africa. Diversity 11, 61. https://doi.org/10.3390/d11040061.

European Commission (EC), 2006. Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions – Thematic Strategy for Soil Protection. Available at: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=CELEX:52006DC0231.

Fang, S., Hou, X., Liang, X., 2021. Response mechanisms of plants under saline-alkali stress. Front. Plant Sci. 12, 667458 https://doi.org/10.3389/fpls.2021.667458.

Fedeli, R., Celletti, S., Loppi, S., Vannini, A., 2023. Comparison of the effect of solid and liquid digestate on the growth of lettuce (Lactuca sativa L.) plants. Agronomy 13 (3), 782. https://doi.org/10.3390/agronomy13030782.

Garbini, G.L., Grenni, P., Rauseo, J., Patrolecco, L., Pescatore, T., Spataro, F., Barra Caracciolo, A., 2022. Insights into structure and functioning of a soil microbial community amended with cattle manure digestate and sulfamethoxazole. J. Soils Sediments 22, 2158–2173. https://doi.org/10.1007/s11368-022-03222-y.

Grenni, P., Barra Caracciolo, A., Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., 2009. Changes in the microbial activity in a soil amended with oak and pine residues and treated with linuron herbicide. Appl. Soil Ecol. 41, 2–7. https://doi.org/10.1016/j.apsoil.2008.07.006.

Grenni, P., Rodríguez-Cruz, M.S., Herrero-Hernández, E., Marín-Benito, J.M., Sánchez-Martín, M.J., Barra Caracciolo, A., 2012. Effects of wood amendments on the degradation of terbuthylazine and on soil microbial community activity in a clay loam soil. Water Air Soil Pollut. 223, 5401–5412. https://doi.org/10.1007/s11270-012-1289-z.

Grenni, P., Barra Caracciolo, A., Patrolecco, L., Ademollo, N., Rauseo, J., Saccà, M.L., Mingazzini, M., Palumbo, M.T., Galli, E., Muzzini, V.G., Polcaro, C.M., Donati, E., Lacchetti, I., Di Giulio, A., Gucci, P.M.B., Beccaloni, E., Mininni, G., 2018. A bioassay battery for the ecotoxicity assessment of soils conditioned with two different commercial foaming products. Ecotoxicol. Environ. Saf. 148, 1067–1077. https://doi.org/10.1016/j.ecoenv.2017.11.071.

Grenni, P., Barra Caracciolo, A., Patrolecco, L., 2019. Site-specific protocols for evaluating environmental compatibility of spoil materials produced by EPB-TBMs. In: Peila, D., Viggiani, G., Celestino, T. (Eds.), Tunnels and Underground Cities: Engineering and Innovation Meet Archaeology, Architecture and Art. CRC Press, pp. 360–366. https://doi.org/10.1201/9780429424441.

Innangi, M., Niro, E., D'Ascoli, R., Danise, T., Proietti, P., Nasini, L., Regni, L., Castaldi, S., Fioretto, A., 2017. Effects of olive pomace amendment on soil enzyme activities. Appl. Soil Ecol. 119, 242–249. https://doi.org/10.1016/j.apsoil.2017.06.015.

Jin, Q., Kirk, M.F., 2018. pH as a primary control in environmental microbiology: 1. Thermodynamic perspective. Front. Environ. Sci. 6, 21. https://doi.org/10.3389/fenvs.2018.00021.

Kirschbaum, M.U.F., 2011. Does enhanced photosynthesis enhance growth? Lessons learned from CO_2 enrichment studies. Plant Physiol. 155, 117–124. https://doi.org/10.1104/pp.110.166819.

Kumar, A., Verma, J.P., 2018. Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol. Res. 207, 41–52. https://doi.org/10.1016/j.micres.2017.11.004.

Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 198, 656–669. https://doi.org/10.1111/nph.12235.

Li, Z.-H., Wang, Q., Ruan, X., Pan, C.-D., Jiang, D.-A., 2010. Phenolics and plant allelopathy. Molecules 15, 8933–8952. https://doi.org/10.3390/molecules15128933.

Li, F., Kautz, T., Pude, R., Köpke, U., 2012. Nodulation of lucerne (Medicago sativa L.) roots: depth distribution and temporal variation. Plant Soil Environ. 58, 424–428.

Liu, C., Liu, Y., Lu, Y., Liao, Y., Nie, J., Yuan, X., Chen, F., 2019. Use of a leaf chlorophyll content index to improve the prediction of above-ground biomass and productivity. PeerJ 6, e6240. https://doi.org/10.7717/peerj.6240.

Liu, D., Ma, Y., Rui, M., Lv, X., Chen, R., Chen, X., Wang, Y., 2022. Is high pH the key factor of alkali stress on plant growth and physiology? A case study with wheat (Triticum aestivum L.) seedlings. Agronomy 12, 1820. https://doi.org/10.3390/agronomy12081820.

Lopes, L.D., Hao, J., Schachtman, D.P., 2021. Alkaline soil pH affects bulk soil, rhizosphere and root endosphere microbiomes of plants growing in a Sandhills ecosystem. FEMS Microbiol. Ecol. 97 (4), fiab028. https://doi.org/10.1093/femsec/fiab028.

Louca, S., Doebeli, M., 2018. Efficient comparative phylogenetics on large trees. Bioinformatics 34, 1053–1055. https://doi.org/10.1093/bioinformatics/btx701.

Mariani, L., Grenni, P., Barra Caracciolo, A., Donati, E., Rauseo, J., Rolando, L., Patrolecco, L., Barra Caracciolo, A., Patrolecco, L., 2020. Toxic response of the bacterium Vibrio fischeri to sodium lauryl ether sulphate residues in excavated soils. Ecotoxicology 29, 815–824. https://doi.org/10.1007/s10646-020-02202-7.

Mariani, L., Grenni, P., Barra Caracciolo, A., Pescatore, T., Spataro, F., Rauseo, J., Narciso, A., Rolando, L., Patrolecco, L., 2022. Use of the Heterocypris incongruens bioassay for assessing ecotoxicity of soils containing the anionic surfactant sodium lauryl ether sulphate (SLES). Ecol. Indic. 145, 109597 https://doi.org/10.1016/j.ecolind.2022.109597.

Matheson, F.E., 2008. Microcosms. In: Jorgensen, S.E., Fath, B. (Eds.), Encyclopedia of Ecology. Academic Press, pp. 2393–2397.

Mazzurco Miritana, V., Massini, G., Visca, A., Grenni, P., Patrolecco, L., Spataro, F., Rauseo, J., Garbini, G.L., Signorini, A., Rosa, S., Barra Caracciolo, A., 2020. Effects of sulfamethoxazole on the microbial community dynamics during the anaerobic digestion process. Front. Microbiol. 11, 537783 https://doi.org/10.3389/fmicb.2020.537783.

McKenna, B.A., Kopittke, P.M., Macfarlane, D.C., Dalzell, S.A., Menzies, N.W., 2019. Changes in soil chemistry after the application of gypsum and sulfur and irrigation with coal seam water. Geoderma 337, 782–791. https://doi.org/10.1016/j.geoderma.2018.10.019.

Milanović, V., Osimani, A., Cardinali, F., Taccari, M., Garofalo, C., Clementi, F., Ashoor, S., Mozzon, M., Foligni, R., Canonico, L., Ciani, M., Aquilanti, L., 2019. Effect of inoculated azotobacteria and Phanerochaete chrysosporium on the composting of olive pomace: microbial community dynamics and phenols evolution. Sci. Rep. 9, 16966. https://doi.org/10.1038/s41598-019-53313-z.

Msimbira, L.A., Smith, D.L., 2020. The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. Front. Sustain. Food Syst. 4, 106. https://doi.org/10.3389/fsufs.2020.00106.

Muscolo, A., Papalia, T., Settineri, G., Romeo, F., Mallamaci, C., 2019. Three different methods for turning olive pomace in resource: benefits of the end products for agricultural purpose. Sci. Total Environ. 662, 1–7. https://doi.org/10.1016/j.scitotenv.2019.01.210.

Oggeri, C., Fenoglio, T.M., Vinai, R., 2014. Tunnel spoil classification and applicability of lime addition in weak formations for muck reuse. Tunn. Undergr. Space Technol. 44, 97–107. https://doi.org/10.1016/j.tust.2014.07.013.

Omer, A.M., Mohamed, M.A., 2012. Effect of olive pomace phenols on the soil fungi and alfalfa yield. Res. J. Agric. Biol. Sci. 8, 55–61.

Onet, A., Dincă, L.C., Grenni, P., Laslo, V., Teusdea, A.C., Vasile, D.L., Enescu, R.E., Crisan, V.E., 2019. Biological indicators for evaluating soil quality improvement in a soil degraded by erosion processes. J. Soils Sediments 19, 2393–2404. https://doi.org/10.1007/s11368-018-02236-9.

Orgiazzi, A., Ballabio, C., Panagos, P., Jones, A., Fernández-Ugalde, O., 2018. LUCAS soil, the largest expandable soil dataset for Europe: a review. Eur. J. Soil Sci. 69, 140–153. https://doi.org/10.1111/ejss.12499.

Padan, E., Bibi, E., Ito, M., Krulwich, T.A., 2005. Alkaline pH homeostasis in bacteria: new insights. Biochim. Biophys. Acta (BBA) Biomembranes 1717, 67–88. https://doi.org/10.1016/j.bbamem.2005.09.010.

Pang, F., Solanki, M.K., Wang, Z., 2022. Streptomyces can be an excellent plant growth manager. World J. Microbiol. Biotechnol. 38, 193. https://doi.org/10.1007/s11274-022-03380-8.

Paraskeva, P., Diamadopoulos, E., 2006. Technologies for olive mill wastewater (OMW) treatment: a review. J. Chem. Technol. Biotechnol. 81, 1475–1485. https://doi.org/10.1002/jctb.1553.

Pinho, I.A., Lopes, D.V., Martins, R.C., Quina, M.J., 2017. Phytotoxicity assessment of olive mill solid wastes and the influence of phenolic compounds. Chemosphere 185, 258–267. https://doi.org/10.1016/j.chemosphere.2017.07.002.

Robles-Aguilar, A.A., Temperton, V.M., Jablonowski, N.D., 2019. Maize silage digestate application affecting germination and early growth of maize modulated by soil type. Agronomy 9 (8), 473. https://doi.org/10.3390/agronomy9080473.

Roy, B., Maitra, D., Ghosh, J., Mitra, A.K., 2022. Unique extremophilic Bacillus: their application in plant growth promotion and sustainable agriculture. In: Malik, J.A. (Ed.), Microbes and Microbial Biotechnology for Green Remediation. Elsevier, Amsterdam, Netherlands, pp. 287–304. https://doi.org/10.1016/B978-0-323-90452-0.00021-9.

Saccà, M.L., Barra Caracciolo, A., Di Lenola, M., Grenni, P., 2017. Ecosystem services provided by soil microorganisms. In: Lukac, M., Grenni, P., Gamboni, M. (Eds.), Soil Biological Communities and Ecosystem Resilience. Springer, Cham, pp. 9–24. https://doi.org/10.1007/978-3-319-63336-7.

Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., Salopek-Sondi, B., 2021. The role of polyphenols in abiotic stress response: the influence of molecular structure. Plants 10, 118. https://doi.org/10.3390/plants10010118.

Santini, T.C., Warren, L.A., Kendra, K.E., 2015. Microbial diversity in engineered haloalkaline environments shaped by shared geochemical drivers observed in natural analogues. Appl. Environ. Microbiol. 81, 5026–5036. https://doi.org/10.1128/AEM.01238-15.

Schloter, M., Dilly, O., Munch, J.C., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ. 98, 255–262. https://doi.org/10.1016/S0167-8809(03)00085-9.

Schmid, J., Sperl, N., Sieber, V., 2014. A comparison of genes involved in sphingan biosynthesis brought up to date. Appl. Microbiol. Biotechnol. 98, 7719–7733. https://doi.org/10.1007/s00253-014-5940-z.

Song, T., Xu, H., Sun, N., Jiang, L., Tian, P., Yong, Y., Yang, W., Cai, H., Cui, G., 2017. Metabolomic analysis of alfalfa (Medicago sativa L.) root-symbiotic rhizobia responses under alkali stress. Front. Plant Sci. 8, 1208. https://doi.org/10.3389/fpls.2017.01208.

Swartz, Talia H., Ikewada, S., Ishikawa, O., Ito, M., Krulwich, T.A., 2005. The Mrp system: a giant among monovalent cation/proton antiporters? Extremophiles 9, 345–354. https://doi.org/10.1007/s00792-005-0451-6.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M, 2014. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. PLoS ONE 9 (8), e105592. https://doi.org/10.1371/journal.pone.0105592.

Tao, J., Chen, Q., Chen, S., Lu, P., Chen, Y., Jin, J., Li, J., Xu, Y., He, W., Long, T., Deng, X., Yin, H., Li, Z., Fan, J., Cao, P., 2022. Metagenomic insight into the microbial degradation of organic compounds in fermented plant leaves. Environ. Res. 214, 113902 https://doi.org/10.1016/j.envres.2022.113902.

Turbé, A., De Toni, A., Benito, P., Lavelle, P., Ruiz, N., der Putten, W.H., Van Labouze, E., Mudgal, S., 2010. Soil biodiversity: functions, threats and tools for policy makers. In: Bio Intelligence Service, IRD, and NIOO, Report for European Commission (DG Environment). https://doi.org/10.2779/14571.

Ugulin, T., Bakonyi, T., Berčič, R., Urbanek Krajnc, A., 2015. Variations in leaf total protein, phenolic and thiol contents amongst old varieties of mulberry from the Gorizia region. Agricultura 12, 41–47. https://doi.org/10.1515/agricultura-2016-0006.

Ye, Y., Doak, T.G., 2009. A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput. Biol. 5, e1000465 https://doi.org/10.1371/journal.pcbi.1000465.

Zeng, Q., An, S., Liu, Y., 2017. Soil bacterial community response to vegetation succession after fencing in the grassland of China. Sci. Total Environ. 609, 2–10. https://doi.org/10.1016/j.scitotenv.2017.07.102.

Zhang, Z., Xu, W., Li, L., Huang, J., Deng, L., Wang, Q., 2021. Effects of temporal conservation measures on water erosion processes of disturbed soil accumulation in construction projects. J. Clean. Prod. 319, 128612 https://doi.org/10.1016/j.jclepro.2021.128612.