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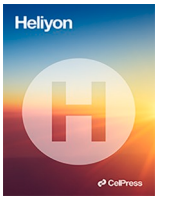

Correlation between microbial communities and volatile organic compounds in an urban soil provides clues on soil quality towards sustainability of city flowerbeds

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ABSTRACT

Soil functionality is critical to the biosphere as it provides ecosystem services relevant for a healthy planet. The soil microbial composition is significantly impacted by anthropogenic activities, including urbanization. In this context, the study of soil microorganisms associated to urban green spaces has started to be crucial toward sustainable city development. Microbes living in the soil produce and degrade volatile organic compounds (VOCs). The VOC profiles may be used to distinguish between soils with various characteristics and management practices, reflecting variations in the activity of soil microbes that use a variety of metabolic pathways. Here, a combined approach based on DNA metabarcoding and GC-MS analysis was used to evaluate the soil quality from urban flowerbeds in Prato (Tuscany, Italy) in terms of microbial biodiversity and VOC emission profiles, with the final aim of evaluating the possible correlation between composition of microbial community and VOC patterns. Results showed that VOCs in the considered soil originated from anthropic and biological activity, and significant correlations between specific microbial taxa and VOCs were detected. Overall, the study demonstrated the feasibility of the use of microbe-VOC correlation as a proxy for soil quality assessment in urban soils.

1. Introduction

In the current Anthropocene era, the importance of soil quality, and its impact on the sustainability of urban environments, is becoming more and more clear as cities continue to develop and expand. As municipalities promote projects implementing green areas intended to restore and enhance ecosystem services, understanding the biological dynamics of urban soil microbiome is becoming a topic of interest not only at scientific level but also for citizen and policy makers [1–3]. The quality of urban soils plays a critical role in

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creating and maintaining urban green spaces, which are essential for promoting environmental sustainability, enhancing human health and well-being, and providing social and economic benefits to urban communities [3]. Urban soils have been defined so far as soils that are heavily mixed with materials that are different from those found in nearby agricultural or forest areas, have a surface layer of more than 50 cm, and have been significantly altered by human activity through mixing, material import and export, and contamination [4]. They are distinguished from rural soils due to the degree of influence of human activities [5]. This classification allows to exclude soils in parks and gardens, since they are regarded as belonging more to agricultural than urban soils [4].

Soils in urban areas are affected by chemical contamination that is recognized as a global threat that involves all environments and is deeply felt in the urban ones, due to the presence of many sources of pollution [6]. For instance, rainwater can dissolve and carry pollutants from the air, including volatile organic compounds (VOCs), and deposit them onto the soil surface [7]. Additionally, road traffic is a source of direct and indirect urban soil contamination because of road dust collection and subsequent atmospheric transfer as well as volatile and particulate emissions from moving cars leading both to deposition into the soil surface and to formation of secondary aerosol [8]. Thus, urban soils are unique and complex systems that are exposed to a multitude of stressors that can have significant impacts on their quality.

Urban soil can be both a sink and source of VOCs [9,10]. Soil VOCs can be divided in two groups: VOCs from anthropic activity origins (AVOCs) and those produced by organisms inhabiting the soil ecosystem, *i.e.*, biogenic volatile organic compounds (BVOCs) [11]. AVOCs, derived from human activities such as combustion, include toxic compounds like BTEX (benzene, toluene, xylene, and related chemicals) and poly-aromatic hydrocarbons (PAHs) [12], and are chemically diverse, encompassing aliphatic and aromatic hydrocarbons, alcohols, alkenes, esters, glycol derivates, aldehydes, ketones, alkynes and halogenated hydrocarbons [13]. Soil microbes are able to degrade some of these toxic compounds, potentially enhancing plant resistance against contaminants, thanks to their presence [14–17]. BVOCs, on the other hand, are produced by soil organisms, especially plants and microbes [18–20] and include a range of compounds like alcohols, ketones, and terpenes [9,21]. BVOCs can be directly released from soils to the atmosphere through microbial degradation of plant waste or soil organic carbon, and unique BVOC profiles can be associated to distinct microbial taxa [22, 23]. In addition, BVOCs can be used by microorganisms for their metabolism or can be emitted and transported in the environment, acting as signals in plant-plant interaction or plant-microbe interaction [20,24]. Soil-related BVOC emissions also contribute to total BVOC ecosystem emissions and have an impact on atmospheric chemistry [25–27]. However, despite the growing recognition of urban soils as critical components of urban ecosystems, there is a gap in our understanding of the complex interactions between soil microbial communities and synthesis and degradation of VOCs in urban environments. The study of the correlations between microbial communities of urban soil and VOCs has been proposed as pivotal to fill this specific gap [9,28]. This topic is particularly important for several reasons, including assessment of soil quality, sustainable urban development and even air quality in cities. The VOCs produced or degraded by microbes in the soil can provide insights into soil quality and the activity of the soil microbial community [28]. Through the comprehension of the correlation between VOCs and microbial communities, the overall status of the soil, that represents a critical factor for protecting and improving the provided ecosystem services, could be better assessed and evaluated. Since urbanization significantly impacted on soil quality, understanding the relationship between VOCs and microbial communities might help policy-makers to take more informed decisions about urban development, promoting sustainable practices and maintaining healthy urban ecosystems. Additionally, soils are a source of VOCs that can contribute to air pollution both directly and through secondary aerosol formation, and affect human health. By understanding the sources and pathways of VOC emissions, strategies to mitigate their impact on the environment could be developed [29].

In this study, whose feasibility was previously demonstrated [28], a combined approach coupling a metabarcoding analysis of microbial communities and VOC emissions in soil was used to evaluate the soil quality of urban flowerbeds in Prato, Italy, in the frame of the project Prato Urban Jungle. The main objective of the project is to create an innovative urban design to re-naturalize certain areas of the Prato municipality in a way that is sustainable and socially inclusive. "*Urban jungles*", or regions with a high density of green, are being developed as an approach to boost sustainable urban land use to accomplish this goal. In addition to recover unused soil and space for community use, the urban jungles would improve plants natural capacity to filter pollutants, by converting undesired and decaying locations into green hotspots.

Thus, "*Urban jungles*" are expected to have net positive impact on soil quality. However, to evaluate the positive effects of the planned activities it is pivotal to evaluate *ex-ante* situation in terms of soil quality, by assessment of soil microbial and VOC diversity. Soils from three different flowerbeds with diverse tree coverage were selected, to assess the role of tree species in shaping soil microbial communities and VOC profiles, and soils from an adjacent park were used to compare data from urban flowerbeds to data from a less disturbed urban area. The overall aim of this work was to identify putative correlation between the composition of the microbial community and the VOC patterns emitted by the selected soils, providing useful information to promote sustainable development in cities.

2. Materials and methods

2.1. Soil sampling

To perform the assessment of the VOCs and the microbial diversity in the selected urban soils, three different sites in the flowerbeds of "Via Turchia", in a council housing complex of Prato municipality, Tuscany region, Italy, were selected. Each site was selected to have a distinct tree canopy, *i.e.*, *Tilia* spp., *Quercus robur*, and *Quercus ilex*, and were located 75 m far from each other (Fig. S1). Additionally, in an adjacent urban park located 70–130 m from the closest flowerbed, these species were selected in three sites for soil sampling for comparison purpose, *i.e.*, flowerbeds *vs* park. Sampling was performed in June 2021. For physical-chemical analyses, four

soil samples, for each selected site (flowerbed with *Tilia*, flowerbed with *Quercus robur,* flowerbed with *Quercus ilex*, and urban park) were used. Soil samples of approximately 500 g were collected from the 10 cm layer and air-dried. Soil chemical properties were determined according to standard analytical procedures (Sparks et al., 2020). For metabarcoding and VOC analysis, three points (point 1 close to the tree, point 2 5 m far from the tree, point 3 10 m far from the tree) for each site were selected, and for each point three soil samples (replicates) were collected (A, B, C). Soil samples were collected using a soil Dutch auger. Small debris and litter material were discarded with a hand trowel, and soil cores up to 18 cm deep (around 300 g) were taken. In detail, 27 samples for VOC and metabarcoding analysis from the three different flowerbeds (flowerbed with *Tilia*, flowerbed with *Quercus robur,* flowerbed with *Quercus ilex)* were collected. Additionally, in the urban park, a single sampling corresponding to soil close to each of the three tree species as in flowerbed was selected, for a total of 3 samplings x 3 replicates (9 samples) for metabarcoding and VOC analysis. A total of 36 samples were collected. Sampling point coordinates are reported in Table S1. Soil samples were stored at 4 °C before VOC and metabarcoding analysis. Meteorological data of relative humidity of the sampling day were retrieved from the local weather station of Prato Università. Soil moisture data were retrieved from the Copernicus Global Land Service SSM (Surface Soil Moisture) product, released daily at 1 km spatial resolution and based on the Sentinel-1 C-SAR satellite [30]. The water content of the soil samples collected for the analysis (expressed as percentage) was also measured as [mass of soil (g) − mass of oven-dried soil (g)/mass of oven-dried soil (g)] × 100 [31].

2.2. VOC analysis

2.2.1. Dynamic headspace sampling of volatile compounds

Before VOC analysis, the collected soil samples were homogenized to create a uniform sample [32] and stabilized at ambient temperature [33]. Volatiles were measured using a dynamic headspace (DHS) technique system followed by gas-chromatography–mass spectrometry (GC–MS) analysis based on the procedure developed in our laboratories for different media [28,34], optimized for determination of polar and nonpolar volatile compounds in the headspace of the soil samples and modified as follows. Optimal headspace parameters were: 300 g of soil samples (mainly backfill) transferred into a 500-ml glass container and kept at room temperature for 5 min to reach equilibrium conditions and to prevent alteration of the volatiles; glass container connected via Teflon tubing to a homemade Zero Air Generator, with the gas inlet located at 1 cm from the top of the extraction vessel; outlet of the extraction system connected to steel tubes packed with Tenax TA 35/60 and Carbograph 1 TD 40/60 ® (Markes International, Ltd, Llantrisant, UK) connected to an external pump (Pocket Pump SKC Inc., USA) adsorbing at flow rate of 200 ml min^{-1} (12 l air collection). To verify the possible presence of VOCs in the ZAG-generated air passing through the entire system, samples of this air flowed into the empty glass container were collected into steel tubes as described above. All the sampled adsorbent tubes were kept at –20 °C until analysis to avoid any chemical alteration and/or artefacts.

2.2.2. Thermal desorption gas chromatography–mass spectrometry (TD-GC–MS) analysis of volatile compounds

GC-MS analysis was carried out as reported in Ref. [35]. In detail, traps were desorbed using a thermal-desorber (Markes International, Series 2 Unity) and analyzed with a 7890A gas chromatograph coupled with a 5975C mass detector (GC–MS, Agilent Technologies, Wilmington, USA). Thermal desorption of the sampling tubes was carried out for 15 min at 280 °C with a helium flow rate of 50 ml min^{-1}, then the cold trap was rapidly heated from - 30 °C to 280 °C and analytes were fast injected onto the capillary column (HP-1, 60 m × 0.25 mm I.D. × 0.25 μm film of polymethylsiloxane; J&W Scientific USA, Agilent Technologies, Palo Alto, CA, USA) via a transfer line heated at 280 °C. Identification was obtained by software comparison of retention times and fragmentation pattern with the NIST 11 database of mass spectra and external reference compounds. The identified compounds, corrected by subtracting the VOCs possibly present in the air coming from the ZAG and from the entire sampling system, were quantified using the external standard calibration procedure. Finally, VOC emissions were expressed as ng kg^{-1} h^{-1}.

2.3. Metabarcoding analysis

2.3.1. DNA extraction and sequencing

Genomic DNA from approximately 400 mg of soil from the same sampling points of those collected from VOC analysis was extracted by using DNeasy PowerSoil Pro Kit (Qiagen) following the manufacture procedure. As soil homogenization is a pivotal step of sample preparation to assess microbial communities in soil [36], soil samples were homogenized by using bead-beating tubes provided by the kit and a Vortex Genie ® 2 (Scientific Industries) for 20 min. After DNA extraction, the DNA concentration of the 36 samples was measured with a Qubit BR dsDNA assay kit (Thermofisher). The 16S V3–V4 and the ITS2-5.8S regions were amplified separately and following the 16S Metagenomic Sequencing Library Preparation protocol (Illumina) with 12.5 ng/sample as starting material. Target-specific primers (Table S2) including the Illumina overhang adapter sequence for subsequent sample indexing via a second round of PCR were used. Sample indexing was performed using the Nextera XT Index Kit v2 (Illumina). Final library concentrations were measured with the Qubit HS dsDNA assay kit (Thermofisher) and used to prepare two equimolar pools (one per each target). The pools were then quantified by Real-Time PCR against a standard curve with the KAPA Library Quantification Kit (Kapa Biosystems). Libraries were sequenced on a MiSeq platform (Illumina) in a 300 paired-end mode. The 16S V3–V4 and the ITS2-5.8S regions were amplified separately for characterization of bacteria and fungi, respectively, following the Metagenomic Sequencing Library Preparation protocol (Illumina). Raw reads were submitted to Sequence Read Archive (SRA) - NCBI (Bioproject ID: PRJNA992400).

2.3.2. Bioinformatic and statistical analysis

The obtained reads were clustered into Amplicon Sequence Variants (ASVs) [37]. Data were analyzed by QIIME2 pipeline and reads from 16S and ITS regions were then classified using the Naïve-Bayes classifiers trained on Silva_v132_99_16S and Unite-v8-99_ITS databases respectively. The analysis on 16S and ITS regions was performed considering all the reads of each sample. The bioinformatic analysis was carried out using the QIIME2_Illumina analysis workflow freely available as a Git repository (https://github.com/swiftbiosciences/q2_ITS). Prior to the analysis, reads were trimmed according to their quality. For the 16S region, the 3′ ends of the forward reads were trimmed at base position 269, whereas the reverse reads were trimmed at position 195. Regarding the ITS, forward and reverse reads were trimmed at position 282 and 196, respectively. Output from QIIME2 (feature table and related taxonomy) were used with Microbiome Analyst [38] for visualization and statistical assessment of data. By setting "4" as the minimum quantity of features (10 % of prevalence in samples), as well as 10 % of features with low variance in samples based on IQR, data were filtered to remove low quality and uninformative features. Data rarefying was also carried out to reduce bias due to various library sizes. To assess both fungal and bacterial diversity, Chao1 and Shannon indexes were used to determine the diversity within the samples (α-diversity), while Bray-Curtis was used to compute the diversity between the samples (β-diversity) and Principal Coordinates Analysis (PCoA) was used to show the results. With adjusted *p*-value cut-off (False Discovery Rate - FDR) set at 0.05 for both analyses (differences were judged significant for *p*-values of 0.05 or less), analysis of similarities (ANOSIM) was done for β-diversity (at feature level). Functional annotation of bacterial taxa was performed by using FAPROTAX, to associate detected bacterial sequences to their putative metabolic functions [39], while FUNGuild was used to assign trait information based on matching to the classification of fungal taxa [40]. Correlation between VOC, fungal and bacterial taxa was performed by Pearson's correlation coefficient (r) using R language v 4.2.1 (package "*ggpubr*"). The database mVOC 3.0 [41] was used to investigate the reported putative association between detected microbial taxa (at genus level) and VOC.

Concerning VOC data, differences in emission rates of VOC categories were assessed by two-way ANOVA without interaction, using as factors the sampling location (the three flowerbeds and the natural park) and the related tree species (*Tilia* spp., *Q. ilex*, *Q. robur*). Tukey post-hoc test was used when ANOVA showed significant differences ($p < 0.05$) among groups. A Principal Component Analysis (PCA) was performed on VOC data to assess the presence of grouped samples and different clusters.

3. Results

3.1. Soil physical-chemical features

The physical-chemical properties of the sampled soils are reported in Table 1. All soil samples could be classified as backfill sandy soils with a low to moderate amount of silt and a low amount of clay, slightly to moderate alkaline pH values and low level of P, and N. Samples from *Tilia* flowerbeds showed and increased amount of N, organic C, and exchangeable Ca and Mg, as well as higher pH (8.04) compared to the other samples (Table 1). The collected urban soil was classified as Calcaric Arenosol [42,43]. During the sampling campaign, the recorded median air relative humidity was 65 % (min 31 % - max 100 %), and the precipitation was 4 mm. Surface Soil Moisture (SSM) reported by Copernicus database was 55 %. Soil water content of the samples was 49.01 ± 8.28 %.

3.2. VOC analysis

Nearly 80 VOCs were identified in the soil samples (Table S3), belonging to different classes. Most abundant chemical classes were acids, alcohols, aldehydes, alkanes, alkenes and arenes, ethers, ketones, terpenes, and terpenoids (Table 2) and further 15 compounds belonging to other different classes (sulphides, furane, pyrrole, phenol, etc.), reported in Table S3. Overall, higher emissions of alcohols, alkanes, alkenes, ketones were detected in samples collected from the park, independently from the tree species, compared to samples from flowerbeds (Fig. 1). Arenes and terpenoids were instead abundant in flowerbed samples (Fig. 1). Acids such as acetic, benzoic and nonanoic, as well as dimethyl disulfide, were high in samples harvested around *Q. robur* in the park (Fig. 1). Terpenes were also particularly detected in samples from the park, specifically in samples close to *Q. ilex*. It should be mentioned that tetrahydrofuran reached high levels in samples coming from soil around *Q. ilex* and *Tilia* in the park (Fig. 1). The PCA on VOC data showed that samples from flowerbed grouped together far from the park samples, except point 2 and 3 of samples of soil close to *Q. robur*. Samples from the park did not cluster together but they were well separated from each other (Fig. 2a). Hierarchical clustering on VOC data, reported in a heatmap (Fig. 2b), showed that samples collected near to *Q. robur*, both in flowerbeds and park, grouped together and separately from those taken close to *Tilia* and *Q. ilex*, except one sample referred to *Tilia* (Tilia 1 flowerbed).

Significant differences in VOC emissions were detected by two-way ANOVA. In detail, the amounts of VOCs belonging to class of

Table 1
Physical and chemical features of soil samples. CEC: Cation Exchange Capacity; EC: Electrical Conductivity; org: organic; ex: exchangeable.

Sample	pH	EC	CEC	Clay	Silt	Sand	P	N	C org	Ca ex	Mg ex	Na ex	K ex
		μS/cm	cmol $^{(+)}$kg^{-1}	%	%	%	mg/kg	%	%	g/kg	g/kg	g/kg	g/kg
Park	7.21	505	11.2	9.25	15.5	75.2	12.6	0.12	1.05	1.30	0.19	0.37	0.19
Tilia flowerbed	8.04	560	13.2	6.65	11.3	82.0	13.1	0.21	2.31	2.21	0.21	0.21	0.17
Q. ilex flowerbed	7.73	628	12.8	14.1	9.80	76.1	12.7	0.14	1.26	1.74	0.15	0.35	0.16
Q. robur flowerbed	7.85	425	11.9	12.3	11.4	76.3	11.5	0.11	0.95	1.44	0.10	0.22	0.13

Table 2
Detected VOC classes expressed as average values (ng kg h^{-1}) and related standard deviation. Letters are reported according to outcomes to Tukey HSD test for significant differences (p-value < 0.05), if significant differences among group means were detected by ANOVA. The category "OTHERS" includes 15 VOC species different from the other VOC classes.

	Q. ilex park	*Q. ilex* flowerbed_1	*Q. ilex* flowerbed_2	*Q. ilex* flowerbed_3	*Q. robur* park	*Q. robur* flowerbed_1	*Q. robur* flowerbed_2	*Q. robur* flowerbed_3	*Tilia* spp. park	*Tilia* spp. flowerbed_1	*Tilia* spp. flowerbed_2	*Tilia* spp. flowerbed_3
Acid	5.8 ± 3.06a	6.59 ± 5.64a	9.25 ± 1.53a	12.78 ± 0.54a	37.47 ± 17.94b	9.45 ± 1.14b	14 ± 5.55b	17.4 ± 3.42b	6.5 ± 1.8a	7.97 ± 6.63a	10.25 ± 13.14a	14.15 ± 7.8a
Alcohol	9.54 ± 12.57a	4.7 ± 1.77b	5.14 ± 2.28b	3.57 ± 1.14b	27.44 ± 11.94a	3.94 ± 2.58b	11.45 ± 4.95 ab	14.61 ± 11.13a	32.57 ± 37.8a	6.73 ± 8.22b	7.35 ± 5.97b	8.16 ± 10.32b
Aldehyde	6.3 ± 6.21	9.95 ± 0.27	8.11 ± 5.94	6.27 ± 5.25	17.83 ± 8.67	10.8 ± 6.12	17.35 ± 4.59	19.91 ± 9.9	11.03 ± 6.03	10.89 ± 7.92	39.55 ± 55.05	7.25 ± 3
Alkane	28.33 ± 42.09	22.39 ± 17.01	12.04 ± 16.86	8.71 ± 12.15	33.8 ± 18.12	15.59 ± 20.28	23.62 ± 14.64	25.38 ± 19.26	33.17 ± 50.16	6.6 ± 9	16.26 ± 18.45	4.63 ± 4.2
Alkene	4.9 ± 4.2a	3.17 ± 2.25a	2.13 ± 1.77a	1.9 ± 1.5a	27.66 ± 14.7b	4.71 ± 3.9a	6.15 ± 3.48a	5.54 ± 3.15a	13.28 ± 4.35b	2.99 ± 0.03a	4.44 ± 2.1a	3.08 ± 0.78a
Arene	113.43 ± 181.95	134.66 ± 180.96	66.53 ± 108.93	23.24 ± 30.87	47.45 ± 58.32	371.77 ± 487.26	251.65 ± 228.03	72.2 ± 77.73	69.4 ± 106.26	18.85 ± 17.73	141.28 ± 175.77	76.62 ± 126.42
Ether	142.75 ± 224.58	0 ± 0	0 ± 0	0 ± 0	23.14 ± 39.27	0 ± 0	0 ± 0	307.4 ± 361.26	35.2 ± 60.84	23.61 ± 29.1	0.07 ± 0	0.06 ± 0.03
Ketone	26.84 ± 22.53a	5.64 ± 6.18b	6.71 ± 4.8b	5.43±3b	15.18 ± 3.51a	4.03 ± 2.76b	7.35 ± 5.16b	8.33 ± 4.53b	31.79 ± 28.62a	4.06 ± 3.9b	2.39 ± 0.84b	3.73 ± 0.06b
Terpene	12.1 ± 18.51	0.64 ± 0.66	0.57 ± 0.57	0.17 ± 0.12	0.22 ± 0.15	0.1 ± 0.12	0.33 ± 0.3	0.26 ± 0.27	1.05 ± 0.57	0.07 ± 0.09	0.12 ± 0.09	0.06 ± 0.06
Terpenoid	0.33 ± 0.15a	0.93±0b	0.73 ± 0.12b	0.71 ± 0.09b	0.26 ± 0.09a	0.19 ± 0.06a	0.29 ± 0.09a	0.33 ± 0.15a	0.47 ± 0.42 ab	0.74 ± 0.15b	0.97 ± 1.08b	1 ± 0.21b
OTHERS	163.14 ± 159.66	34.19 ± 17.23	27.75 ± 13.24	25.05 ± 7.52	120.99 ± 119.48	33 ± 25.11	394.33 ± 343.37	37.66 ± 21.39	200.15 ± 227.32	13.48 ± 3.45	80.48 ± 106.77	17.04 ± 2.71

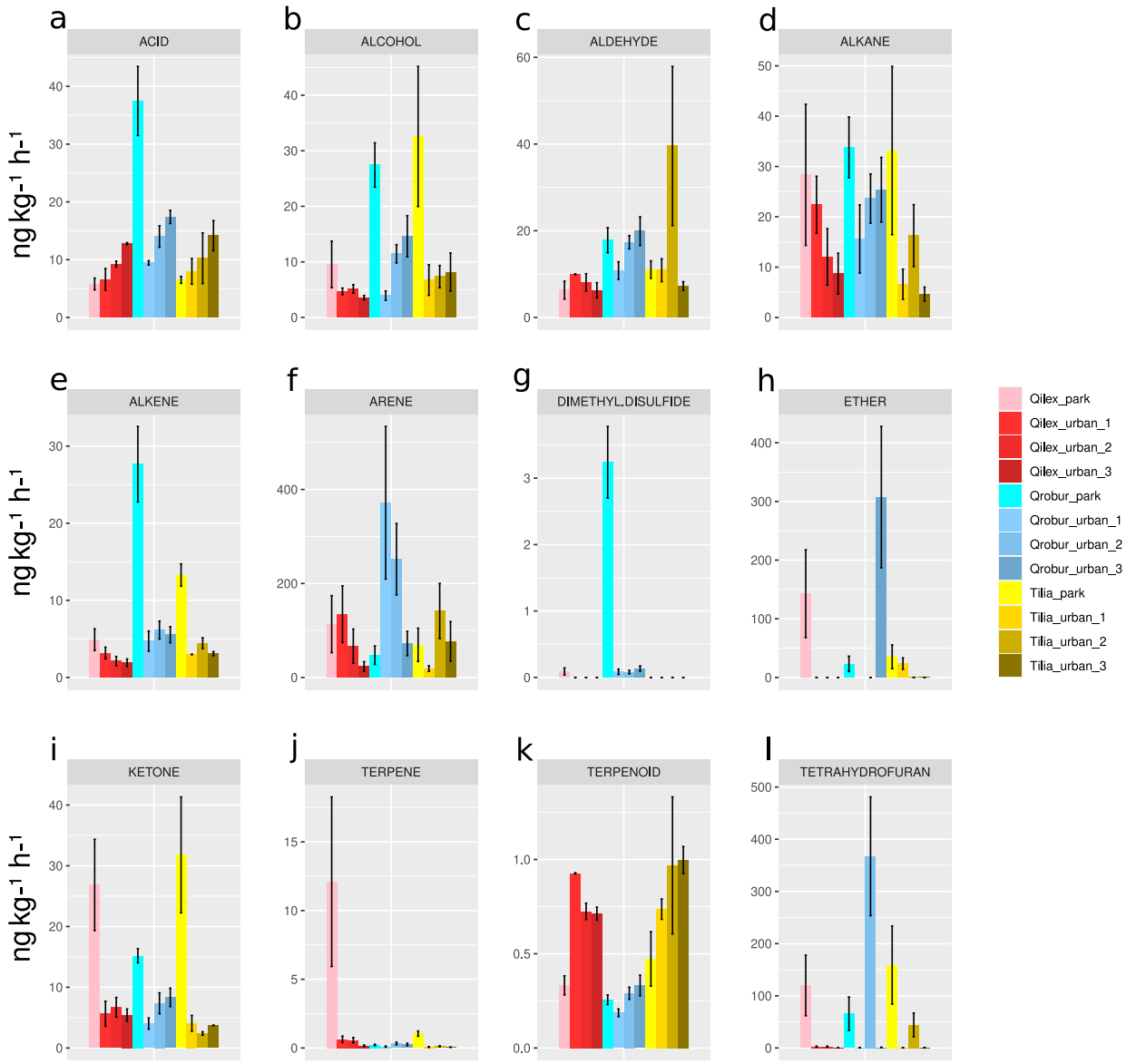

Fig. 1. Barplots of VOC emission from the selected soil samples. VOC species are reported as divided by class with the exception of dimethyl sulfide and tetrahydrofuran. Error bars represent standard errors (SE). Acid (a), alcohol (b), aldehyde (c), alkane (d), alkene (e), arene (f), dimethyl sulfide (g), ether (h), ketone (i), terpene (j), terpenoid (k), and tetrahydrofuran (l) contents are reported.

alcohol, alkene and ketone were significantly different between samples collected in flowerbeds and those collected in the urban park (*p*-value 0.0281, 1.77 E^{-05}, 4.52 E^{-05}, respectively). All these three classes were more abundant in park samples compared to flowerbeds (Fig. 1 and Table 2).

By contrast, acids and terpenoids were different among tree species (*p*-value 0.00793 and 0.00252, respectively), with higher level of acids and lower level of terpenoids in *Q. robur* samples in comparison with *Tilia* and *Q. ilex* (Fig. 1 and Table 2).

3.3. Microbial community assessment

Metabarcoding of the 16S and ITS genes generated a total of 4,052,368 and 2,772,991 reads after quality filtering, respectively, across the three urban soil sites. Rarefaction curves of both bacterial and fungal ASVs reached a plateau, confirming sufficient sequencing depth for obtaining a whole picture of microbial diversity in the analyzed soil samples. For bacterial data, 1727 ASVs were considered for diversity analysis. The bacterial communities in all samples were dominated by Actinobacteria, Proteobacteria, Acidobacteria, and in less percentage Chloroflexi, Firmicutes, Planctomycetes, Verrucomicrobia and Gemmatimonadetes (Fig. 3a). A relative high presence of Rokubacteria has been observed for some samples collected in the park close to *Quercus ilex* trees (Fig. 3a).

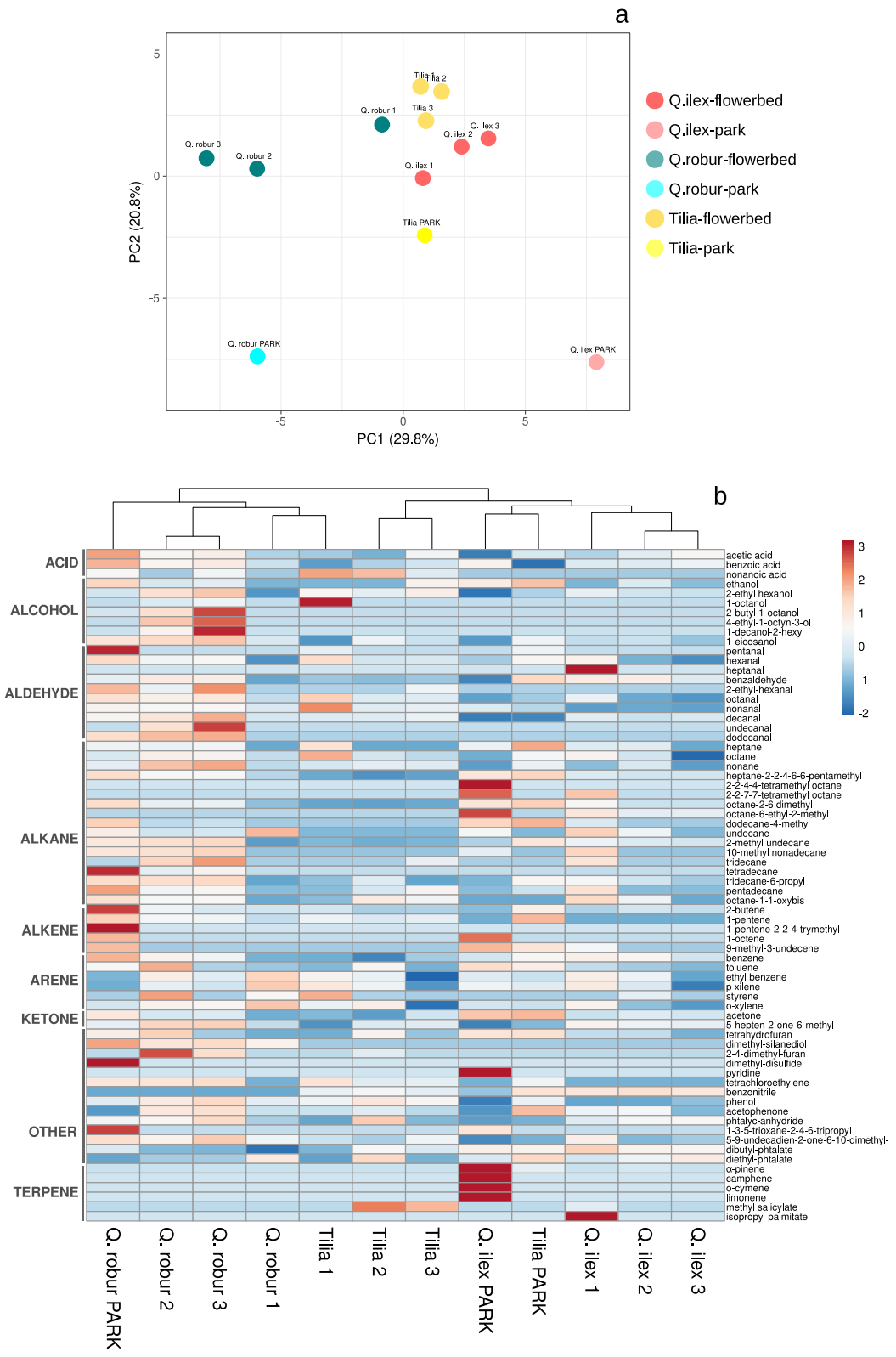

Fig. 2. Total VOC emission profiles. In (a), principal component analysis (PCA) plot of component area normalized data of VOC profiles. In (b), heatmap of the detected VOC species in the selected sampling points. In (b) original values are ln(x)-transformed, rows are centered and unit variance scaling is applied to rows; columns are clustered using correlation distance and complete linkage.

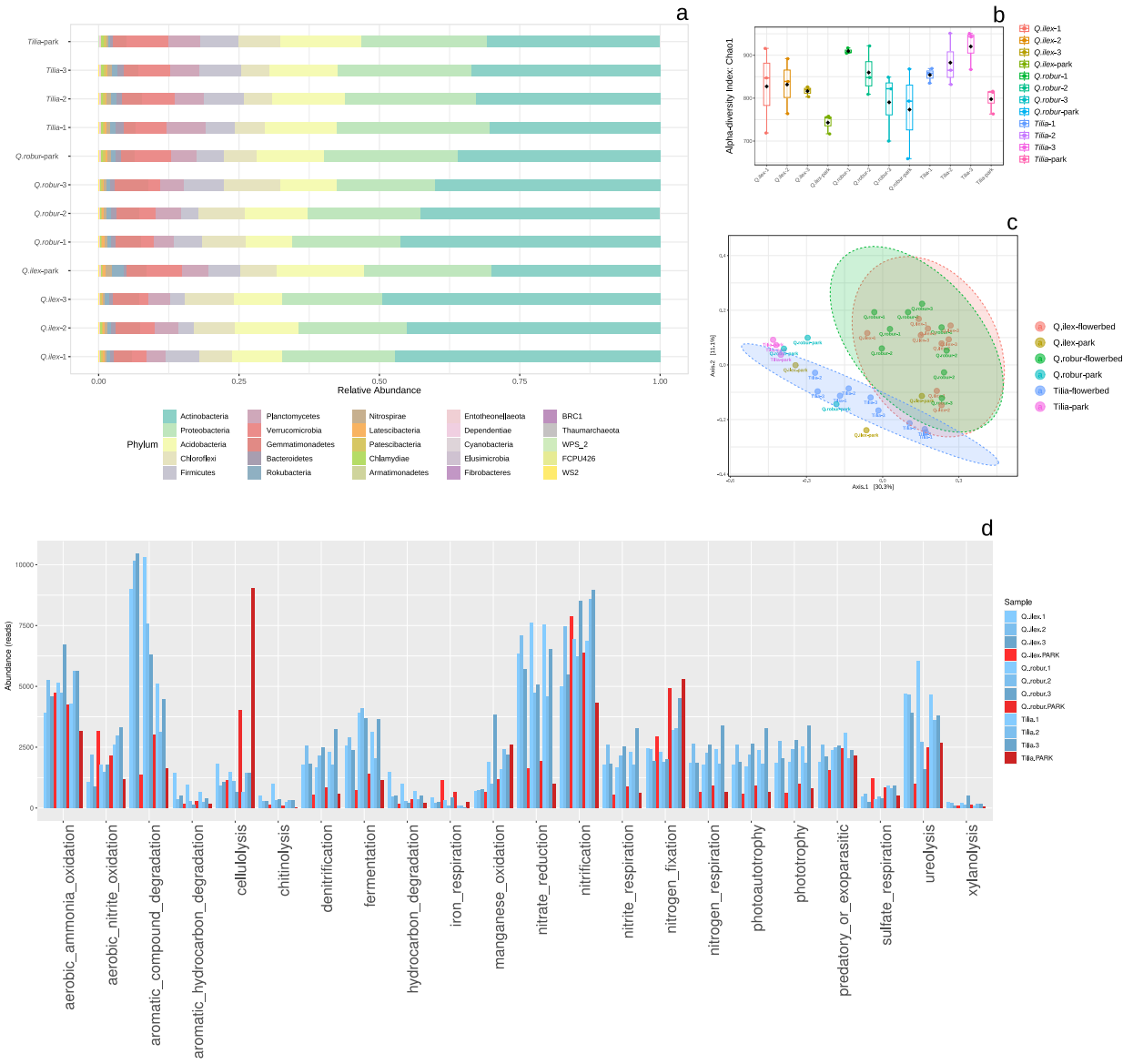

Fig. 3. Results of metabarcoding analysis related to bacteria of soil samples. In (a), stacked taxa barplots of bacterial relative abundance at phylum level in soil samples. In (b) and (c), α- and β-diversity of soil samples calculated with Chao1 diversity index and Bray–Curtis dissimilarity index, respectively. In (d), outcomes of FAPROTAX functional assignment as barplots, where in y-axis is reported the actual abundance and in x-axis the functional classes are listed.

Among Actinobacteria, relevant families were Solirubrobacteraceae (8.47 % of Actinobacteria), Streptomycetaceae (7.80 %), Gaiellaceae (7.40 %), and Nocardioidaceae (7.23 %) (Table S4). Within Proteobacteria, Xanthobacteraceae (21.3 %), Nitrosomonadaceae (6.6 %). Acidobacteria phylum included high percentage of ASVs with not assigned families (98.36 %). Acidobacteria were more abundant in the park samples (T-test FDR 0.019), while Actinobacteria were less detected (T-test FDR 0.018), compared to flowerbed samples.

Alpha-diversity of bacterial communities showed significant differences among samples (Chao1 index, ANOVA F-value 2.230, *p*-value 0.04). According to this richness index, a higher diversity in flowerbed samples was found when compared to samples from urban park, with the highest α-diversity occurring in samples close to *Tilia* in flowerbed, followed by a sample close to *Q. robur* flowerbed (*Q. robur* 1; Fig. 3b). On the bases on the β-diversity calculated with Bray-Curtis dissimilarity, the bacterial communities in *Q. robur* and *Q. ilex* samples in flowerbeds were more similar to each other than to the bacterial community in *Tilia* samples, and separated from soil samples coming from the park (ANOSIM R 0.57, p-value < 0.001; Fig. 3c). Functional annotation of bacterial communities by FAPROTAX allowed to identify several categories and the most represented are reported in Fig. 3d. High abundance of sequences of bacteria associated with putative "aromatic compound degradation", "nitrate reduction", "nitrification", "aereobic ammonia

oxidation", and "ureolysis" abilities were detected in flowerbed samples, while samples from the park showed abundance in bacteria putatively capable of "cellulolysis", "nitrification", and "nitrogen fixation" (Fig. 3d).

Concerning ITS data, 576 ASVs were defined after filtering, and fungal communities were dominated by Ascomycota, Basidiomycota, and Mortierellomycota (Fig. 4a). Nectriaceae (6.82 % of Ascomycota), Pseudoeurotiaceae (8.26 %) and Aspergillaceae (8.88 %) were the main Ascomycete families; among Basidiomycetes, key families were Cortinariaceae (9.34 % of Basidiomycota), Hymenogastraceae (7.24 %) and Inocybaceae (9.06 %). Mortierellaceae comprised the 100 % of the identified Mortierellomycota fungi (Table S5). No significant differences in α-diversity within samples was observed for fungi, both for Chao1 and Shannon indexes (Fig. 4b). Diversity of fungal communities as determined by β-diversity showed two distinct clusters, one including samples collected in *Quercus* flowerbeds and one with samples collected in *Tilia* flowerbed (Fig. 4c). Samples from the park grouped separately from each other and from the flowerbed samples (Fig. 4c). Guild assignments of trophic modes of detected fungal taxa showed a clear dominance of saprotrophs in all samples, followed by symbiotroph (95 % of Ectomycorrhizal fungi and about 4.2 % of arbuscular mycorrhizal fungi, 324,694 and 14,304 sequences, respectively; Fig. 4d). About 78 % (753,540) of the total sequences (960,884) assigned to Ectomycorrhizal and Arbuscular mycorrhizal fungi were associated to samples close to *Tilia* in flowerbed (Table S6). Comparison by MicrobiomeAnalyst showed that Basidiomycota and Glomeromycota were significantly more abundant in samples close to *Tilia* tree compared to samples close to *Q. ilex and Q. robur* trees (T-test FDR 1.2214 E^{-4} and 3.5633 E^{-3}, respectively).

3.4. Correlation between VOC emissions and microbial communities

Correlation between identified VOCs and microbial communities allowed to detect several bacterial and fungal taxa positively correlated to specific VOCs. Results of correlation analysis at genus level is showed in Fig. 5 and outcomes from correlation between detected microbial species and those reported in mVOC database 3.0 is reported in Table 3, at order level. At genus level, ten bacterial

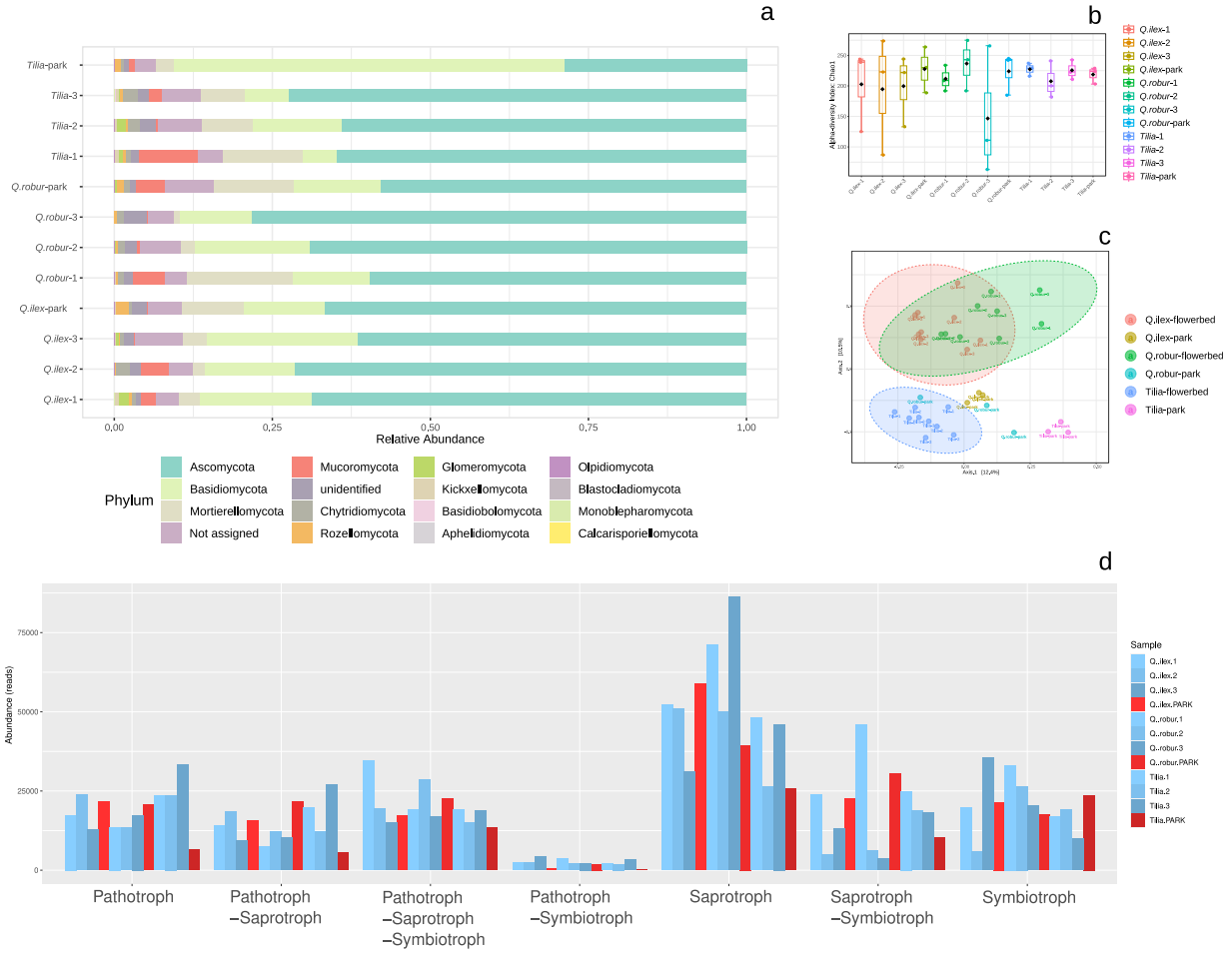

Fig. 4. Results of metabarcoding analysis related to fungi of soil samples. In (a), stacked taxa barplots of fungal relative abundance at phylum level in soil samples. In (b) and (c), α- and β-diversity of soil samples calculated with Chao1 diversity index and Bray–Curtis dissimilarity index, respectively. In (d), results of FUNGuild assignment as barplots, where in y-axis is reported the actual abundance and in x-axis the detected trophic guilds are listed.

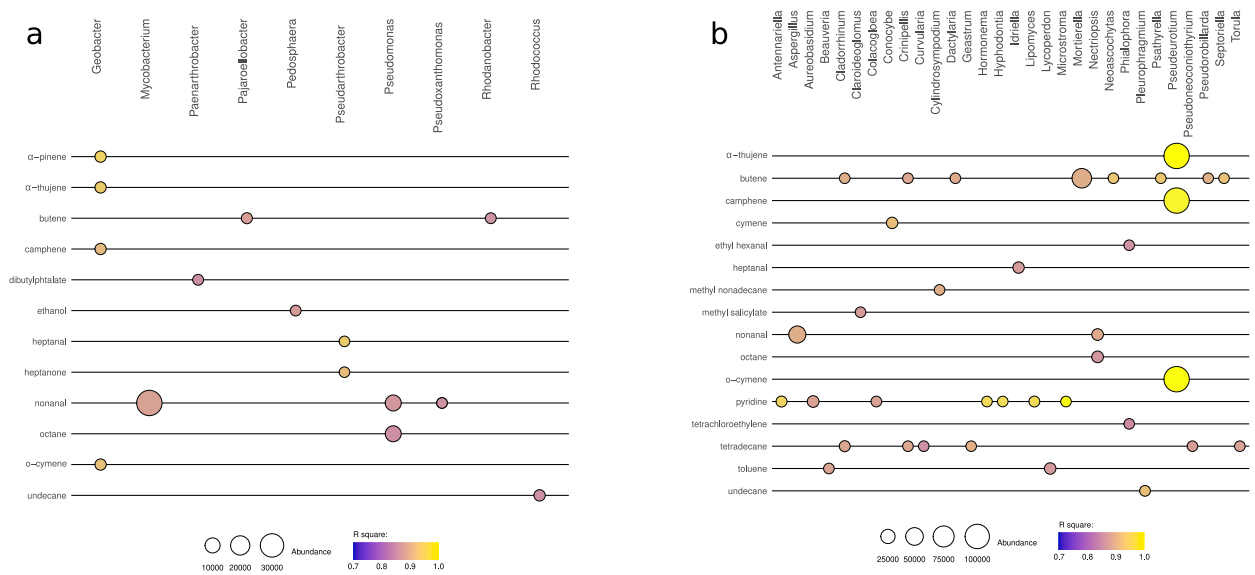

Fig. 5. Bubble plot of detected significant correlation between bacterial (a) and fungal (b) taxa with VOC species. Size of bubbles represents actual abundance of microbial genera, while color intensity showed the R-square values (ranging from 0.7 to 1.0). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

taxa were found to be correlated with twelve VOC (Fig. 5a). Among them, *Geobacter*, *Pseudarthrobacter* and *Pseudomonas* correlated with at least two VOC species, with *Geobacter* showing higher R-square values with four VOCs, *i.e.*, α-pinene, α-tujene, camphene and *o*-cymene (Fig. 5a). Nonanal was found to be correlated with three bacterial taxa. Thirty fungal taxa were identified as correlated with sixteen VOC species (Fig. 5b). Fungal clades *Cladorrhinum*, *Crinipellis*, *Nectriopsis*, *Phialophora*, and *Pseudeurotium* were positively associated with at least two VOCs (Fig. 5b). Pyridine, butene and tetradecane showed a correlation with more than five fungal taxa (Fig. 5b).

4. Discussion

4.1. Soil from flowerbed harbored higher microbial diversity compared to the urban park

In recent years, it has been suggested that the restoring and the rewilding of urban green spaces will avoid degradation of microbial communities associated with urban areas, in line with the "Microbiome Rewilding Hypothesis" [44]. However, urban soils may also harbor rich microbial communities, as observed by Scholier and colleagues in a compelling comparative study between urban and natural forests [45]. A similar outcome was observed in our work, where bacterial α-diversity increased in flowerbeds, which can be considered areas with higher level of anthropogenic disturbance compared to the urban park. It has been documented that soil alkalinity plays a role in structuring urban soil microbial communities [45]. In our study site, soil pH of flowerbed samples was slightly higher than in the park. Soil alkalinity has been frequently reported in urban areas [46], and it could be explained by presence of ash emissions and dust from roadways [47,48], or by rainwater flowing through structures as pipes and gutters [49]. In addition, presence of tree species producing base-rich litter, such as linden trees, may have affected the soil pH, as observed in natural forests [50]. For example, in our study, the flowerbed close to *Tilia* trees showed the highest pH compared to the other two flowerbeds. In addition, soil from flowerbed with *Tilia* trees showed a relative high amount of organic carbon and nitrogen, compared to the other flowerbeds where the oak plants were present. Hence, the peculiar microbial communities in soil collected close to *Tilia* trees, as highlighted by beta diversity analysis on both bacterial and fungal ASVs, may be also explained by the high pH and by the abundance of organic matter.

4.2. VOCs in soil from flowerbed originated from anthropic and biological origin

The plethora of detected VOCs from soil collected from the selected flowerbeds suggested the presence of a complex substrate, *i.e.*, urban soils, where belowground processes, such as microbial respiration and exchange of VOCs, occurred [51]. However, and unexpectedly, it has been recently demonstrated that the amounts of various VOCs released from soils rose while the amount of microbial diversity in the soil decreased [52]. In our study, on average, VOC emissions from soil samples of flowerbeds was limited if compared to those from the urban park. Particularly, emission of VOCs belonging to alcohol, alkene and ketone classes was significantly reduced in flowerbeds, despite a higher microbial α-diversity, supporting the observations by Abis and colleagues [52].

By using data from metabarcoding analysis and VOC quantification, significant correlations between several microbial taxa and specific VOCs in the considered soils was inferred. Both fungi and bacteria were found to have a potential role in the generation or

Table 3
Comparison between microbial orders detected in the study and reported in database (mVOC 3.0) as correlated to specific BVOCs.

VOC	Type	Bacterial order in database (mVOC 3.0)	Bacterial order detected as correlated in this study	Fungal order in database (mVOC 3.0)	Fungal order detected as correlated in this study
α- thujene	BVOC	NA	Chthoniobacterales Tistrellales Desulfuromonadales Rokubacteriales	Agaricales Boletales Xylariales	Thelebolales Glomerellales Coniochaetales Sordariales
camphene	BVOC	NA	Tistrellales Desulfuromonadales Chthoniobacterales	Agaricales Boletales Cantharellales Eurotiales Hypocreales Polyporales Tuberales	Thelebolales Coniochaetales Sordariales
cymene	BVOC	NA	Gemmatimonadales	Agaricales Hypocreales Xylariales Tuberales Eurotiales	Agaricales
ethanol	BVOC	NA	Pedosphaerales Blastocatellales	Agaricales Pucciniales Saccharomycetales Tuberales Hypocreales	NA
heptanal	BVOC	NA	Micrococcales	Eurotiales Polyporales Agaricales Hypocreales Tuberales Polyporales	Xylariales Capnodiales
heptanone	BVOC	Streptomycetales	Micrococcales	Polyporales Tuberales	NA
methyl nonadecane	BVOC	Pseudomonadales	NA	NA	Venturiales
methyl salicylate	BVOC	Myxococcales	Xanthomonadales	NA	Glomerales
nonanal	BVOC	NA	Corynebacteriales Pseudomonadales Xanthomonadales	Hypocreales Eurotiales Agaricales Tuberales	Eurotiales Hypocreales
o-Cymene	BVOC	Enterobacterales	Chthoniobacterales Tistrellales Desulfuromonadales Rokubacteriales	Eurotiales Hypocreales Polyporales	Thelebolales Glomerellales Coniochaetales Sordariales
pyridine	BVOC	Enterobacterales	NA	Agaricales	Capnodiales Dothideales Dothideales Saccharomycetales Hypocreales Hymenochaetales Microstromatales

degradation of these VOCs [41]. However, both I) the emission of a certain VOC by specific microbial taxa or II) the stimulation of the growth of the microbe when that VOC concentration increases may result in a positive correlation [52]. Our approach based on searching on available VOC database (mVOC3.0) allowed to identify VOC species positively correlated with several fungal taxa, thus suggesting that some detected BVOCs, *i.e.*, α-thujene, heptanal, heptanone, methyl-salycilate, nonanal, pyridine, were putatively emitted by fungi. It is well known that fungi may release complex VOC bouquets, which can affect the growth and overall development of nearby organisms, including plants and bacteria [53]. Abundance of monoterpenoids may be correlated with presence of fungal saprotrophs [23]. Interestingly, in the selected site, large presence of saprotrophs, as highlighted by the FUNGuild assignment, may explain this class of detected BVOCs.

α-thujene is a monoterpene commonly described as produced by plants, especially conifers and *Eucalyptus*, and can be found in many essential oils [54]. The ability of plants to withstand bacterial and fungal disease is also a key function of plant terpenes [55]. However, it has been reported that terpenes are also produced by some fungal species [56,57]. In our study, α-thujene, along with camphene and *o*-cymene, were found to be correlated with the abundance of the ascomycete *Pseudeurotium* spp. This fungal genus has been documented to persist in oil-contaminated environments [58] and to include species able to growth on aromatic compounds [59]. Hence, it is more likely that correlation of this taxa with the emission of several terpenic compounds may result from the ability of these fungi to overcome the putative antifungal activity of these compounds rather than to produce it. Presence of these BVOCs in the soil samples may be linked to emission of terpenic compounds by plant roots [60].

Heptanal is a naturally occurring product of the oxidation of lipids [61]. Due to the presence of yeasts, acetic acid and lactic acid bacteria, this reaction often occurs during fermentation (for example, during the fermentation process for manufacturing bread; [62]). Members of Micrococcoacee were reported to form heptanal as a secondary metabolite [63] and correlation between a member of this family, *i.e., Pseudarthrobacter*, and this VOC may suggest the ability of this genus to produce it. In fungi, heptanal has been reported as produced by *Trichoderma* spp. as antifungal compound against *Neolentinus lepidus, Gloeophyllum trabeum*, *Postia placenta* and *Trametes versicolor* [64]. In our study, a link between heptanal and the plant pathogen *Idriella* spp. was discovered. Therefore, it is possible to speculate that this fungus plays a part in the synthesis of this aldehyde, or, alternatively, that heptanal production by bacteria was stimulated by the presence of the fungus. An important BVOC found to be correlated with the presence of mycorrhizal fungal taxa, *i.e.*, *Claroideoglomus* genus, was methyl salicylate. This ester is synthesized by plants *via* the salicylic acid pathway, possibly playing a role in systemic acquired resistance (SAR) under stress conditions [65]. Interestingly, previous studies documented that methyl salicylate emissions increased substantially in plants under high availability of arbuscular mycorrhizal fungi [66]. It could be hypothesized that abundance of the arbuscular mycorrhizal fungus *Claroideoglomus* in urban soils may be linked to methyl salicylate production by nearby plants, or that its presence played a role in the stimulation of its emission. It has been reported that arbuscular mycorrhizal fungi may affect plant VOC emission and that methyl salicylate emission from stressed plants inoculated with arbuscular mycorrhizal fungi increased [67]. An additional BVOC correlating with some bacterial and fungal taxa was nonanal. Nonanal can be produced by a variety of sources, including biological, chemical, and anthropogenic processes [68]. Soil microbes have been shown to produce nonanal through the degradation of organic matter in the soil. Specifically, certain species of bacteria, fungi, and actinomycetes are known to produce nonanal as part of their metabolic processes. In our study, nonanal was correlated with the presence of *Pseudomonas* and *Mycobacterium* genera, and several studies have reported the production of this compound by these bacterial taxa [68–70]. Nonanal is deemed to have an antifungal activity [68]. Lastly, an important significant correlation was detected in our study between the VOC pyridine and several fungal taxa, including both Ascomycetes and Basidiomycetes. Pyridine can be produced by fungi [71], but it could also be a constituent of pollutants as pyridine-based polyurethanes [72]. The observed fungal taxa involved in the generation or degradation of this VOC, regardless of whether it is a real BVOC or derived from anthropogenic origin, should be further investigated.

On the other hand, the presence of bacterial taxa functionally assigned to aromatic compound degradation in flowerbeds may be also indicative of the abundance of potential degraders, suggesting that some AVOCs can be putatively degraded by specific microbial taxa detected in the analyzed soil. Some of the VOCs that have been found and correlated with microbial taxa may indeed have anthropic origins, *i.e.*, dibutyl phthalate, undecane, toluene. Phthalate esters, such as dibutyl phthalate, are typical environmental pollutants and constituents of many polymers [73]. *Microbacterium* spp., *Pandoraea* spp., *Escherichia coli*, *Mycobacterium* spp., *Pseudomonas* spp., and *Sphingomonas* spp. are only a few of the bacteria that may break down phthalate esters [74,75]. One of the most widely used plasticizers is dibutyl phthalate, which is embedded into high-molecular weight polymers like polyvinyl chloride (PVC) [73]. In our study, dibutyl phthalate was found to be highly correlated with abundance of *Paenarthrobacter* genus. It is worth noting that, in contaminated soils, a strain of *Paenarthrobacter* was isolated and characterized as one of the most effective phthalic acid esters degrading bacteria [76], suggesting that the presence of this AVOC in our study site favored this bacterial taxon capable of di-n-butyl phthalate degradation. A similar hypothesis can be inferred for undecane, belonging to the class of alkanes, hydrocarbons with only single bonds between carbon atoms. Undecane is a colorless liquid with a strong odor that is commonly found in petroleum and crude oil, as n-undecane was one of the main VOC constituents in the diesel exhaust [77]. Undecane is considered a VOC because it can easily vaporize and enter the atmosphere, where it can react with other chemicals to form ground-level ozone and contribute to air pollution [77].

4.3. Microbial communities and VOC emissions from soil seem to be associated with the close tree species

The analysis of metabarcoding data highlighted that different microbial communities in urban soils may be correlated with the tree species present in the area, providing useful information for the more suitable species that could be used in the urban green planning. In our study, fungal communities of soil close to linden trees comprised a large presence of symbiotic fungi, including ectomycorrhizal

and arbuscular mycorrhizal fungi. Additionally, soil samples close to *Tilia,* both from park and from flowerbeds, showed a reduced emission of acids and an increased amount of terpenoids. Notably, some terpenoids produced by trees have been demonstrated to have a beneficial impact on human health [78]. This finding raises the question of whether specific plant species recruit certain microbial taxa that can generate or degrade specific VOCs. This hypothesis is supported by studies showing that plants release specific VOCs that can attract or repel specific microbial taxa [79,80]. For example, the species *Thymus vulgaris* releases VOCs that attract specific types of bacteria, which in turn enhance the plant resistance to herbivores [81]. Furthermore, a previous study by Prescott and Grayston [82] showed that the microbial communities in the soil of different tree species were also distinct from each other.

Despite limited on a specific urban area, our findings might have several implications for understanding the relationships between plants and microbial communities in urban environments. First, they suggest that the tree species present in urban areas may have a significant impact on soil microbial communities. This finding is important because microbial communities play a crucial role in maintaining soil health and fertility, and therefore have a direct impact on the health and vitality of urban ecosystems. Second, results suggest that different microbial communities in their associated soil showed the potential to generate or degrade specific types of VOCs. This finding might have important implications for developing sustainable strategies for managing VOC emissions in urban areas, and might boost other research studies in other different urban areas to confirm our outcomes. Although previous studies have suggested that plant roots can be the primary source of VOC emissions from soil [80], our work showed that soil microbes may also play a significant role in this process. The observed emissions were correlated with the abundance and diversity of soil microbial communities independently from the sample origins, thus corroborating the hypothesis that co-occurrence of microbes and VOC species represent a clue of the potential of microbial communities to produce and/or degrade VOCs.

5. Conclusions

In conclusion, our study has shown a significant correlation between microbial communities and specific VOCs in urban soils. The study also demonstrated the feasibility of the use of microbe-VOC correlation as a proxy for soil quality assessment in urban soils. Furthermore, results suggest that the tree species present in urban areas may have a significant impact on the microbial communities in the soil and may have the potential to recruit specific microbial taxa, *e.g.*, the fungi belonging to *Pseudeurotium* spp. and the bacterial taxa *Paenarthrobacter* and *Pseudomonas* spp., that can generate or degrade specific VOC classes.

Data availability statement

Raw reads used in this study were submitted to NCBI Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) with Bioproject ID PRJNA992400. All other data presented in the study were included in the supplementary material.

CRediT authorship contribution statement

Fabiano Sillo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Luisa Neri:** Writing – review & editing, Methodology, Investigation, Data curation. **Alice Calvo:** Writing – review & editing, Investigation. **Elisa Zampieri:** Writing – review & editing, Investigation. **Gianniantonio Petruzzelli:** Investigation. **Irene Ferraris:** Investigation, Formal analysis. **Massimo Delledonne:** Investigation, Formal analysis. **Alessandro Zaldei:** Writing – review & editing, Methodology, Conceptualization. **Beniamino Gioli:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Rita Baraldi:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Raffaella Balestrini:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23594.

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