New BIOTECHNOLOGY 78 (2023) 131–140

Contents lists available at ScienceDirect

New BIOTECHNOLOGY

journal homepage: www.elsevier.com/locate/nbt

Effects of municipal waste compost on microbial biodiversity and energy production in terrestrial microbial fuel cells

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ARTICLE INFO

Keywords:
Exoelectrogenic bacteria
Bacterial community
Microbial activity
16 S rRNA gene sequencing
Electricity production

ABSTRACT

Microbial Fuel Cells (MFCs) transform organic matter into electricity through microbial electrochemical reactions catalysed on anodic and cathodic half-cells. Terrestrial MFCs (TMFCs) are a bioelectrochemical system for bioelectricity production as well as soil remediation. In TMFCs, the soil is the ion-exchange electrolyte, whereas a biofilm on the anode oxidises organic matter through electroactive bacteria. Little is known of the overall microbial community composition in a TMFC, which impedes complete exploitation of the potential to generate energy in different soil types. In this context, an experiment was performed to reveal the prokaryotic community structure in single chamber TMFCs with soil in the presence and absence of a municipal waste compost (3% w/v). The microbial community was assessed on the anode and cathode and in bulk soil at the end of the experiment (54 days). Moreover, TMFC electrical performance (voltage and power) was also evaluated over the experimental period, varying the external resistance to improve performance. Compost stimulated soil microbial activity, in line with a general increase in voltage and power. Significant differences were observed in the microbial communities between initial soil conditions and TMFCs, and between the anode, cathode and bulk soil in the presence of the compost. Several electroactive genera (*Bacillus*, *Fulvivirga*, *Burkholdeira* and *Geobacter*) were found at the anode in the presence of compost. Overall, the use of municipal waste compost significantly increased the performance of the MFCs in terms of electrical power and voltage generated, not least thanks to the selective pressure towards electroactive bacteria on the anode.

1. Introduction

Bioelectrochemical systems (BES) are capable of converting chemical energy into electrical energy [1]. They rely on the biological activity of living organisms (electroactive or electrogenic bacteria) for reducing pollutants, recycling elements, synthetizing new products, and generating electricity [2]. Electrogenic bacteria or electroactive bacteria (EAB) can develop biofilms on electrodes and catalyze oxidations on the bioanode and/or reductions on the biocathode [1]. In the context of bioremediation, BES technologies have attracted a lot of interest in recent years for their biodegradation/bioremoval of several contaminants such as chlorinated compounds [3,4] and heavy metals [5–7]. BES are an ecofriendly technology, with zero pollution, long technical life, and sustainability [1–3], and can have multiple applications (e.g. electricity, hydrogen and chemicals production [8,9]). BES technology is in line with the circular economy model because waste can be used as the fuel material, converting it to bioenergy [10]. Microbial Fuel Cells (MFCs) are a type of BES which transforms organic waste into electricity through microbial electrochemical reactions catalyzed in the anodic and cathodic regions [11–15].

Abbreviations: ASV, amplicon sequencing variants; BES, Bioelectrochemical system; DAPI, 4′,6-Diamidino-2-phenylindole dihydrochloride; DDE, dichlorodiphenyldichloroethylene; DHA, Dehydrogenase Activity; EAB, Electrogenic or Electroactive Bacteria; EET, Extracellular electron transfer; MFC, Microbial Fuel Cell; OCV, Open Circuit Voltage; PAHs, polycyclic aromatic hydrocarbons; TMFC, terrestrial microbial fuel cell; TPF, 2,3,5- triphenyl formazan; TTC, triphenyltetrazoliumchloride.

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https://doi.org/10.1016/j.nbt.2023.10.009
Received 25 January 2023; Received in revised form 12 October 2023; Accepted 21 October 2023
Available online 22 October 2023

Electroactive biofilms (also termed electrochemically active biofilms) can develop in both natural ecosystems (soils, sediments, seawater or freshwater), and in a wide range of different microbe-rich environments, such as sewage sludge, activated sludge or industrial and domestic effluents [16]. The composition of these biofilms is not completely known, however specific anaerobic bacterial strains (e.g. *Geobacter sulfurreducens, Rhodoferax ferrireducens, Shewanella* sp.) with a catalytic ability, and a capacity to exchange electrons with solid substrates (i.e. electrodes) have been identified [4,9,17]. These natural microorganisms are able to generate electricity through various metabolic processes [18]. More recently, EAB activity in Terrestrial MFCs have been tested as a promising variant for bioelectricity generation. A terrestrial MFC comprises two electrodes separated by a layer of soil (the electrolyte) and connected through an external electrical circuit (usually a wire or electrical load). EAB growing in anaerobic conditions develop a biofilm on the anode (located at the bottom of an MFC) and can catabolize (oxidize) organic compounds (including several contaminants), producing and releasing protons (H^+), electrons (e^-) and carbon dioxide (CO_2). Currently, about 100 microorganisms have been described as EABs, able to perform extracellular electron transfer (EET) [19] which coordinate their development, activity and mobility with advantageous cell-to-cell interactions. The EET pathways of the gram-negative *Geobacter sulfurreducens* and *Shewanella oneidensis* have been investigated; these bacteria can perform anaerobic respiration utilizing a metal such as iron (III) or manganese (IV) as a terminal electron acceptor [20]. Other bacteria such as *Klebsiella, Azonexus, Comamonas, Petrimonas* and *Acidivorax* have been also found to show EET capacity, however the final electron acceptors have not been well identified [18].

Electrons from bacteria on the anode are transferred using different mechanisms, such as the electron transport chains through membrane cytochrome or conductive pili [21]. Subsequently, electrons are transported from the anode to the cathode (where oxygen acts as an electron acceptor) through an external circuit. Protons flow from the anode to the cathode through the soil. The latter provides the biofilm bacterial community and the organic matter [22]. Carbon-based materials, such as graphite fiber brushes, rods, felts and fabrics, are used to design electrodes because they have high performance, low-cost and strong biocompatibility and a high electrical conductivity [23].

Terrestrial MFCs have been used for restoring soil or sediment from phenolic compound [24], polycyclic aromatic hydrocarbon [25], PAH [26] and dichlorodiphenyldichloroethylene (DDE) contamination [22,27]. However, the efficiency of terrestrial MFCs still needs to be improved especially if compared to that of conventional liquid-based MFCs. In fact, terrestrial MFCs are more complex compared to other MFCs where the electrolyte is a liquid (e.g. water or wastewater). Owing to soil heterogeneity and its variable abiotic factors (e.g. pH, texture, organic carbon content and water content) terrestrial MFC performance can vary significantly [4,28,29]. In particular, organic matter content can be a key factor in MFC electricity production and durability as well as in decontamination process effectiveness [30]. Recent studies report that adding an external carbon source, such as glucose [31] or compost [22] to soil, can significantly improve performance of terrestrial MFC electroactive bacteria [32]. Compost is an organic fertilizer obtained from the treatment of organic waste. It is commonly produced by aerobic degradation of plant and food waste and organic materials (such as municipal waste). Compost is rich in organic carbon, active microbial communities and microelements, features which make it suitable for a wide variety of agronomic uses. It is well known that compost increases water retention, soil organic matter content, nutrients and cation exchange capacity [33,34]. For example, in soil MFCs [35] a cattle manure compost was added, enriched with 0.5 g/ml of urea, for increasing their power production. In another work, [36], two sets of MFCs were tested using soil amended with 2 type of compost from vegetable waste with different C:N ratio and then added a saline solution (5 g/L of NaCl); the authors obtained the best electrical performances (W/m^2) using the compost with the highest nitrogen concentration.

Although MFCs have been investigated in terms of energy production and engineering design, and some electroactive bacteria identified, current knowledge of overall microbial community composition inside a terrestrial MFC and its distribution between the anode, cathode and soil is very limited.

In this context, the structure and functioning of a microbial community in the presence/absence of a municipal waste compost were investigated in single-chamber terrestrial MFCs over 54 days. The bacterial community on the anode and cathode and in bulk soil were analyzed in terms of microbial activity (dehydrogenase activity) and total microbial abundance (DAPI counts). Moreover, bacterial composition was characterized by sequencing its 16 S rRNA gene (Miseq Illumina). The electrical performance was also evaluated measuring daily voltage and power over the experimental period.

2. Material and methods

2.1. Terrestrial MFC set-up

The soil was sampled from the first 30 cm of an abandoned agricultural field located 30 km north of Rome. It had a neutral pH (7), with 1.35% organic carbon and 0.15% total N, and the texture was 28% sand, 24% silt and 48% clay. The soil was air dried and stones, gravel and roots were removed. A municipal solid waste compost (organic carbon content: 26%), produced and supplied by Progeva Spa (Laterza, TA, Italy), was used. The compost quality was certified on the basis of ISO/IEC 17025, and the main characteristics were discussed in a previous report [37]. The compost (54 g) was added and mixed to 1746 g of dry soil to obtain 3% of compost (after adding the compost organic carbon and total nitrogen were 1.9% and 0.22%, respectively). A further 1800 g of soil were used without adding compost. About 600 g of soil were employed for each microbial fuel cell microcosm (MFC). Finally, the soil was saturated with water (30% of the water holding capacity).

Each MFC consisted of a single chamber with inert graphite electrodes (anode and cathode), which have been shown to be appropriate conductors and an easily available material [22]. Microcosms were set up as follows: 1 cm of wet soil layer was packed at the bottom of each MFC. The anode was then placed on top of the soil and after that, each cell was filled with 5 cm of soil. The soil and anode were squeezed in order to form a smooth layer and remove air bubbles. The cathode was then placed on top of the soil and exposed to air. A total of 6 terrestrial MFCs were assembled (3 with only soil: TMFC; 3 with soil and compost: TMFC+Compost). Some soil samples in the absence/presence of compost were collected before setting up the MFCs to assess the initial microbial community; they were termed Soil and Soil+Compost, respectively. At day 54, all the MFCs were disassembled, and the soil sampled in each cell in 3 different points: one in close contact with the anode (Anode), another in close contact with the cathode (Cathode) and a third one in the middle of each MFC (Bulk soil). At the end of the experiment (54 days) aliquots of initial soil samples from the Cathode, Bulk and Anode TMFC and TMFC+Compost conditions were used for microbiological analyses. Moreover, electrical measurements (power and voltage) were carried out daily over the experimental period.

2.2. Total microbial abundance and dehydrogenase activity

The total microbial abundance (N. cells/g soil) was estimated using the epifluorescence direct count under a microscope and using DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride) dye as the DNA intercalant. Formaldehyde-fixed soil samples (1 g for each replicate condition, in 3 sub-replicates) were used and processed as reported in detail in previous works [38,39].

The microbial activity was measured as dehydrogenase activity (DHA), which reflects the overall microbial respiration rate and therefore the biological oxidation of organic matter [40,41]. The method

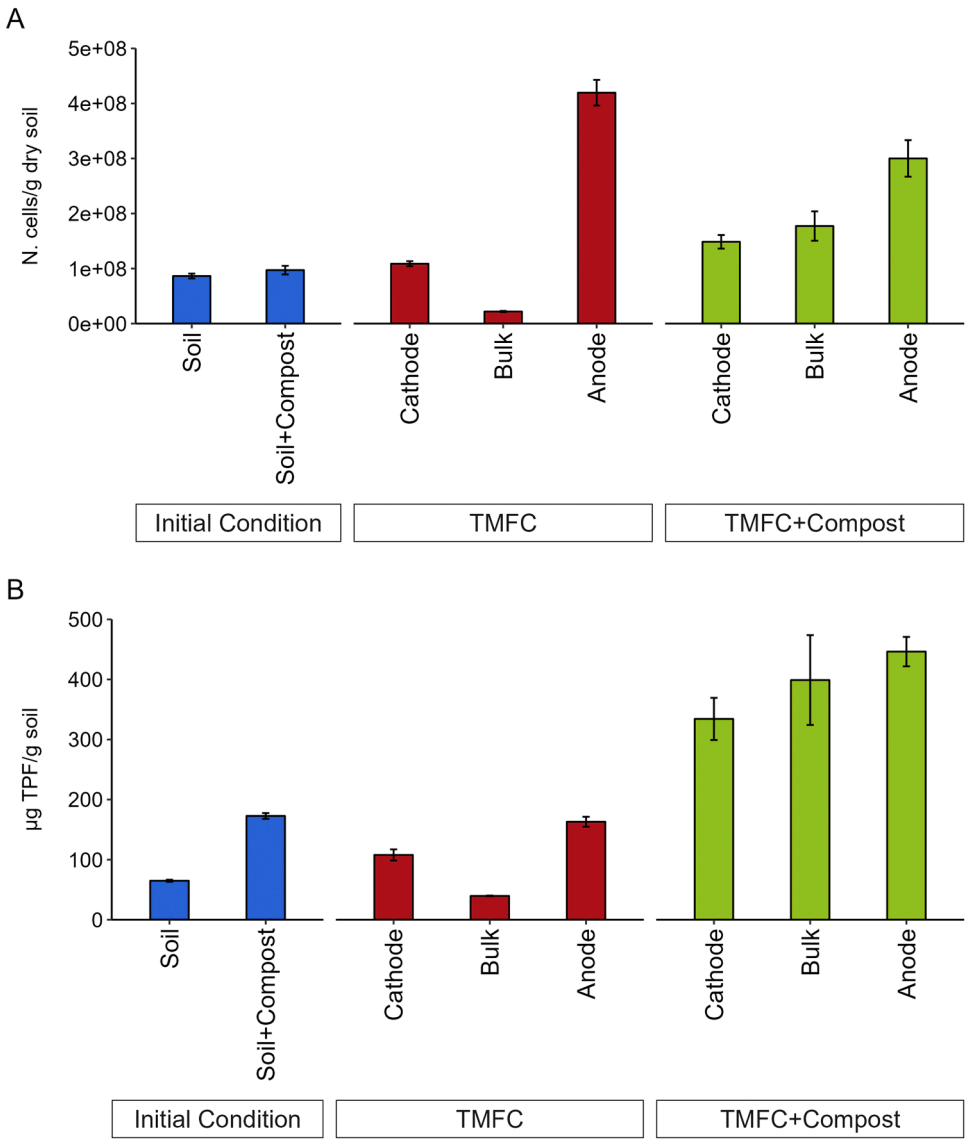

Fig. 1. Soil microbial community. **A.** Total Microbial Abundance (N. cells/g dry soil) in initial soil conditions (Soil and Soil+Compost, blue bars), TMFC (red bars), and TMFC+Compost (green bars). Vertical bars represent the standard error. **B.** Total dehydrogenase activity, (DHA, µg TPF/g soil) in initial soil conditions (Soil and Soil+Compost, blue bars), TMFC (red bars), and TMFC+Compost (green bars). Vertical bars represent the standard error.

applied is based on extraction and colorimetric determination of the color intensity of the 2,3,5-triphenyl formazan (TPF) produced from the reduction of the colorless 2,3,5-triphenyltetrazoliumchloride (TTC) in soil samples (6 g for each replicate condition, in 3 sub-replicates), 24 h after an incubation at 37°C in the dark [41]. Soil dehydrogenase activity was expressed as µg TPF/g dry soil and was measured with a Thermo Multiskan FC Microplate Photometer (Thermo Fisher Scientific; Waltham, MA, USA).

2.3. Prokaryotic community composition: DNA extraction, sequencing of 16 S rDNA and bioinformatic analyses

The effect of compost on the bacterial community structure and its possible modifications on Anode and Cathode and in Bulk soil was evaluated using a metabarcoding approach. Metabarcoding is the large-scale taxonomic identification of complex environmental samples via analysis of DNA sequences for short regions of genes [42], such as the regions of the 16 s rRNA gene.

Soil DNA was extracted from the initial soil (Soil and Soil+Compost) and from MFC (3 replicates for each sampling point: Anode, Cathode and Bulk soil) using the DNeasy PowerSoil kit (Qiagen, Valencia, CA, USA), following the manufacturer's recommendations. A DNA-free sample was also analyzed 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 Fisher Scientific, USA). DNA extracted was stored at −20 °C until sequencing.

The DNA extracted was used as the template for sequencing the hypervariable V3-V4 region of 16 S rRNA with MiSeq Illumina, using the 341F and 805R primers (Table S1). Nucleotide sequences were deposited in GenBank (accession number **PRJNA918324**).

The raw sequences were imported and demultiplexed using QIIME2 platform v2019.11 [43] and denoised with the DADA2 plug-in, as previously described [44]. The primers were removed using the DADA2 commands: "trim- left-f" for the forward primer and "trim-left-r" for the reverse one. These commands remove the sequences from its beginning to a specific position. The exact length of the primers was 17 nucleotides for the forward one and 21 nucleotides for the reverse one [45].

The amplicon sequencing variants (ASV) obtained with denoising process were sorted using the Silva 132 database (https:// www.arb-

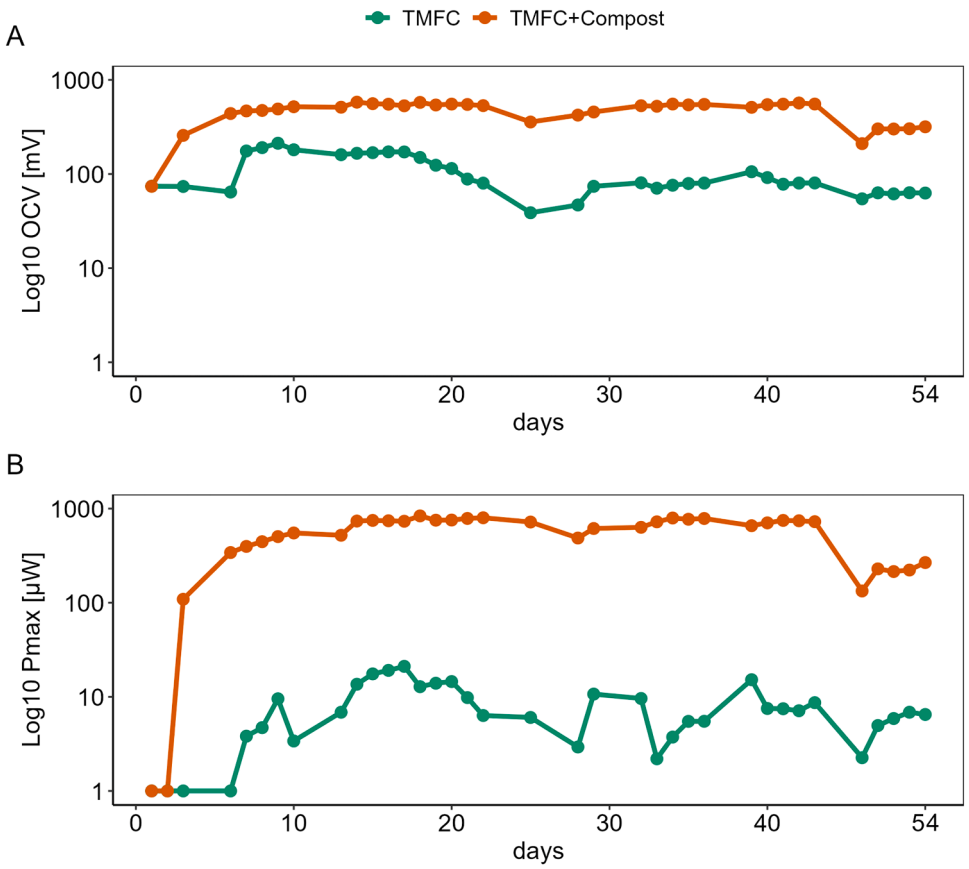

Fig. 2. Electrical parameters in TMFC and TMFC+Compost measured daily over the experimental period. **A:** Open Circuit Voltage (OCV) values reported as mV, measured just before closing the circuit. **B:** Maximum Electric power generated (Pmax, µW) obtained daily in closed circuit conditions. Pmax represents the maximum value recorded in TMFCs during the discharge phases for variable external resistors. The results on the y-axis are reported in a log scale in order to capture the difference in order of magnitude between the two configurations (TMFC and TMFC+Compost).

silva.de) with a naive Bayes classifier trained on the amplified regions with 80% confidence [46].

2.4. Electrical measurements

The electrical measurements were carried out daily to evaluate the MFCs' performance. A data logging device (measurement station device), previously designed [22], was implemented for measuring and obtaining (using Ohm's law) the main electrical parameters such as voltage (V) and current (µA) [47]. The current is also reported as current density (A/m^2), when divided by the electrode surface. The station device also controlled and modulated the external resistive load. Power generation (µW) and power density (W/m^2) were also calculated. The operating conditions of the device varied when obtaining an open or closed-circuit. During open circuit operation, a 'recharge' period was in action and the cell voltage increased, eventually reaching a stable voltage. When the external circuit was closed (by modulating the external resistance), electrons flowed from the anode towards the cathode and the voltage diminished following the polarization curve (discharge period).

In order to capture the differences between the electrical results in the presence and absence of compost, the Open Circuit Voltage values (OCV, mV) and the electric power generated (µW) are reported in the logarithm base 10 scale. Because the MFC maximum electrical output was obtained when the internal resistance was close to the external one [48], the power produced was calculated by closing the electrical circuit and varying the external resistances (112.5, 300.8, 530.8, 990.6, 2983.3, 4976.0 and 9957.7 Ohm). This made it possible to identify the external resistance value at which the maximum electrical power (Pmax) was generated.

The daily electrical measurement phase consisted of several successive steps. At each one, the circuit was closed over one of the 7 resistance values for 15 s. Between two successive measurements with different resistance, the circuit remained open for 250 s to recover the undisturbed open circuit voltage condition. Due to the significant difference in magnitude between the test conditions, voltage was plotted on a logarithmic scale.

2.5. Diversity indices and statistical analyses

The diversity of the prokaryotic community was analyzed using the Evenness and Shannon diversity indices, while the Chao 1 index [49] was used as an estimator of potential richness. All the statistical analyses and graphic elaboration were performed using R (4.0.4 version https://www.r-project.org). The effects of compost on the prokaryotic community in the initial conditions (Soil and Soil+Compost) and TMFCs and the differences between Anode, Cathode and Bulk soil inside each cell, were evaluated with a principal-coordinate analysis (PCoA) of the ASVs, based on Bray-Curtis distances. A multivariate ANOVA with permutations (PERMANOVA) was applied in order to assess significance. Pairwise PERMANOVA [50] was performed using the function *pairwise.perm.manova* from the package *RVAideMemoire* [51] in order to evaluate the significance of ASV changes in the prokaryotic composition in the different experimental conditions. A one-way ANOVA together with TukeyHSD as the post-hoc test [52] was performed to find significant differences among experimental conditions within the Alpha-diversity indices (Chao1, Shannon, and Evenness), and for microbial abundance, dehydrogenase activity and prokaryotic (genera and classes)

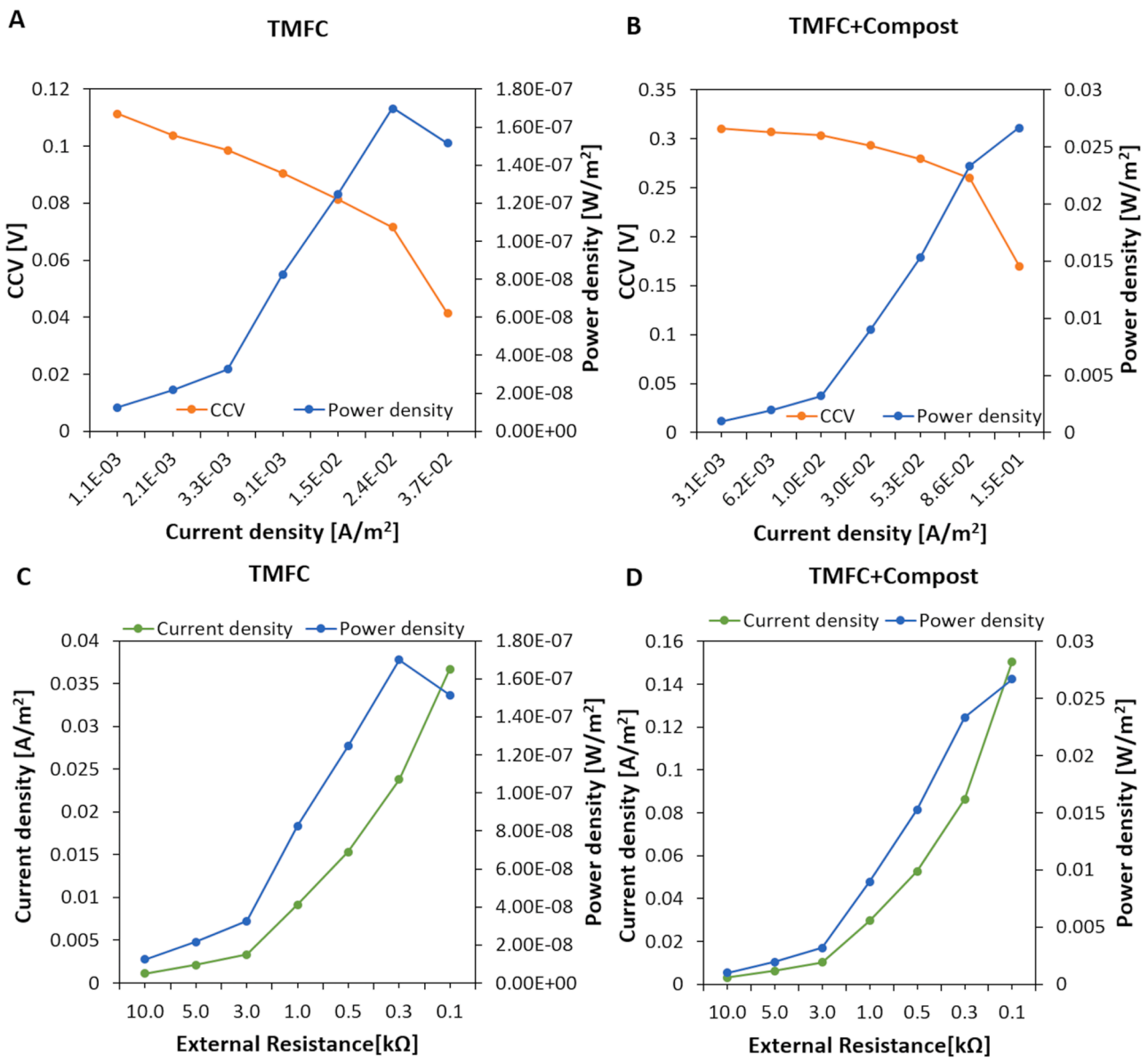

Fig. 3. Polarization curves, power density and current density detected at day 52 in TMFC and TMFC+Compost. **A and B**: Polarization curves (orange) obtained plotting the closed circuit voltage (CCV, expressed as V) and the Current density (A/m^2). In blue, Power density (W/m^2). **C and D**: Current density (A/m^2, green) and power density (W/m^2, blue) obtained as a function of the external resistance in TMFC and TMFC+Compost, respectively.

taxa.

The abundances of the prokaryotic community were normalized to *z-scores* and displayed in a heatmap generated by *Complex Heatmap* [53]. In the heatmap, bacterial genera and experimental conditions were grouped in accordance with hierarchical clustering dendrograms, which are shown at the top and on the left side of the heatmaps.

3. Results and discussion

An overall increase in microbial abundance and activity was observed on the cathodes and anodes of the MFCs, with values significantly higher than those in the initial soil (Fig. 1A,B).

The highest microbial abundance values were observed on the anodes in both the TMFC and TMFC+Compost conditions. This result was presumably due to bacteria migration from soil to the electrodes by quorum sensing [54] for the forming of bioactive biofilms [4], confirming that the bio-electrochemical reactions are triggered on the anode.

A positive effect on microbial activity was found when adding compost (Fig. 1B, TMFC+Compost) not only on the anode, but also on the cathode and in the bulk soil and this promoted an overall increase in active microbial populations in the overall microbial fuel cell. The effectiveness of organic amendments in increasing microorganism abundance and organic matter content has been also demonstrated in other works [37,39].

The effect of compost on microbial activity was reflected in the higher performance of the TMFC+Compost than TMFC, as shown by the electric power and voltage values (Fig. 2A,B). The MFCs started to produce electricity (open circuit voltage, OCV) in the same way (day 0: 74 mV in both TMFC and TMFC+Compost); see Fig. 2A. However, in the presence of compost (TMFC+Compost) a sharp increase of up to 467 mV (5 days) in OCV values was observed, while, during the same time, TMFC only reached 175 mV. The OCV remained stable in the 480–600 mV range in TMFC+Compost and with values significantly higher than those recorded in TMFC (range: 80–210 mV). The performance of the cells dropped after 45 days in both TMFC and

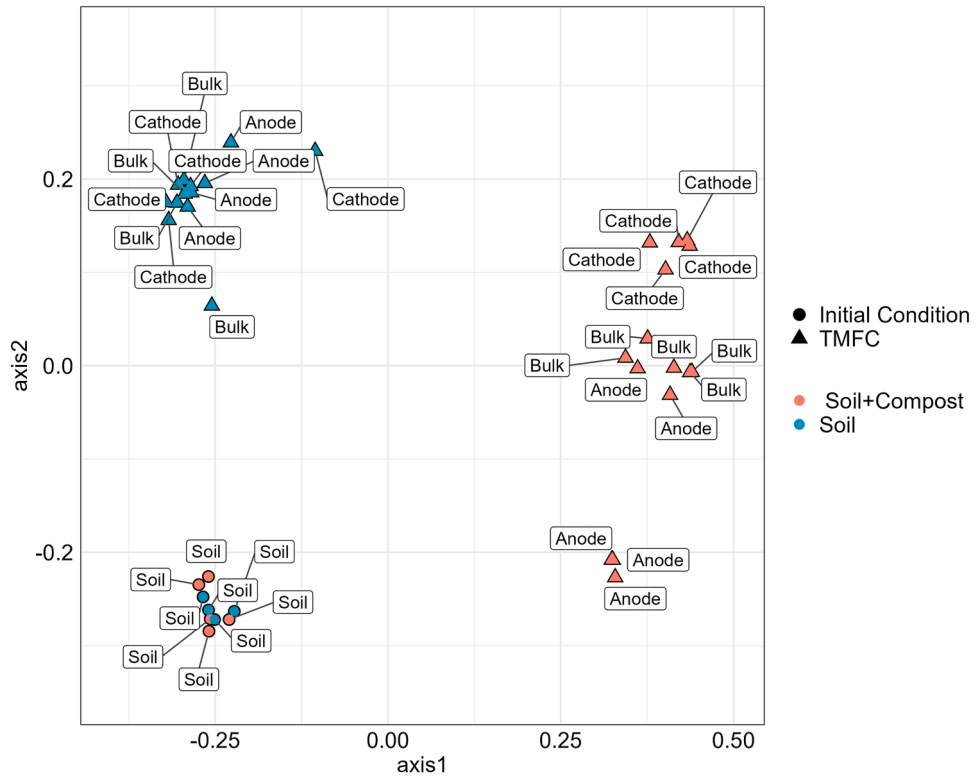

Fig. 4. Principal component analysis based on Bray-Curtis distance matrix calculated for ASV distribution. In pink the TMFC amended with compost; in light blue the TMFC without compost. Dots are for initial soil conditions (Soil, Soil+Compost); triangles for TMFC.

TMFC+Compost and for this reason the experiment was disassembled at day 54.

In line with the voltage levels, the electrical power values were significantly ($p < 0.05$) higher (ranging from 700 to 800 μW, with maximum peaks of 836 μW, Fig. 2B) in the presence of compost than in the TMFC (range: 10 and 20 μW; Fig. 2B). Interestingly, between days 14 and 45 of the experiment, the MFCs showed a stable operating phase in terms of OCV and electrical productivity (Figure2A and 2B), and the polarization curves remained relatively stable (see for example Supplementary Materials, Fig. S1 for day 41).

At day 52, close to the end of the experiment, the electrical power generated (Pmax in Fig. 2B) reached its maximum with external resistance values of about 300 Ω and 113 Ω (Fig. 3C,D) were registered with the measurement station device resistors for TMFC and TMFC+Compost, respectively (see also Fig. S2 for day 51). The internal resistance of the TMFC was estimated by analyzing the slope of the ohmic region in the polarization curves (orange lines in Fig. 3A,B). In TMFC (without compost) this value was about 320 Ω, close to the external resistance of about 300 Ω required for maximum power (Fig. 3C, left side). In the presence of compost, the internal resistance was 75 Ω (Fig. 3B, right side), and this value was also close to the external resistance of about 113 Ω, required for obtaining the maximum power (Fig. 3D, right side) [48]. The latter result is in line with the highest microbial activity in the TMFC+Compost. The compost, rich in organic matter, was presumably used as a substrate by microorganisms and favored bacterial development. Bacteria increased organic compound oxidation, releasing H^+ and electrons and supporting the generation of an electric current towards the anode, as previously reported [22,55,56]. Interestingly, positive correlations ($p < 0.001$, R: 0.80) were found between voltage (OCV) and microbial activity (DHA) and power and microbial activity (DHA).

3.1. Microbial community diversity and composition

The PCoA (Fig. 4), based on Bray-Curtis distances of the ASVs of the prokaryotic community shows that there are significant differences among the experimental conditions. A shift in ASV distribution was observed between the initial soil (Soil and Soil+Compost) and the MFCs.

Table 1
Diversity indices (Chao1, Shannon and Evenness) estimated on ASV (Amplicon Sequences Variant) abundances that have passed the denoise step in initial condition (Soil and Soil+Compost) and in TMFC and TMFC+Compost.

		Chao01 ± e.s.	Shannon (H) ± e.s.	Evenness (E) ± e.s.
	Soil	440 ± 45.80	8.70 ± 0.13	0.90 ± 0.02
	Soil+Compost	544 ± 62.04	7.17 ± 0.15	0.92 ± 0.01
TMFC	Cathode	826 ± 102.20	7.74 ± 0.16	0.84 ± 0.02
	Bulk	582 ± 57.77	7.72 ± 0.21	0.87 ± 0.02
	Anode	696 ± 78.22	8.71 ± 0.19	0.83 ± 0.02
TMFC	Cathode	807 ± 188.45	8.01 ± 0.18	0.86 ± 0.01
+ Compost	Bulk	806 ± 127.95	8.13 ± 0.52	0.86 ± 0.01
	Anode	708 ± 74.79	8.28 ± 0.13	0.83 ± 0.02

The TMFCs selected and activated soil microbial populations involved in electrochemical activity, inducing a change of ASV distribution and an increase in the potential number of species, as shown by the Chao1 index values (Table 1). Indeed, significant ($p < 0.05$) differences were found between the initial soil samples (Soil and Soil+Compost, dots), where the prokaryotic community was dominated by *Actinobacteria* (40% in Soil and 38% in Soil+Compost) followed by the *Alphaproteobacteria*, *Bacilli* and *Gammaproteobacteria* classes (Fig. 5).

Actinobacteria numbers decreased in the TMFCs (Fig. 5, TMFC and TMFC+Compost), and other classes, such as *Gammaproteobacteria*, *Deltaproteobacteria* and *Bacterioidetes* (gram-negative), increased. Interestingly, adding compost not only positively influenced the performance of the microbial fuel cells (Fig. 2A,B), but also promoted a different ($p < 0.05$) distribution of the microbial populations with respect to the other condition (Fig. 4, TMFC+Compost, pink triangles vs TMFC, blue triangles). A co-dominance of *Actinobacteria*, *Alphaproteobacteria* and

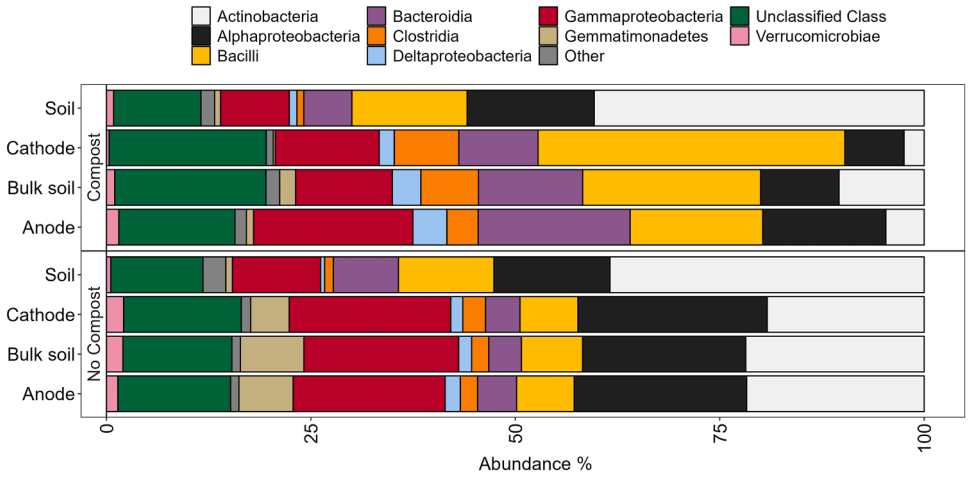

Fig. 5. Class relative abundance (% of ASV) at the end of the experiment, in both starting conditions (Soil and Soil+Compost) and in TMFC (TMFC and TMFC+Compost) Anode, Bulk and Cathode.

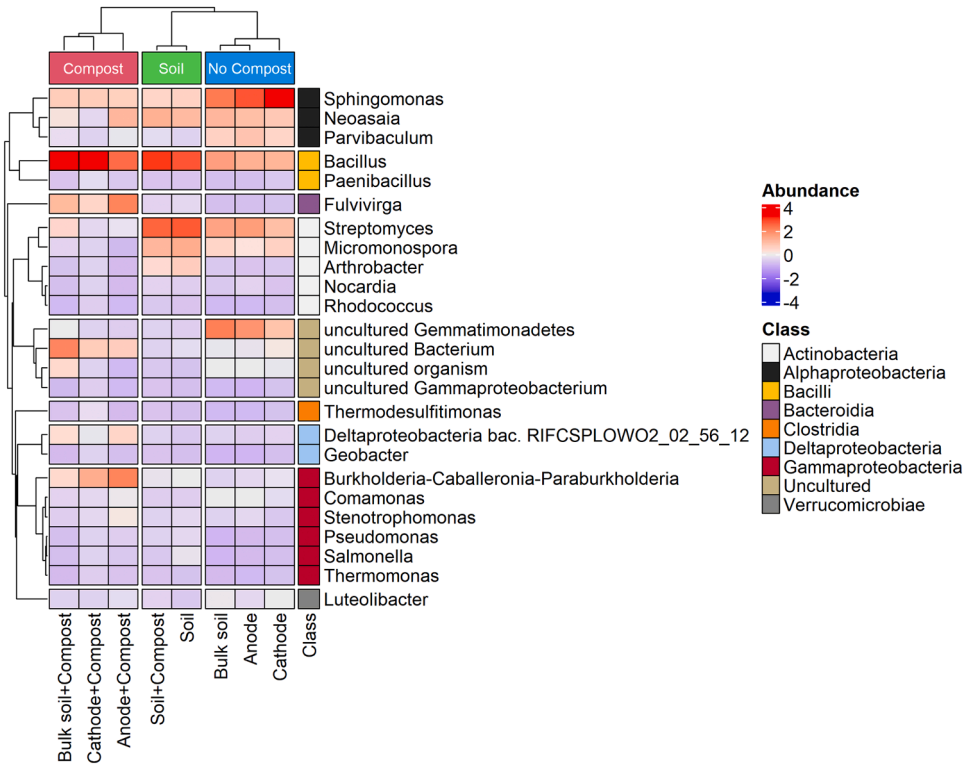

Fig. 6. Heatmap of main bacterial genera identified by ASVs. The values are normalized by z-score. Higher genera abundances are in red, while low abundances are in blue. Genera and conditions are grouped in accordance with a hierarchical clustering dendrogram, at the top and on the left of the heatmap.

Gammaproteobacteria (ca. 20% each) was observed in the TMFC condition, with no significant differences inside each cell and among the three regions analyzed (Anode, Bulk and Cathode, Fig. 5).

Bacilli, *Bacteroidia*, *Clostridia*, *Deltaproteobacteria* and *Gammaproteobacteria* were in higher abundance in TMFC+Compost than in TMFC (Fig. 5). These classes include several bacterial genera recognized for their exoelectrogenic abilities [57–59], such as *Geobacter*, a genus belonging to *Deltaproteobacteria*, which was found in the TMFC+Compost, in particular on the anode, with higher values than in other points ($p < 0.05$). More specifically, the main bacterial genera identified in the MFCs are shown in Fig. 6. The heatmap reports the most abundance genera (25 genera with at least 1% of abundance within the overall dataset, net of "unclassified" ones). The identified genera cover 60–70% of total sequences (ASV). The abundance is reported with different colour: from blue the lowest value, to red colour the highest one.

The initial soil conditions were dominated by *Bacillus* (19% Soil; 22% Soil+Compost) and *Streptomyces* (19% Soil; Soil +Compost 20%; Fig. 6), both gram-positive bacteria. In both TMFC and TMFC+Compost, a significant ($p < 0.01$) shift in overall genera distribution was found, in line with the classes observed (Fig. 5).

In the TMFC condition, the dominant genus was *Sphingomonas* (*Alphaproteobacteria*, 18%), followed by *Bacillus* (10.5%) and *Streptomyces* (*Actinobacteria*, 10.5%), without any significant differences between anode, bulk and cathode. On the other hand, a significant ($p < 0.05$) difference between bacterial genera was found in TMFC+Compost between anode, bulk and cathode.

In the TMFC+Compost, where the highest values for electrical power were observed, (100 times higher than in TMFC, Fig. 2), the dominant bacteria was *Bacillus*. In particular on the anode *Bacillus* was at 16%, *Fulvivirga* at 14% and *Burkholdeira* at 13% (Fig. 6). *Bacillus* is able to grow in the absence of oxygen by using nitrate or nitrite as terminal electron acceptors and producing a biofilm [60], and it is biocompatible with the carbon electrodes of terrestrial MFCs, resulting in a more efficient electron transfer and energy gain [61]. Indeed, *Bacillus thuringiensis* inoculated in MFCs was found to form a biofilm on the electrodes [62,63].

Fulvivirga, the second most abundant genus on the anode in TMFC+Compost, belongs to the *Bacteroidia* class, which is able to metabolize several organic compounds and comprises bacteria reported to be found on the anodes of microbial fuel cells [58]. The presence of this genus may therefore have improved the availability of some metabolic and electrogenic substrates [64,65], which, in turn, increased bacterial activity in the high-performing TMFC+Compost. *Fulvivirga* has also, in other studies, been found to be able to accept electrons from cathodes [21,66], and this is the first time in which it has been observed on an anode. The fact that well-known exoelectrogenic bacteria, such as *Shewanella oneidensis* and *Geobacter sulfurreducens*, have protein complexes as both electron donors on the anode (exoelectrogenic) and electron acceptors on the cathode (electrotrophic) [67], does not exclude *Fulvivirga* as a possible exoelectrogenic bacterium. The latter considerations need to be better investigated.

Finally, *Burkholdeira* has been reported to be an exoelectrogenic genus [59] and it has been found on the anode of microbial fuel cells where it was able to use organic carbon as an electron donor and to perform denitrification, producing nitrogen and maintaining an anaerobic environment on the anode [68]. This bacterium which is commonly found in soil and has a wide metabolic capacity to degrade various chemicals, such as triazines [69,70] and hydrocarbons [71–73], may be a good potential candidate for both producing energy and degrade contaminants in TMFC applications.

4. Conclusions

The overall results show how MFC technology can also be applied to recovering poor soils, through adding organic compounds such as compost, in order to stimulate the naturally occurring exoelectrogenic bacteria to produce electrical power. The use of municipal waste compost proved to be effective and necessary by significantly increasing the performance of the microbial fuel cells, and also achieving a lower internal resistance, while otherwise there would have been low electricity and power output. In addition, the slight increase in organic matter made it possible to select different microbial communities (including chemical degrading bacteria) in the different compartments of the microbial cell, a phenomenon not observed in the absence of compost. Several advantages of applying compost from municipal organic waste were found, including its low cost and the use of a by-product in line with the circular economy, while achieving results comparable to terrestrial microbial cells with more expensive carbon substrates such as acetate, lactate and glucose.

This study forms a starting point for subsequent bioremediation applications with terrestrial microbial cells in contaminated soils that are poor in organic matter. This trait can also be associated with the removal of organic contaminants and heavy metals inside TMFCs in a bioeconomy context and the possibility of their application as a nature-based solution.

Funding

This research was partially funded by the PHOENIX COST Action CA19123: Protection, Resilience, Rehabilitation of damaged environment, https://www.cost-phoenix.eu.

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.

Acknowledgments

Special thanks to Claudio Scardamaglio for his help in metagenomic data processing. Moreover, the Authors thank Francesca Falconi (CNR-IRSA) for performing the organic carbon and nitrogen analyses of soil.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbt.2023.10.009.

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