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Bacterial community structure of two Mediterranean agricultural soils amended with spent coffee grounds

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1. Introduction

Natural and anthropogenic causes of soil quality decline include land use, soil overexploitation, deforestation, climate change and heavy rainfall. As a result, soil degradation processes, such as acidification or salinization, and loss of the soil structure or organic matter (Muñoz-Rojas et al., 2015), represent a serious challenge for environmental and socio-economic sustainability (Boardman, 2006).

Soil quality is assessed in regard to global improvement of chemical (organic carbon, pH, electrical conductivity, available NPK, cation exchange capacity), physical (soil structure, aggregates) and biological (microbial communities) parameters (Acevedo et al., 2005). Different organic and inorganic soil conditioners have been studied to improve soil quality and increase crop yield, such as inorganic fertilizers (Obi and Ebo, 1995), or biofertilizers (N-fixing, and P- and K-solubilizing bacteria; Jilani et al., 2007).

Waste from plant and animal based agriculture has also been used to improve soil quality, including the addition of: pig manure and rice straw to agricultural soil (Hui et al., 2017); pearl millet residue and cattle manure as a fertilizer in a sandy soil (Suzuki et al., 2017); and mushroom compost, green manure, cattle manure and rice straw as organic amendments in a low-productivity paddy soil (Mi et al., 2016).

Coffee is the largest traded product after petroleum (Nabais et al., 2008), and as a consequence produces a large amount of waste. Two kg of wet spent coffee grounds (SCG) are produced from every kg of coffee (Pfluger, 1975). Spent coffee grounds is the powdered organic remnant from steaming the ground coffee, accounting for > 50% of the initial coffee ground volume (Tsai et al., 2012). The solid acidic residue has a fine particle size with high humidity content (80–85%) and organic load (Mussatto et al., 2011). Spent coffee grounds can increase soil organic matter content (Murthy and Naidu, 2012), and improve soil structure by allowing for an appropriate balance of water retention and drainage (Kasongo et al., 2013; Hardgrove and Livesley, 2016). Therefore, the use of SCG as an alternative soil conditioner may well have "Triple Bottom Line" advantages, having social, environmental/ecological and financial benefits.

Addition of organic and inorganic substances has a direct effect on soil microbial abundance, diversity, and metabolism (Wang et al.,2013); therefore microbial observation could be used as an indicator of soil quality (Giacometti et al., 2013). Although the effects of SCG addition on the soil microbiota have not been described previously, there are several studies on the phytotoxic effect of SCG supplementation on heavy metal contaminated water/soils (Kim et al., 2014) and horticulture soils (Hardgrove and Livesley, 2016; Cervera-Mata et al., 2017). Chelating substances within the SCG, such as caffeine, melanoidins and polyphenols, are thought to cause this phytotoxicity (Jiménez-Zamora et al., 2015).

Unlike open airfield tests, microcosm studies help minimize environmental heterogeneity, hence numerous soil studies use this method (Sun et al., 2017; Casanovas-Massana et al., 2018). In Cervera-Mata et al. (2017), 2 Mediterranean agricultural soils (*Vega* and *Red* soil) with low organic matter content, were amended with different concentrations of SCG, and soil microcosms were set-up with *Lactuca sativa* var. *longifolia* ("Little Duende" lettuce, 30-day grown plant). Soil respiration rates demonstrated that SCG stimulated microbial activity, concurrently, high fungal hyphae densities were observed in microcosms treated with SCG. Bacterial activity was not determined in the study. Total Nitrogen content increased with time after addition of SCG, and it was suggested that nitrogen-fixing bacteria might also have similarly increased. The aim of the current study was to test whether the addition of SCG as a soil conditioner improved the physicochemical properties of the soil and what effect it had on microbial groups. The study of the effects of SCG on the soil microbiota would fill the current knowledge gap regarding their reuse in soil quality enhancement systems, by analysing links between microbial diversity and functionality.

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Table 1
Physicochemical properties of spent coffee grounds and poor Mediterranean Red and Vega soils (0–20 cm). †Data obtained from Cervera-Mata et al. (2017).

Sample	Gravel (> 2mm) (%)	Sand (%)	Silt (%)	Clay (%)	†pH	†EC_{25} (dS/m)	†OC (%)	†Total N (%)	†C/N	†Av. P (mg/Kg)	†Av. K (mg/Kg)	†CAC (%)
SCG	–	–	–	–	5.8	4.5	59.38	1.867	32	228	3072	nd
Red Soil	6.0	38.8	18.0	43.2	7.2	0.6	1.16	0.113	10	51	248	1.6
Vega Soil	2.0	12.1	29.0	58.0	8.2	1.3	1.36	0.105	13	69	453	39.0

SCG – spent coffee grounds; EC_{25} – electrical conductivity measured at 25 °C; OC – organic carbon; Av – available; CAC – carbonates as $CaCO_3$. Total N – Total nitrogen; C/N – carbon:nitrogen ratio; nd – Not detected.

2. Material and methods

2.1. Soil and SCG

Arable top soils (0–20 cm) from 2 different Andalusian (Southern Spain) agricultural fields were collected: *Vega* soil (37°14′7.1″N, 3°45′40.7″W) used for farming corn, alfalfa and horticultural crops under irrigation; and *Red* soil (36°59′2.8″N, 3°36′54.3″W) used for cereal crops under rain-fed conditions. *Vega* soil was classified as brownish-grey (Munsell 10YR 5.5/2) TypicCalcixerept, clayey, illitic and mesic, while *Red* soil was red (Munsell 2.5YR 4/6) Calcic Rhodoxeralf, clayey, mixed and mesic (Soil Survey Staff, 2015). The soil samples were air-dried at room temperature and sieved (< 2 mm). Spent coffee grounds were obtained by grinding 50 g of coffee beans (100% Arabica, Café Cumbal, S.A.) before mixing with 1 L boiling distilled water, and then filtrating and air drying the residue. The physicochemical characteristics of the SCG are described in Table 1.

2.2. Experimental design in soil microcosms

Soil microcosms were set up with 2 different soils (*Vega* soil and *Red* soil), 3 SCG doses (0, 2.5 and 10% w:w) and 3 incubation times (0, 30 and 60 days). A total of 54 microcosms were set-up, with 18 experimental scenarios performed in triplicate. Microcosms with 0 concentration of SCG at day 0 (C_0_T_0) were the control samples. The soil and SCG mixtures (400 g) were transferred to 300 ml capacity PVC pots, closed with fibre glass mesh at the base. The pots were covered with a dish to exclude light and prevent unwanted plant growth, while allowing air exchange. The microcosms were incubated in a growth chamber at 22/18 °C (day/night) and a relative humidity of 60–80%. The moisture of the pots were maintained between field capacity and permanent wilting point by assessing pot weights (Dumroese et al., 2015); distilled water was used for irrigation to prevent leaching and water stress during all the experiment.

2.3. Physicochemical analysis

Soil was characterized by the "Methods of Soil Analysis" of the American Society of Agronomy and Soil Science Society of America (Soil Survey Staff, 2014) as previously described (Cervera-Mata et al., 2017). pH was measured in 1:2.5 (w/w) soil-water and in 1:5 (w/w) SCG-water suspensions. Electrical conductivity at 25 °C (EC_{25}) was quantified in the extract of the 1:5 (w/w) water suspensions. Organic Carbon (OC) and carbonates as $CaCO_3$ (CAC) were determined by hot wet oxidation (Tyurin's method) and the Bernard's calcimeter method, respectively. Total nitrogen was measured with a Truspec CN analyser (LECO Corporation, Saint Joseph, MI, USA), and available phosphorus was gauged by the Olsen Watanabe method with a Helios alpha spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Available potassium was extracted with 1 N ammonium acetate pH 7, and measured with a PFP7 flame photometer (Jenway, Stone, UK). Available Cu, Fe and Zn were determined by the Linsay and Norvell's method with an 1100 B atomic absorption spectrophotometer (Perkin-Elmer Inc., Waltham, MA, USA).

2.4. Total phenolic content

The Folin-Ciocalteu assay was used to measure total phenolic content. A 1 g soil sample was mixed with 20 ml methanol/water (50:50 v/v) and stirred for 1 h. The mixture was maintained in the dark at room temperature for 24 h, followed by filtration through a 0.22 µm nylon filter. Ninety µl filtered sample was added to 570 µl dH_2O and 45 µl of Folin-Ciocalteu reagent, followed by 180 µl 10% Na_2CO_3 (w/v) after 10 min. The reaction was kept in the dark for 60 min, and then its absorbance was measured at 725 nm. A solution of gallic acid was used as a standard, and total phenolic acid levels were expressed as mg of gallic acid equivalents per g of soil (mg GAE/g).

2.5. Microcosm sampling and DNA extraction

Three replicate soil mixtures for each condition were homogenized, and used to extract total DNA (0.5 g soil mixed fraction), using the FastDNA® SPIN Kit for Soil with the FastPrep® 24-Instrument (MPBiomedicals, Germany) according to the manufacturer's protocol. This kit was used due to its high reproducibility (Purswani et al., 2011). The DNA obtained was resuspended in 100 µl DES (DNase/pyrogen-free Water) and stored at −20 °C until further use.

2.6. Polymerase chain reaction amplification and 454-pyrosequencing

DNA samples collected at 30 and 60 days of incubation for each treatment were amplified by polymerase chain reactions (PCR) and nucleotide sequences of partial bacterial 16S rRNA genes were determined by 454-pyrosequencing. DNA extraction of an initial soil sample (C_0_T_0) was also performed for each soil. The 454-pyrosequencing was conducted by Research and Testing Laboratory (Lubbock, USA) on a GS FLX + platform (Roche, Branford, USA), using a previously described procedure (Dowd, 2008) and primers 28F/519R targeting the V1–V3 hypervariable regions of bacterial 16S rRNA genes (28F: GAGTTTGATCNTGGCTCAG, 519R: GTNTTACNGCGGCKGCTG). PCR amplification conditions for pyrosequencing were: 94 °C for 3 min; 40 cycles of 94 °C for 30 s, 60 °C for 40 s and 72 °C for 1 min; with an elongation step at 72 °C for 5 min.

2.7. Pyrosequencing post run analysis

Reads were trimmed and denoising was performed with the USEARCH clustering algorithm (Edgar, 2010) at a 4% divergence. Sequences of < 100 bp and clusters with < 2 members (i.e. singletons) were discarded. Operational Taxonomic Unit (OTU) selection was performed with the UPARSE OTU selection algorithm (Edgar, 2013), followed by Chimera checking with the UCHIME chimera detection software (Hamady et al., 2010) executed in *de novo* mode. Taxonomic affiliation was assigned to each OTU using the RDP Classifier (Wang et al., 2007) with a minimum confidence of 80%.

2.8. Sample and clustering of bacterial taxa

Analysis of the microbial community relative abundance at genus level (> 0.5%) for all samples was done to characterize the microbial

community structure. Heat maps were developed using the R packages Pheatmap and RColorBrewer to represent the abundance of each genus in the samples. To confirm these results, analysis of abundant matrices was used to perform Analysis of Similarity (ANOSIM) with a Primer 6 based Bray–Curtis dissimilarity statistic derived from relative abundance. Primer-E, Version 6.0 software (Plymouth Routines in Multivariate Ecological Research Ltd, Plymouth, UK) was used for cluster analysis. The significance of the cluster groups was tested using SIMPROF (similarity profile) analysis. Non-metric multi-dimensional scaling (NMDS) analysis was performed using the same software. To carry out all analysis, data were transformed in sample resemblance matrices using the Bray-Curtis coefficient of similarity (Fig. A1).

2.9. Species richness analysis and diversity indices

Rarefaction curves and alpha-diversity indices were calculated using the PAST free software (Hammer et al., 2001). Based on the relative abundance data, several diversity indices, such as the Shannon, Simpson and Chao indices were used to describe the microbial communities found in this study (Hill, 1973; Chao, 1984).

2.10. Potential plant growth promoting bacteria and other functional groups

Our abundance data were cross referenced to previous reports and known genera in order to find potential plant growth promoting bacteria (PGPB) within the microbial communities in this study (Table A1). The same approach was also taken to identify potential phenolic, crude oil and solvent degraders.

Core microorganisms were defined as those OTUs that were present in all samples and times for each soil, and their abundances were documented with time.

2.11. Consensus networks for microbial interactions

MetaMIS software (Shaw et al., 2016) was used to infer microbial interactions among all samples within a soil. Relative abundance data was used (Relative abundance default settings, high > 0.01, and rare < 0.001), and concordant pairs (P) = 0.86 was established for consensus networks. The consensus networks displayed interactions that had more consensus directions among interaction networks. This analysis allowed for the observation of interactions between highly abundant families and rare/low abundant microbes. The minimum model for consensus networks was used for each network. Cytoscape was used to draw and analyse the networks (Shannon et al., 2003).

2.12. Statistical analysis

One-way analysis of variance (ANOVA) was performed using the software package Statgraphics Plus Version 3.0 (Scientific Time Sharing Corporation, Inc., Rockville, MD, USA) in order to identify the effect of different amounts of SCG, and physicochemical and biological properties within the same incubation time and soil. A significance level of 95% ($p < 0.05$) was selected.

Multivariate redundancy analysis (RDA) was performed in order to establish the relationships between the relative abundance of bacterial members in the 2 soils studied and physicochemical parameters. The physicochemical data used to perform the RDA, are shown in Table 2.RDA was calculated through 499 unconstrained Monte Carlo simulations using the Canoco for Windows 4.5 software.

3. Results

3.1. Physicochemical effect of SCG on soil

Both *Vega* and *Red* soils were of a clayey textural class, although they differed in their total content of fine particles (< 2 mm, lime + clay), *Vega* soil 87.9% and *Red* soil 61% (Table 1). The soils were also unlike each other in their CAC), 39.0% in *Vega* soil and1.6% in *Red* soil. The organic carbon and the macro-nutrients N, P, and K contents were higher in *Vega* than in *Red* soil. For SCG, carbonate content was not detected, and its contents of C, N, C/N, K and P were much higher than the soils tested (Table 1).

Physicochemical parameters and phenolic acid concentrations showed clear parametric increases in initial SCG-soils due to the SCG added (Table 2). The pH values for *Red* and *Vega* soils averaged 7.1 and 8.1, respectively throughout, showing the lowest pH values with 10% SCG-soils. However, soil pH at 30 days (T_{30}) was increased in all pots containing SCG compared to the initial pH recordings (T_0). The concentrations of OC, total N, P, K and C/N were significantly higher in soils with SCG at T_0 compared to control pots (C_0_T_0). Addition of 10% SCG increased the OC at T_0 in *Vega* and *Red* soils by 441 and 517%, respectively, and increased the total N by 275% (on average) in both soils. Total N did not increase with time in our microcosms of SCG-soils. The initial C/N values in 10% SCG-soils increased (9 units) in both soils when 10% SCG was added; however, it decreased slightly with time due to the mineralization of SCG. Concentration increases of available macronutrients (P and K) were also observed with the addition of SCG. Carbonates (as CAC) decreased significantly after the addition of SCG due to the increase in total volume and the absence of these in SCG (Table 1).

The highest total phenolic acid concentrations in T_0 soils were registered with 10% SCG. The lowest total phenolic acid concentrations were measured in 0% SCG soils. Though phenolic acids were traceable in *Red* soils at C_0T_0 (0.341 mg/g), SCG addition was the primary source. Phenolic acid reduction at T_{60} for *Vega* and *Red* soils were 99% and 80%, respectively.

3.2. Ecological parameters: Richness and diversity indices

Microbial community structure of each soil illustrated by alpha-diversity indices are shown in Table 3. Positive correlations were observed between the Chao1 index and % content of SCG in the soils. This tendency was observed in both soils; however, SCG increased species richness in *Vega* soil more than in *Red* soil.

Shannon–Wiener (H′) index gave a measure of the diversity, and was sensitive to loss of rare species. H′ was expressed with a positive number, which in most natural ecosystems was between 0.5 and 5, although its normal range was between 2 and 3. In our experiments, H' values ranged from 3.7 to 4.9 in *Red* soil and from 4.9 to 5.7 in *Vega* soil; therefore, high diversity was observed in both soils, though maximum diversity was detected in soils that were treated with 2.5% SCG.

Finally, Simpson index measured the probability that 2 individuals taken randomly from the same system represent the same type, insensitive to the loss of rare species. As such, it was a measure of species dominance (D) within a sample, where a value of D close to 1 indicated monoculture dominance. Our data showed that Simpson index (1-D) values were near to 1, that monoculture dominance was low within the community, and that fluctuations after the addition of SCG were very low. The latter indicates a stable microbial community structure to all the pots.

In both soils, 5–11% of the total diversity at the genus levels was from core microorganisms. The abundance of the core microorganisms varied with time and SCG content of the soils. Their initial relative abundance was ~50%, which decreased with increasing SCG (Fig. 1).

3.3. Variation of bacterial community structure with increasing SCG

The abundance phyla profiles over the time of the experiment, and for different SCG percentages and soils were largely unchanged, probably as a result of the relatively small sampling time. Observation of abundance data of genus clustered OTU-samples in time, showed large dynamic community changes (Fig. 2), though soil samples were

Table 2
Evolution of physicochemical parameters in poor Mediterranean *Red* and *Vega* soils after spent coffee ground amendment.

Soil	Incubation time	% SGC	pH	EC (dS/cm)	OC (%)	N (%)	C/N	K assim (mg/Kg)	CAC (%)	Av. P (mg/Kg)	Av. Cu (mg/Kg)	Av Zn (mg/Kg)	Av Fe (mg/Kg)	Total phenolic acid (mg GAE/g)
Red	0	0	7.2	0.6	1.16	0.113	10.0	248	1.6	51.0	3.78	1.97	4.27	0.341
		2.5	7.1*	0.6	2.31*	0.148	16.0*	324*	0.9*	55.0*	5.08*	2.60*	5.39*	2.371*
		10	6.5*	0.7*	6.00*	0.314*	21.0*	482*	0.8*	64.0*	6.74*	3.38*	6.46*	6.309*
	30	0	7.4	0.6	1.03	0.087	12.0	249.8	0.8	36.2	4.11	2.12	5.71	0.069
		2.5	7.4	0.2*	2.48*	0.158*	15.9*	282.1*	1.1	42.9*	4.81*	2.42*	6.58*	0.157*
		10	6.7*	0.3*	5.4*	0.274*	19.6*	404.8	1.0	45.9*	4.60	2.37*	7.92*	0.866*
	60	0	7.5	0.9	1.19	0.109	11.0	247.5	0.8	45.6	4.48	2.32	5.63	< 0.001
		2.5	7.5	0.3*	2.08*	0.158*	13.7*	289.2*	0.7	46.4	4.30	2.18	6.86*	< 0.001
		10	6.7*	0.3*	5.54*	0.290*	19.1*	423.3*	1.2*	51.1*	4.71	2.17	8.53*	0.921*
Vega	0	0	8.2	1.3	1.4	0.105	13.0	453	39.0	69.0	4.58	0.89	6.86	< 0.001
		2.5	7.9*	1.5*	2.96*	0.138*	21*	482*	37.6*	79.0*	4.95*	0.96*	6.15*	1.049*
		10	7.6*	1.6*	6.18*	0.286*	13	688*	35.5*	87.0*	5.47*	1.2*	3.46*	4.121*
	30	0	8.21	0.9	1.4	0.094	14.9	447.2	39.3	45.6	4.34	0.82	11.46	< 0.001
		2.5	8.31	0.6*	2.6*	0.139*	18.4*	511.9*	39.0	50.4*	4.29	0.90	10.54	< 0.001
		10	8.0*	0.8	5.7*	0.275*	21.0*	635.3*	38.0	56.1*	4.40	1.10*	8.41*	0.419*
	60	0	8.2	1.0	1.4	0.090	15.7	458.6	40.2	46.7	3.71	0.71	6.72	< 0.001
		2.5	8.4*	0.6*	2.4*	0.160*	14.9	509.3*	38.3*	47.2	4.24	0.83	7.91	< 0.001
		10	8.1	0.9	5.5*	0.284*	19.6*	638.6*	36.0*	50.7	4.23	0.99*	7.02	< 0.001

SCG-spent coffee grounds; EC_{25}-electrical conductivity measured at 25 °C; OC-organic carbon; Av- available; CAC-carbonates as $CaCO_3$.
Total N – Total nitrogen; C/N – carbon:nitrogen ratio; nd – Not detected. *Statistically different ($p < 0.05$) to 0% SCG, for each incubation time.

clustered by SCG% using NMDS analysis (Fig. A1).

The thermophilic and radiotolerant *Rubrobacter* was overall the most abundant genus observed in both soils; however, there was a negative correlation between their relative abundance and SCG% (Fig. 2). This effect was also observed for clusters vi and x, e.g. for *Blastococcus* in both soils. Conversely, positive correlations existed between SCG% and abundance data in genus clusters ii, iii, ix and xi. Among the genera shared by both soils that were observed to increase with SCG% were: *Pseudomonas, Clostridium, Caulobacter, Variovorax, Rhizobium, Ohtaekwangia,* and *Phenylobacterium*. As high phenolic acid concentrations were incorporated into soils via SCG addition (Table 1), abundance dynamics of bacteria that were potential phenolic degraders were closely examined (Fig. 3). Potential phenolic acid degraders included members of the genera *Caulobacter, Pseudomonas, Achromobacter* and *Rhodococcus* (Table A1). Notably, their relative abundances increased at T_{30}, which coincided with the largest decrease in phenolic acid levels (Table 2).

Relevant genera belonging to the potential PGPB were also tracked to detect agricultural relevant communities (Fig. 3). Direct correlations were observed between SCG% and PGPB abundance. At T_{30}, both soils showed an increase in PGPB relative abundance, and although contradictory PGPB soil shifts were observed at T_{60}, their relative abundance (> 10%) was still high. Mineralization of SCG requires the chemical transformation of organic compounds, hence we also observed the relative abundance of potential crude oil and solvent degraders. Among these 2 groups, only solvent degraders had a significant and constant increase in abundance in soils with 10% SCG.

3.4. Redundancy analysis of environmental variables and bacterial community structure

Redundancy analyses showing correlations between physicochemical and biological factors were performed on phyla (Fig. 4) and genera (Fig. A2) abundances. The RDA based on phyla abundance data showed that samples were grouped by SCG% in both soils, just as in the NMDS analysis (Fig. A1). Similarities between the 2 soils were observed, with phenolic acid concentrations being positively correlated with *Firmicutes*, *Planctomycetes*, and *Proteobacteria*, while *Acidobacteria*, *Actinobacteria* and *Chloroflexi* were observed to correlate with pH.

Fig. A2 shows correlations between phenolic acid concentrations and genera; the strongest correlating bacteria were *Rhodococcus*, *Variovorax*, and *Caulobacter* in *Red* soil. Additionally, genus correlations with total N were observed with *Promicromonospora, Clostridium, Rhizobium* and *Caulobacter* in *Red* soils. In Vega soil, phenolic acid concentrations were correlated with total nitrogen, and in turn with a large number of genera, including: *Achromobacter*, *Klebsiella*, *Mycobacterium*,

Table 3
Microbial community diversity indices of SCG amended soils. Ecological indices Chao1, Shannon-Wiener (H'), and Simpson (1-D) were calculated for samples describe the community in terms of richness, diversity and dominance of both soils in time and SCG%. Confidence intervals (CI, 95%) are reported.

Soil	Incubation time	% SCG added	Chao-1	CI	Shannon_H	CI	Simpson_1-D	CI
RED	0	0	224.3	211–257	4.293	4.295–4.467	0.9623	0.9614–0.9724
		0	265.9	231.6–280.5	4.61	4.593–4.737	0.9775	0.976–0.9827
	30	2.5	225.3	188.1–243.4	4.608	4.565–4.708	0.9834	0.9819–0.9867
		10	149.1	122.2–163.1	3.675	3.613–3.805	0.9438	0.9358–0.9534
		0	237.1	193–240	4.405	4.39–4.559	0.9669	0.9646–0.9775
	60	2.5	262.1	216.5–266.7	4.88	4.834–4.94	0.9889	0.9874–0.9904
		10	242	221.8–267	4.437	4.412–4.53	0.9781	0.9765–0.9808
VEGA	0	0	362.8	311.8–376.4	5.078	5.06–5.166	0.9892	0.9884–0.9912
		0	433.6	390.7–458.4	5.158	5.154–5.272	0.9854	0.9847–0.9891
	30	2.5	703	704.3–745.8	5.69	5.657–5.713	0.9926	0.9923–0.9931
		10	625.1	599.5–654.5	5.15	5.133–5.2	0.9859	0.9853–0.9868
		0	505.8	487.5–545.9	5.496	5.473–5.556	0.9915	0.9909–0.9929
	60	2.5	551	517.1–582.2	5.504	5.485–5.57	0.9919	0.9915–0.9931
		10	469.3	450.5–509.4	4.939	4.931–5.014	0.9843	0.9837–0.9858

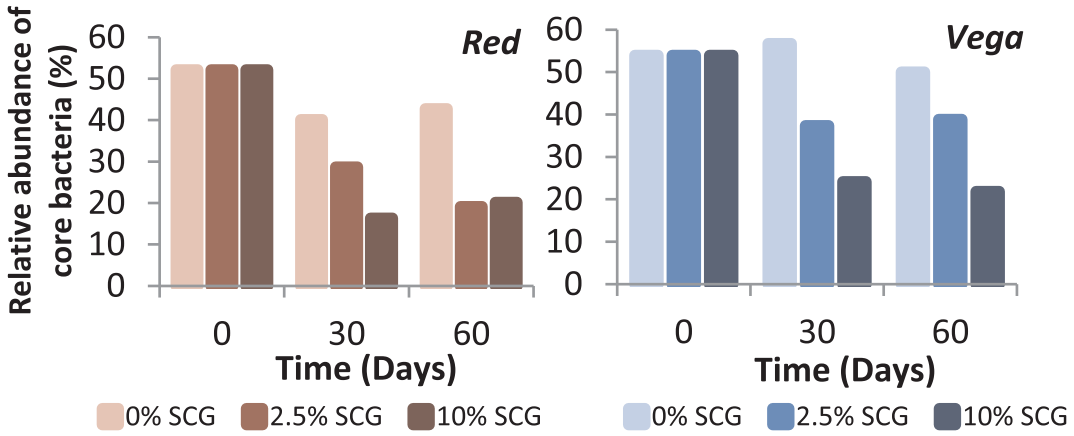

Fig. 1. Relative abundance of core microorganisms. Indirect correlation between abundance of core microorganisms and SCG% is observed in both soils. The percentage of core microorganisms within the total number of OTUs: in Vega soil − 11.4%; in Red soil − 5.3%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Geobacter, *Pelosinus*, *Chlostridium*, *Caulobacter*, *Olivibacter*, *Devosia*, *Pseudomonas*, *Brevundimonas*, *Promicromonospora*, *Vampirovibrio*, *Flavobacterium,Ohtaekwangia, Bradyrhizobium, Castiobacter, Opitutus, Asticcacaulis, Steroidobacter, Phenylobacterium, Terrimonas* and *Rhizobium*.

3.5. Microbial interaction networks

The high number of microbial correlations with phenolic acids and total N in *Vega* soil required further study; therefore, the microbial interactions between genera where built into consensus networks (Fig. 5). Consensus microbial interactions of each soil over time were observed for *Red* and *Vega* soils (Fig. 5). Comparison of soil networks was performed with Cytoscape (Table A2). The clustering coefficients for these networks were not correlated with SCG%. This was also the case for the average number of neighbors; however, it was clear that highest connectivity between neighbors was observed with $C_{2.5}$ soils. Furthermore, the average number of neighbors of key players (largest nodes/label) increased for SGC-soils compared to C_0 soils. Interaction numbers within each consensus network was also similar between these 2 soils when compared to their respective C_0 soil samples.

Across soils, the microbial interaction key player was *Rubrobacter*, and *Pseudomonas* was also highly correlated with C_{10} soils. Other highly associated bacterial genera included *Bradyrhizobium* and *Microvirga* for *Red* soil, and *Streptomyces* and *Ramlibacter* for Vega soil. *Caulobacter* was also highly connected to all SCG-soil networks. Genera *Achromobacter*, *Pseudomonas*, *Caulobacter* and *Rhodococcus* described in the literature as phenol degraders (Table A1), shared negative interactions. These were likely to be mutualism or competition interactions, although strong predation effects were calculated with *Achromobacter* (data obtained with MetaMIS).

Among the PGPBs described in the literature were *Bradyrhizobium*, *Rhizobium*, *Pseudomonas*, *Nitrospira* and *Streptomyces*. Their modelled microbial interactions showed that mainly mutualism or competition interactions took place, although predation was calculated for *Bradhyrhizobium* and *Streptomyces* in *Red* C_{10} and *Vega* $C_{2.5}/C_{10}$ soils. Other calculated predation interactions included: *Massilia* in SGC-*Red* soils; *Nocardiodes* in *Red* $C_{2.5}$ and *Vega* C_{10} soils; *Phenylobacterium* in *Red* C_{10} soils; and *Achromobacter* and *Micromonospora* in *Red* $C_{2.5}$ soils. *Afibia* was the only genus (*Red* $C_{2.5}$ soil) to have a calculated commensalism/amenalism trait.

4. Discussion

Spent coffee grounds increase the availability of organic content and macronutrients NPK when amended to soil. Thus, their use as soil conditioners on poor soils are sought after, especially on agriculturally exploited soils within the Mediterranean area, such as *Vega* and *Red* soil.

The physicochemical parameter dynamics during the experimental period described soil transformation due to the addition of SCG. The difference in pH increase in SCG amended microcosms during the cultivation time (Table 2) was attributed to the respective buffering capacity of the soils, which was higher for the *Vega* soil due to the abundance of carbonates (Brady and Weil, 2002). Additionally, the pH increase with time was in accordance with previous studies where soil was amended with other organic substrates (Teutscherova et al.,2017). Overall, the difference in initial soil pH might be the basis for the different SCG assimilations observed, such that with a higher initial pH SCG assimilation rates were greater. A decrease of P and K over the cultivation time was also detected, and this could be explained in different ways. The decrease of available P might be due to precipitation as calcium phosphates in carbonate-rich soils, such as *Vega* soil, or it might complex with free iron (hydroxides) in those rich in iron oxides (*Red* soils are rich in hematite; Navarro and Navarro, 2013). Cruz and Cordovil (2015) observed the same effect for SCG; however, they attributed P immobilisation to soil microorganisms. A possible cause for the K decrease could be the incorporation of this element in the illites interlayer (phyllosilicates derived from mica), causing illite reconstruction to mica (Calero et al., 2013). The illites are one of the most common clay minerals in Mediterranean soils. Phenolic acid concentrations (Table 2) were consistent with studies carried out by Cruz et al. (2015) and Baslam et al., 2013, which analysed the effect of SCG addition on lettuce growth. The phytotoxic effects recorded in these studies increased with SCG%, since seed plantation was performed without prior SCG mineralization.

During our study, we allowed for a SCG mineralization period of up to 60-days, and monitored both phenolic acid concentrations and microbial soil dynamics of the 2 poor soils.

Among the biological parameters analysed, variations in soil microbial diversity were found to be similar to data reported previously. Bastida et al. (2017) observed that organic amendments such as sludge and compost, increased the bacterial Shannon index. Wu et al. (2014) also obtained an increase in this index after cattle manure compost amendment of an agricultural soil. Shannon and Simpson indices in our study (Table 3) were also within the higher range of those found in other soil amendment reports, such as for manure and straw compost (Xi et al., 2016) and tea compost (Vela-Cano et al., 2018). Therefore, although no composting was performed before addition of SCG to the soil, in time, the SCG seemed to be undergoing a natural degradation process (Table 2).

Indices showed an increase in diversity, richness and evenness of the bacterial community structures with increasing SCG%, even for SCG

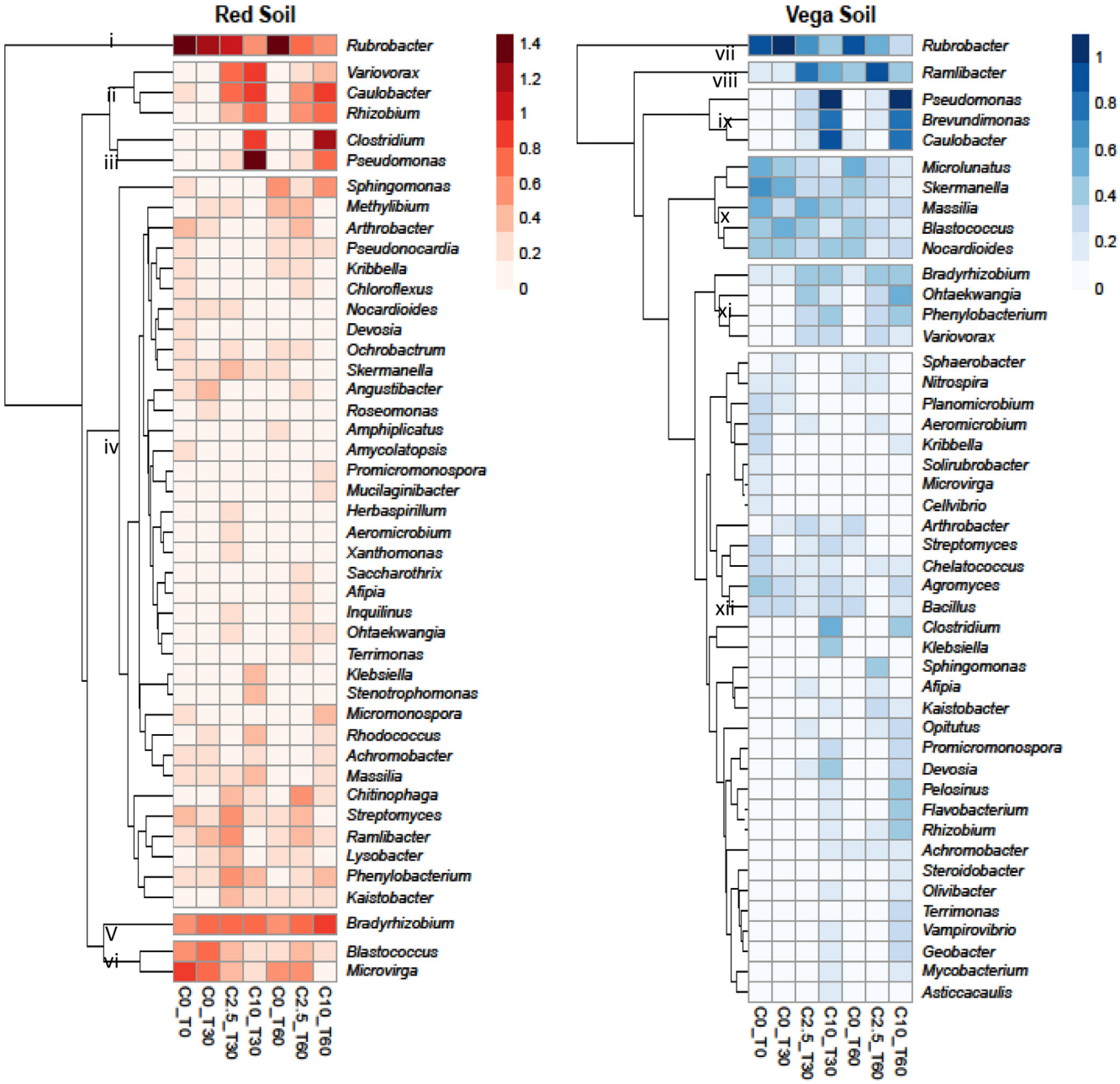

Fig. 2. Cluster analysis of bacteria in SCG amended soils. Only OTUs belonging to known genera and relative abundance of > 0.5% (in at least 1 sample) were included. Trees were cut at 6 groups in rows. Within each sample, the color scale indicates the $\log_{10}$ of relative abundance %. Columns and rows are clustered using Euclidean distance.

concentrations tested to be phytotoxic.

Microbial dynamics at the phyla level showed that *Proteobacteria* and *Actinobacteria* were the most abundant in all experimental scenarios in the current study, and this is a general observation in agricultural and non-agricultural soils (Tian et al., 2015; Ding et al., 2016). Our data did not reveal significant phyla abundance changes across treatments, which was likely due to the short time period of the experiment (Marschneret al., 2003; Dolfinget al., 2004); however, when bacteria were classified at the genus level important dynamics were observed with respect to SCG and time.

Positive correlations of known phenolic acid degraders (*Achromobacter*, *Caulobacter* and *Pseudomonas*) and phenolic acid reduction were observed for both soils (Fig. A2). Other genera that produce a similar correlation were: *Olivibacter* (alkaline olive mill waste; Ntougias et al., 2007); *Phenylobacterium* (aromatic contaminated soils; Lingens et al., 1985), known to live in high phenolic acid environments; and *Geobacter,* whose genome has been shown to contain phenol degradation pathways (Schleinitz et al., 2009). The calculated microbial interactions between the latter genera were mainly mutualistic and competitive interactions, with the exception of *Phenylobacterium* which was found to have a predation effect. Abundances of phenolic acid degrading bacteria (Fig. 3) were not only positively correlated with phenolic acid reduction, but also with SCG%. Hence, decline in abundance of phenolic degrading bacteria could be indicative of phenolic acid decrease, with the inference that these soils were less phytotoxic at T_{60}.

The abundance of solvent degrading bacteria (Fig. 3) in *Red* C_{10} soil increased continuously up to T_{60}, and phenolic acid removal efficiency was below the microbial capacity of *Vega* soils; therefore, accumulation of metabolites might be the reason for this constant increase. As such,

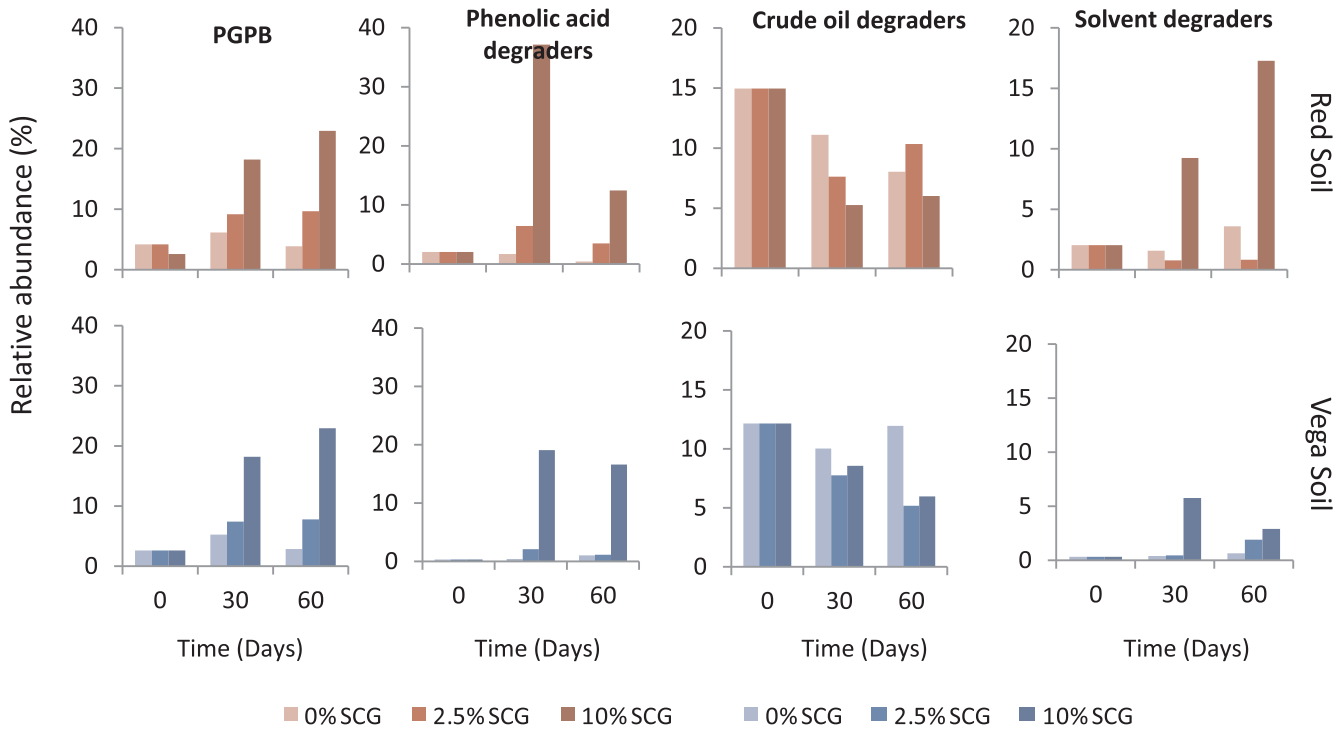

Fig. 3. Relative abundance of potential functional groups. Genera and literature were cross referenced and abundances were added accordingly. Genera for each group are detailed in Table A1.

complete phenolic acid degradation in *Red* soils might take longer than 60 days.

Plant-growth promoting bacteria (PGPB) actively mineralize organic matter, are involved in nitrogen processes and nutrient acquisition, and also promote plant growth via different mechanisms such as phytohormones or increased nitrogen availability by nitrogen fixation. Plant defense mechanisms are also performed by some PGPB through microbial antagonism. PGPB abundance increased with increasing SCG%, and was approximately 20% of the total microbiota at T_{60}. This increase was not accompanied by an increase in total N with time, such as that observed in planted pots (Cervera-Mata et al., 2017). Nitrogen-fixing bacteria were not among the PGPBs detected in our study; hence, addition of SCG did not result in an increase of total N, and only the association between root nodules and nitrogen-fixing microorganisms conferred total N increase in the study of Cervera-Mata et al. (2017). A PGPB abundance of > 20% might be indicative of optimal plantation conditions after SCG amendment; however, it is not a microbial indicator of total nitrogen concentrations and further studies are required

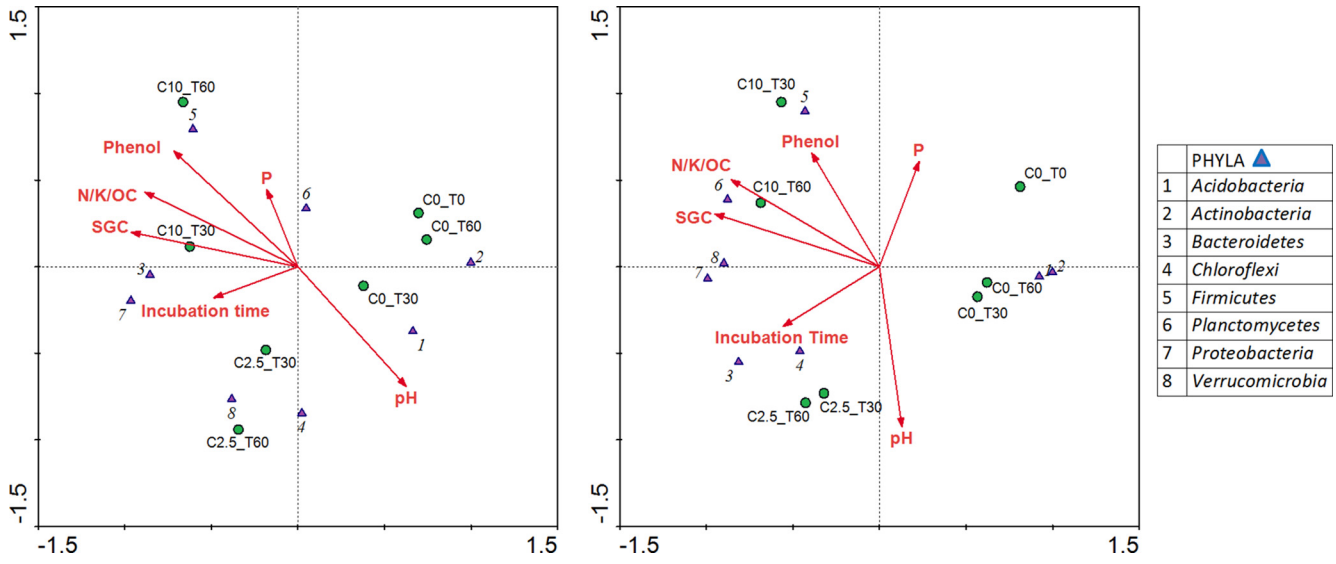

Fig. 4. Inference of bio and physicochemical correlations among whole communities in *Vega* (left) and *Red* (right) soils using multivariate redundancy analysis plots. Samples are represented by circles, environmental parameters by arrows, and phyla are represented by triangles. A total of 499 unconstrained Monte-Carlo simulations were performed. Cumulative percentage variance: Red Soil (x axis = 78.5%, y axis = 93.7%); Vega Soil (x = 75.7%, y axis = 95.9%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

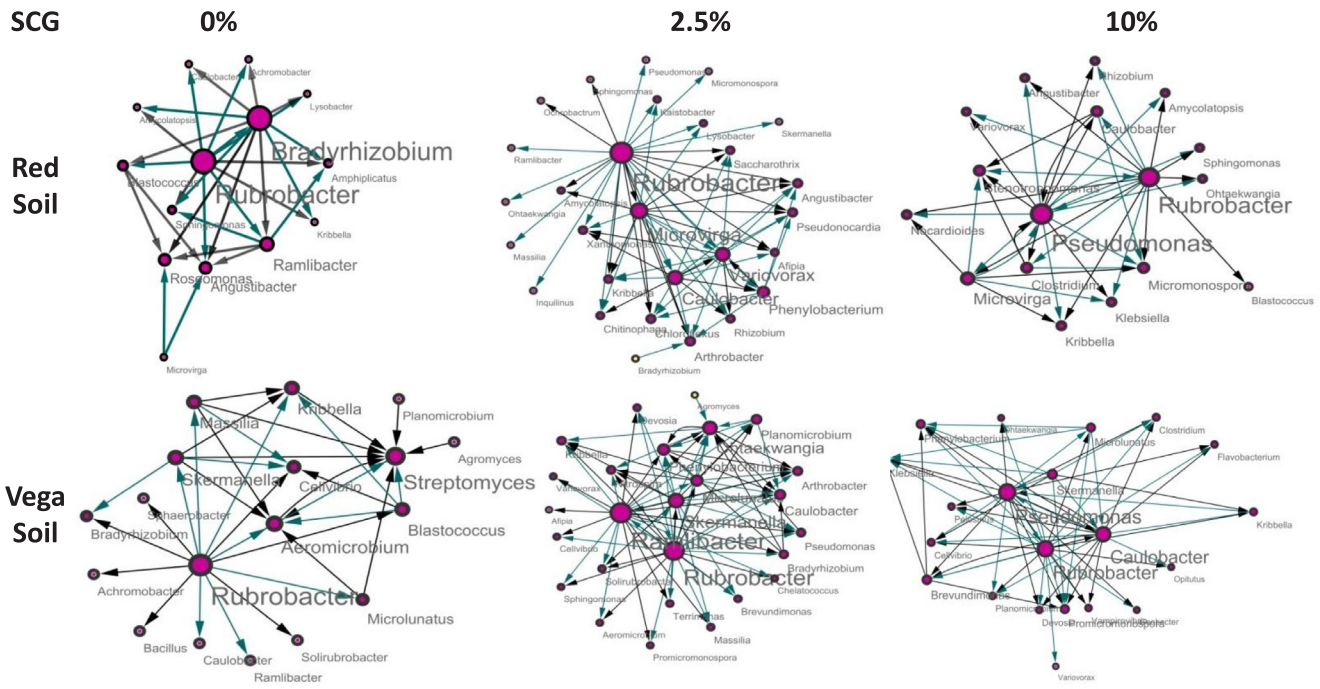

Fig. 5. Microbial-interaction consensus networks for *Red* and *Vega* soils at different SCG%. Inference of microbial interactions using Lotka-Volterra models for 16S-rRNA microbial abundance profiles were calculated in METAMIS, and consensus networks [P (concordant pairs) = 0.86]. Both consensus networks were drawn and analyzed in Cytoscape. Network analysis for each soil condition is presented in Table A3. The red (or blue) arrow represents the activation (or repression). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to define such a measure.

Schloter et al. (2018) included in their description of soil bioindicators the need to define beneficial microbial groups and their traits by using several criteria, such as their universal presence, their contribution of functions to the geochemical cycles linked to plant growth, and to report soil disturbances that might cause their deterioration or harm. Consequently, a limitation in the use of phenolic acid degraders or PGPBs as microbial indicators for SCG-soil improvement is that their functionality is dependent on population dynamics and not an absolute count. Moreover, different soil types assimilate SCG at different rates, e.g. *Vega* soil assimilates SCG through removal of phenolic acids better than *Red* soil.

The relative abundance of the core microorganisms stabilized during the 30–60 day period, after experiencing a rapid decline. This raises the question as to whether high levels of these core microorganisms are an indicator of poor soil quality. Among the core microorganisms was the thermophilic and radiotolerant *Rubrobacter*. Its negative correlation with SCG% suggested this amendment might have a slight toxic effect on the bacterium (Fig. 2); however, its declining numbers might also be explained by competition resulting from the increase in abundance of other bacteria.

The differences in the microbial interaction networks found between 0 and 10% SCG reflected the complex microbial changes occurring due to changes in nutrient and physicochemical parameters. Both soils were highly clustered (clustering coefficient > 0.5) in C_{10} soil networks as a result of increased microbial interactions compared to the C_0 soil networks (Table A2, Fig. 5). Further microbial network analysis could be performed to study the effects between microbial network connectivity dynamics and soil recovery.

Overall, the use of SCG as a biofertilizer is an excellent sustainable solution. The addition of SCG not only increases nutrient value but enhances PGPBs, which are known to aid plant growth. Further work is needed to define seeding and plantation times after SCG amendment of soils; however, our study indicates that for maximal nutrient incorporation and reduced concentrations of phenolic acid and their likely toxic metabolites a good starting point is > 30 days for *Vega* soil, and > 60 days for *Red* soil, after addition of 10% SCG. In addition, the presence of more intricate microbial networks with increasing SCG% might suggest a more active soil.

5. Conclusion

Spent coffee grounds are a good soil conditioner due to their contribution in increasing essential macronutrients. Physicochemical parameters showed that a SCG mineralization period decreased phenolic acids, thus decreasing overall phytotoxicity. Furthermore, we suggest that microorganisms such as phenolic acid degraders and PBPBs could be used to evaluate SCG mineralization in poor soils.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.01.006.

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