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Soil microbial biomass and bacterial diversity in southern European regions vulnerable to desertification

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ABSTRACT

Soil functionality is strongly dependent on the soil microbiota, which in turn is affected by soil quality and climate. Among global change factors, desertification is the most threatening ecosystem change affecting southern Europe, but the effects on the soil microbiota are largely unknown. In this study, we investigated soil microbial biomass and bacterial diversity in regions of southern European countries (Spain, Portugal and Italy), most under desertification risk, and related to key soil chemical-physical indicators and land use. Soil microbial biomass was positively related to soil organic carbon (SOC), but bacterial diversity was negatively correlated with it. pH was the most influencing factor affecting soil alpha-diversity, while SOC, pH and cation exchange capacity drive the biogeographic patterns of bacterial communities. 16S rRNA gene metagenomics allowed the identification of a core microbiota of the analyzed semi-arid soils, including Proteobacteria, Actinobacteria, Acidobacteria and Firmicutes. We identified, at regional scale, a few rare multi-extremophilic endemic genera, which could reveal functional strategies to be exploited for arid land restoration. This work creates the baseline for the necessary monitoring of soils facing climate change and for the evaluation of the efficacy of adaptation measures in semi-arid European soils.

1. Introduction

Mediterranean soils are experiencing increasingly negative effects of climate change due to drought and extreme weather phenomena. Such climate change effects are exacerbated by intensive agriculture and overgrazing, that are inducing soil erosion, compaction, salinization and loss of soil functional properties, a critical issue particularly on dry and semi-dry conditions covering large regions of the Mediterranean (Yang et al., 2020, Stolte et al., 2016). Dry conditions are generally estimated by the Aridity Index (AI: the ratio of annual precipitation to annual potential evapotranspiration) and AI values between 0.2 and 0.5 indicate semi-arid regions (Middleton and Thomas, 1992). In such regions, soil degradation can rapidly degenerate to desertification, with profound changes on soil physico-chemical properties such as organic matter reduction and biodiversity loss (Wiesmeier, 2015, Stolte et al.,

2016). Soil organic carbon (SOC) reduction is of particular concern because, beyond being the major source of nutrients, it exerts numerous positive effects on soil physicochemical properties as well as on soil's regulatory ecosystem services, including the reduction of CO_2 emissions (Tecon and Or, 2017). Such changes are expected to cause also a dramatic shift in the microbial diversity and biomass although the magnitude of this influence is still far from being understood (Maestre et al., 2015, Bastida et al., 2021).

SOC depletion is usually associated with a lower soil biological activity and diversity but no studies report a specific threshold level of SOC associated to a critical dysfunctionality of the soil microbial community (Stolte et al., 2016). Recently, a SOC concentration of 20 g kg^{-1} was indicated as a threshold value below which any additional decline of SOC might result in a steep variation of many key soil parameters in semi-arid regions (Grilli et al., 2021), indicating that this concentration

is critical for soil ecosystem services.

SOC turnover is dependent on soil microbial community dynamics (Terhonen et al., 2019, Su et al., 2020) and the ratio between the fraction of C retained in microbial biomass and the C fraction that is lost by microbial heterotrophic respiration (referred to as carbon use efficiency, CUE) is expected to be reduced under dry conditions and high temperatures (Domeignoz-Horta et al., 2020). Thus, global warming in semi-arid regions is expected to increase the respiration rates of soil microbes because the processes they mediate are temperature sensitive, thus contributing to increase carbon loss to the atmosphere (Timmis et al., 2019). On the other hand, increased temperature can also increase rates of microbial turnover in soil, increasing the stability of soil organic matter (Hagerty et al., 2014).

There is growing awareness of the fundamental role played by the soil microbiota, with its highly diverse and unique metabolisms and functions, in providing ecosystem services that support soil quality and plant productivity (Saccá et al., 2017, Bünemann et al., 2018, Steidinger et al., 2019, Yang et al., 2020). For this reason, soil biodiversity monitoring is a method to be used to assess soil quality and to inform management and policy to contrast climate change, especially in semi-arid regions (Dubey et al., 2019). Actually, the patterns of microbial biomass and soil bacterial diversity in relationship to the main soil biotic and abiotic features, land use, biogeography and global change factors are still far to be known (Hagan et al., 2021). Below-ground microbial diversity seems not to respond consistently to the abiotic environment (Hendershot et al., 2017), indicating that much remains to be learned about drivers of below-ground diversity, especially in arid soils. Moreover, many of the drivers co-vary and feedback on each other (Hagan et al., 2021) thus it is still unclear which are potential drivers of microbial diversity and which factors are instead regulated by below-ground diversity. In Europe, a recent study has reinforced the contrasting relationship between soil physicochemical properties, land use and bacterial diversity, also supporting spatial structuration of bacterial communities (Plassart et al., 2019). The paper provides also specific bacterial indicators of land use and soil conditions but only four sampling sites were located in the Mediterranean, and only two of them in areas under desertification risk (Plassart et al., 2019).

The present study specifically targets on soils threatened by desertification in Mediterranean climatic areas of southern Europe (ECA, 2018), with the aim to: i) investigate the relationships between soil chemical-physical parameters, land use type and microbial biomass and bacterial diversity; ii) identify the core microbiota in European semi-arid soils; iii) create a baseline of data to allow the monitoring of bacterial communities in soils facing climate change and desertification. For this purpose, soil microbial biomass and bacterial diversity were estimated on a large set of soils from vulnerable regions of Italy (Sicily), Spain (Extremadura) and Portugal (Alentejo), most of which characterized by an Aridity index below 0.5, where an assessment of chemical-physical parameters was previously carried out (Grilli et al., 2021). A molecular fingerprinting method based on ribosomal 16S-23S genes intergenic spacer length polymorphisms (Automated Ribosomal Intergenic Spacer Analysis, ARISA) was used to determine bacterial diversity, while a more in depth analysis of the bacterial community structure and composition was done on a subset of soils by high throughput metagenomics 16S rRNA gene sequencing. Microbial biomass and diversity were related to the main soil chemical-physical parameters (pH, SOC, CEC, TN), and land cover.

2. Materials and methods

2.1. Soil sampling from the study sites

Top soil was sampled (0–10 cm) from 30 sites, situated in 10 municipalities, either private farms or public areas, in southern Portugal (Alentejo, PT), Central Spain (Extremadura, SP) and southern Italy (Sicily, IT) during the Spring 2018 (Grilli et al., 2021) (Table S1). Soil

properties data were obtained from Grilli et al., 2021 and refereed to the same soil samples, which were split at the moment of sampling. The sites included different land covers/uses, grouped in 4 main dominant types (Grilli et al., 2021): trees, pastures dominated by grass cover, shrublands and croplands. In each site, from 2 to 4 composite samples (according to soil heterogeneity and width), for a total of 69 composite samples, were collected in sterile plastic bags and kept refrigerated. Once in the laboratory soil samples were subjected to DNA extraction and a sub-sample was oven dried at 65 °C up to constant weight, to relate the estimate of the microbial biomass to the dry weight of the soil samples.

2.2. Metagenomic DNA extraction from soil samples and estimation of microbial biomass

The total soil DNA was extracted from aliquots of 0.5 g of fresh soil samples with the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Germany) using the FastPrep instrument, according to the manufacturer's instructions, with exception in the last step, where the nucleic acids were eluted as described in Novara et al., 2020. Metagenomic DNA were checked for quality and concentration using NanoDrop-ND-1000 spectrophotometer (NanoDrop Technologies, LLC Wilmington, DE) and stored at −20 °C until further processing. Soil Microbial biomass (SMB) was estimated as micrograms of total metagenomic double stranded DNA (dsDNA) extracted from 1 g of dry soil.

2.3. Automated ribosomal intergenic spacer analysis (ARISA)

The soil bacterial communities were investigated using automated ribosomal intergenic spacer analysis (ARISA). Soil DNA extracted as described above, was used as a template in PCR amplifications using universal primers ITSF/ITSReub targeting the 16S-23S rRNA ITS (Cardinale et al., 2004). ITSReub were labelled with the phosphoramidite dye HEX (6-carboxy-1,4-dichloro-2,4,5,7-tetra-chlorofluorescein). ARISA-PCR was performed following the method of Cardinale et al., 2004, with modifications. Reaction mixtures contained OneTaq Standard Reaction Buffer 1 X (NEB), a 200 μM concentration of dNTPs mixture (Invitrogen), a 250 μM concentration of each primer, 1U of OneTaq DNA Polymerase (NEB), and approximately 10 ng of template metagenomic DNA in a final volume of 50 μl. Reaction mixtures were held at 94 °C for 30 sec, followed by 30 cycles of amplification at 94 °C for 30 sec, 55 °C for 1 min, and 68 °C for 1 min and a final extension of 68 °C for 5 min. The best concentration of labelled PCR product was tested and estimated by comparing it to known quantities of PCR products and by run a dilution series on Bioanalyzer ABI PRISM® 310 Genetic Analyzer. After determining the optimal dilution ratio, the same dilutions for all ITS products was utilized. Based on these estimates, a standardized amount (between 0.5 and 4 μl) of PCR product was used for ARISA. Amplified ITS fragments were separated on a capillary electrophoresis Bioanalyzer ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Run conditions were performed as described elsewhere (La Marca et al., 2021). The output of ARISA was analyzed by the Peak Scanner software vs1.0 and the biodiversity indices were calculated using the PAST 3.0 software (http://folk.uio.no/ohammer/past). Fragments in the range between 50 and 1000 bp were analysed and exported into spreadsheets for subsequent analysis, the fluorescence threshold was set at 50 relative fluorescence units (RFU). A bin size of 5 bp was employed to minimise the inaccuracies in the ARISA profiles.

2.4. Illumina High-Throughput analysis of 16S rRNA gene and data processing

The V3-V4 hypervariable region of prokaryotic 16S rRNA gene, for the simultaneous detection of bacteria and archaea, was sequenced using Miseq Illumina with 300 bp paired-end reads. PCR amplification was performed in a 25 μl reaction volume containing 10 ng of

metagenomic DNA, 1 × MightyAmp buffer ver. 2, 0.5 μl of MightyAmp DNA polymerase (TaKaRa Bio, Japan), 0.25 mM of Pro341F/Pro805R primers (Takahashi et al., 2014). The PCR procedure was as follows: an initial cycle of 2 min at 98 °C, followed by 35 cycles of annealing beginning at 65 °C and ending at 55 °C for 15 sec (the temperature was lowered 1 °C every cycle), and extension at 68 °C for 30 sec. Sequences obtained from Illumina Sequencing were processed using the QIIME2 software package version 2018.4 (Caporaso et al., 2010). To find the reads containing adapters and to remove the adapters Cutadapt software version v2.9 was used. Quality check, trimming, error rate estimation, dereplication and read merging were done using the DADA2 a R packages wrapped in QIIME2 (Callahan et al., 2016). To remove chimeras from the Illumina sequenced FASTQ files the "consensus" method implemented in DADA2 was used. For taxa assignment the QIIME2 q2-feature-classifier plugin that was trained on the Greengenes database version 13–8 with a 99 % Operational Taxonomic Units (OTUs) full-length sequences. QIIME2 taxa barplot command and ggplot2 were used for visualization of the taxonomic composition of the samples.

The 16S rRNA gene sequences were deposited into the NCBI short reads archive (SRA) database under BioProject number PRJNA805054.

2.5. Statistical analysis

To test the effects of soil physical–chemical properties (SOC, pH, TN and CEC) on SMB, richness and diversity we used multiple linear regression. We first performed ordinary least square regression and checked the residuals of the models for normality with a Shapiro-Wilk test. To test the differences across the three countries and five land uses we used ANOVA with Tukey HSD post-hoc test for richness and Kruskall-Wallis with Dunn post-hoc test with Bonferroni correction for SMB and Shannon diversity index. We also tested for differences in richness (*t* test), SMB and diversity (Wilcoxon test) across two groups of sites, those containing SOC < than 20 g kg^{-1} soil (n = 31) and those with higher values (n = 38), following the threshold reported in Grilli et al. (2021) as critical for desertification.

Multivariate analysis of community structure and (beta)diversity was performed on the ARISA-based dataset and soil variables using Canonical Analysis of Principal Coordinates CAP with PRIMER v7 (Clarke and Gorley, 2015) with the PERMANOVA+ add-on package (Clarke and Gorley, 2015).

To check for differences in the relative abundancies of bacterial phyla between countries and soil use, we used ANOVA and post hoc Tukey HSD tests for the normally distributed data (after Shapiro-Wilk test on the residuals) and Kruskal-Wallis with post hoc Dunn test for the non-normally distributed phyla (Bacteriodetes, Chloroflexi e Firmicutes). We also compared the relative abundances between sites with more (n = 14) and less (n = 6) than 20 g kg^{-1} of SOC.

We used multiple regressions to test the relationship of soil physical–chemical variables (pH, SOC, TN, CEC) with the relative abundance of the most represented phyla. For Bacteroidetes, Chloroflexi and Firmicutes we fitted generalized linear models with negative binomial distribution and log link functions to control for the overdispersion found in the Poisson models. For the other taxa we used ordinary least square regressions. After fitting the models, we checked for multicollinearity among the predictors using the Variance Inflation Factor, with all values below 4.3. Statistical analysis was performed with R v 3.6.1 (R core Team 2019).

To check for the relationship between aridity index (the ratio of annual precipitation to annual potential evapotranspiration) and SMB, richness and Shannon diversity, the sites were classified in two groups: semi-arid (aridity index > 0.2 and < 0.5) or not-arid (>0.65), accordingly to the Aridity Index rank (Middleton and Thomas, 1992). Differences across the two groups were assessed with Wilcoxon rank test for SMB and Shannon index and t tests for richness.

Table 1

Summary of the regression testing the effects of SOC, pH, N and CEC on soil microbial biomass (SMB), richness (estimated as observed bacterial OTUs), and diversity (Shannon index) calculated on ARISA profiles. dsDNA. R^2 = adjusted fit of the model. Significant p values in bold characters.

		Estimate	SE	t	p
SMB	Intercept	−19.156	28.005	−0.68	0.496
R^2 = 44	SOC	0.572	0.177	3.23	**0.002**
	pH	7.013	4.089	1.71	0.091
	TN	10.012	3.974	2.52	**0.014**
	CEC	−0.889	0.798	−1.11	0.270
Richness	Intercept	−3.368	6.723	−0.50	0.618
R^2 = 0.49	SOC	0.099	0.042	2.33	0.023
	pH	4.676	0.982	4.76	**<0.001**
	TN	−2.249	0.954	−2.36	0.021
	CEC	0.334	0.192	1.74	0.086
Shannon	Intercept	2.505	0.241	10.389	**<0.001**
R^2 = 0.21	SOC	0.002	0.001	1.478	0.144
	pH	0.089	0.035	2.557	**0.013**
	TN	−0.067	0.034	−1.943	0.056
	CEC	0.005	0.007	0.701	0.486

3. Results and discussion

3.1. Soil microbial biomass

The total soil microbial biomass (SMB) of 69 soil samples from 30 study sites (Table S1, Fig. S1) across southern Europe Mediterranean regions was estimated using total soil dsDNA as a proxy (Dequiedt et al., 2011,Fornasier et al., 2014). The amount of extracted metagenomic dsDNA from soils of the study sites ranged from 7.09 to 208.6 μg g^{-1} soil dry weight and varied significantly across sites (Fig. S2a. Kruskall-Wallis χ^2 = 44.92, p < 0.001), although the most significant differences were found within Sicilian sites and between Sicilian and Extremadura (SP) sites (Dunn test p < 0.05; Fig. S2). SMB was positively related to SOC and also to TN (Table 1), coherently with the positive correlation between SOC and TN (Plassart et al., 2019, Grilli et al, 2021). SMB was significantly higher in non-arid sites than in semiarid sites (Fig. S3, soils with AI > 0.65, Wilcoxon X^2 = 11.61, p < 0.001), as a result of higher plant productivity, increased access to substrates due to soil moisture, and higher microbial growth rates, in accordance with the results found in other studies (Maestre et al., 2015,Ren et al., 2018). SMB also varied across cover with coniferous stand soils displaying the highest SMB, and croplands the lowest (Dunn test p < 0.05, (Fig. 1, Table S1,S2). Microbes in croplands are generally affected by cultivation practices that disrupt soil aggregates, where they live, and by inorganic fertilization, that increases nutrient availability, promoting microbial community shifts towards more copiotrophic taxa, with higher respiration rates (Leff et al., 2015). Since most SOC formation is mediated by microbial activity (Gleixner, 2013), a reduction in microbial biomass and, eventually, in its substrate use efficiency, in tilled soils, may diminish the overall potential for SOC formation (Cotrufo et al., 2013). For these reasons sustainable soil management, based on reduced tillage, organic fertilization and cover crops, could help to maintain crop yield while avoiding further SOC depletion in semi-arid soils (Novara et al., 2020).

Interestingly, high SMB was detected under the coniferous forest of the driest investigated area in Lampedusa (IT1, aridity index: 0.29), where high SOC levels were also detected (Grilli et al., 2021) in agreement with other studies on SOC under pine tree woods (Xie et al., 2013). The levels of microbial biomass under coniferous stands are controversial because other studies report that soils under pine plantations, in semi-arid soils, present markedly smaller and less active microbial communities (Goberna et al., 2007, Grilli et al., 2020, Rutigliano et al., 2004), generally explained by the lower availability and degradability of organic substrates, such as lignin (Dequiedt et al., 2011). However the
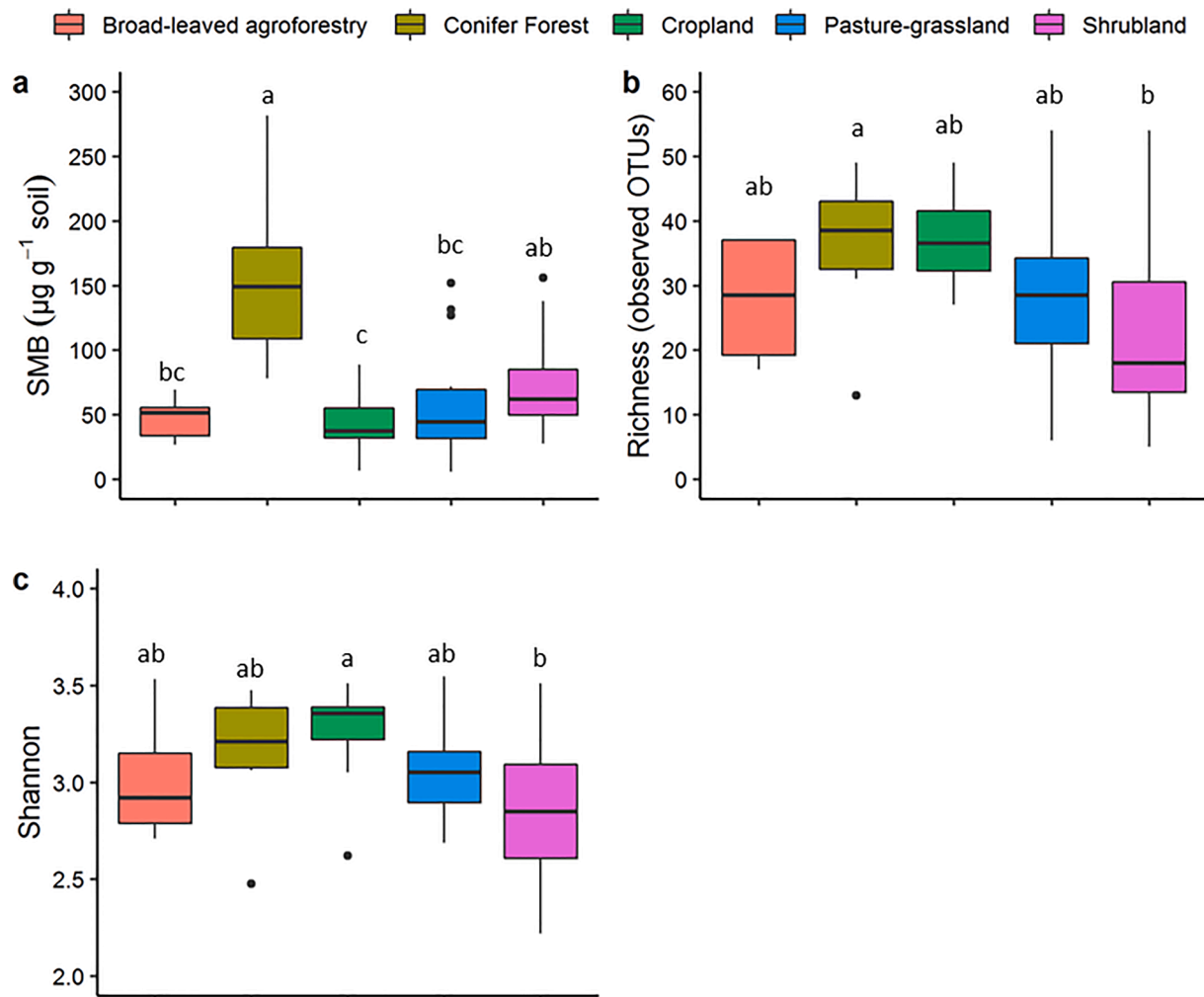
Fig. 1. Boxplots (median. interquartile and range) of a) SMB, b) richness and c) Shannon index across the five land uses in the farms of Sicily (Italy), Extremadura (Spain) and Alentejo (Portugal). SMB was estimated as total soil dsDNA, diversity indices were based on ARISA.

involvement of bacteria in wood litter decomposition, including cellulose and aromatic compounds derived from lignin degradation, is more important than was previously assumed (Lladó et al., 2017). As bacteria, more than fungi, can modulate at community level their carbon use efficiency, the specific composition of the soil microbiota could explain SOC accumulation in dry soils. Drought could act on bacteria by reducing the microbial respiration rate (Ullah et al., 2021), and increasing the stability of soil organic matter (Hagerty et al., 2014). Moreover bacteria could persist in soils for long periods in a dormancy condition as spores and cysts and be revived when environmental conditions change (Bastida et al., 2016, Tecon and Or, 2017). All this could contribute to higher resilience to drought of coniferous forest ecosystems in respect to other soil uses (Moreno et al., 2019, Cotrufo et al., 2019). We found the lowest SMB levels in soils with SOC < 20 g kg^{-1} (Wilcoxon Z = -4.73, p < 0.001), the threshold value below which any additional decline of SOC concentrations might result in a steep variation of most soil parameters (Grilli et al., 2021), indicating that this concentration is also critical for key microbe-driven soil ecosystem services (Bastida et al., 2021).

3.2. Relationship between bacterial diversity and edaphic variables

Molecular fingerprinting of the soil bacterial communities, was obtained by the automated ribosomal intergenic spacer analysis (ARISA) on a subset of 69 soil DNA samples from the 30 study sites. Average OTU richness ranged from 9.0 (S.E. 0.5) to 46.5 (0.26) and Shannon index ranged from 2.34 (0.26) to 3.55 (0.2). Soil bacterial richness and Shannon index significantly differed across the sites (Fig. S2, ANOVA F = 33.08, p < 0.001, Kruskall-Wallis χ^2 = 20.68, p < 0.001) with soils of Sicily presenting higher richness than most of the other sites (p < 0.05 after Tukey HSD). Richness and Shannon index also varied across land uses (F = 4.79, p = 0.002; χ^2 = 21,76, p < 0.001), although only between coniferous and shrublands (richness) and croplands and shrublands (Shannon) the difference was significant (after Tukey HSD; Fig. 1). Bacterial richness was the highest in coniferous soils while Shannon index was the highest in croplands; both indexes showed the lowest values in shrubland soils.

Among the analyzed soil variables, pH was the most influencing factor affecting soil bacterial richness and Shannon index with significant positive relationship (p < 0.001 and p < 0.05, respectively). Shannon bacterial diversity tended to be higher in low-SOC soils (Z = 2.28, p = 0.02), while bacterial richness was not significantly influenced by SOC levels (t = -1.72, p = 0.08). Coherently, semi-arid sites (AI < 0.65) presented higher bacterial diversity (richness t = -5,04, p < 0.0001; Shannon index X^2 = 11.78, p < 0.001) than non-arid sites (AI > 0.65) (Fig. S3). Several studies have reported such a negative relation between soil bacterial diversity and total C, leading to the conclusions that, a decline in SOC, as a result of soil degradation and aridity, leads to an increased bacterial diversity (Delgado-Baquerizo et al., 2017, Delgado-Baquerizo et al., 2018, Qi et al., 2018) especially in arid soils
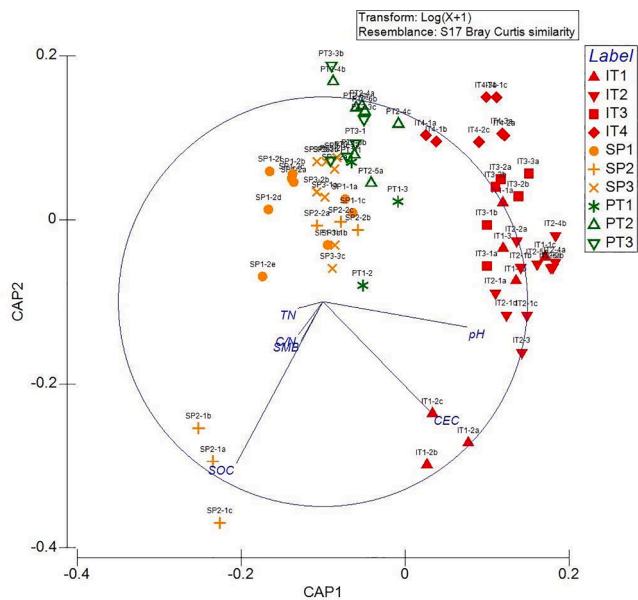
Fig. 2. Canonical Analysis of Principal Coordinates (CAP) calculated on the ARISA profiles of 69 soil samples of the ten farms located in Sicily (Italy), Extremadura (Spain) and Alentejo (Portugal) and the relative main chemical physical soil variables. Labels of bacterial communities are described in Table S1.

(Bastida et al., 2021). Reduction of soil C content result in an increase of bacterial diversity probably due to decrease of dominant taxa and consequently, to release of subordinate taxa which contribute to increasing bacterial diversity. In soil with high C content, the abundance of some dominant taxa is promoted; these taxa may suppress the total bacterial diversity by preventing the establishment of other species by competitive exclusion mechanisms (Delgado-Baquerizo et al., 2017, Delgado-Baquerizo et al., 2018, Bastida et al., 2021). In the semi-arid

soils, however, a large part of the biomass, contributing to the extracted dsDNA, could be dormant, thus metagenomics could overestimate the real expressed soil functional microbial diversity. In this respect metaproteomics could be a better predictive indicator of ecosystem functionality than DNA diversity (Bastida et al., 2016).

The Canonical Analysis of Principal coordinates (CAP) indicated that beta-diversity is related to the main soil parameters (Grilli et al., 2021, Fig. 2), mainly pH, SOC and CEC, as already reported in other studies (Delgado-Baquerizo et al., 2017, Qi et al., 2018).

The similarities among the bacterial communities across all sites reflected their biogeography confirming that soil bacterial communities were spatially structured (Lladó et al., 2017, Plassart et al., 2019). Interestingly, the coniferous forest soils (SP2-1 and IT1-2) displayed the highest distance from their respective biogeographic cluster, indicating the strong influence of conifer cover on the soil bacterial assemblage.

3.3. Soil bacterial community structure in the different European Mediterranean sites

Illumina sequencing of the 16S rRNA gene was carried out on a subset of 22 soil DNA samples. The subset was selected to include different soil covers in the three regions, and two replicates (a and b) for each site. Only one sample (IT1-2 replicate b) gave no results, due to low quality DNA that hampered further processing. The subset of 21 soil DNA samples was sequenced and resulted (after denoising and chimeras check) in a minimum of 13,482 to a maximum of 73,358 reads, with an average of 44,922 reads for each sample (Table S2). Richness, assessed on bacterial observed OTUs clustered at 97 % (OTU_{97}), ranged from 107 to 581 OTU_{97} (Table S2), both extremes were observed in two croplands in central Sicily (Italy).

OTU_{97} richness, was positively correlated to the alpha diversity based on ARISA profiles (Pearson tests r = 0.36, p = 0.12) considering all data, and the correlation improved and was significant (r = 0.56, p = 0.013) excluding the outlier IT4-1a. This significant correlation shows that the two data sets of bacterial diversity were congruent (Terrat et al., 2015).
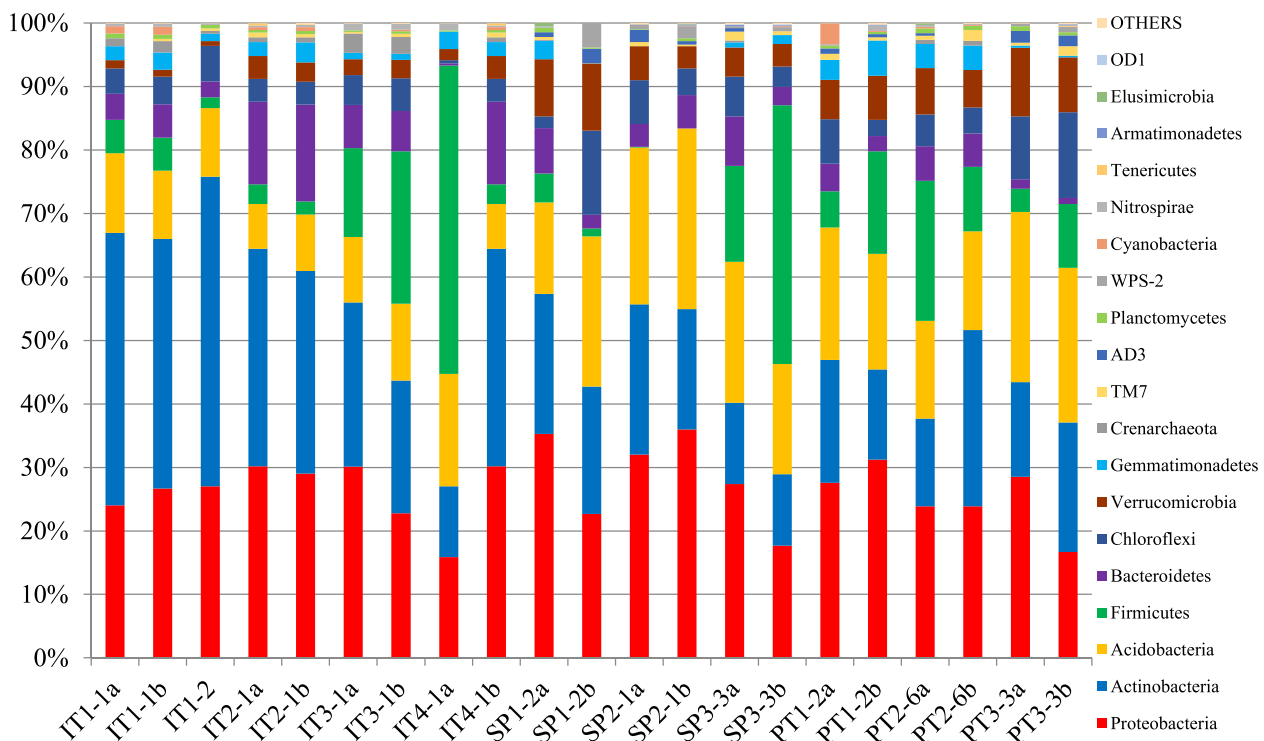

Fig. 3. Relative abundance of soil bacterial and archaeal phyla in the semi-arid soils of Southern Europe. Others includes phyla <0.1 %.

Table 2

Summary of the OLS and GLM models testing the effects of cation exchange capacity (CEC), pH, total nitrogen (TN) and soil organic carbon (SOC) on the relative abundances of the 7 most abundant phyla. In bold characters the significant values ($p < 0.05$).

Phylum		Estimate	SE	t/z	p
Acidobacteria	Intercept	26.78	4.25	6.30	**<001**
	CEC	−0.28	0.11	−2.49	**0.024**
	pH	−1.60	0.68	−2.35	**0.032**
	TN	0.42	0.42	0.98	0.340
	SOC	0.04	0.02	1.76	0.098
Actinobacteria	Intercept	−5.66	9.41	−0.60	0.556
	CEC	0.67	0.25	2.68	**0.016**
	pH	2.97	1.51	1.97	0.066
	TN	−1.23	0.94	−1.31	0.210
	SOC	0.05	0.04	1.10	0.287
Bacteroidetes	Intercept	1.57	0.99	1.58	0.114
	CEC	0.05	0.03	1.97	**0.049**
	pH	−0.04	0.16	−0.23	0.821
	TN	−0.10	0.10	−1.01	0.313
	SOC	0.00	0.00	−0.46	0.648
Chloroflexi	Intercept	6.46	0.92	6.98	**<001**
	CEC	0.07	0.03	2.69	**0.007**
	pH	−0.70	0.16	−4.39	**<001**
	TN	−0.11	0.09	−1.30	0.194
	SOC	−0.01	0.00	−2.21	**0.027**
Firmicutes	Intercept	0.55	1.06	0.52	0.602
	CEC	−0.11	0.03	−3.95	**< 001**
	pH	0.39	0.16	2.37	**0.018**
	TN	0.39	0.12	3.19	**0.001**
	SOC	−0.02	0.01	−2.37	**0.018**
Proteobacteria	Intercept	17.09	7.16	2.39	0.030
	CEC	0.16	0.19	0.84	0.413
	pH	0.41	1.15	0.36	0.727
	TN	−0.02	0.72	−0.03	0.979
	SOC	0.05	0.03	1.57	0.136
Verrucomicrobia	Intercept	17.37	3.40	5.11	**< 001**
	CEC	0.02	0.09	0.21	0.836
	pH	−1.87	0.54	−3.43	**0.003**
	TN	0.20	0.34	0.60	0.557
	SOC	−0.03	0.02	−1.76	0.098

All soils contained the main typical soil bacterial phyla (Delgado-Baquerizo et al., 2018) but with different abundances in respect to European soils of other climatic zones (Plassart et al., 2019) and also in respect to desertified soils of other continents (Qi et al., 2018).

The most abundant bacterial phyla were Proteobacteria (average relative abundance 26.6 %), Actinobacteria (24.2 %), Acidobacteria (16.6%), Firmicutes (11.3%), Bacteroidetes (5.5%), Chloroflexi (5.4%) and Verrucomicrobia (5.0%) (Fig. 3). The dominance level of these phyla is more similar to previous characterizations of world's dry forests and drylands (Delgado-Baquerizo et al., 2018) than non-Mediterranean European soils (Plassart et al., 2019). In Southern European soils Proteobacteria were less abundant, and not always the dominant phylum, while Actinobacteria and Acidobacteria were much more abundant than in higher European latitudes (Plassart et al., 2019). Archaea were below 2%, mainly represented by Crenarchaeota. Beyond Proteobacteria, the phyla Acidobacteria, Verrucomicrobia and Chloroflexi were more abundant in the soils of Extremadura (SP) and Alentejo (PT) while Actinobacteria were more abundant in Sicilian soils (IT) (Fig. S4). The overall relative abundance of the 7 main phyla did not vary across soils (Kruskal-Wallis $X^2 = 0.76$, $p = 0.68$) or land use ($X^2 = 1.09$, $p = 0.89$).

Considering each phylum separately, however, the abundance of 5 out of the 7 dominant phyla was significantly related to cation exchange capacity (CEC), with positive and negative relationships (Table 2). Soil CEC is considered strongly correlated with the organic carbon content (Plassart et al., 2019) as well as to the physical (structural stability), chemical (nutrient availability), and biological characteristics of soils (Hazelton and Murphy, 2007). Four phyla were related to pH, two positively, Firmicutes and Verrucomicrobia, and two negatively, Acidobacteria and Chloroflexi. Interestingly, the phylum Firmicutes was significantly related to all edaphic variables (SOC, pH, TN, and CEC), conversely Proteobacteria was not significantly related to any of the analyzed variables. In particular the relative abundance of Firmicutes was negatively related to SOC, in agreement with the role of Firmicutes as responsible for enhanced mineralization of dissolved organic carbon and increased soil respiration (Fierer et al., 2007, Whitman et al., 2016).

Conversely, Acidobacteria and Chloroflexi, more abundant in acidic soils of broad leaved agroforest in Spain and Portugal, could increase the mineralization of dissolved organic carbon; consequently the soils with an abundance of these phyla have low carbon turnover and low carbon dioxide emission, resulting in higher carbon sequestration capacity (Singh et al., 2010). In soils of Sicily the most abundant phylum was Actinobacteria (Tecon and Or, 2017). The definition of this phylum as copiotroph or oligotroph is controversial. Some studies associate it with copiotrophy, others suggest that it is better suited to oligotrophic conditions probably because it includes members that are able to use both labile and recalcitrant substrates (Ho et al., 2017). In areas with SOC below 20 g kg^{-1}, Actinobacteria ($t = 2.70$, $p = 0.014$), Bacteroidetes ($Z = -2.10$, $p = 0.035$) and Proteobacteria ($t = 2.91$, $p = 0.012$) were significantly less abundant, suggesting a copiotrophic lifestyle of these three phyla, including Actinobacteria.The correlation of edaphic variables with soil bacterial abundance and lifestyle, however, depends on the taxonomic level considered (Ho et al., 2017). For example, CEC effects was not significant only for two phyla (Table 1), while for the remaining five the effects were both positive and negative. Additionally, we found compositional turnovers when considering the most abundant order and genera of the same phyla, with consequent variation in the responses to edaphic variables (Table 3, Table S3). In general, the correlation between the chemico-physical parameters and the five most abundant orders within each phylum, confirmed the prevalent role of pH in modulating the distribution of bacterial taxa in soils, with positive and negative correlations (Table S3). Within Actinobacteria, two orders (Acidimicrobiales and Solirubrobacterales) showed positive and significant relation to SOC. In turn, Proteobacteria, Rhodospirillales and Rhizobiales were positively related to SOC suggesting that they may contribute to C sequestration in soils of semi-arid regions. Only 38 % of the sequences (on average) was identified at the genus level (Fig. 4).

Most of the genera with abundancies above 1 % were correlated to soil pH with the exclusion of those within the phylum Firmicutes (Table 3). Conversely, in the Firmicutes, 4 out of the 6 most abundant genera (*Bacillus, Ammoniphilus, Solibacillus* and *Sporosarcina*) were negatively correlated to SOC and 3 also to CEC, with *Ammoniphylus* and *Bacillus* showing highly significant negative correlations (Table 3). This negative correlation with SOC of Firmicutes, was also confirmed at the order level (Table S3). *Bacillus* and *Ammoniphilus* are known copiotrophs meaning that they rapidly consume the substrate (Ranney and Clark, 2016) thus giving low contribution to SOC accrual. The genus *Bacillus* in particular was dominant in low-SOC pastures, broad-leaved forest soils of Portugal and in a cropland of central Sicily, where *Opuntia ficus indica* is organically cultivated with no-tillage and mulching using *Opuntia* cladodes (M. Russo personal communication). The abundancy of copiotrophs in this cropland could be the result of abundant fresh organic matter (but with low molecular diversity) resulting from mulching of *Opuntia* cladodes. Probably introducing a greater molecular diversity by mulching with more than one organic source, may increase the metabolic demand, and thus potentially limit soil C loss by respiration (Lehmann et al., 2020). However, before considering Firmicutes as markers of low quality soils, we should also consider the role of *Bacillus*

Table 3

Spearman correlation coefficients between the relative abundance of the most abundant genera and main soil parameters: cation exchange capacity (CEC), pH, total nitrogen (TN) and soil organic carbon (SOC) obtained in the sampled soils. Only significant correlations (p-value < 0.05) are shown. Blue and red colors indicate negative and positive relationships, respectively, with color intensity representing the degree of significance (p < 0.05 > 0.01, <0.01 > 0.001 and <0.001).

Phylum	Genera	CEC	pH	TN	SOC
Acidobacteria	Candidatus Koribacter	−0.52	−0.69		
	Candidatus Solibacter	−0.46	−0.78		
Actinobacteria	*Arthrobacter*		0.66		
	Mycobacterium		−0.48		
	Pseudonocardia		0.68		
	Rubrobacter	0.48	0.84		
	Streptomyces	−0.55		−0.6	−0.6
Alphaproteobacteria	*Balneimonas*		0.86		
	Bradyrhizobium		−0.66		
	Kaistobacter				−0.54
	Rhodoplanes		−0.66		0.48
	Skermanella		0.88		
Bacteroidetes	*Adhaeribacter*		0.83		
	Flavisolibacter		0.48		
	Rhodocytophaga	0.57	0.7		
Betaproteobacteria	*Burkholderia*		−0.82		
Deltaproteobacteria	Candidatus Entotheonella	0.63	0.84		
	Geobacter				
Firmicutes	*Alicyclobacillus*				
	Ammoniphilus	−0.77			−0.63
	Bacillus	−0.57			−0.71
	Planifilum			−0.47	
	Solibacillus				−0.55
	Sporosarcina	−0.51			−0.52
Gammaproteobacteria	*Pseudomonas*		0.62		
	Steroidobacter		0.84		
Nitrospirae	*Nitrospira*		0.73		
Verrucomicrobia	Candidatus Xiphinematobacter				
	DA101	−0.57	−0.51		

as a plant growth promoter and P solubilizing agent, especially valuable in arid soils (Ayangbenro and Babalola, 2021). Only two genera were positively correlated with CEC, namely *Rubrobacter* (Actinobacteria) and Candidatus Entotheonella (Deltaproteobacteria) both detected in italian soils and both also positively related to soil pH. A negative correlation with SOC and also to CEC was observed at level genus within Actinobacteria, only with the genus *Streptomyces* that was highly abundant in low SOC soils.

3.4. The core microbiota of semiarid Mediterranean soils

The soil core microbiota was identified as phyla and genera that exist in all the samples with at least 1 % of the relative abundance within each sample, respectively (Qi et al., 2018). At the phylum level the core microbiota of semi-arid soils under desertification risk of southern Europe (considering exclusively those from sites with an AI < 0.65, Table S1), was composed of four phyla Proteobacteria, Actinobacteria, Acidobacteria and Firmicutes (Fig. 3). At genus level we were unable to define a common core microbiota, due to high variability of edaphic parameters. At regional level *Bacillus, Rubrobacter* and *Balneimonas* were the (identified) core microbiota in Sicilian soils (AI 0.29–0.49) (Fig. 4). While *Bacillus* is an ubiquitarious genus, *Rubrobacter*, belonging to a deep evolutionary line of descent in the class of Actinobacteria is known for its multi-extremophilic growth conditions. Although it was not possible to classify it at species level, this genus is highly represented in extremely hot, arid and/or acidic ecosystems or habitats with severe radiation/desiccation conditions, such as deserts, volcanic areas and other arid regions (Novara et al., 2020, Kouřilová et al., 2021). Its presence was recently associated to high polyphenol oxidase activities and accumulation of polyhydroxyalkanoates that could function as protection against stress factors (Moreno et al., 2019). *Balneimonas* (Bradyrhizobiaceae) is also associated to arid soils where it produces extracellular material that contributes to the formation of soil crusts (Matthews et al., 2019, Khan and Khan, 2020).

The core microbiota of Alentejo soils (AI 0.35–0.38) beyond *Bacillus,* included: DA101*,* Candidatus Solibacter (Acidobacteria), and *Rhodoplanes* (Fig. 4). The phylotype DA101 within the, as yet, underdescribed soil phylum Verrucomicrobia, has been reported in a wide range of soils and ecosystem types throughout the world. In this work it resulted negatively correlated to CEC, coherently with its previous identification as low fertility specialist (Fierer et al., 2013). Similarly, Candidatus Solibacter belonging to slow growing oligotrophs adapted to resource limitations (Lladó et al., 2017), is strongly negatively correlated to soil pH and CEC (Tables 2 and 3). This genus, reported as dominant in arid soils (Khan and Khan, 2020), was abundant also in coniferous forest soils of our study sites. *Rhodoplanes* is a phototrophic (Khan and Khan, 2020), nitrogen fixing genus (Buckley et al., 2007).These traits, together with the positive correlation with SOC, suggest a pivotal role of this genus in low nutrient Mediterranean semi-arid soils where it may be involved in carbon and nitrogen fixation. *Rhodoplanes* was also the only genus represented above 0.8 % in all investigated soils. Thus, *Rhodoplanes* could be considered "the rare endemic genus" within arid Mediterranean soils where drought reduces the dominance of common bacterial species and allow rare soil bacteria suited for resource limited environments to proliferate (Vohník et al., 2013, Bickel and Or, 2021).

4. Conclusions

This work provides the first large scale overview of the soil bacterial diversity across European Soils threatened by desertification in the Mediterranean regions.

Overall, the results obtained so far, suggest that the impact of desertification and soil degradation on the soil microbiota, could be fundamentally different from previous knowledge for well-studied plants and animals (Zhou et al., 2020). According to our results, increasing aridity (that means lowering the AI) due to rain reduction and increased temperatures, will lead to a reduction in both SOC and microbial biomass with consequential effects on soil ecosystem services (Bastida et al., 2021). Conversely bacterial diversity, that did not correlate to the soil organic carbon content, nor to cation exchange
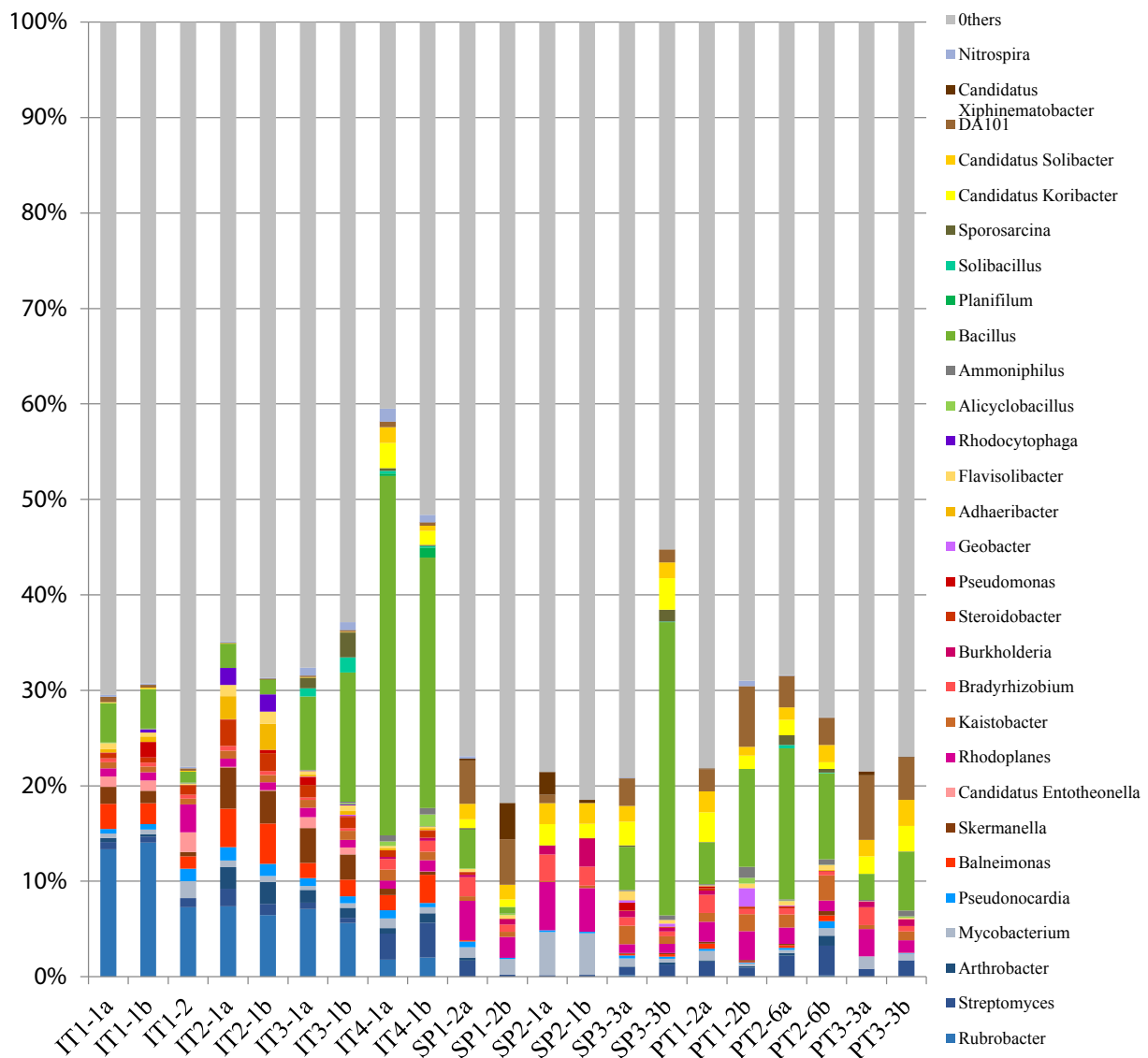
Fig. 4. Relative abundance of bacterial genera in the soil communities of desertified ecosystems in Souther Europe. Genera whose abundance is above 1% in at least one sample are shown. Others includes 292 genera with abundances below 1% in all sites.

capacity, will be affected in terms of community structure and composition but not necessarily as alfa diversity. Our study reveals divergent patterns of bacterial alpha diversity in semi-arid and non-arid sites, with bacterial diversity (OTU richness and Shannon diversity) showing increasing trends, from non -arid toward semi-arid the sites. These results support hypothesis that aridity have effects on the soil bacterial OTU richness and bacterial diversity directly. It appears that microbial biomass rather than diversity is the soil biological index most appropriate to indicate the negative effect of desertification on soil biology. The main edaphic driver of bacterial diversity, in the analyzed sites, was pH, an intrinsic soil parameter that was confirmed positively related to biodiversity. Beyond soil pH, SOC and CEC exerted the strongest influence on the soil bacterial community structure, which was also influenced by biogeography, as bacterial community similarity decreased significantly with increasing geographic distance.

Among soil cover, croplands displayed the lowest microbial biomass while shrublands hosted the lowest diversity. In contrast, coniferous forest soil displayed higher beta-diversity irrespectively of aridity index levels and biogeography than generally reported, and this could contribute to higher resilience to drought of these ecosystems in respect to other soil covers. In this sense, future research might investigate the

effects of historical land-use legacies as well as vegetation spatio-temporal changes including more pristine areas as control (Bueno et al. 2020) in order to have a deeper understanding on microbial composition and dynamics across different land-uses.

Overall, it appears difficult to describe a core microbiota of Mediterranean semi-arid soils because microbial responses to increasing warming and drought across soils and regions can be different and related to other edaphic and environmental parameters. Future modifications induced by global change factors in the microbial community structure may have different impact on soil functionality by affecting the abundance of relevant phyla such as Actinobacteria, Bacteroidetes and Proteobacteria and by selecting different resistant and resilient bacterial taxa, such as the rare multi-stress resistant genera that make up the (identified) core microbiota of semi-arid European soils. The genome of the core members *Rhodoplanes, Rubrobacter, Balneimonas,* and phyloptype DA101 endowed with highly specific traits to cope with low nutrient, dry, harsh conditions, could reveal ecofunctional strategies that can be exploited for arid land reclamation. Even more could be revealed by metagenomics and metaproteomics approaches that will shed light also on the taxa that were not identified by 16S amplicon metagenomic sequencing.

The results of this investigation will be useful as baseline for the necessary monitoring of European soils facing the intensification of climate change and for the evaluation of the efficacy of adaptation measures to contrast desertification.

CRediT authorship contribution statement

V. Catania: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **R.S. Bueno:** Conceptualization, Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. **R. Alduina:** Investigation, Writing – review & editing. **E. Grilli:** Investigation, Writing – review & editing. **T. La Mantia:** Investigation, Writing – review & editing. **S. Castaldi:** Investigation, Writing – review & editing. **P. Quatrini:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109725.

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