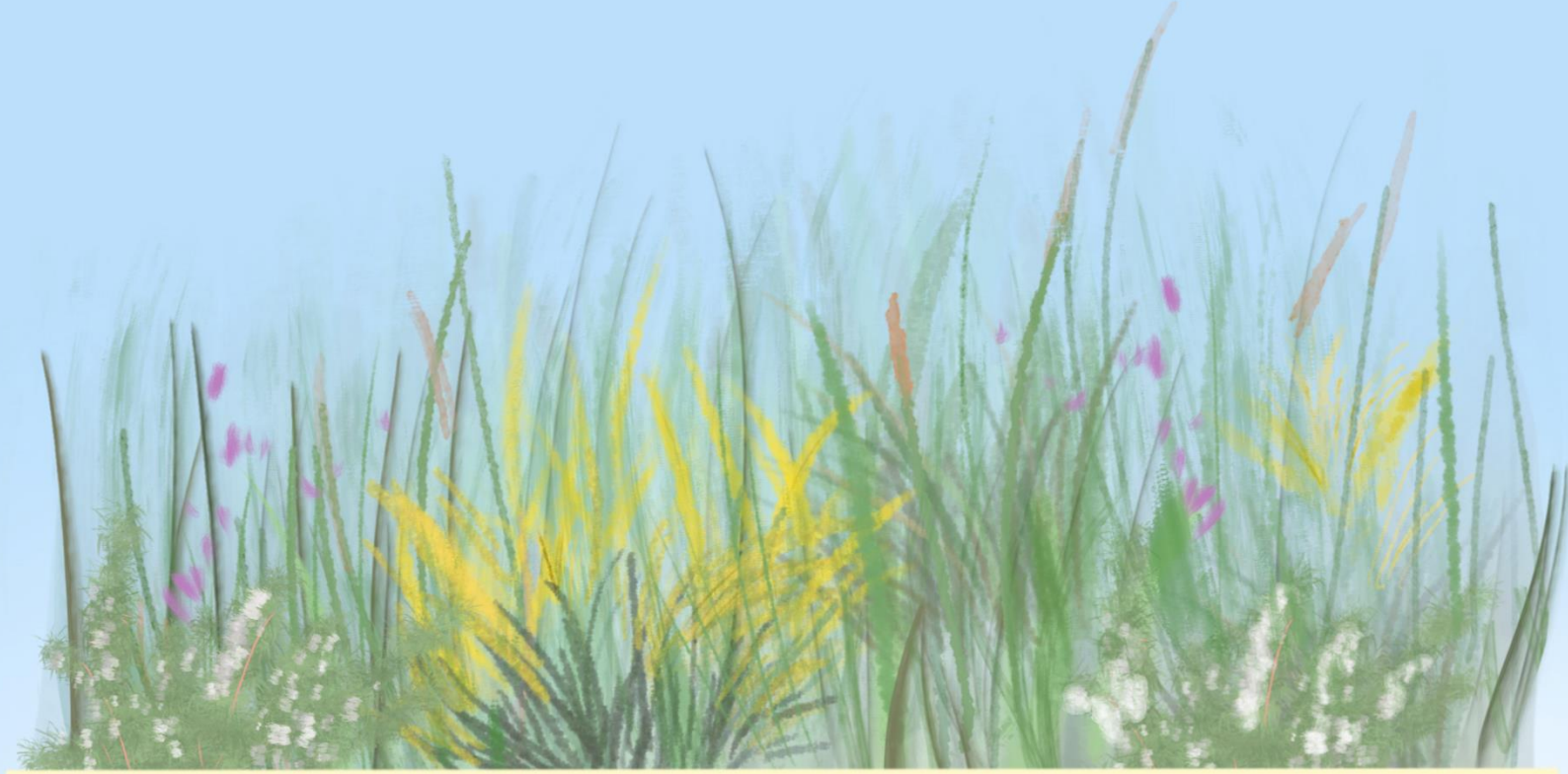

Estudio de perfiles taxonómicos y funcionales de comunidades microbianas en suelos restaurados en ambientes semiáridos

Study of taxonomic and functional profiles of microbial communities in restored soils in semi-arid environments

Doctorado en Ciencias Aplicadas al Medio Ambiente

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Universidad de Almería
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La Tesis Doctoral titulada “Estudio de perfiles taxonómicos y funcionales de comunidades microbianas en suelos restaurados en ambientes semiáridos”, realizada por la Graduada en Biología Dña. Natalia Rodríguez Fernández para optar al grado de Doctor por la Universidad de Almería dentro del Programa de Doctorado en Ciencias Aplicadas al Medio Ambiente, se presenta bajo la modalidad de “Tesis por compendio de publicaciones”, con la aprobación de los Directores y el Departamento de Agronomía de la Universidad de Almería.

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Soria, R., Tortosa, A., **Rodríguez-Berbel, N.**, Lucas-Borja, M.E., Ortega, R., Miralles, I., 2023. Short-term response of soil bacterial communities after prescribed fires in semi-arid Mediterranean forests. *Fire*. 6 (4), 145. DOI: 10.3390/fire6040145

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ÍNDICE

Resumen

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Resumen

Abstract

Regardless of the great social importance of economic activities such as agriculture and mining, the negative effects on ecosystems should not be forgotten. In particular, soil degradation decreases soil quality and reduces the abundance and diversity of soil microorganisms, affecting biogeochemical cycles (C, N and P). Semi-arid Mediterranean soils are particularly vulnerable to these activities; their limiting climatic conditions and low fertility favor degradation processes, hindering the natural recovery of degraded soils. Also, the production of waste derived from human activities is a social challenge and an opportunity that must be seized in order to change to a circular economy model and achieve Sustainable Development. In this context, a potential solution to reuse waste and accelerate the restoration of degraded soils could be the use of organic remains to improve the physical, chemical and biological properties of semiarid soils. Therefore, this Doctoral Thesis proposes the use of organic amendments as a strategy to recover the quality and microbiological activity of semiarid soils degraded by agricultural and mining activities.

For this purpose, different organic amendments obtained from recycled wastes of different origin (plant residues, domestic solid wastes, sewage sludge, vermicompost and animal manures) and chemical composition were applied in three experimental areas, one agricultural and two mining sites. Four studies were conducted at different time intervals after the addition of different types of amendments: i) short-medium term (baseline, 3 and 12 months) on abandoned agricultural soils; ii) short term (6 months) on limestone quarry soils; iii) medium term (18 months) on limestone quarry soils; iv) long term (10 years) on limestone quarry soils. For each sampling, a set of physico-chemical (pH, nutrient content, etc.) and biological (basal respiration, enzymatic activities involved in C, N and P cycles, etc.) indicators were analyzed in the restored soils as well as in control soils without amendments and in surrounding natural soils. Moreover, using DNA sequencing metagenomic techniques, changes in the diversity and composition of microbial communities (bacteria and fungi) in all soils were studied.

The results of this thesis showed that all soils restored with organic amendments improved the analyzed properties in the short, medium and long term. Furthermore, the heterogeneous composition and origin of the amendments, with different proportions of labile and resilient C content, differentially influenced the edaphic communities developed in the restored soils of the three study areas.

In agricultural degraded soils, each amendment allowed the proliferation of specific taxa involved in C, N and P cycling, in addition to a short-medium term bacterial succession possibly due to the increase of nutrients that favored the proliferation of copiotrophic phyla 3 months after the addition of the amendments that were replaced by oligotrophic phyla at 12 months. Likewise, the bacterial community at the genus level of soils amended with vermicompost and both types of manures derived from animal remains differed to a greater extent from control and natural soils after 12 months, while greenhouse vegetable compost was in an intermediate position.

The improved quality of restored quarry soils increased bacterial diversity in the short term (6 months) and fungal diversity in the medium term (18 months) compared to unamended soils, although without reaching the values of natural reference soils. In the short term, the establishment of two patterns of bacterial co-occurrence was observed, with some taxa being more abundant or almost exclusive to restored soils, while others followed these premises for untreated soils. It is particularly noteworthy that even 18 months after quarry soil restoration, control soils still lacked fungal communities, while the application of amendments benefited their proliferation in restored soils. After 10 years of quarry soil restoration, chemical properties, diversity and bacterial composition of the soils treated with domestic solid waste more closely resembled the natural reference soils. In contrast, sewage sludge treatment placed the soils in an intermediate position between control and natural soils. Also, two patterns of co-occurrence were found in the bacterial communities, one shared by the natural soils and the soils treated with domestic solid waste, and a second pattern in the control soils.

The studies of the present Thesis highlight that restoration with organic amendments improves in the short, medium and long term the physico-chemical and microbiological quality of soils degraded by agriculture and mining in semi-arid conditions. However, the organic nature of the amendments applied developed different stages of recovery in each soil, emphasizing the importance of selecting an adequate treatment that favors the natural recovery of the soils, as well as revealing that the study of microbial communities is key to understanding the evolution of restoration. Studies based on the functional diversity of microbial communities as well as longer-term studies are needed to understand the ecosystem response after restoration with organic amendments under semi-arid climate.

Resumen

A pesar de la gran importancia social de actividades económicas como la agricultura o la minería, no podemos olvidar que también conllevan efectos negativos sobre los ecosistemas. En concreto, la degradación del suelo disminuye la calidad y reduce la abundancia y diversidad de los microorganismos edáficos afectando a los ciclos biogeoquímicos (C, N y P). Los suelos semiáridos mediterráneos son especialmente vulnerables a estas actividades, sus condiciones climáticas limitantes y su baja fertilidad favorecen los procesos de degradación obstaculizando la recuperación natural de los suelos degradados. También, la producción de residuos derivados de actividades humanas supone un reto social y una oportunidad que debemos aprovechar para cambiar a un modelo de economía circular y alcanzar un Desarrollo Sostenible. En este contexto, una solución potencial para reutilizar residuos y agilizar la restauración de suelos degradados, podría ser el aprovechamiento de restos orgánicos para mejorar las propiedades físicas, químicas y biológicas de suelos semiáridos. Por ello, esta Tesis Doctoral propone el uso de enmiendas orgánicas como estrategia de recuperación de la calidad y la actividad microbiológica de suelos semiáridos degradados por actividades agrícolas y mineras.

Con este fin, se aplicaron diferentes enmiendas orgánicas obtenidas a partir de residuos reciclados de distinto origen (restos vegetales, residuos urbanos, lodos de depuradora, vermicompost y estiércoles animales) y composición química en tres zonas experimentales, una agrícola y dos explotaciones mineras. Se realizaron cuatro estudios en diferentes escalas temporales tras la adición de diferentes tipos de enmiendas: i) a corto-medio plazo (inicio, 3 y 12 meses) en suelos agrícolas abandonados; ii) a corto plazo (6 meses) en suelos de cantera caliza; iii) a medio plazo (18 meses) en suelos de cantera caliza; iv) a largo plazo (10 años) en suelos de cantera caliza. En cada muestreo, se analizaron un conjunto de indicadores físico-químicos (pH, contenido en nutrientes, etc.) y biológicos (respiración basal, actividades enzimáticas implicadas en ciclos de C, N y P, etc.) tanto en los suelos restaurados como en suelos control sin enmiendas y en suelos naturales de los alrededores. Además, mediante técnicas metagenómicas de secuenciación de ADN, se estudiaron los cambios en la diversidad y composición de las comunidades microbianas (bacterias y hongos) de todos los suelos.

Los resultados de esta Tesis mostraron que todos los suelos restaurados con enmiendas orgánicas mejoraron las propiedades analizadas tanto a corto, como a medio y largo plazo. Además, la heterogénea composición y origen de las enmiendas, con

diferentes proporciones de contenidos de C lábil y resiliente influenció diferencialmente sobre las comunidades edáficas desarrolladas en los suelos restaurados de las tres áreas de estudio.

En suelos degradados por agricultura, cada enmienda permitió la proliferación de taxones específicos involucrados en los ciclos de C, N y P, además de una sucesión bacteriana a corto-medio plazo debida posiblemente al incremento de nutrientes que favoreció la proliferación de filos copiotróficos pasados 3 meses de la adición de las enmiendas que fueron reemplazados por filos oligotróficos a los 12 meses. Asimismo, la comunidad bacteriana a nivel de género de los suelos enmendados con vermicompost y ambos tipos de estiércoles derivados de restos animales se diferenció en mayor medida de los suelos control y naturales tras 12 meses, mientras que el compost vegetal de invernadero se situó en una posición intermedia.

La mejora de la calidad de los suelos restaurados de cantera incrementó la diversidad bacteriana a corto plazo (6 meses) y fúngica a medio plazo (18 meses) en comparación con los suelos no enmendados, aunque sin alcanzar los valores de los suelos naturales de referencia. A corto plazo, se observó el establecimiento de dos patrones de coocurrencia bacterianos, siendo algunos taxones más abundantes o casi exclusivos de suelos restaurados, mientras que otros siguieron estas premisas para los suelos sin tratamiento. Resulta especialmente destacable que incluso 18 meses después de la restauración de suelos de cantera, los suelos control seguían sin presentar comunidades fúngicas, mientras que la aplicación de enmiendas benefició su proliferación en los suelos restaurados. Tras 10 años de la restauración de suelos de cantera, las propiedades químicas, diversidad y composición bacteriana de los suelos tratados con residuos urbanos se asemejaron más a los suelos naturales de referencia. En cambio, el tratamiento de lodos de depuradora situó los suelos en una posición intermedia entre los suelos control y los suelos naturales. También, se encontraron dos patrones de coocurrencia en las comunidades bacterianas, uno que compartieron los suelos naturales y los tratados con residuos urbanos y, un segundo patrón en los suelos control.

Los estudios de la presente Tesis destacan que la restauración con enmiendas orgánicas mejora a corto, medio y largo plazo la calidad físico-química y microbiológica de suelos degradados por agricultura y minería en condiciones semiáridas. Sin embargo, la naturaleza orgánica de las enmiendas aplicadas desarrolló estadios de recuperación diferentes en cada suelo destacando la importancia de seleccionar un tratamiento

adecuado que favorezca la recuperación natural de los suelos además de, poner de manifiesto, que el estudio de las comunidades microbianas es clave para entender la evolución de la restauración. No obstante, es necesario ampliar estos conocimientos mediante estudios basados en la diversidad funcional de las comunidades microbianas así como estudios a más largo plazo para comprender la respuesta del ecosistema tras restauraciones con enmiendas orgánicas bajo clima semiárido.

Introducción

1. El suelo y su importancia

La Pedosfera se define como "la estructura que anida todos los ecosistemas terrestres" (Etchevers Barr et al., 2008) estando revestida por lo que denominamos suelo. La Pedosfera está ligada a la Biosfera, la Litosfera, la Hidrosfera y la Atmósfera (Figura 1). La Biosfera está compuesta por todos los seres vivos que habitan la Tierra, mientras que la Litosfera conforma la parte sólida del planeta. Así cómo la Hidrosfera alberga el agua existente en el planeta y la Atmósfera estaría compuesta por la fracción gaseosa que rodea al conjunto, incluidos los llamados "espacios vacíos" en el suelo (Molina, 2017) (Figura 1). En consecuencia, el suelo es un medio complejo conformado por tres fases: sólida, líquida y gaseosa, pudiendo definirse como "una cubierta delgada en la superficie terrestre, de unos pocos centímetros a varios metros, que constituye una interfase que permite intercambios entre la Biosfera, la Litosfera, la Hidrosfera y la Atmósfera" (Porta Casanellas et al., 2008).

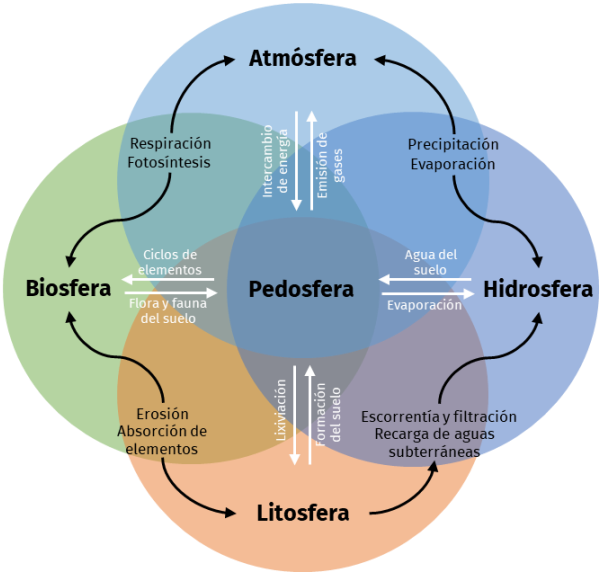

Figura 1. Diagrama de las cuatro dimensiones la pedosfera. Adaptado de UNESCO (2007).

Por las razones antes mencionadas, el suelo es un recurso dinámico y vivo (Lal, 2015) que desempeña un papel multifuncional de vital importancia para los ecosistemas (Porta Casanellas et al., 2008; Rodríguez-Berbel et al., 2020). Entre sus principales funciones podemos encontrar la conservación de la calidad del aire y el agua, el mantenimiento de la productividad animal y vegetal o el soporte físico para las actividades humanas, características que lo convierten en un elemento crucial en la salud de los seres vivos (Porta Casanellas et al., 2008; Singer and Sojka, 2002). Los suelos

juegan un papel fundamental en el calentamiento global, dado que son el principal reservorio terrestre de carbono (C) y nitrógeno (N) (Schaufler et al., 2010), otorgándoles la capacidad de actuar como fuente o sumidero de gases de efecto invernadero (dióxido de carbono –CO_2–, metano –CH_4– y óxido nitroso –N_2O–) (Oertel et al., 2016). Sin embargo, los cambios en la dinámica del suelo pueden afectar a los stocks de estos elementos influyendo en las concentraciones de gases de efecto invernadero (Schaufler et al., 2010). Según el Grupo Intergubernamental de Expertos sobre el Cambio Climático (IPCC), la temperatura del planeta experimentará un incremento de al menos 1.5 ºC en los próximos 20 años (IPCC, 2020), siendo vital la necesidad descender inmediatamente las emisiones de CO_2 para mantener el calentamiento global por debajo de las predicciones (IPCC, 2022). Por ello, es imprescindible la protección de los suelos dado que, para conseguir un suelo equilibrado y funcional que cumpla las necesidades de los organismos que habitan en él, es menester largos periodos de tiempo considerándose un recurso natural no renovable a escala humana (Lal, 2015; Porta Casanellas et al., 2008).

2. Formación del suelo

La edafogénesis o formación del suelo radica en una serie de procesos sucesivos mediante los cuales un material originario (una roca *in situ*, un material transportado o un suelo anterior) es transformado en un suelo que puede estar constituido por uno o más horizontes (Barrett and Schaetzl, 1998) que se distinguirán entre ellos por propiedades visibles (color, textura, estructura, etc.; White, 2006). Estos procesos o condiciones pueden cambiar a lo largo del tiempo resultando en la complejidad evolutiva de los suelos (Porta Casanellas, 2003). La mezcla de procesos físicos, químicos y biológicos que actúan sobre los materiales minerales y orgánicos del suelo favorecen la presencia de un tejido poroso en ellos. Esta porosidad permite la retención de agua y aire en las suelos, a la vez que crea hábitats favorables para el desarrollo de la vida vegetal, animal y microbiana (White, 2006).

Por ende, los suelos son un sistema físico dinámico procedente de la combinación de diversos factores formadores o factores ecológicos de formación, que cambiaran en función de la ubicación (Porta Casanellas, 2003). Los factores formadores que intervienen en el desarrollo del suelo son el clima, el relieve, la roca madre, los organismos y el tiempo (Jenny, 1941; Figura 2). Porta Casanellas et al. (2008) describió el *clima* como el aporte de agua y energía al material originario; el *relieve o geomorfología* como la

posición que ocupa el suelo en un lugar concreto; la *roca madre* (material originario o material parental) como el material a partir del cual se forma el suelo; los *organismos* como agentes que actúan sobre la roca o material originario contribuyendo a su mezcla y disgregación; y, el *tiempo*, como la prolongación a lo largo de los años de la acción de los distintos factores (Figura 2). Por tanto, la interacción entre estos cinco factores determinará la dirección, velocidad y duración de la formación del suelo (Porta Casanellas et al., 2008). Asimismo, otros autores han puesto de manifiesto la influencia de nuevos elementos como la hidrología o la influencia humana en el desarrollo de los suelos (Blume et al., 2016; Gaucher, 1981), introduciendo el uso de conceptos como suelos antrópicos (Anthrosol^{WRB}) o suelos tecnogénicos (Technosol^{WRB}).

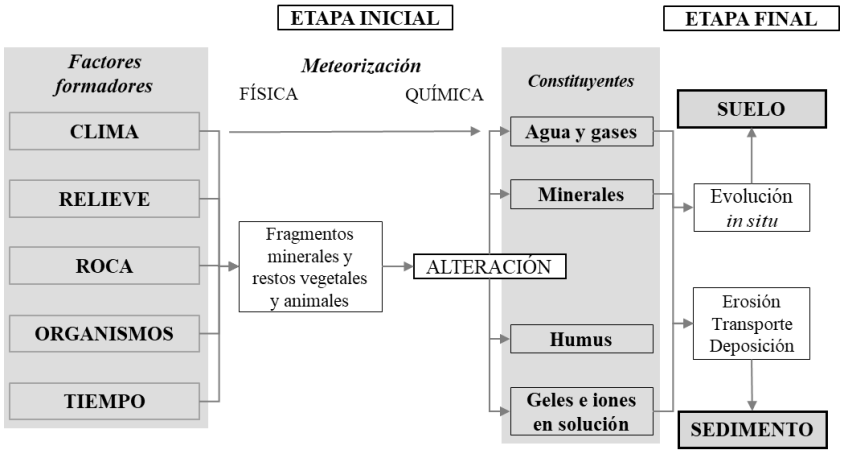

Figura 2. Diagrama de la formación de suelo. Adaptado de Dorronsoro (Universidad de Granada).

El tiempo actúa sobre los otros factores estableciendo diferentes procesos específicos que darán lugar a la diferenciación de horizontes y a la gran diversidad de suelos. Por consiguiente, el tiempo se considera una variable independiente, mientras que el resto de los factores dependerán, en mayor o menor medida, unos de otros. Aunque todos tienen mucha importancia, en algunas ocasiones un factor puede dominar en el desarrollo del suelo (Figura 3). Por ejemplo, la vegetación es dependiente del clima y el relieve, ya que condicionarán la temperatura y el agua del suelo a la vez que, el tiempo condicionará la evolución de la vegetación (Porta Casanellas et al., 2008). Los procesos de formación del suelo, desencadenados por las complejas interacciones de los factores implicados (Figura 2Figura 3), se podrían dividir en procesos de transformación (meteorización de rocas, transformación de minerales, descomposición de la materia orgánica o humificación) y en procesos de translocación (percolación, ascenso de agua, lixiviación, salinización, etc.) (Blume et al., 2016). Como resultado, debido a la

variabilidad espacial y a la variabilidad espacio-temporal a la que los suelos están expuestos (Porta Casanellas et al., 2008), su evolución dependerá de los diferentes procesos que lo conformen, afectando de igual forma a las funciones o servicios ecosistémicos potenciales que puedan ofrecer. Por lo que el desarrollo de la vida en la Tierra está estrechamente ligada a su evolución (Blume et al., 2016).

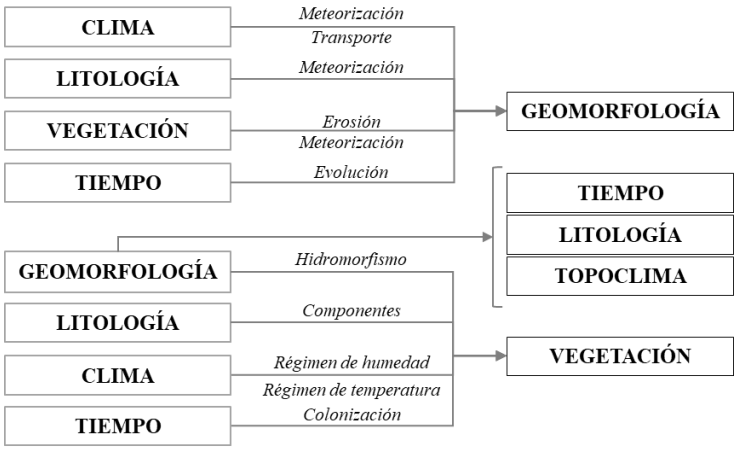

Figura 3. Diagrama de los factores influyentes en la formación del suelo. Adaptado de Porta Casanellas (2008).

2.1. Factores formadores en Almería

Dentro del continente europeo, España cuenta con la mayor extensión de clima árido o semiárido (Porta Casanellas, 2003), destacando el sudeste de la Península Ibérica, donde se ubica provincia de Almería entre las latitudes 37º 52' y 36º 40' y las longitudes 1º 37' y 3º 07' (Simón Torres et al., 2005). La provincia de Almería, desde hace millones de años, ha experimentado una evolución climática y geológica que ha proporcionado al territorio una gran variedad morfológica, litológica y biológica (García Lorca, 2009). La heterogeneidad de sus ecosistemas unida a las condiciones ambientales desfavorecedoras y al bajo contenido en materia orgánica (MO) de los suelos secos (Bastida et al., 2006; Bukar et al., 2019; Solé-Benet et al., 2009) repercuten negativamente a la recuperación natural de éstos haciéndola extremadamente complicada (Rodríguez-Berbel et al., 2021), siendo la aridez el factor condicionante para su desarrollo (Porta Casanellas, 2003). Por tanto, estas peculiaridades convierten a Almería en un escenario clave para el estudio de zonas áridas.

2.1.1. Características climatológicas

El clima de Almería se define como subdesértico, mediterráneo, cálido y seco (Lázaro et al., 2004). Además de estar condicionado por la precipitación y la temperatura

como en cualquier otro lugar, también se encuentra supeditado al acusado relieve (MAGRAMA, 2004). La insolación media es de 2.800 h año $^{-1}$ con zonas que superan las 3.000 h anuales (Instituto Geográfico Nacional, 2021; MAGRAMA, 2004) junto a una evaporación potencial media de 1.200 mm año $^{-1}$ (Instituto Geográfico Nacional, 2021).

La mayor parte del territorio cuenta con una pluviométrica media anual de 150 - 350 mm (Figura 4a), llegando a valores de 650 mm año^{-1} en la proximidades de Sierra Nevada (Simón Torres et al., 2005). Las precipitaciones se registran principalmente en los meses de otoño e invierno como episodios torrenciales, desencadenando inviernos suaves y veranos largos y secos que inducen a largos periodos de estrés hídrico (Gallardo, 2016). En cuanto a las temperaturas medias anuales, oscilan entre 16-18ºC, aunque en algunas zonas de menos altitud pueden superar los 18ºC (Figura 4b) (Instituto Geográfico Nacional, 2021). Como consecuencia a estas condiciones climatológicas, la formación del suelo se ve enormemente ralentizada (Porta Casanellas, 2003).

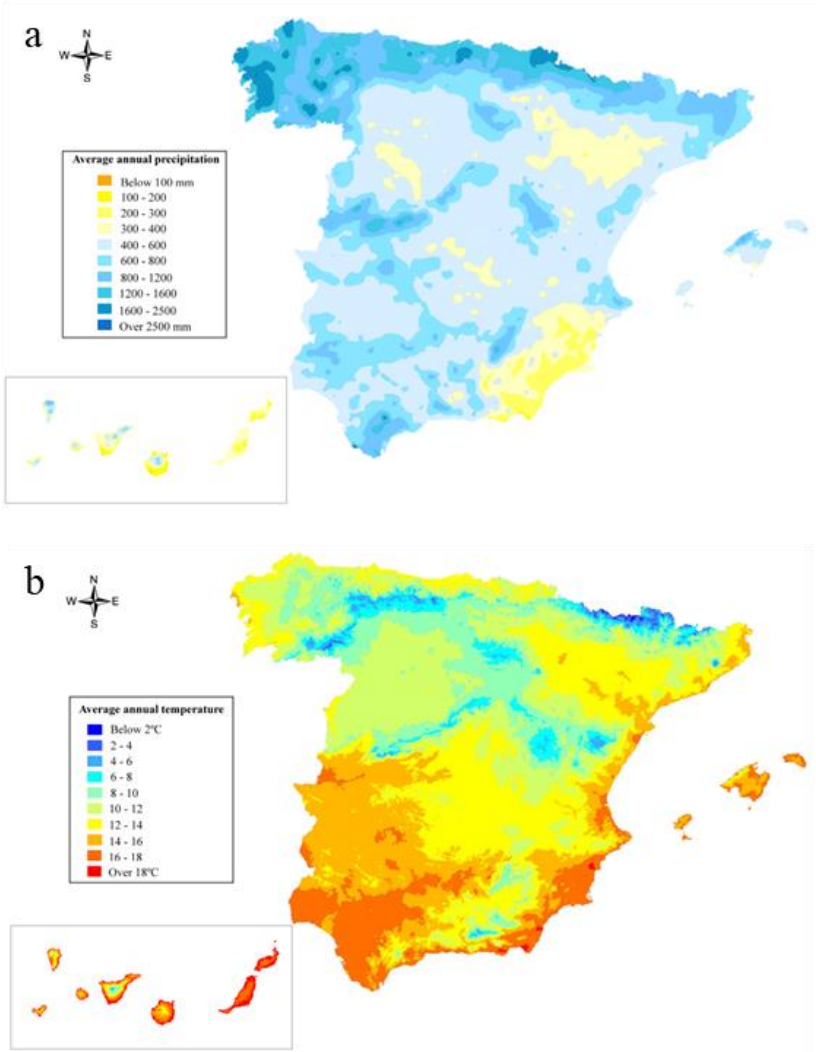

Figura 4. Precipitaciones (a) y temperaturas (b) medias anuales registradas mediante métodos de interpolación geoestadística (kriging) de 1803 y 4189 estaciones, respectivamente, pertenecientes a la Agencia Estatal de Meteorología (AEMET). Fuente: Infraestructura de Datos Espaciales de España (IDEE), Infraestructura de Información Espacial de Europa (INSPIRE), Ministerio de Agricultura, Pesca y Alimentación (MAPAMA) en Rodríguez-Berbel et al. (2022).

2.1.2. Características geológicas

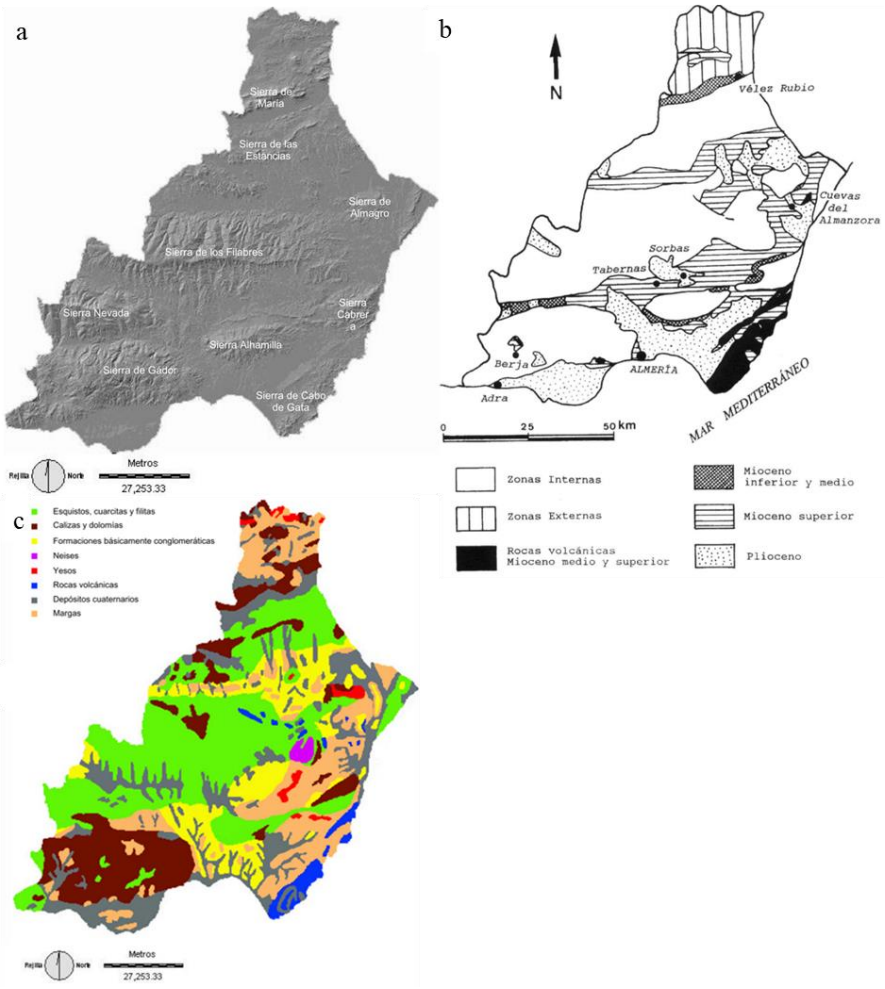

Figura 5. Modelo digital del terreno (a), esquema geológico (b) y mapa litológico (c) de la provincia de Almería. Fuente: Proyecto LUCDEME. Mapa de suelos de Almería (MAGRAMA, 2004) *y* Simón Torres et al. (2005).

El territorio almeriense se encuentra ubicado en las zonas Bética y Prebélica de las Cordilleras Béticas (MAGRAMA, 2004), otorgándole una acusada geología muy variada y compleja. La Zona Bética ocupa la mayor parte del territorio albergando: Sierra Nevada y Sierra de los Filabres (complejo Nevado-Filábride); las Sierras de Gádor, Alhamilla, Cabrera, Almagro y Estancias (complejo Alpujárride); y, el borde Norte de la Sierra de las Estancias y algunas zonas de Sierra Cabrera (complejo Maláguide) (Figura 5a; MAGRAMA, 2004). La roca madre o materiales parentales de las Zonas Internas de las Cordilleras Béticas proceden de la Edad Paleozoica y Triásica, mientras que las Zonas Externas pertenecen al Trías Medio, Jurásico, Cretácico y Eoceno. Asimismo, también

encontramos materiales de las grandes Cuencas Neógenas (Mioceno inferior y medio, Mioceno superior y Plioceno) y rocas volcánicas del Mioceno medio y superior (Figura 5b; Simón Torres et al., 2005). En cuanto a la composición litológica destacan la presencia de esquistos, cuarcitas y filitas, seguido por calizas y dolomías y, formaciones conglomeráticas (**¡Error! No se encuentra el origen de la referencia.**c).

2.1.3. Características geomorfológicas

La altitud media provincial es de 860 m, superando el 31% del territorio los 1.000 m, mientras que los relieves de mayor altitud se ubican en Sierra Nevada, Filabres, Gádor y María sobrepasando los 2.000 m hasta el nivel del mar (Figura 6a) (Instituto de Estudios Almerienses, 2016). Por tanto, podríamos diferenciar tres grandes sectores: sierras, depresiones neógenas y zonas costeras (Simón Torres et al., 2005). Debido a la diferencia de altitudes, el área provincial presenta gran cantidad de zonas escarpadas o muy escarpadas con pendientes superiores al 15% (Figura 6b) (MAGRAMA, 2004). Sólo el 12% de la superficie muestra un relieve llano o casi llano, mientras que el 65.5% tiene una pendiente entre el 6-13% (Simón Torres et al., 2005).

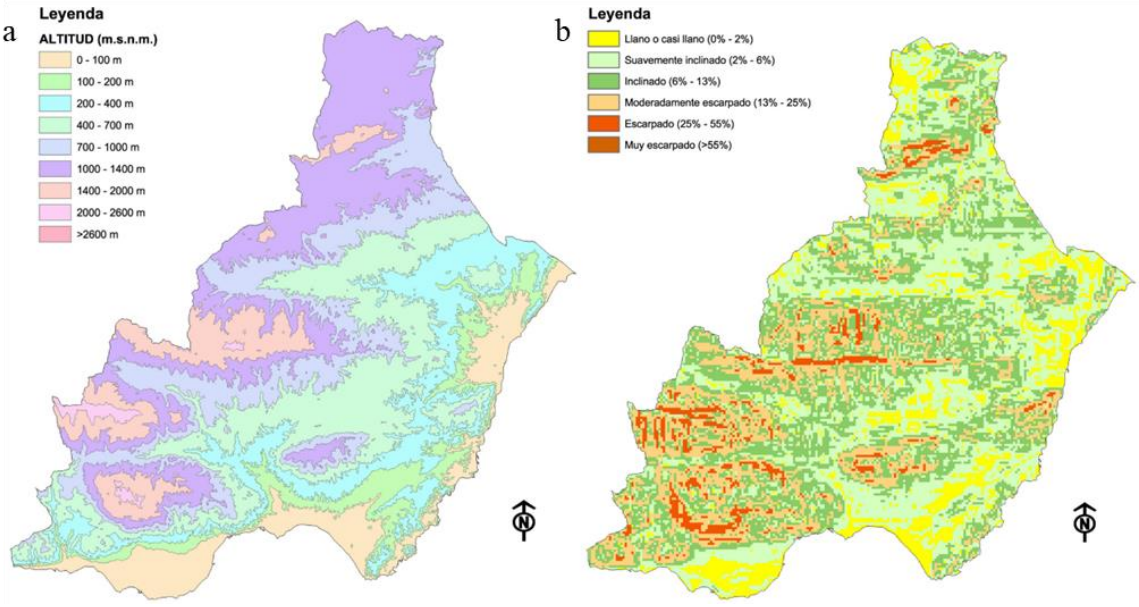

Figura 6. Distribución altitudinal a intervalos de cota de 100 m (a) y distribución de pendientes según la clasificación FAO (b) en la provincia de Almería. Fuente: Simón Torres et al. (2005).

2.1.4. Características biológicas

Almería presenta cuatro termotipos o pisos bioclimáticos (Figura 7a). Las áreas de estudio se encuentran ubicadas en el piso Termomediterráneo, caracterizado por la

presencia de arbustos espinosos y matorral subdesértico, así como por la escasez o ausencia de bosques (Simón Torres et al., 2005).

Respecto a las series de vegetación, ambas zonas de estudio (más información en *4.1. Zonas de estudio*) se localizan entre dos series de vegetación. Por un lado, Enix se localiza sobre la interfase entre la serie de encinares béticos basófilos y la serie semiárida de lentisco, mientras que la zona de Gádor se encuentra sobre la serie semiárida del lentisco y la vegetación tabernense sobre margas salinas (Figura 7b).

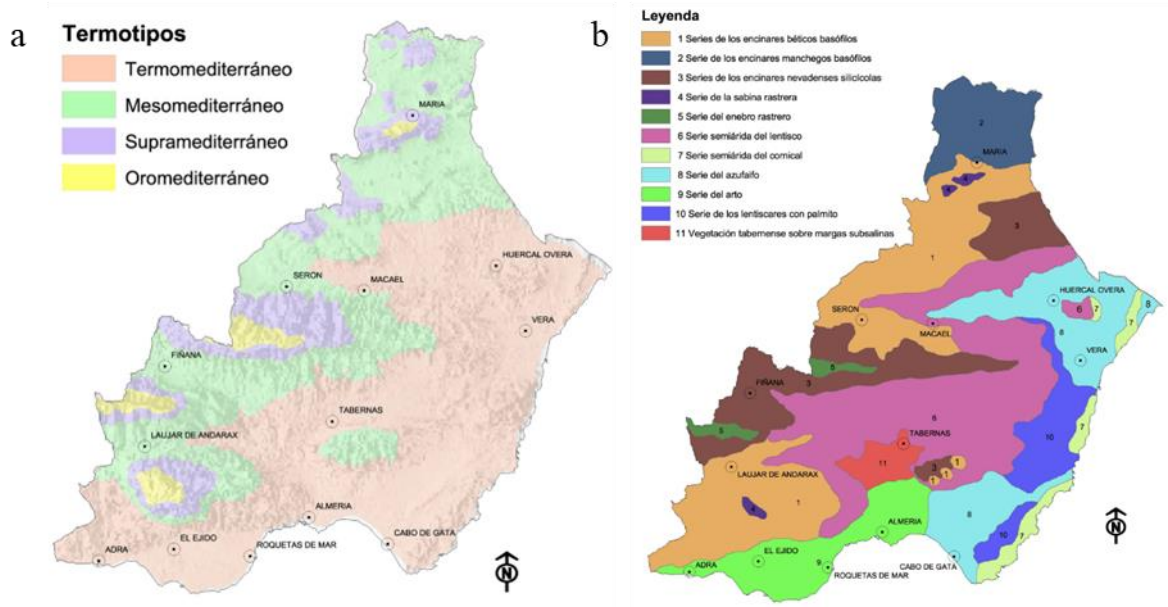

Figura 7. Pisos bioclimáticos (a) y series de vegetación (b) de la provincia de Almería. Fuente: Simón Torres et al. (2005).

2.1.5. Factores antrópicos

A lo largo de la historia, el consumo de recursos naturales (madera, matas o esparto), así como la minería y la agricultura han sido las principales actividades industriales en la provincia. Las diversas explotaciones que han sufrido los ecosistemas almerienses han provocado la deforestación de la mayor parte del territorio y, consecuentemente, la transformación del suelo (Simón Torres et al., 2005). En la actualidad, Almería cuenta con la mayor concentración de cultivos bajo plástico e intensivos ocupando 31.614 ha del territorio (Rodríguez-Berbel et al., 2022a), destacando las zonas del Campo de Níjar y el Campo de Dalias (Simón Torres et al., 2005).

3. Degradación del suelo

La degradación del suelo se define como "un cambio en la salud del suelo resultando en una disminución de la capacidad del ecosistema para producir bienes o prestar servicios" (FAO, 2023), conllevando la pérdida de fertilidad y la disminución a largo plazo de la funcionalidad y productividad de los ecosistemas (Bai et al., 2008; López-Bermúdez and García-Gómez, 2006). Entendiéndose la salud del suelo como la capacidad de un suelo para funcionar como un sistema vivo y definiéndose como "la capacidad de un suelo para sostener la productividad biológica, mantener la calidad ambiental, y promover la salud de plantas y animales" (Doran and Parkin, 1994). Se estima que a nivel mundial, un tercio de los suelos están moderada o altamente degradados a causa de factores como la erosión, pérdida de materia orgánica, salinización, acidificación, compactación, incendios o contaminación (metales pesados, agroquímicos, medicamentos, etc.) (Cantera et al., 2015). La mayoría de los suelos degradados o perturbados se caracterizan por el déficit de materia orgánica (MO) en comparación con las áreas adyacentes no perturbadas. Particularmente, la pérdida de la capa superficial del suelo resulta en la reducción del depósito de MO de éste, conllevando la disminución de la capacidad de retención de agua debido a la falta de MO y al aumento de escorrentía. Por consiguiente, la capacidad del suelo de regenerar MO se ve dificultada debido a la alteración de los regímenes de nutrientes, agua y temperatura, a la vez que afecta negativamente a la actividad microbiana (Larney and Angers, 2012).

Una de las principales causas de la degradación del suelo son las condiciones socioeconómicas (Martínez-Valderrama et al., 2016). Existen dataciones que indican que, desde el Neolítico, la región mediterránea ha sufrido efectos destructivos como consecuencia del impacto de las actividades humanas (agricultura, deforestación, sobrepastoreo, minería, incendios forestales, etc.) (López-Bermúdez and García-Gómez, 2006; Rodríguez-Berbel et al., 2022a; Yassoglou, 2000), lo que ha significado cuantiosos cambios en el uso de la tierra a lo largo de la historia (Rodríguez-Berbel et al., 2022b). Cuando hablamos de "uso del suelo", nos referimos al empleo de la tierra física y sus recursos por los seres humanos con el fin de obtener diferentes beneficios, incluyendo el mantenimiento de la masa forestal, agricultura o paisajismo, además de su papel como depurador natural (Porta Casanellas et al., 2008). Asimismo, los procesos de degradación están directamente relacionados con el cambio climático y los cambios en el uso del suelo, considerándose los principales promotores del cambio global (Castillo-Monroy et al.,

2011). Esta problemática se agrava en climas semiáridos y, especialmente, en los ecosistemas semiáridos, a causa de las escasas precipitaciones torrenciales, la elevada radiación solar y la baja cobertura vegetal que facilitan los procesos erosivos (Bruneel et al., 2019; Miralles et al., 2009; Rodríguez-Berbel et al., 2020b). En consecuencia, las áreas semiáridas experimentan la aceleración de los procesos de degradación y desertificación debido a los cambios en el uso del suelo, la pérdida de cubierta vegetal, las restricciones climáticas, la pérdida de materia orgánica, la erosión del suelo, los incendios forestales o la intensificación de la agricultura (Sciortino, 2001; Symeonakis et al., 2007).

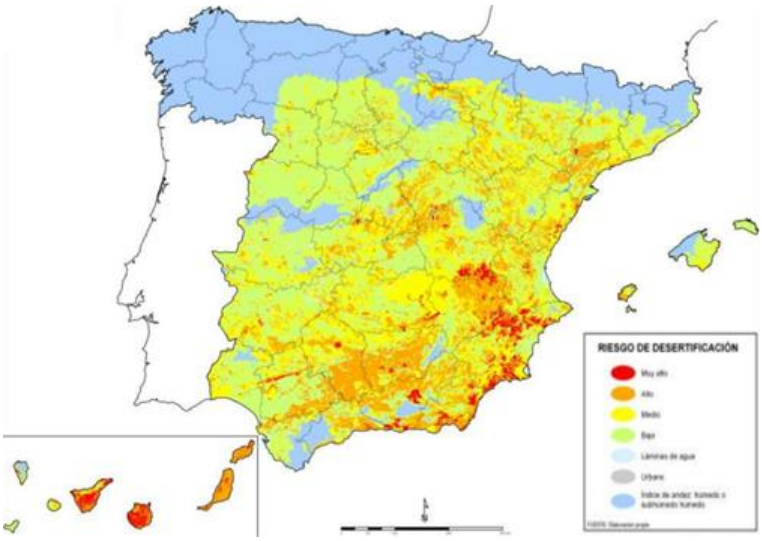

Figura 8. Mapa de riesgo de desertificación en España. Fuente: MAGRAMA (2016).

Los procesos de degradación están directamente relacionados con la desertización (Sciortino, 2001). El proceso de desertización podría definirse como "un proceso de degradación leve o severa del medio que involucra un continuo cambio, especialmente de los recursos vegetales y del suelo como resultado de las actividades humanas" (López-Bermúdez and García-Gómez, 2006). Por ello, la desertización es uno de los principales problemas de degradación en las regiones secas (Figura 8), dado que la combinación de condiciones climáticas, geográficas, socioeconómicas y el manejo de recursos naturales provocan la reducción de la productividad de estos ecosistemas suponiendo la pérdida de los servicios ecosistémicos (Rodríguez-Berbel et al., 2022a; van Leeuwen et al., 2019). Consecuentemente, los suelos mediterráneos son especialmente vulnerables a los procesos de desertificación (Figura 8), dado que las condiciones de aridez y sequía unida a las labores antrópicas inducen su aceleración (López Bermúdez, 2002).

Conocer los sistemas de explotación de recursos que ha experimentado un ecosistema a lo largo de la historia junto con el entendimiento del medio natural (estructura, dinámica, evolución) y la vulnerabilidad de las áreas afectadas es de vital importancia para comprender los procesos de desertificación que puedan experimentar (López-Bermúdez and García-Gómez, 2006). Por ende, es necesaria la selección de una estrategia de uso adecuada con el objetivo de prevenir la degradación del suelo, llevándola a cabo teniendo en cuenta factores como sus características, su distribución, su comportamiento así como los cambios esperables (López-Bermúdez and García-Gómez, 2006).

3.1. Suelos degradados por agricultura

Desde la antigüedad, la agricultura ha sido una de las principales actividades socioeconómicas del ser humano. En la actualidad, unas 50 millones de hectáreas del territorio español se destinan a este propósito (MAPA, 2020). Por ello, la exposición continúa de los suelos a procesos degradativos unida a otros procesos de degradación íntimamente relacionados con las actividades agrícolas, como la compactación, salinización, erosión o la contaminación por metales pesados, agroquímicos y/o microplásticos (Cuevas et al., 2019; Pulido et al., 2018; Symeonakis et al., 2007), vulneran los servicios ecosistémicos de los mismos.

La agricultura intensiva perturba las funciones de los ecosistemas, dado que el manejo durante largos periodos de tiempo del mismo cultivo generan una disminución progresiva de su fertilidad (Duval et al., 2015), dado que al aportar poco carbono al suelo la reserva de materia orgánica disminuye. Este descenso de la materia orgánica del suelo (MOS) y su calidad en los suelos ocasionan cambios en las comunidades edáficas además de la pérdida de diversidad biológica (Vázquez et al., 2020; Wang et al., 2017). Por tanto, en los agroecosistemas, el contenido en materia orgánica (MO) es un determinante esencial, pues está ligado a la calidad y salud del suelo así como relacionado con los ciclos de nutrientes, la actividad biológica y la productividad agrícola (Li et al., 2022). La combinación de explotaciones de tierras fértiles con un manejo inadecuado y las previsiones de procesos de desertificación por el cambio climático (Wijesekara et al., 2016), podrían conducir al deterioro funcional de los suelos, perdiendo la capacidad de regular el ecosistema y propiciar su degradación (Rodríguez-Berbel et al., 2023),

resultando, en ocasiones, en el abandono de estas tierras agrícolas por falta de productividad.

3.2. Suelos degradados por minería

Las explotaciones mineras han impulsado la degradación de 800.000 Km^2 a nivel mundial afectando gravemente a la calidad y fertilidad de los suelos (Cherlet et al., 2018). Unida a la degradación, estas actividades provocan alteraciones en el paisaje con negativos impactos visuales y grandes cambios ecológicos en los sistemas dañados (Luna et al., 2017; Sheoran et al., 2010). En concreto, la minería a cielo abierto conlleva la degradación severa del suelo como resultado de la pérdida total de éste (Luna et al., 2016a), ya que implica la eliminación de la cubierta vegetal y de los horizontes organominerales (Kasting and Siefert, 2002; Luna et al., 2017). Por ello, este tipo de prácticas supone una considerable amenaza para los ecosistemas, dado que remueve el suelo provocando cambios en las propiedades físicas, químicas y biológicas (Rodríguez-Berbel et al., 2020), además de perturbar la estabilidad suelo-planta (Luna et al., 2016a) e inducir la lixiviación de nutrientes por erosión (Li et al., 2018). Asimismo, las modificaciones en las condiciones edáficas reducen su productividad además de generar alteraciones directas sobre las comunidades microbianas del suelo (Chen et al., 2020; Rodríguez-Berbel et al., 2022b, 2021, 2020).

Por tanto, siguiendo las razones antes mencionadas, las áreas que han sido perturbadas por minería y, en especial aquellas ubicadas bajo clima semiárido, requieren el impulso de una restauración ecológica para recuperar la calidad del suelo, suponiendo con ello, el restablecimiento de la cubierta vegetal y la comunidad edáfica.

4. El suelo en regiones semiáridas

Se estima que el 40% de la superficie terrestre posee condiciones climáticas áridas o semiáridas (Granados-Sánchez et al., 2013), conformando un extenso territorio ocupado por tierras secas, siendo de vital importancia estudiar su evolución y comportamiento.

Un suelo en condiciones ideales está constituido por cuatro componentes: material mineral (45%), materia orgánica (5%), aire (25%) y agua (25%) (Acosta, 2006). En suelos secos, los minerales predominantes se caracterizan por presentar una estructura sencilla, tales como calcita, dolomita, yeso o sales solubles (halita, tenardita, mirabilita, epsomita, etc.). En los suelos mediterráneos áridos y semiáridos dominan los minerales de arcilla,

generalmente heredadas. En ellos, abundan los horizontes en los que el yeso o la calcita se observan en proporciones mayores al 60%, estando condicionadas las propiedades físicas y químicas de estos suelos y la vegetación que sustentan por estos elementos, así como por la salinidad (Porta Casanellas, 2003). Asimismo, los suelos áridos y semiáridos se identifican por un bajo contenido de MO con valores que varían entre menos del 1% y el 3%. El porcentaje de la MOS viene determinado por diferentes factores como la vegetación, el clima, las propiedades físico-químicas de los suelos o la microbiota edáfica (Porta Casanellas, 2003).

4.1. Zonas de estudio

Las zonas de estudio se encuentran situadas en el centro sur de la provincia de Almería (Figura 9). Ambas, han sufrido algún tipo de explotación antrópica que ha desencadenado en el deterioro de las características físicas, químicas, bioquímicas y microbiológicas de los suelos (Rodríguez-Berbel et al., 2023, 2022, 2021, 2020).

Figura 9. Localización de las áreas de estudio. Fuente: Google Earth.

4.1.1. Enix: Suelos agrícolas abandonados

Al otro lado de la Sierra de Gádor (cara sur-oeste), dentro del término municipal de Enix (36°53′20″ N, 02°36′14″ O) a 977 m.s.n.m y a unos 10 km lineales del mar, se seleccionó una zona experimental en cultivos de secano abandonados sobre terrazas sin pendiente. Los suelos de estudio y de las zonas colindantes que no han sufrido explotación agraria se desarrollaron sobre rocas calizas, dolomías y filitas clasificadas como

Leptosoles (MAGRAMA, 2004). Las labores agrícolas en la zona fueron destinados a cultivo de uva, almendro y cereal, siendo abandonados por falta de productividad hace al menos 50 años (Rodríguez-Berbel et al., 2023).

La flora de las áreas naturales limítrofes no cultivadas está compuesta fundamentalmente por: *Retama sphaerocarpa* L. Boiss., *Anthyllis cytisoides* L., *Macrochloa tenacissima* (o *Stipa tenacissima* L.) (L.) Kunth., *Thymus zygis* L., *Cistus albidus* L., y algunos ejemplares de *Pinus halepensis* Mill. (Figura 10).

Figura 10. Vegetación más abundante en las zonas no expuestas a explotación agrícola: Retama sphaerocarpa L. Boiss. (a), Anthyllis cytisoides L. (b), Stipa tenacissima L. (c), Thymus zygis L. (d), Cistus albidus L. (e) y Pinus halepensis Mill. (f). Fuente fotográfica: Flora Vascular de Andalucía (2021).

4.1.2. Gádor: Suelos degradados por minería

Se instalaron dos áreas experimentales, una en 2008 (Gádor-2008) y otra en 2018 (Gádor-2018), en una cantera caliza propiedad de la empresa minera CEMEX España Operaciones S.L.U.

Ambas zonas de estudio se ubican a 15 km al norte de Almería dentro del término municipal de Gádor (36°55′20″N, 2°30′29″W) a 362 m.s.n.m., entre la cuenca intermontana terciaria formada por margas Tortonienses (Mioceno superior), lutitas

calcíticas-yesíferas y areniscas calcáreas y la Sierra de Gádor conformada por dolomías y calizas cenozoicas. La litología de las áreas de estudio se compone primordialmente por areniscas calcáreas que se superponen sobre margas. En las áreas circundantes que no han sufrido perturbaciones de la extracción de áridos, los suelos son principalmente Leptosoles y Regosoles calcáricos (Figura 11) (FAO-IUSS-ISRIC Working Group WRB, 2015) sobre (a) lutitas calcíticas y yesíferas (margas), (b) areniscas calcáreas y (c) depósitos de taludes alimentados principalmente por la suelos poco profundos sobre calizas y dolomitas de relieves superiores (Luna et al., 2016b, 2016a; Rodríguez-Berbel et al., 2022, 2021, 2020).

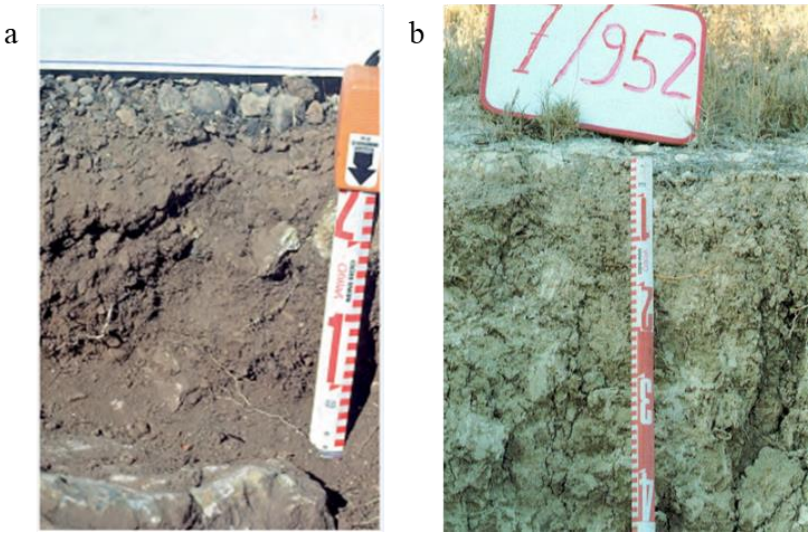

Figura 11. Perfil de suelos: Leptosol (a) y Regosol calcárico sobre margas (b). Fuente: Instituto de Estudios Almerienses (2016) y MAGRAMA (2004).

Los Leptosoles, abundantes en la Sierra de Gádor, se caracterizan por ser suelos poco desarrollados de poca profundidad (pocos centímetros) asentados sobre rocas duras, como calizas compactas. Generalmente, aparecen ligados a zonas erosionadas (Instituto de Estudios Almerienses, 2016). En cuanto a los Regosoles calcáricos, son suelos con bajo contenido en materia orgánica, fuertemente carbonatados ricos en Ca^{2+} y Mg^{2+}, K^{+} y Na^{+}, con textura arcillo-limosa (MAGRAMA, 2004).

En la Figura 12 se muestra la vegetación nativa que rodea las zonas de estudio. Está conformada por pastizales compuestos por la gramínea *Macrochloa tenacissima* (o *Stipa tenacissima* L.) (L.) Kunth. y algunos arbustos perennes enanos como *Anthyllis terniflora* (Lag.) Pau. *A. cytisoides* L., junto con otras especies como *Ulex parviflorus* Pourr., *Maytenus senegalensis* (Lam.) Exell., *Rhamnus lycioides* L. y *Pistacia lentiscus* L. (Luna et al., 2016b; Rodríguez-Berbel et al., 2021).

Figura 12. Vegetación más abundante en las zonas que no han sufrido explotación minera. Macrochloa tenacissima (L.) Kunth. (a), Anthyllis terniflora (Lag.) Pau. (b) Anthyllis cytisoides L. (c), Ulex parviflorus Pourr. (d), Maytenus senegalensis (Lam.) Exell. (e), Rhamnus lycioides L. (f) y Pistacia lentiscus L. (g). Fuente fotográfica: Flora Vascular de Andalucía (2021); Herbari Virtual del Mediterrani Occidental de la Universidad de las Islas Baleares (2023).

5. Materia orgánica en los suelos

La materia orgánica del suelo (MOS) hace referencia a la agrupación de todos los componentes orgánicos del mismo, estando constituida principalmente por carbono (C), oxígeno (O) y nitrógeno (N) y, en menor proporción por azufre (S), fósforo (P) e hierro (Fe), entre otros elementos. Habitualmente, la MOS está compuesta por materia orgánica fresca no humificada procedente mayoritariamente de la biomasa vegetal o necromasa (parte aérea y raíces), así como por restos de animales y microorganismos. Por lo general, la MO no humificada está constituida por sustancias fácilmente biodegradables como glúcidos, proteínas, péptidos, aminoácidos o ácidos orgánicos de bajo peso molecular que perdurarán poco tiempo en el suelo (Porta Casanellas et al., 2008; Schnitzer and Khan, 1978). Además de, por sustancias más complejas como las ligninas, conllevando una degradación será más lenta (Porta Casanellas et al., 2008). El aporte continuo de MO al suelo, forma una MOS con una composición muy heterogénea compuesta en un 60-80% por sustancias en diferentes estados de alteración (Porta Casanellas, 2003; Porta Casanellas et al., 2008). Las diferentes alteraciones que experimenta la MOS vienen

determinadas por las condiciones del medio, pudiendo agruparse en: descomposición (fragmentación y catabolismo), mineralización (transformación a formas inorgánicas) y humificación (reorganización y neoformación de productos orgánicos; Figura 13).

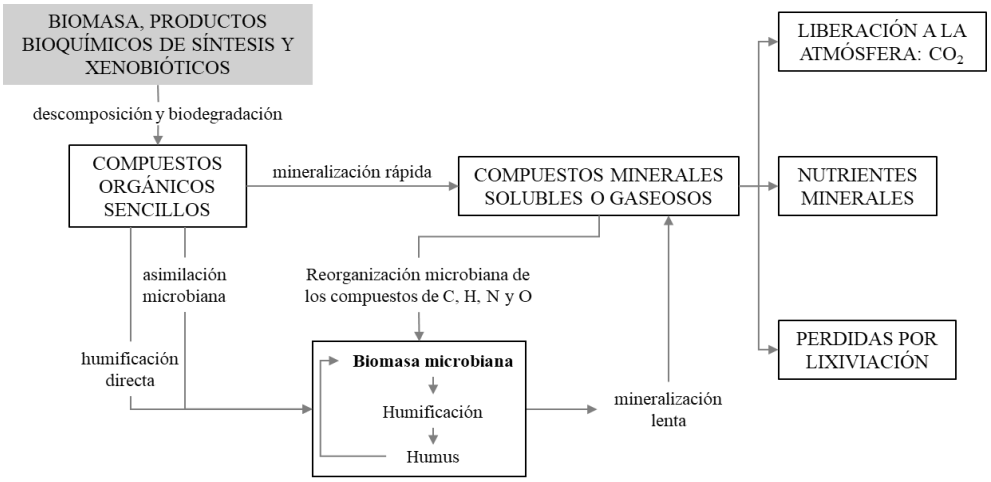

Figura 13. Esquema conceptual de los procesos que perciben la materia orgánica desde su incorporación al suelo y sus interrelaciones. Adaptado de Porta Casanellas (2008).

5.1. Descomposición y mineralización de la materia orgánica

La descomposición de la MO consiste en el fraccionamiento progresivo en partículas de menor tamaño de la necromasa mediante procesos bióticos y abióticos (Cotrufo et al., 2010), tales como lixiviación (liberación de compuestos solubles), fragmentación y catabolismo. Sin embargo, la alteración de la MOS está íntimamente ligada a la actividad microbiológica (bacterias, hongos, algas y protozoos), así como por la mesofauna edáfica (lombrices, moluscos, nematodos y pequeños artrópodos). Como consecuencia, la velocidad de la descomposición de la MO estará condicionada por la calidad de la necromasa aportada, es decir, existirá una relación directamente proporcional entre el aporte de MO lábil y la rapidez en el flujo de nutrientes y en la contribución energética de los organismos edáficos. Durante la descomposición, las sustancias solubles liberadas como azúcares o aminoácidos pueden pasar a formar parte de sustancias húmicas o ser lixiviadas rápidamente (Porta Casanellas, 2003).

Simultáneamente a la descomposición de la MO, en el medio edáfico se llevan a cabo procesos de mineralización sobre la MO incorporada y la ya presente en el suelo. Los procesos de mineralización están determinados por diversos factores abióticos (pH, temperatura, humedad, textura, etc.), que repercuten en las poblaciones microbianas (factores bióticos) condicionando las reacciones químicas y bioquímicas que tendrán lugar en los suelos. Durante la mineralización, los elementos que constituyen las

sustancias orgánicas pasan de formar parte de compuestos inorgánicos (sólidos o gaseosos) pudiendo disolverse, adsorberse o ser liberados a la atmósfera como gas. Uno de los factores condicionantes de estos procesos es la calidad de la MO, dado que la dinámica de la mineralización dependerá de la relación entre compuestos lábiles y compuestos recalcitrantes, así como de la presencia de inhibidores microbianos como los antibióticos. Por consiguiente, la calidad de la MO incorporada determinará la fracción del C orgánico que será devuelto a la atmósfera como CO_2 (Porta Casanellas, 2003). Asimismo, es importante conocer el contenido de C orgánico que alberga un suelo, dado que es un indicador esencial de calidad, ya que a partir de éste es posible estimar la capacidad de un suelo para funcionar eficientemente como componente de un ecosistema equilibrado (Berryman et al., 2020).

No obstante, en los ecosistemas terrestres, los procesos biológicos están condicionados por la limitación de nutrientes, principalmente de nitrógeno (N) y fósforo (P). Por ende, la disponibilidad de N es importante para predecir el comportamiento metabólico en los suelos, dado que los procesos de descomposición de la MO aumentan su demanda requiriendo, por tanto, la presencia de este elemento para la transformación metabólica (Porta Casanellas, 2003). Parte del N puede perderse del suelo liberándose a la atmósfera por volatilización (He et al., 2013), liberándose como amonio (NH_4^+). El amonio también puede ser oxidado a nitrato (NO_3) o ser reducido a óxido nitroso (N_2O) y/o dinitrógeno (N_2) en condiciones anóxicas. Por tal razón, la relación entre el contenido de carbono orgánico y nitrógeno (relación C/N) es un indicador de calidad ampliamente utilizado. Generalmente, la MO fresca muestra valores altos para la relación C/N que disminuyen progresivamente a medida que es transformada en humus. Sin embargo, la actividad microbiana es favorecida en ecosistemas constituidos por compuestos hidrosolubles fácilmente metabolizables y ricos en nitrógeno disponible, es decir, con valores bajos de C/N (Porta Casanellas, 2003). Siguiendo esta premisa, se podría pronosticar que medios con MO rica en N o con aportes de N mineral tendrán un efecto positivo sobre el crecimiento microbiano resultando en una descomposición más rápida (Cotrufo et al., 2013).

Asimismo, otro componente esencial para los seres vivos es el fósforo (P). Un suelo puede contener gran cantidad de P, sin embargo la biodisponibilidad del ortofosfato soluble suele ser baja para el desarrollo de los organismos (Bergkemper et al., 2016) considerándose como el nutriente menos accesible del suelo (Vitousek et al., 2010). Los

suelos semiáridos conformados por rocas calizas, dolomitas y filíticas, como las zonas de estudio, se caracterizan por un alto contenido en ión calcio (Ca^{2+}) que, junto con el pH alcalino puede afectar el equilibrio de especiación del ion ortofosfato (PO_4^{3-}) provocando que P precipite como fosfato tricálcico ($Ca_3(PO_4)_2$), inmovilizándolo en el suelo y dando como resultado una baja disponibilidad de P (Tunesi et al., 1999). Este proceso natural hace que el P asimilable se encuentre en muy bajas concentraciones en suelos sin ningún aporte orgánico.

En consecuencia, tras la incorporación de MO fresca, se aprecia una primera etapa muy activa correspondiente a la liberación de sustancias orgánicas lábiles (azúcares, aminoazúcares, aminoácidos y ácidos orgánicos), seguida de una segunda etapa en la que incrementa la actividad biológica mineralizándose otras sustancias como celulosa o hemicelulosa, mientras que los materiales recalcitrantes permanecen en el sustrato (Tabla 1). Por ejemplo, en medios biológicamente activos se produce una mineralización rápida de la MO que puede durar unos 12 meses mientras que, en medios anaerobios (carentes de O_2), los procesos de mineralización son más lentos llegando incluso en ocasiones a observarse la formación de turba debido a la acumulación de la MO (Porta Casanellas, 2003). Por lo que, con el objetivo de modelizar los procesos implicados en la transformación de la MO, diversos autores han propuesto el estudio de propiedades bioquímicas como la determinación de actividades enzimáticas clave, como β-glucosidasa, ureasa o fosfatasa, implicadas en rutas de interés de los ciclos de C, N y P, respectivamente (Bastida et al., 2008, 2006; Gautam et al., 2020; Ros et al., 2003; Sinsabaugh et al., 1994).

Tabla 1. Mineralización microbiana de los componentes de la materia orgánica fresca. Adaptado de Porta Casanellas (2008).

Componente	**Mineralización**
Azúcares	*Microbiana rápida*
Proteínas	70-85% del C orgánico pasa a CO_2 en 6-12 meses
Hemicelulosa Celulosa	*Microbiana intermedia*
Lignina Compuestos fenólicos Grasas y ceras	*Microbiana lenta*

Por tanto, el equilibrio en el ciclado de nutrientes (C, N y P) se mantiene gracias a la mineralización de la MO, pudiendo conocerse la tasa de mineralización de un suelo

mediante el estudio de las emisiones de CO_2 (Bastida et al., 2013b; Porta Casanellas, 2003; Soria et al., 2021b, 2021a). La velocidad o tasa de mineralización de la MO representa el porcentaje de carbono orgánico inicial mineralizado en un período de tiempo determinado. La liberación de CO_2 es empleada como indicador de la actividad biológica de un medio determinado, ya que está relacionada con la actividad respiratoria y con la eficacia relativa de los microorganismos en los procesos de descomposición. La medición de la emisión de CO_2 pueden realizarse *in vivo* tomando mediciones directamente en campo mediante el empleo de un analizador de gases (Curiel Yuste et al., 2007; Emmerich, 2003; Soria et al., 2021b, 2021a); o, *in vitro* en el laboratorio a través del estudio de la cinética de la mineralización mediante mediciones secuenciales del CO_2 liberado por la actividad biológica denominadas incubación aeróbica o respiración basal (RB) (Bastida et al., 2007b; Miralles et al., 2012b; Ortega et al., 2023; Rodríguez-Berbel et al., 2021). Estas técnicas son una buena herramienta para determinar el efecto de la adición de diferentes tipos de productos orgánicos compostados al suelo, como residuos de cosechas, estiércoles, lodos, etc., sobre la actividad biológica, permitiendo evaluar la capacidad de un suelo para actuar como depurador natural (Porta Casanellas, 2003).

5.2. Humificación de la materia orgánica

La MO humificada está constituida por macromoléculas orgánicas de estructura compleja, diversa y estable, derivadas de la metabolización microbiana (actividad enzimática) y del metamorfismo orgánico durante los procesos de degradación de restos vegetales y animales (Schnitzer, 1999). Estas sustancias húmicas se diferencian en ácidos fúlvicos, ácidos húmicos y humina conformando la fracción orgánica del suelo con actividades fundamentales en procesos físicos, químicos y biológicos (MacCarthy, 2001).

Del mismo modo que la relación C/N, la relación entre el contenido de ácidos húmicos y ácidos fúlvicos (relación AH/AF) es usada como indicador de la humificación en suelos (MacCarthy, 2001; Miralles et al., 2020b; Zancada et al., 2004). Los valores de este ratio permiten conocer las condiciones en las cuales se están llevando a cabo los procesos de humificación (Porta Casanellas, 2003).

5.3. Papel de la materia orgánica en el suelo

La MOS presenta una importante influencia sobre multitud de propiedades físicas, químicas y biológicas de los suelos (Tabla 2), por lo que su cantidad y calidad está directamente relacionada con la funcionalidad de éstos (Porta Casanellas, 2003). Al

mismo tiempo, también participa en diversas interacciones como condicionante del funcionamiento biogeoquímico y como componente clave en la sostenibilidad de los ecosistemas, definiendo los procesos edafogénicos que producen diferentes tipos de humus o como sumidero de C presentando beneficios directos frente al cambio climático (Porta Casanellas, 2003). Considerándose, por tanto la MOS, como un indicador de calidad, fertilidad y productividad del suelo (Boudjabi and Chenchouni, 2022; Figueiredo et al., 2019; Gregorich and Carter, 1997). Por tanto, la materia orgánica juega un papel fundamental en los suelos, actuando como materia prima para la formación de sustancias húmicas además de, como fuente de energía para las comunidades edáficas (Porta Casanellas et al., 2008).

Tabla 2. Funciones de la materia orgánica del suelo (MOS) sobre las propiedades físicas, químicas y biológicas. Adaptado de Porta Casanellas (2008).

Propiedades físicas
• Formación y estabilidad de agregados.
• Porosidad y aireación del suelo.
• Movimientos del agua y retención hídrica en el suelo.
• Prevención de procesos erosivos.
Propiedades Químicas
• Procesos de intercambio iónico.
• Actúa como tampón en los cambios de pH.
• Estabilización de nutrientes orgánicos (N, P y S).
• Formación de complejos organominerales.
• Capacidad de depurar y regular la movilidad y biodisponibilidad de productos tóxicos.
Propiedades biológicas
• Formación del suelo.
• Reserva metabólica de C, macro- y micronutrientes.
• Efecto biofumigante o antibiótico frente a patógenos.
• Contiene reguladores del crecimiento de plantas.
• Disminuye o inhibe los efectos de las perturbaciones ambientales contribuyendo a la resiliencia de los ecosistemas.

6. Microbiota edáfica

El microbioma del suelo conforma un recurso biológico de vital importancia a causa de su implicación en el funcionamiento y productividad de los ecosistemas (Barea et al., 2011). Los microorganismos son responsables de la salud de la plantas y la calidad del suelo (Barea et al., 2005), debido al papel fundamental que juegan en los ciclos biogeoquímicos de nutrientes, la descomposición de la materia orgánica y la estructura del suelo (Bainard et al., 2016; Chaparro et al., 2012). La heterogeneidad del suelo permite el asentamiento de comunidades edáficas muy diversas, pudiéndose encontrar entre 10.000 y 50.000 especies diferentes en 1 g de suelo (Schloss and Handelsman, 2006). Esta diversidad es esencial para conservar la fertilidad y la funcionalidad del suelo (Xue et al., 2017), ya que los microbios regulan los procesos biológicos que suceden en él (Nannipieri et al., 2017). Por tanto, las comunidades de bacterias y hongos desempeñan una labor de vital importancia sobre las propiedades físicas, químicas y biológicas de los suelos (Fierer, 2017; Hart et al., 2019) llevando a cabo funciones clave en los ecosistemas edáficos (Bukar et al., 2019; Rodríguez-Berbel et al., 2022b).

La mayoría de los grupos microbianos son sensibles a las alteraciones del medio provocadas por perturbaciones tanto naturales como antropogénicas (Allison and Martiny, 2008), pudiendo provocar la pérdida de biodiversidad afectando negativamente a su productividad y funcionalidad (Bukar et al., 2019), especialmente debido a los cambios producidos por los procesos de degradación y desertificación que se ven agravados en los ecosistemas áridos y semiáridos. Los impulsores principales de las comunidades bacterianas del suelo incluyen factores abióticos, como el clima o las propiedades físico-químicas del suelo, así como bióticos, es decir, la comunidad vegetal (Bainard et al., 2016). En cuanto al clima, la respuesta de las comunidades microbianas que residen en los suelos vendrá determinada por el ecosistema (Curiel Yuste et al., 2007; Trasar-Cepeda et al., 1998). Por ejemplo, en ecosistemas áridos y semiáridos, el desarrollo microbiano es dependiente de la humedad y la temperatura, resultando en la estimulación de la actividad microbiana tras pulsos de lluvias que suelen darse en periodos cortos e irregulares a lo largo del año (Soria et al., 2021b). La variación en la humedad del suelo tiene un efecto directo sobre las comunidades bacterianas debido al estrés fisiológico, pero también un efecto indirecto al regular la disponibilidad de nutrientes, seleccionando las bacterias mejor adaptadas a las condiciones del suelo (Fierer et al., 2003). Esto significa que, bajo clima semiárido, los procesos de descomposición y

mineralización de la MOS sólo se producirán cuando haya humedad disponible para la comunidad microbiana (Huxman et al., 2004).

Del mismo modo, los cambios en las propiedades físicas (textura), químicas (pH o conductividad eléctrica) o en el contenido de nutrientes (C, N y P) pueden modificar la composición de la comunidad microbiana (Chaparro et al., 2012), aunque la respuesta de los microorganismos a estos cambios es variada (Bastida et al., 2008; Dick, 1997; Torres et al., 2016). Tanto el pH como el contenido en N disponible del suelo son considerados como los promotores de mayor influencia sobre las comunidades bacterianas y fúngicas (Fierer and Jackson, 2006; Lauber et al., 2008; Rodríguez-Berbel et al., 2022b).

Además, la eficacia de la comunidad edáfica para adaptarse al consumo de recursos podría generar también cambios en la composición microbiana (J. Guo et al., 2018), dado que la composición química del C de los aportes de materia orgánica podría influenciar en el uso de éstos (Martens, 2000). Generalmente, las comunidades bacterianas tienen una mayor preferencia por las fuentes de carbono lábil, aunque también existen bacterias con preferencias por compuestos recalcitrantes (Goldfarb et al., 2011). En cambio, la comunidad fúngica es considerada como generalista, presentando una participación activa en el ciclo del C siendo capaz de degradar compuestos de MO recalcitrante (Paula et al., 2020), favoreciendo la presencia de nutrientes lábiles para las plantas y otros microorganismos lo que permite procesos de sucesión microbiana (Kabel et al., 2020; Paterson et al., 2008). Del mismo modo, los microorganismos también pueden clasificarse según su velocidad de crecimiento. Los microbios que presentan un crecimiento rápido tras el aporte de materia orgánica son llamados copiotróficos, mientras que los organismos oligotrofos o de lento crecimiento más lento son capaces de vivir en ambientes con bajos niveles de nutrientes (Fierer et al., 2007; Semenov, 1991; Zelenev et al., 2005). Por estos motivos, las comunidades microbianas se consideran mejores indicadores de la calidad del suelo que las propiedades físico-químicas (Deng et al., 2020; Van der Heijden et al., 2008).

Los microorganismos pueden encontrarse en cualquier parte del suelo, aunque es junto a las raíces de las plantas donde se concentran la mayoría de ellos. La aporte de materia orgánica procedente de las raíces senescentes junto con los exudados radiculares (aminoácidos y azúcares) favorecen el desarrollo de las poblaciones microbianas (Ortega et al., 2017) además de establecer interacciones con el sistema radicular (Whipps, 2001). Las interacciones microorganismo-planta pueden clasificarse en tres categorías: neutral,

negativa o positiva (Whipps, 2001). La mayoría de los microorganismos no presentan efectos visibles sobre la fisiología de la planta estableciendo interacciones neutras, mientras que otros pueden afectar negativamente al crecimiento o a la fisiología de la planta, considerándose fitopatógenos (Beattie, 2006). Además de los organismos parásitos y causantes de enfermedades, también pueden producir sustancias fitotóxicas pudiendo inhibir el crecimiento vegetal (Martínez-Viveros et al., 2010). Aquellos que producen interacciones positivas se denominan promotores del crecimiento de las plantas (PGP), pudiendo incrementar el crecimiento de las plantas mediante la fijación de N_2, la solubilización de nutrientes, la promoción del desarrollo de micorrizas, la regulación de producción de etileno en raíces, liberación de fitohormonas, la producción de antibióticos, la exclusión de patógenos y/o la eliminación de sustancias fitotóxicas (Ali et al., 2022; Devi et al., 2020; Ortega et al., 2017; Pal and Mc Spadden Gardener, 2006). La mayoría de los organismos bacterianos caracterizados como PGP corresponden a los géneros *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* o *Rhizobium* (Antoun and Kloepper, 2001; Saharan and Nehra, 2011). También existen algunos hongos con propiedades PGP pertenecientes a los géneros *Verticillium*, *Trichoderma*, *Paecilomyces* o *Beauveria* (Devi et al., 2020).

Por tanto, la composición y diversidad de la comunidad microbiana es fundamental para la salud del suelo y el ciclado de nutrientes. La microbiota tiene la capacidad de desencadenar mecanismos de movilización de nutrientes que aumentan la concentración y disponibilidad de éstos para las plantas (Moreno-Lora et al., 2023), favoreciendo la cobertura vegetal y pudiendo evitar la degradación de los ecosistemas. Sin embargo, aún se conoce poco sobre la estructura de las comunidades microbianas en regiones áridas y semiáridas (Neilson et al., 2012).

7. Enmiendas orgánicas

La gestión sostenible del suelo recoge, como uno de sus principios básicos, que las actividades humanas realizadas en el presente no deben ser perjudiciales para las generaciones futuras (FAO, 2017). Sin embargo, los suelos degradados no recuperados amenazan la provisión de sus servicios ecosistémicos y, por tanto, el bienestar de las generaciones venideras (Larney and Angers, 2012).

En la actualidad, el aumento de la producción de residuos orgánicos urbanos e industriales supone un reto para la Economía Circular. Según el Ministerio para la

Transición Ecológica y el Reto Demográfico del Gobierno de España, la Economía Circular se define como "aquella en la que el valor de los productos, los materiales y los recursos se mantienen en la economía durante el mayor tiempo posible, y en la que se reduce al mínimo la generación de residuos" (MITECO, 2023). Siguiendo esta premisa, una solución ideal podría ser la reutilización de restos orgánicos como enmiendas orgánicas para la conservación de recursos naturales con el objetivo de poner en valor estos desechos, contribuyendo a la Estrategia Europea de Economía Circular (Hueso-González et al., 2018) y apoyando los esfuerzos de la Unión Europea (UE) para lograr una economía sostenible y eficiente en el uso de recursos (MITECO, 2023). Por tanto, se considera como enmienda orgánica cualquier material de origen vegetal o animal capaz de ser aplicado en el suelo con el fin de mejorar sus propiedades físicas, químicas y/o microbiológicas a la vez que incrementar su fertilidad (He and Zhang, 2014).

Las enmiendas se caracterizan por su alto contenido en nutrientes esenciales, pudiendo favorecer su biodisponibilidad en el suelo para el crecimiento vegetal y microbiano a corto, medio y largo plazo (Abbasi and Khizar, 2012; Bergkemper et al., 2016). Asimismo, la selección de una enmienda apropiada dependerá de la dosis aplicada, la tipología y composición del material aportado, las características del suelo receptor, las condiciones climáticas y las necesidades de la vegetación. Por ejemplo, en regiones mediterráneas, el uso de restos orgánicos frescos (sin tratamiento previo) podría llevar a condiciones no deseadas por la liberación excesiva de nutrientes o la aparición de fitotoxicidad (Huerta et al., 2011). Por tal razón, el compostaje podría ser una opción para la estabilización de estos residuos evitando las derivaciones de su aplicación en crudo. El proceso de compostaje se define como "una técnica mediante la cual se crean las condiciones necesarias para las que a partir de residuos orgánicos los organismos descomponedores fabriquen un abono de elevada calidad" (MARM, 2011). En consecuencia, el compost generado presenta una materia orgánica estabilizada que ha sido transformada por microorganismos en un producto combinado de compuestos similares a las sustancias húmicas del suelo, estando libre de patógenos y pudiendo ser almacenado sin ocasionar inconvenientes. Asimismo, debe estar exento de contaminantes o presentarlos en tasas muy bajas para que su aplicación no sea perjudicial, sino que sea beneficiosa para el suelo y el crecimiento vegetal. Como resultado, la capacidad de la MO aportada con la adición de enmiendas para perdurar en el tiempo en el suelo dependerá

de su estabilidad y, ésta a su vez, dependerá del tipo y el desarrollo del proceso de compostaje (Huerta et al., 2011).

Por consiguiente, la fuente orgánica de los residuos y la producción de las enmiendas le otorgarán una composición química variada (Antil et al., 2011), es decir, en función de la naturaleza del residuo (animal o vegetal) y del tiempo de compostaje, la enmienda orgánica resultante presentará diferentes proporciones de C lábil y resiliente, así como diferentes concentraciones de otros nutrientes (N, P, K, etc.). Las enmiendas orgánicas procedentes de residuos animales como los lodos de depuradora o los estiércoles presentan mayor contenido en MO lábil (Antil et al., 2011), compuesta por carbohidratos, proteínas y lípidos libres así como por N y P (Almendros et al., 2000, 1990; Shou et al., 2019), pudiendo conllevar un consume rápido de éstos nutrientes. Mientras que, los residuos vegetales o los residuos sólidos urbanos compostados son ricos en MO resiliente más difícilmente degradables como ligninas o polímeros húmicos (Argyropoulos and Menachem, 1997; Stevenson, 1994) y en N, K o P (Huerta et al., 2011). Además del aporte de materiales orgánicos, los parámetros ambientales de clima, temperatura y humedad, entre otros, determinarán la disponibilidad de nutrientes (C, N y P) aportados en los suelos (Bastida et al., 2017; González-Ubierna et al., 2012; Ros et al., 2003). Por lo que otra posibilidad de aplicación podría ser la combinación de compost de diferentes naturalezas. La mezcla de dos tipos de compost podría favorecer una composición química equilibrada entre MO lábil y resiliente pudiendo garantizar que la materia orgánica aportada no sea consumida a corto plazo permitiendo una actividad microbiana estable a lo largo del tiempo.

En las regiones semiáridas, el descenso del contenido de materia orgánica de los suelos como consecuencia de la degradación supone un grave problema para su fertilidad (Diacono and Montemurro, 2011). Por ende, el aprovechamiento de enmiendas orgánicas o residuos orgánicos compostados para la recuperación de suelos degradados podría ser una buena estrategia para mejorar la calidad y fertilidad de estos suelos a la vez que se favorece la Economía Circular. Por ello, el uso de enmiendas orgánicas apoyaría el desarrollo de actividades secundarias generando, de forma simultánea, un nuevo entorno económico para empresas que composten estos residuos a la vez de poner en valor estos desechos orgánicos otorgándoles una segunda vida. Por lo que el empleo de estos residuos orgánicos compostados podría ser una estrategia para reducir la acumulación de residuos

aumentando el contenido de MO a la vez que se mejoran las propiedades físicas, químicas y biológicas en suelos degradados.

8. Restauración con enmiendas orgánicas en clima semiárido

La aplicación de enmiendas orgánicas mejora las propiedades físicas, químicas y biológicas del suelo, favoreciendo la transformación de la materia orgánica y la estabilización vegetal (Bukar et al., 2019; Luna et al., 2016b). Asimismo, la adición de estos residuos orgánicos compostados benefician la estructura física del suelo, dado que reduce los procesos de degradación como la escorrentía o la erosión, así como la fertilidad del suelo mejorando la biomasa y actividad microbiana (Saison et al., 2006). Sin embargo, el microbioma del suelo responde rápidamente a los cambios (Ros et al., 2003), siendo alterado por los procesos de degradación, las prácticas antropogénicas (Luna et al., 2016b; Rodríguez-Berbel et al., 2022b) y por los procesos de restauración (Bastida et al., 2007a, 2017; Rodríguez-Berbel et al., 2020).

El funcionamiento heterogéneo de las tierras secas junto con las limitaciones físicas y químicas de las propiedades del suelo y las características climáticas, hacen particularmente compleja llevar a cabo una restauración ecológica exitosa en ecosistemas degradados por actividades humanas usando enmiendas orgánicas (Costantini et al., 2016). Los tratamientos de restauración basados en la aplicación de enmiendas orgánicas podrían influir en la composición de las comunidades microbianas del suelo a través de su efecto sobre las propiedades físico-químicas (Bukar et al., 2019; Lauber et al., 2009; Rodríguez-Berbel et al., 2020, 2023), dependiendo también de la composición de materia orgánica añadida a los suelos (Rodríguez-Berbel et al., 2022b; Ye et al., 2019). Tras la aplicación de enmiendas orgánicas, el ciclo natural del C junto con la transformación de las sustancias orgánicas añadidas provoca un aumento de la emisión de CO_2 en el suelo conocido como “priming effect” (PE) o efecto cebado (Kuzyakov, 2010). Durante el efecto cebado se produce un aumento de la actividad microbiana para degradar los compuestos orgánicos lábiles y recalcitrantes adicionados teniendo un impacto directo sobre la emisión de CO_2 (Blagodatskaya and Kuzyakov, 2008). Además, en función de la procedencia orgánica de las enmiendas orgánicas tendrán una composición química diferente, pudiendo tener un efecto directo sobre las emisiones de CO_2 (Blagodatskaya et al., 2014), siendo de mayor magnitud tras la aplicación de enmiendas ricas en C lábil (Soria et al., 2021b). Sin embargo, la dinámica del carbono y la actividad microbiana de

suelo también se ven afectadas por factores abióticos como el pH o la conductividad eléctrica (Blagodatskaya and Kuzyakov, 2008).

Por las razones antes mencionadas, es necesario profundizar en los efectos de la aplicación de enmiendas orgánicas sobre la proliferación y actividad de las comunidades microbianas en suelos degradados. Dado que pese a la importancia de la diversidad microbiana en la multifuncionalidad del suelo, aún existen pocos conocimientos sobre la dinámica a corto, medio y largo plazo de las comunidades microbianas en restauraciones de suelos degradados por actividades humanas bajo condiciones semiáridas.

Objetivos

El objetivo marcado en esta Tesis Doctoral es estudiar la respuesta de la microbiota edáfica a la restauración de suelos degradados por actividades agrícolas y mineras empleando diferentes enmiendas orgánicas. Para llevar a cabo este propósito, se establecieron los siguientes objetivos específicos:

- Ensayar distintas enmiendas procedentes de diferentes residuos orgánicos compostados como tratamientos de restauración con el objetivo de beneficiar la evolución de la materia orgánica del suelo además de acelerar su recuperación.

- Conocer la relación entre los procesos físico-químicos, bioquímicos y microbianos que intervienen en el suelo y en la vegetación a corto-medio plazo, así como en los suelos naturales del entorno.

- Monitorizar la evolución de la estructura de las comunidades microbianas (bacterias y hongos) a corto, medio y largo plazo de los suelos restaurados tras la aplicación de las enmiendas orgánicas.

- Estudiar las comunidades microbianas implicadas en procesos de biodegradación y/o humificación de la materia orgánica del suelo.

- Dilucidar las comunidades bacterianas y fúngicas de los suelos que podrían participar en el desarrollo vegetal como promotoras del crecimiento vegetal.

- Determinar el tratamiento más beneficioso para la recuperación de las propiedades físico-químicas, bioquímicas y microbianas de los suelos restaurados hacia el estado natural de referencia.

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Resumen:

Se evaluó la restauración de suelos agrícolas abandonados improductivos bajo un clima semiárido en el sureste de España. Se aplicaron cuatro enmiendas orgánicas procedentes de diferentes residuos compostados (residuos de cultivos de invernadero; vermicompost de estiércol de oveja-vaca y restos vegetales; gallinaza; y estiércol de oveja-pollo y restos vegetales), se instalaron parcelas control sin tratar y se seleccionaron suelos naturales no explotados como ecosistema de referencia. A lo largo de tres campañas de muestreo (inicial, 3 meses y 12 meses), se observaron cambios significativos en las propiedades fisicoquímicas del suelo, el contenido de nutrientes (carbono -C-, nitrógeno -N- y fósforo -P-) y la composición bacteriana de los suelos restaurados con respecto a los suelos de control y naturales, especialmente a los 3 meses. El aumento de nutrientes lábiles provocó la proliferación de filos copiotróficos a los 3 meses, que, tras su consumo, fueron sustituidos por filos oligotróficos a los 12 meses. Se identificaron taxones específicos implicados en los ciclos de C, N y P para cada suelo. En cuanto a la composición bacteriana del suelo de vermicompost, sólo los estiércoles de gallina y oveja-gallina fueron más disímiles a los suelos control y natural después de 12 meses, mientras que el compost de cultivos de invernadero mostró una posición intermedia entre ambos. Los resultados indicaron que los restos de cultivos de invernadero compostados podrían ser un tratamiento óptimo para la recuperación a corto plazo de las propiedades fisicoquímicas, el contenido en nutrientes y la composición bacteriana de suelos degradados por la agricultura en zonas semiáridas.

Palabras clave: Restauración ecológica; Carbono; Nitrógeno; Fósforo; Análisis metagenómico; Composición de la comunidad bacteriana.

Material suplementario disponible en ANEXO I y en https://www.mdpi.com/article/10.3390/agronomy13010086/s1

Short-Term Dynamics of Bacterial Community Structure in Restored Abandoned Agricultural Soils under Semi-arid Conditions

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Abstract

The restoration of unproductive abandoned agricultural soils under a semi-arid climate in southeastern Spain was evaluated. Four organic amendments from different composted wastes (greenhouse crop residues; worm compost from sheep–cow manure and plant remains; chicken manure; and sheep–chicken manure and plant remain) were applied, untreated control plots were installed, and natural unexploited soils were selected as a reference ecosystem. Through three sampling campaigns (initial, 3 months, and 12 months), significant changes were observed in soil physico-chemical properties, nutrient content (carbon -C-, nitrogen -N-, and phosphorus -P-), and bacterial composition of the restored soils with respect to control and natural soil, especially at 3 months. The increase of labile nutrients caused the proliferation of copiotrophic phyla at 3 months, which, after their consumption, were replaced by oligotrophic phyla at 12 months. Specific taxa involved in C, N, and P cycles were identified for each soil. For the soil bacterial composition of vermicompost, only chicken and sheep–chicken manures were more dissimilar to control and natural soils after 12 months, while greenhouse crop compost showed an intermediate position between them. Results indicated that composted greenhouse crop remains could be an optimal treatment for the short-term recovery of physico-chemical properties, nutrient content, and bacterial composition of agriculture-degraded soils in semi-arid areas.

Keywords: ecological restoration; carbon; nitrogen; phosphorus; metagenomic analysis; bacterial community composition

1. Introduction

The exploitation of fertile soils for agricultural activities generates degraded soils susceptible to abandonment due to lack of productivity. These soils tend to lose their capacity to regulate the ecosystem, which, together with functional deterioration, leads to serious biodiversity declines (Stanturf, 2021). In arid and semi-arid areas, the particular climatic factors, such as low rainfall and weather irregularity (Cano Navarro et al., 2017) as well as the low organic matter content of soils (Bastida et al., 2013b; García et al., 1994), aggravate this problem, making natural restoration processes more difficult (Lasanta et al., 2019). In agroecosystems, a crucial determinant of soil quality is the organic matter content, given that it is intimately linked to biological activity, nutrient cycling, and crop productivity (Li et al., 2022). For these reasons, to successfully recover

these soils, restoration techniques must be employed that are oriented not only to repairing the physical and chemical soil properties, but also to re-establishing the biological communities (Cano Navarro et al., 2017), as there is increasing evidence of the fundamental role played by the microbial composition in soil fertility (Gao et al., 2020). Therefore, the application of composted organic wastes could be a good strategy to avoid the abandonment of non-productive land under a semi-arid climate, because their high organic matter content (Calleja-Cervantes et al., 2015) favors soil fertility (Wijesekara et al., 2016) and could accelerate the recovery of these degraded soils, in addition to contributing to the European Circular Economy Strategy through the reuse of organic waste produced by local industrial activities in the restoration of semi-arid degraded soils. The addition of organic amendments benefits the physical, chemical, and biological soil properties (Bastida et al., 2017; Rodríguez-Berbel et al., 2021, 2020; Soria et al., 2022), favoring the supply of nutrients and improving soil functionality (Li et al., 2021; Rodríguez-Berbel et al., 2021b; Soria et al., 2022; Tejada et al., 2006). The organic compounds that make up these composted residues depend on their organic provenance and are directly related to their nutrient content (C, N, and P) (Wang et al., 2022), causing stoichiometric variations in the C:N:P ratio (Xiao et al., 2015) of the amended soils.

Previous studies have suggested significant differences in the structure of soil bacterial communities after soil restoration, which together with soil characteristics determine the bacterial structure of the soil (Bastida et al., 2017, 2008; Rodríguez-Berbel et al., 2020). Thus, soil bacterial composition is explained not only by edaphic variables (Rodríguez-Berbel et al., 2021; Xue et al., 2020) but also by the nutrients provided from the different origins of the organic amendments used (Li et al., 2017; Singh and Prabha, 2019). As a consequence, the behavior of the soil microbiome responds to the availability of nutrients (Singh and Prabha, 2019), and these changes will alter the C, N, and P cycles affecting the biological and physico-chemical reactions carried out by soil micro-organisms (Neemisha and Sharma, 2022). Consequently, the type of organic matter (Li et al., 2017) together with the capacity and efficiency of the micro-organisms in the decomposition of the organic matter provided (Wang et al., 2022) could have a differential effect on the structure of the soil bacterial community, influencing the proliferation of some taxa versus others better adapted to the applied amendment (Bastida et al., 2008). Therefore, bacterial succession induced by substrate availability could have possible beneficial effects on the functionality and stability of the agroecosystem

(Francioli et al., 2016). However, there are few studies that support short-term monitoring of restorations with the organic amendment of semi-arid abandoned agricultural soils. Likewise, the differential effect of the type of organic amendment applied on the physico-chemical properties and nutrient content of the soil as well as their impact on soil bacterial communities at the genus level have not yet been explored.

Therefore, three sampling campaigns were carried out at the beginning, 3 and 12 months of the restoration of an abandoned agricultural soil using different organic amendments with the main objective of studying the changes in soil physico-chemical properties and nutrient content, and their impact on soil bacterial composition. The working hypothesis was that the application of organic composted residues will improve soil quality and fertility by enhancing the physico-chemical properties and nutrient content of the soil, and consequently, altering the structure of the soil bacterial community involved in C, N, and P cycling. This research should result in a better understanding of the changes produced after the application of different organic amendments, facilitating their appropriate selection for the recovery of the bacterial community in abandoned agricultural soils under a semi-arid climate.

2. Materials and Methods

2.1. Site Details and Field Experimental Design

An old dry farming area located in the Sierra de Gádor range (Almería, SE Spain) was selected to conduct the restoration task. The experimental site was located in abandoned cultivation terraces without slope, with soils developed on limestones, dolomite, and phyllites rocks classified as Leptosols (Peinado and Sierra, 2004). Grapes, almonds, and cereals were grown on these soils, being abandoned due to lack of productivity more than 50 years ago. It is located at an altitude of 977 m. a. s. l. (36°53′20″ N, 02°36′14″ W) and at approximately 10 linear km from the sea. The climate of the area corresponds to the semi-arid Mediterranean climate (with rainfall of 398.50 mm yr^{-1} over the last 24 years (Junta de Andalucía, 2021); temperatures of 17 °C yr^{-1} with long dry periods and high solar radiation). Through randomly distributed linear transects, a vegetation inventory was carried out in the natural areas that have never undergone agricultural exploitation. The vegetation was mainly composed of *Retama sphaerocarpa* (L.) Boiss., *Anthyllis cytisoides* L., *Stipa tenacissima* L., *Thymus zygis* L., *Cistus albidus* L., and some spare specimens of *Pinus halepensis* Mill., among others.

The restoration work was carried out in the first week of October 2020. Initially, soil decompaction was performed, and subsequently, 15 experimental plots of 35 m² (7 m × 5 m) were designed, 3 replicates per treatment, and 3 untreated control plots (CON). A previous selection of amendments from different types of organic wastes was carried out in order to determine if the composition of nutrients derived from the source of origin affect in a dissimilar way the recovery of semi-arid restored soils. A compost of different plant remains was used as resilient organic matter (Rodríguez-Berbel et al., 2021; Soria et al., 2022). Animal manure rich in labile organic matter, such as chicken manure, was used to provide organic matter rich in N and P (Wongkiew et al., 2021). A vermicompost from the bio-oxidative digestion of some species of earthworms and their digestive microbiota was chosen because of its benefits in promoting plant growth and against pathogens (Munoz-Ucros et al., 2020) as well as for the contribution of diverse organic matter rich in N, P, and micronutrients (Naseer et al., 2019). Lastly, an amendment consisting of a mixture of animal manure and plant debris was chosen in order to obtain a balanced composition between labile and resilient organic matter. Finally, four types of composted organic amendments were added, mixed, and homogenized to a depth of 20 cm using a rotary tiller. The composted amendments consisted of both plant and animal wastes: (i) compost from fruit and vegetable greenhouse crop residues (GC); (ii) organic compost derived from floor-raised chicken manure on ecological farms (CM); (iii) vermicompost from sheep and cow manures with vegetable remains (VC); and (iv) certified organic compost derived from sheep manure, chicken manure, and vegetable waste from grass and straw (SC). A single dose was used to increase the initial organic matter content by 1.5% above the degraded soil without amendment. To contribute to soil restoration, four species of native aromatic plants, such as *Rosmarinus officinalis* L. Schleid., *Lavandula latifolia* Medik., *Thymus zygis* L., and *Anthyllis cytisoides* L., were selected for their ability to adapt to climatic conditions and for their biotechnological potential in the pharmaceutical and cosmetic industry (Gupta et al., 2021). In addition, the suitability of the introduced plants was consulted with the flora technicians of the region and contrasted with the Forest Restoration Models of the Andalusian Government (Costa Pérez and Valle Tendero, 2004). Subsequently, 15 plants of each aromatic species were planted in each experimental plot (60 plants per plot) using a planting frame of 60 cm × 60 cm. In addition, they were planted adjacent to natural vegetation that has never been submitted to agricultural activities, and preserved natural vegetation soils were selected as reference ecosystem (NAT) (Miralles et al., 2009). One week after completion

of the restoration (19 October 2020; t1), three months (29 January 2021; t3), and one year later (22 October 2021; t12), soil samples composed of 10 random subsamples were collected from each experimental plot at a depth of 0–10 cm. The samples were transported to the laboratory cold, and DNA extraction analyses were immediately performed. Then, soil samples were air-dried, sieved (Ø 2 mm) and stored at 4 °C for physico-chemical property and nutrient content (C, N, and P) analyses.

2.2. Soil Physico-Chemical Properties Measurements

The pH and electrical conductivity (EC) were determined in a distilled water solution (1:2.5 and 1:5 w/v, respectively) measured with a pH meter (LAQUA PH1100, Horiba, Tokyo, Japan) and conductivity meter (LAQUA EC1100, Horiba, Tokyo, Japan). Total organic carbon (TOC) was determined by colorimetric method described by Mingorance et al. (Mingorance et al., 2007) and measured with a spectrophotometer, Spectronic Helios Gamma UV-Vis (Thermo Fisher Scientific, Waltham, MA, USA), and total nitrogen (TN) with an elemental analyser TCD detector (ELEMENTAR Rapid N; Elemental Analysen systems GmbH, Hanau, Germany). Available phosphorus (AP) was determined by Olsen and Watanabe (1957) method (Olsen and Watanabe, 1957). The results of TOC, TN, and AP contents were used to calculate the carbon–nitrogen (C:N), carbon–phosphorus (C:P), and N:P ratios (Boudjabi and Chenchouni, 2022). Finally, available water (AW) was calculated from the pF values at −33 and −1500 KPa using the Richards membrane method (Richards, 1941).

2.3. Bacterial Community Composition and Sequencing Data Processing

Total soil DNA was extracted from each sample (n = 18) using the commercial DNA Power Soil kit (QIAGEN, Hilden, Germany) and quantified by ND-2000 Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA), owned by the “Nucleic Acid Analysis Service” located at the “Central Research Services” of the University of Almería. The V4–V5 regions of the bacterial 16S rRNA were amplified by polymerase chain reaction (PCR) and sequenced by Illumina MiSeq platform. Then, QIIME2 software (version 2019.7) was used for sequence processing following the protocol established by Comeau et al. (Comeau et al., 2017) to identify the soil bacterial community at phylum level and genus level or the next upper taxonomic level identified in the different soils analyzed at each sampling time (t1, t3, and t12). Soil bacterial taxa with a relative abundance ≥ 0.1% in all samples were selected.

2.4. Statistical Analysis

Measurements of selected physico-chemical and bacterial soil properties analyzed were performed in triplicate for each treatment. A permuted multivariate and univariate analysis of variance (PERMANOVA) was used to determine the differences as a function of treatment and elapsed time for chemical properties and nutrient stoichiometry (Euclidean distance) as well as for bacterial community structure (Bray–Curtis) in restored, natural, and control soils (Anderson, 2017), which included two factors: soil treatment and time of field sampling. In cases where PERMANOVA detected a significant effect ($p < 0.05$), the origin of differences was assessed by comparing treatment pairs with post PERMANOVA (Monte Carlo) test pairs, and results with $p < 0.05$ were reported as significant. Differences in physico-chemical soil properties and stoichiometric relationships between treatments were evaluated by principal component analysis (PCA). A previous distance-based linear modelling analysis (DistLM) was performed on genus-level relative abundance data of bacterial taxa and abiotic variables (physico-chemical properties and nutrient stoichiometry) of the soil to determine the relative importance in the interrelationships between soil bacterial composition and edaphic conditions in the different sampling campaigns (t1, t3, and t12). Then, a distance-based redundancy analysis (dbRDA) was employed on bacterial taxa data to build a regression model against the abiotic variables. The Akaike Information Criterion (AICc) was employed for selecting the best model, and the stepwise approach for building the model was followed (H Akaike, 1974). PERMANOVA, PCA, DistLM, and dbRDA statistical analysis were performed with PRIMER-E + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, United Kingdom). To identify those bacterial taxa at the genus or the next upper taxonomic level identified that were specifically associated with each treatment (restored, control, and natural reference soil), an indicator species analysis (ISA) was conducted using the IndicSpecies R package, using the *multipatt* function (9999 perm) (De Caceres and Jansen, 2016). Pearson correlations ($p < 0.05$) were calculated using *corrplot* and *RColorBrewer* R packages in order to evaluate the interrelationships between the bacterial taxa identified by species-index analysis and soil physico-chemical properties and stoichiometric components.

3. Results

3.1. Changes in Soil Physico-chemical Properties and Stoichiometry Nutrients over Time

The application of organic amendments progressively changed the physico-chemical soil properties (EC, pH, and AW), increased nutrient content (TOC, TN, and AP), and modified the stoichiometry of nutrients (C:N, C:P, and N:P). The multivariate PERMANOVA analysis applied to soil properties and nutrients stoichiometry showed that the treatments were significantly different ($p < 0.05$) for both time and treatment factors, but not in the interaction between them (Table S1a). All treated soils showed a significantly higher ($p < 0.05$) EC than CON and NAT in all soil sampling campaigns, being significantly higher at t1 just after amendment application (Table 1). The treatments significantly decreased the pH with respect to CON soils, with SC and CM having significantly lower values ($p < 0.05$) at t1, while the pH of GC and VC was similar to CON and NAT (Table 1). t3 and t12 had similar trends, except for CM showing the lowest significant pH values at t12. Restored and control (CON) soils showed significantly lower water availability (AW) than in the reference soils (NAT) just after the start of the experiment (t1); however, this became similar over time (Table 1). All restored soils had significantly higher TOC, TN, and AP content than CON, and were similar to NAT in all sampling campaigns, except for AP, which also showed significantly higher values ($p < 0.05$) than NAT during all sampling campaigns (Table 1). The TOC values were maintained in VC and GC soils in all sampling times, while there was a significant decrease in CM over time and a slight increase in SC at t12 (Table 1). VC and CM presented significantly higher TN content, followed by SC, and then, lastly, GC. This trend was maintained in the restored soil during all sampling campaigns. On the other hand, VC, SC, and CM had the significantly highest AP values and GC the smallest in all periods of study, but significantly lower values were presented at t1, while its content increased at t3 and t12 (Table 1). Regarding the stoichiometric nutrient ratios, all restored soils showed a C:N ratio significantly superior to CON and similar to NAT, and so, the results at t1, t3, and t12 experienced a comparative decline over time (Table 1). NAT and CON presented high C:P values together with GC, being significantly lower in VC, SC, and CM, which decreased further through time. This trend was clearest at t1, but GC had C:P values similar to CON and below NAT at t3 and t12 (Table 1).

Table 1. *Soil physico-chemical properties and main nutrient ratios of newly reclaimed abandoned agricultural soils with* incorporation *of different organic amendments.*

		GC	VC	SC	CM	CON	NAT
t1	*EC (mS cm^{-1})*	0.98 ± 0.01 aA	0.63 ± 0.8 bA	0.93 ± 0.09 abA	1.02 ± 0.11 aA	0.10 ± 0.00 cA	0.09 ± 0.00 cA
	pH	8.13 ± 0.04 abA	8.25 ± 0.01 acA	8.07 ± 0.02 bA	8.01 ± 0.01 bA	8.44 ± 0.08 cAB	8.31 ± 0.07 acA
	TOC (%)	2.55 ± 0.24 aA	4.01 ± 0.55 aA	2.81 ± 0.30 aAB	2.80 ± 0.13 aA	1.78 ± 0.12 bA	4.25 ± 0.59 aA
	TN (%)	0.29 ± 0.02 aA	0.39 ± 0.02 bA	0.367 ± 0.04 abA	0.370 ± 0.01 bA	0.210 ± 0.01 cA	0.393 ± 0.04 abA
	AP (%)	0.004 ± 0.00 aA	0.028 ± 0.0 b0A	0.019 ± 0.00 bA	0.023 ± 0.00 bA	0.002 ± 0.00 aA	0.004 ± 0.00 aA
	C:N	8.63 ± 0.45 aA	10.25 ± 0.86 bA	7.67 ± 0.25 abA	7.56 ± 0.19 bA	8.52 ± 0.20 cA	10.78 ± 0.48 abA
	C:P	581.75 ± 148.35 aA	141.75 ± 5.77 bA	141.84 ± 10.34 bA	120.35 ± 8.87 bA	730.80 ± 132.85 aA	1022.07 ± 100.00 aA
	N:P	66.15 ± 13.31 aA	13.95 ± 0.82 bA	18.54 ± 1.65 bA	15.87 ± 0.79 bA	86.57 ± 17.95 aA	95.69 ± 12.89 aA
	AW (%)	9.66 ± 0.26 aA	9.19 ± 0.39 aA	9.52 ± 0.24 aA	9.47 ± 0.30 aA	9.12 ± 0.37 aA	13.02 ± 1.01 bA
t3	*EC (mS cm^{-1})*	0.20 ± 0.01 aAB	0.24± 0.01 abB	0.43 ± 0.06 bB	0.72 ± 0.16 bA	0.09 ± 0.01 cA	0.07 ± 0.01 cA
	pH	8.38 ± 0.14 abcA	8.35 ± 0.05 aA	8.04 ± 0.04 bA	8.05 ± 0.08 bcB	8.34 ± 0.07 acB	8.03 ± 0.05 bB
	TOC (%)	2.17 ± 0.23 abA	3.20 ± 0.13 cA	2.48 ± 0.04 aA	2.74 ± 0.27 acAB	1.64 ± 0.18 bAB	3.44 ± 0.14 cA
	TN (%)	0.27 ± 0.02 aA	0.35 ± 0.00 bA	0.34 ± 0.01 bA	0.32 ± 0.04 abA	0.14 ± 0.01 cA	0.33 ± 0.01 abA
	AP (%)	0.015 ± 0.00 aB	0.12 ± 0.00 bB	0.05 ± 0.00 cB	0.07 ± 0.02 abcB	0.007 ± 0.00 dA	0.004 ± 0.00 dA
	C:N	8.12 ± 0.43 aA	9.15 ± 0.23 bA	7.29 ± 0.38 bA	8.68 ± 0.54 abA	8.84 ± 0.43 cA	10.36 ± 0.03 abA
	C:P	139.91 ± 3.24 aB	26.58 ± 0.96 bB	54.05 ± 7.14 cB	42.63 ± 9.13 bc B	246.07 ± 40.96 aB	840.35 ± 184.24 dA
	N:P	17.3 ± 1.04 aB	2.90 ± 0.16 bB	7.35 ± 0.58 cB	4.90 ± 1.03 bcB	28.43 ± 6.31 aB	81.18 ± 18.03 dA
	AW (%)	10.70 ± 0.36 aA	8.42 ± 2.13 abA	9.65 ± 0.41 aA	10.08 ± 0.27 aA	10.43 ± 1.68 abA	13.12 ± 0.24 bA
t12	*EC (mS cm^{-1})*	0.32 ± 0.06 aB	0.45 ± 0.08 abA	0.47 ± 0.04 aB	1.30 ± 0.30 bA	0.10 ± 0.01 cA	0.08 ± 0.00 cA
	pH	8.76 ± 0.16 aB	8.55 ± 0.14 abB	8.11 ± 0.00 cA	7.67 ± 0.11 dC	8.54 ± 0.01 aA	8.19 ± 0.02 bAB
	TOC (%)	2.61 ± 0.22 abA	3.47 ± 0.27 aA	2.86 ± 0.05 aB	2.23 ± 0.12 bB	1.31 ± 0.05 cB	3.03 ± 0.03 aA
	TN (%)	0.27 ± 0.01 aA	0.39 ± 0.01 bA	0.34 ± 0.01 bA	0.33 ± 0.04 abA	0.16 ± 0.00 cA	0.28 ± 0.00 aA
	AP (%)	0.01 ± 0.00 aB	0.158 ± 0.02 bB	0.055 ± 0.00 cB	0.141 ± 0.02 bB	0.003 ± 0.00 dA	0.004 ± 0.00 dA
	C:N	9.51 ± 0.29 aA	8.70 ± 0.36 bA	8.42 ± 0.27 bA	6.86 ± 0.66 abA	8.14 ± 0.26 cA	10.7 ± 0.37 aA
	C:P	214.96 ± 41.2 aAB	23.22 ± 4.40 bB	51.78 ± 4.08 cB	16.42 ± 2.43 bC	457.89 ± 141.61 adAB	728.85 ± 133.25 dA
	N:P	22.36 ± 3.75 aB	2.63 ± 0.42 bB	6.13 ± 0.29 cB	2.48 ± 0.52 bB	55.19 ± 15.86 adAB	67.34 ± 11.16 dA
	AW (%)	10.63 ± 1.59 abA	8.91 ± 1.33 abA	8.42 ± 1.23 abAB	7.37 ± 0.67 aB	8.49 ± 1.17 abA	11.02 ± 0.07 bA

EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio; AW: available plant water. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months. The lowercase letter indicates significant differences ($p < 0.05$) between treatments at the same sampling time. The uppercase letter indicates significant differences ($p < 0.05$) of the same treatment between different sampling times (t1, t3, and t12).

These results were confirmed by the PCA performed shown in Figure 1. The first two component axes accounted for 40.7% and 25.7% of the total variance of the soil properties and stoichiometry of nutrients, explaining a total of 66.4% (Figure 1). The samples for soil physico-chemical properties and nutrients stoichiometry were approximately grouped, where the distance between samples represented the extent of the differences. PC1 clearly differentiated soils treated with compost derived from animal waste (CM, SC) and vermicompost (VC). On the opposite side (negative PC1 values), NAT and CON soils were clearly located, while vegetable composts (GC) were in an intermediate position between the two previous clusters (Figure 1a). On the other hand, PC2 separated the control soils in its positive values, and practically all the samples of the GC treatment were separated from the rest of the treatments and NAT soils, which were mainly located in the negative values of PC2 (Figure 1a). Therefore, the groups emphasized that, in the short term (Figure 1b), an improvement of soil quality is related to the treatment, as they were positioned closer to NAT soils. In addition, the arrow lines of AP, TN, TOC, and EC influenced mainly SC, CM, and VC, while the AW and stoichiometric ratios (C:N, C;P, and N:P) were declined for NAT, and the pH for CON soils (Figure 1; Table S2).

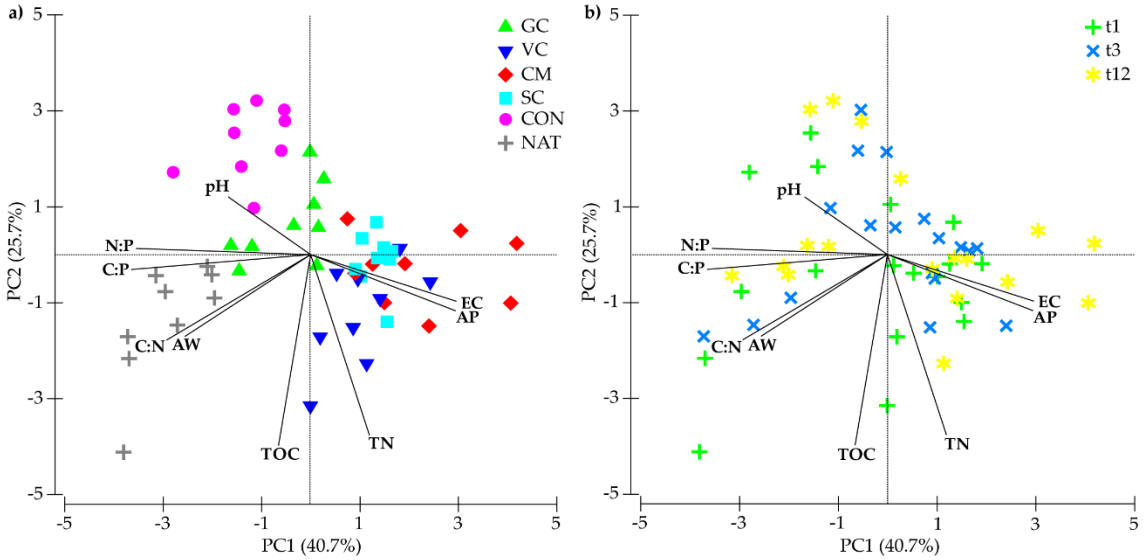

Figure 1. *Principal component analysis (PCA) of soil physico-chemical properties and stoichiometry of nutrients (C, N, and P) between treatments (a) and between soil sampling campaign (b) EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio; AW: available plant water. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months.*

3.2. Bacterial Community Composition

The composition of the bacterial soil community at the phylum level varied significantly ($p < 0.05$) between the different treatments (GC, VC, SC, CM, CON, and NAT) and campaigns (t1, t3. and t12; Table S1b). Proteobacteria (31.9%), Bacteroidetes (21.8%), Planctomycetes (13%), Actinobacteria (10.6%), Acidobacteria (10.1%) and Firmicutes (4.1%) were the most abundant bacterial phyla in the soil samples (Figure S1). The phylum Proteobacteria was the most abundant in all treatments, being the most abundant at 3 months of the restoration (t3) in vegetable compost soils (GC; 37.9%), and t3 and t12 in SC soils (35.4% and 38.6%, respectively). Bacteroidetes phylum showed the highest abundance, mainly at t3 in soils treated with manures (SC = 47.5% and CM = 48.9%). GC and vermicompost (VC) treatments favored the presence of Planctomycetes and Acidobacteria (Figure S1). The abundance of Planctomycetes in GC and VC soils increased over time (18.1% and 16.2%, respectively), but without reaching the values recorded in CON (19.8%) and NAT soils (15%). The VC treatment mainly favored the phylum Chloroflexi (t1 = 5.4%; t3 = 2.9%; t12 = 6.1%) with respect to the rest of restored, CON (t12 = 3.7%), and NAT (3.5%) soils, while Actinobacteria decreased at t3 (6.4%) and increased at t12 (18.2%) in VC soils, approaching the values of the reference soils (NAT; 18.5%). The application of GC (23.9%), SC (12.4%), and CM (11.9%) promoted the presence of Firmicutes at t1 with respect to CON (2.2%) and NAT soils (1.3%; Figure S1). Finally, CON and NAT soils showed scarce changes in the relative abundance of over time.

At the genus level or the next upper taxonomic level identified, a total of 181 bacterial taxa were found. The bacterial soil membership at the genus level was significantly different ($p < 0.05$). both among the different treatments and among three monitoring campaigns performed (Table S1c).

3.3. Relationships among Soil Chemical Properties, Nutrient Stoichiometry, and Bacterial Communities

The DistLM analysis in combination with the best linear distance model (R^2 = 0.37; AICc = 345.14) explained that all abiotic soil variables studied (physico-chemical properties and nutrient stoichiometry) were key in the development of the soil bacterial community (Table S3). Moreover, dbRDA results revealed a distinct clustering of soil bacterial communities for soils restored with organic amendment, control, and natural reference soils (Figure 2 and Table S3). The soil bacterial taxa of the VC, CM, and SC

treatments were separated from NAT and CON soils (positive and negative dbRDA1 values, respectively; Figure 2a), while GC-treated soils were placed in an intermediate position between the two previous groups. Furthermore, the results of dbRDA1 in this study revealed that EC and PA exerted a significant influence on the bacterial composition of soils treated with animal-derived composts (VC, CM, and SC; positive dbRDA1 values). In contrast, for negative dbRDA1 values, samples collected at t1 and t3 from exclusively plant-derived compost (GC) and CON and NAT soils were influenced by pH, TN, TOC, and nutrient stoichiometry (C:N, C:P, and N:P; Figure 2b). Regarding the dbRDA2 axis, control and VC-treated soils had positive values influenced by AP, C:N, pH, and TN, while N:P, TOC, and AW were negatively influencing GC-treated soils, and SC and CM in campaign t1 (Figure 2a). Regarding the timing of the different campaigns, the soil bacterial community of the treatments at t1 showed a more similar behavior, clustering around the origin of the co-ordinates (Figure 2b). However, at t3 and t12, the bacterial composition was better differentiated among treatments, especially in soils treated with VC, CM, and SC (Figure 2b).

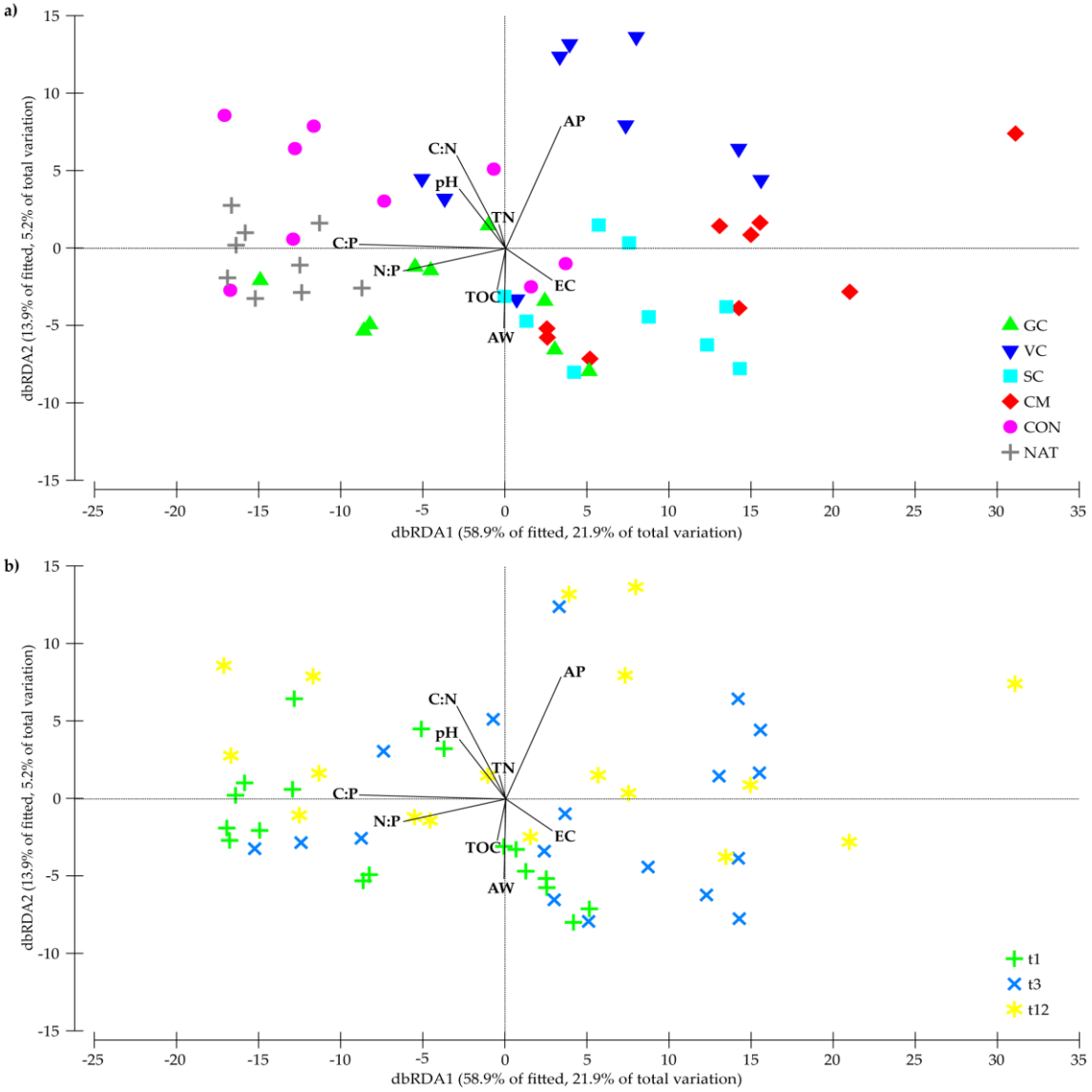

***Figure 2**. Distance-based redundancy analysis (dbRDA) of soil bacterial community and physico-chemical properties for restored, control, and natural reference soils (a), and for soil sampling campaign (b). EC: electrical conductivity; AW: available plant water; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months.*

3.4. Indicator Bacterial Communities in Different Restored, Control, and Reference Soils

Soil bacterial taxa sensitive to restoration strategies using organic amendments were identified through the analysis of indicator species for the total number of sampling campaigns over time (Figure 3). The results showed that, among the bacterial taxa identified, 53 were potential indicator species for soils treated with organic amendments employed together with CON and NAT soils (Table S4). Specifically, five indicator bacterial taxa belonged to CG treatment, seven to VC, 10 to CM treatment, and three to

SC treatment. Also, five bacterial taxa were obtained as indicator species from the control soils (CON), while the reference soils (NAT) showed 23 different taxa (Figure 3 and Table S4).

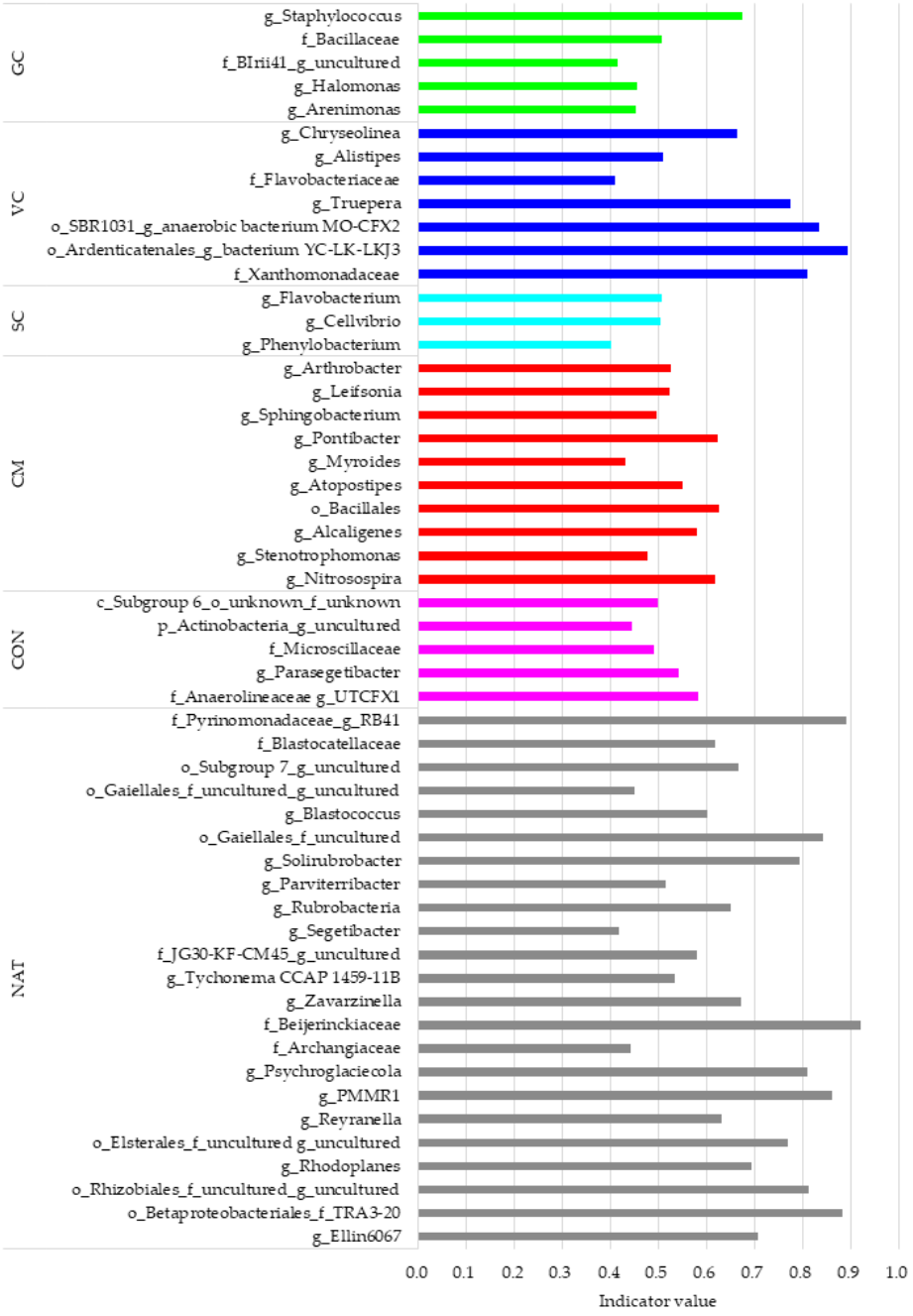

***Figure 3**. Soil bacterial taxa selected by indicator species analysis for different treatments (restored, control, and natural reference soils). GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil.*

3.5. Correlation Analysis between Bacterial Taxa from Indicspecies Analysis and Soil Parameters

Different correlation patterns were found among the 53 selected as treatment indicator physico-chemical properties (EC, pH, TOC, TN, AP, AW) and stoichiometric nutrient ratios (C:N, C:P, N:P) in the different sampling campaigns (t1, t3, and t12) (Figure 4). Overall, the highest number of significant correlations ($p < 0.05$) was found at time t3, followed by t12, while it was lower at t1. The bacterial taxa of the amended soils clearly differed from those belonging to the natural reference soils (NAT) in terms of their relationship with the soil properties studied (Figure 4). Specifically, in CM-treated soils, the bacterial groups identified with the highest significant positive correlations were related to EC for sampling times t3 and t12 (Figure 4). *Alcaligenes*, *Atopostipes*, and *Sphingobacterium* showed high significant positive correlations with EC. Other bacteria indicative of CM treatment in addition to EC also showed correlations with AP, especially at t12, such as *Arthrobacter*, *Nitrosospira*, *Pontibacter*, *Leifsonia*, and *Myroides*, the last two being also correlated with TOC at t1 (Figure 4). Similarly, in VC-treated soils, bacteria such as *Truepera*, *Alistipes*, *o_SBR1031_g_anaerobic bacterium MO-CFX2*, *o_Ardenticatenales_bacterium YC-LK-LKJ3*, and genera belonging to the Xanthomonadaceae and Flavobacteriaceae families had an intense positive significant correlation with AP during the whole study period. In addition, the last four also correlated with TOC at t1. Regarding CG and SC treatments, the trends were clear regarding the high number of negative correlations with stoichiometric ratios, coinciding in this aspect as VC and CM; however, GC showed that some bacterial taxa such as *Staphylococcus* had significant positive correlations with pH in t3. Other taxa, such as *UTCFX1* and c_Subgroup 6_o_unknown_f_unknown, followed the same trend for pH in CON soils (Figure 4). On the other hand, the bacterial taxa belonging to the NAT soils had an opposite affinity with the soil properties. The NAT soil indicator bacteria showing the highest significant correlations with the stoichiometric ratios C: N, C:P, and N:P (Figure 4), and which were also negatively correlated with EC and AP, included the genera *Blastococcus*, *RB41*, *Segetibacter*, *Tychonema CCAP 1459-11B*, *Psychroglaciecola*, *PMMR1*, *Reyranella*, *Rhodoplanes*, *Zavarzinella*, and *Ellin6067* (Figure 4).

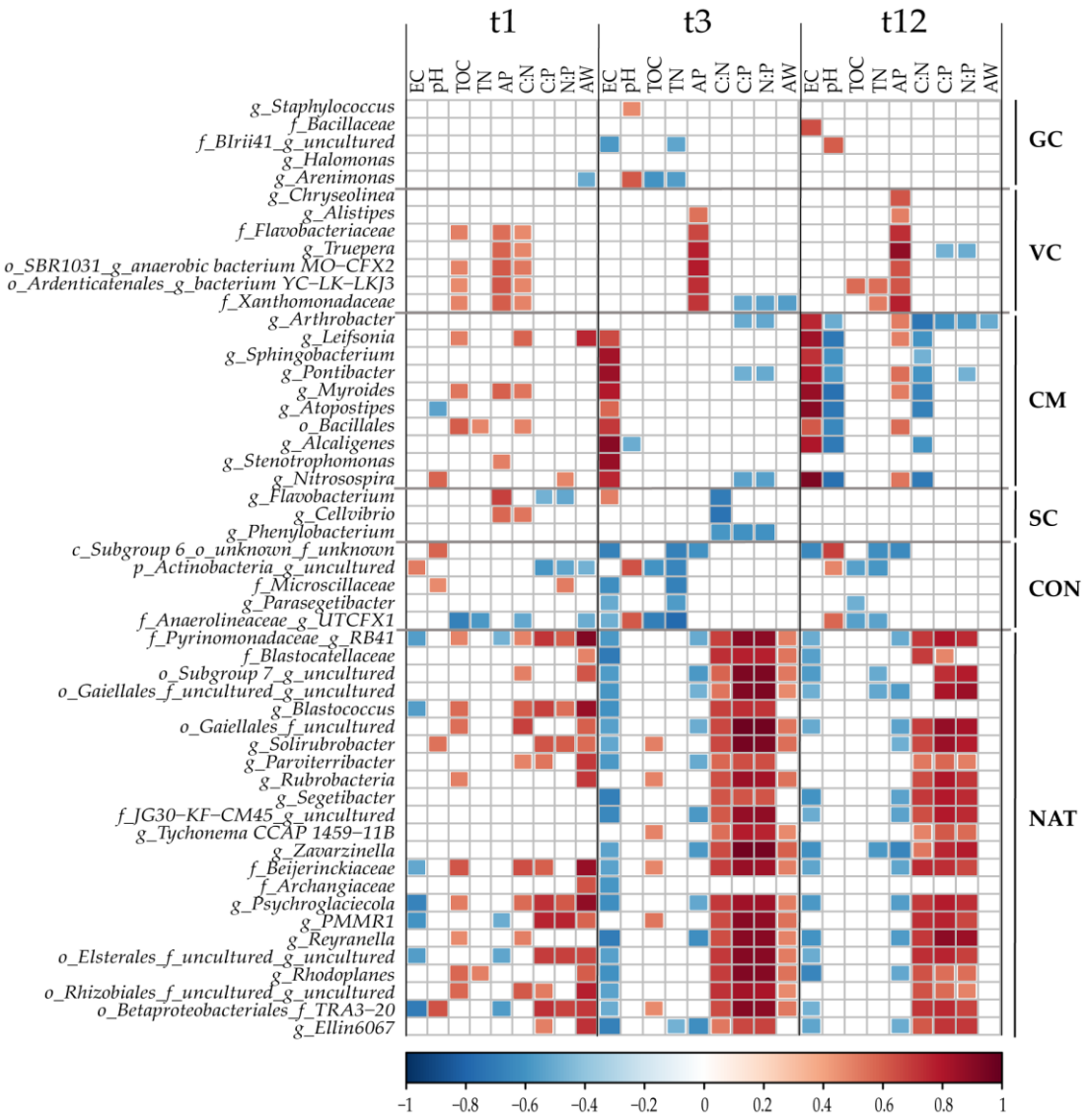

***Figure 4**. Significant Pearson correlation ($p < 0.05$) between soil physico-chemical properties and the groups of bacterial taxa selected as indicators of each restored, control, and natural reference soils, for each sampling campaign (t1, t3, and t12). EC: electrical conductivity; AW: available plant water; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil.*

4. Discussion

Soil physico-chemical properties and bacterial metagenomic analysis at the phylum and genus level provided evidence on the impact of the addition of organic amendments from different origins on soil bacterial communities. Our results provide information to facilitate the selection of organic amendments which could favor the recovery of abandoned agricultural soils in a semi-arid climate by studying the short-term response of edaphic bacterial populations.

The organic amendment application modified the physico-chemical properties of the restored soils, differentiating them from control soils and natural soils over time (Table S1a). The most marked changes were observed between the beginning and 3 months after restoration (t1 and t3), with the greatest alterations in soil bacterial communities (Figure 2b). Calcareous soils in semi-arid regions, such as the study area, are characterized by a low nutrient content (Bastida et al., 2013b; García et al., 1994). Thus, the composted waste used produced a significant increase in TOC, TN, and AP for all treatments, causing a nutrient stoichiometric imbalance (C:N, C:P, and N:P; Table 1). These changes were more notable in soils amended with animal compost (VC, SC, and MC), which also suffered an increase in salinity compared to untreated (CON) and reference soils (NAT; Table 1). Decomposition of added organic matter could lead to immobilization of available nutrients, causing competition for mineral N and available P between soil organisms, and leading to modifications in the bacterial community (Lv et al., 2014). Previous studies have shown that compost from plant waste has a higher organic matter resilience than compost from animal waste (González-Ubierna et al., 2012; Soria et al., 2022). This could explain the milder changes in nutrient content in the very short term (t1 and t3) after plant compost (GC) application, while after 12 months of restoration, the values of the stoichiometric nutrient ratios were in an intermediate position between the rest of the restored soils and the NAT soils (Table 1 and Figure 1).

Total and available nutrient contents could have contributed to the change in the bacterial community composition of the restored soils, mainly between t1 and t3 (Table S1b and S1c). Changes in the bacterial community structure favor the decomposition of organic amendments (Li et al., 2015; Wang et al., 2022), consequently favoring the relationships in mineral nutrients (Francioli et al., 2016). Therefore, the bacterial communities of the soils together with the new characteristics of the restored soils could have generated notable impacts over time in the soils of our study. These were more evident in the soils amended with compost from manure (VC, CM, and SC) after 12 months of restoration (Figure 2a,b). The influence exerted by the stoichiometry of soil nutrient content may have produced changes in the composition and functionality of the soil decomposer bacterial community (Wang et al., 2022), which could reflect differences in nutrient demand and nutrient availability (Khan et al., 2016). The use of composted waste to reclaim degraded soils could not only favor a more efficient soil decomposer community, but could also benefit the presence of genera categorized as plant-growth-

promoting rhizobacteria (PGPR) (Kadoglidou et al., 2014). In this study, some of the taxa identified as indicator species for restored soils, such as *Alcaligenes*, *Arthrobacter*, *Staphylococcus,* or *Leifsonia,* are considered PGPR (Naseer et al., 2019; Ortega et al., 2017; Pratap et al., 2016; Zheng et al., 2020).

The increase of the copiotrophic phyla Proteobacteria and Bacteroidetes (Fierer, 2017) three months after the application of organic amendments, as well as the high abundance of Firmicutes at the beginning of the restoration, suggests high rates of mineralization of nutrients easily assimilated by the bacterial community (Goldfarb et al., 2011), in agreement with other authors who have previously observed similar behaviors after the addition of organic wastes (Calleja-Cervantes et al., 2015; Li et al., 2017). The application of GC favored the presence of indicator taxa involved in the nitrogen cycle such as the Proteobacteria *Halomonas* and the Firmicutes Bacillaceae (Guo et al., 2013; Xu et al., 2019), as well as the Proteobacteria *Arenimonas* that present different catalytic activities capable of metabolizing a large number of substrates (Li et al., 2017). The presence of these bacteria could have favored the improvement in stoichiometric ratios, bringing these soils closer to the values of natural soils. The increase in TN and AP after the application of CM and SC composts could have benefited the presence of bacterial taxa with copiotrophic lifestyles, such as the genera *Sphingobacterium* and *Flavobacterium* belonging to the phylum Bacteroidetes (Neher et al., 2020), capable of degrading organic compounds (Zhang et al., 2020). Likewise, the positive correlations with nutrient content (TOC, TN, and AP) presented by Proteobacteria *Cellvibrio* (SC indicator species; Figure 4 and Table S4) could indicate that the SC treatment favored the proliferation of micro-organisms with a heterotrophic metabolism due to their involvement in the degradation of organic compounds (Akyol et al., 2019) such as polysaccharides (Wongkiew et al., 2021) or cellulose (Miranda-Carrazco et al., 2022), suggesting a high degradation of the organic matter contributed 3 months after restoration (Figure 4). The CM treatment may have led to a selection of bacterial taxa adapted to increased salinity and decreased pH (Figure 4), as EC and pH together with nutrient content are the most influential chemical properties on the soil bacterial composition (Goldfarb et al., 2011; Khan et al., 2016; Miralles et al., 2020b; Rodríguez-Berbel et al., 2020), suggesting that soil characteristics were the main driving forces behind edaphic biodiversity (Liang et al., 2022). Moreover, positive correlations with TN and AP in CM soils of the Proteobacteria *Alcaligenes* (order Burkholderiales) able to degrade both labile

and recalcitrant C (Goldfarb et al., 2011) and fix N (Miralles et al., 2021); as well as of the order Bacillales (Firmicutes) able to secrete organic compound degrading enzymes (Ye et al., 2019) and involved in C and P cycling (Lin et al., 2019) could favor biogeochemical cycles and consequently improve plant yield by repairing soil fertility. Similarly, the indicator bacterial taxa for the VC treatment showed significantly positive correlations with AP, as well as positive correlations with TOC and TN (Figure 4). Among the VC indicator taxa, the Flavobacteriaceae (Bacteroidetes) and Xanthomonadaceae (Proteobacteria) families are distinguished for their involvement in the digestion of organic macromolecules and lignin, respectively (Goldfarb et al., 2011; McBride, 2014). Li et al. (2017) found that unclassified members of the family Xanthomonadaceae increased with amendments of manure and straw. In addition, the significantly positive correlations between VC indicator taxa with PA, together with their high content, could suggest that the vermicompost presented a high stage of maturation, since mature composts represent an important source of available P (Elbl et al., 2019). Regarding the copiotrophic phyla detected in untreated soils and natural soils (CON and NAT, respectively), the indicator bacterial taxa belonging to the phylum Bacteroidetes as the family Microscillaceae in CON soils, and in NAT soils, the genus *Segetibacter* or the Proteobacteria *Psychroglaciecola* have been previously observed in Mediterranean soils near the study area (Miralles et al., 2020b; Rodríguez-Berbel et al., 2020).

Despite the fact that most of the indicator bacterial taxa of the study soils belonging to oligotrophic phyla were identified in the untreated and natural reference soils (Table S4), the restoration also produced changes in oligotrophic phyla such as Actinobacteria and Planctomycetes, which suffered a decrease at t3 and proliferated at t12 in the restored soils (Figure S1). These changes in relative abundance between copio- and oligotrophic phyla could indicate a succession of soil bacterial communities towards more oligotrophic metabolisms (Bastida et al., 2017; Fierer et al., 2007; Li et al., 2017) due to short-term consumption of easily assimilable nutrients, mainly in soils treated with animal-derived compost (VC, SC, and CM), given that in these same soils, the phylum Acidobacteria decreased in abundance over time, increasing only in GC soils (Figure S1), suggesting a decrease in the mineralization of C (Fierer et al., 2007; Goldfarb et al., 2011). Soil enrichment with poultry manure compost (CM soils) favored the presence of PGPR genera (phylum Actinobacteria) such as *Arthrobacter* and *Leifsonia* involved in N fixation and P cycling, respectively (Miralles et al., 2021; Pratap et al., 2016). The

presence of these indicator bacteria together with those previously described in these soils could suggest that metabolisms involved in N and P cycling are taking place in CM soils that could be reflected in the slight loss of N and increase of assimilable P over time (Table 1). As for the indicator taxa of the VC treatment, bacteria belonging to the oligotrophic phyla Deinococcus-Thermus and Chloroflexi were found, which could be involved in the decomposition of recalcitrant organic matter (Goldfarb et al., 2011). In the control soils, all those bacteria that were not able to be identified at lower levels taxonomically were encompassed in the slow-growing oligotrophic phylum Actinobacteria (Bastida et al., 2013b; Zhang et al., 2019). This indicator taxon presented significantly negative correlations with TOC and TN, and positive correlations with pH, suggesting the adaptation of the bacterial community to the higher pH and low nutrient conditions of untreated soils (Table 1). As for the indicator taxa from natural soils, most of them presented significantly positive correlations with stoichiometric nutrient ratios (C:N, C:P, and N:P) and useful water, suggesting greater adaptation to the conditions of the reference soils. Some of these bacterial taxa, such as *Blastococcus,* were also frequently found in other semi-arid soils characterized by low organic matter content and adaptations to extreme conditions (Miralles et al., 2020b; Rodríguez-Berbel et al., 2021).

The results of this experimental case for the reclamation of abandoned agricultural soils have shown that the bacterial community structure of the soils was influenced by the type of organic amendment applied. The most drastic changes occurred between the beginning and 3 months after restoration, as a consequence of the alterations in the abiotic properties generated (nutrient content and stoichiometric ratios). It was also observed that the organic origin of the composted residues used generated differences in the bacterial composition of the restored soils, control soils, and natural reference soils.

5. Conclusions

Studies of the evolution of abandoned soil restorations through the application of organic amendments are important to reveal soil bacterial patterns and to identify edaphic factors and nutrient availability that could influence soil biodiversity. The results of this research revealed that the addition of composted residues modified the physico-chemical properties of the soils by increasing the nutrient content and varying their stoichiometric ratios with respect to untreated soils and natural reference soils, especially during the 3 months of the restoration. These alterations could have interfered with the development of the bacterial community in the different soils studied. This could be explained by the

fact that the bacterial composition of each treatment was significantly different from the rest. Additionally, the results revealed that throughout the study period the soils restored with plant compost (GC) presented an intermediate behavior between the reference soils (untreated and natural soils) and the soils treated with vermicompost and animal compost (SC and CM), the latter behaving differently from the former mainly at 12 months of restoration. At the phylum level, the results suggested the existence of a short-term bacterial succession between the increase after 3 months in the relative abundance of faster-growing phyla (Proteobacteria, Bacteroidetes, and Firmicutes), which, after 12 months of restoration, were replaced by slower-growing phyla (Actinobacteria, Planctomycetes, and Acidobacteria). Likewise, the organic matter provided by each amendment could have favored the presence of specific bacterial taxa for each of the study soils with important implications in the C, N, and P cycles, as well as the establishment of bacterial genera considered PGPR such as *Alcaligenes*, *Arthrobacter*, *Staphylococcus*, or *Leifsonia*. In conclusion, the results indicated that composted greenhouse crop residues favored a soil bacterial community and nutrient content closer to reference soils than other organic amendments applied. Therefore, it could be an optimal treatment for the short-term recovery of agriculturally degraded soils in semi-arid areas.

Supplementary Materials: The following supporting information can be downloaded at: **https://www.mdpi.com/article/10.3390/agronomy13010086/s1**, Table S1. Results of different multivariate PERMANOVA analysis ($p < 0.05$; 999 perm) by the factors treatment (GC, VC, SC, CM CON and NAT soils) and time (t1, t3 and t12) for the data of soil physico-chemical properties and bacterial composition (phylum level and genus or the next upper taxonomic level identified); Table S2. Component loadings on all principal-component-analysis (PCA) solution for physico-chemical soil properties and nutrient stoichiometry ratios in restored, natural and control soils; Table S3. Distance-based redundancy analysis results, explanatory variance and contribution of relative abundance of bacterial taxa and abiotic properties (physico-chemical properties and nutrient stoichiometry) of the soil; Table S4. Results of the analysis of indicator species at the phylum and genus or the next upper taxonomic level identified for each type of restored, control and natural reference soil.

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2. Rodríguez-Berbel et al. 2021. Science of the Total Environment.

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Resumen:

La selección de una enmienda orgánica adecuada para la recuperación de suelos semiáridos degradados por la minería es clave para el éxito de una restauración ecológica. El objetivo de esta investigación es estudiar las respuestas a corto plazo de las propiedades fisicoquímicas, bioquímicas y biológicas, así como los cambios de una comunidad bacteriana del suelo a nivel de género tras la aplicación de cinco tipos de enmiendas orgánicas en una cantera de caliza en Almería (SE, España). También se analizó la relación entre los taxones bacterianos con las propiedades bioquímicas y fisicoquímicas y el efecto de imprimación de los suelos restaurados. Seis meses después de la aplicación de enmiendas orgánicas, los valores de diferentes estados del suelo, tales como carbono orgánico total, nitrógeno total, fósforo asimilable y formas de materia orgánica lábil (carbohidratos y polifenoles), respiración basal (BR) y actividades enzimáticas aumentaron significativamente respecto a los suelos no restaurados. De forma similar, todas las enmiendas orgánicas produjeron un efecto positivo en la mineralización de la materia orgánica del suelo, siendo significativamente mayor ($p < 0,05$) en los suelos tratados con lodos de depuradora. La diversidad bacteriana fue mayor en los suelos restaurados que en los de control. La restauración provocó cambios en la composición de las comunidades bacterianas del suelo a nivel de filo y género. Se observó que las comunidades bacterianas del suelo estaban significativamente relacionadas con varias propiedades físicas, químicas y bioquímicas del suelo, estableciéndose dos patrones de co-ocurrencia diferentes entre los suelos restaurados y los no restaurados. Un primer patrón de co-ocurrencia bacteriana mostró correlaciones positivas significativas con el pH y la relación C/N y negatividad con el resto de las propiedades del suelo. El segundo patrón bacteriano se correlacionó positivamente con carbohidratos, μg de C, efecto de cebado, BR, β-glucosidasa y fosfatasa y negativamente con pH y relación C/N. Se concluyó que las comunidades bacterianas del suelo están claramente influenciadas por los tipos de enmiendas orgánicas aplicadas. Taxones bacterianos como *Taibaiella* o *Pseudomonas* podrían desempeñar funciones clave en el ciclo del carbono en suelos restaurados.

Palabras clave: Enmiendas orgánicas; Estudio del amplicón del ARNr 16S; Respiración del suelo; Actividad enzimática; Ciclo del carbono; Patrones de co-ocurrencia bacteriana del suelo; Terrenos secos.

Material suplementario disponible en ANEXO I y en

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Quarry restoration treatments from recycled waste modify the physicochemical soil properties, composition and activity of bacterial communities and priming effect in semi-arid areas

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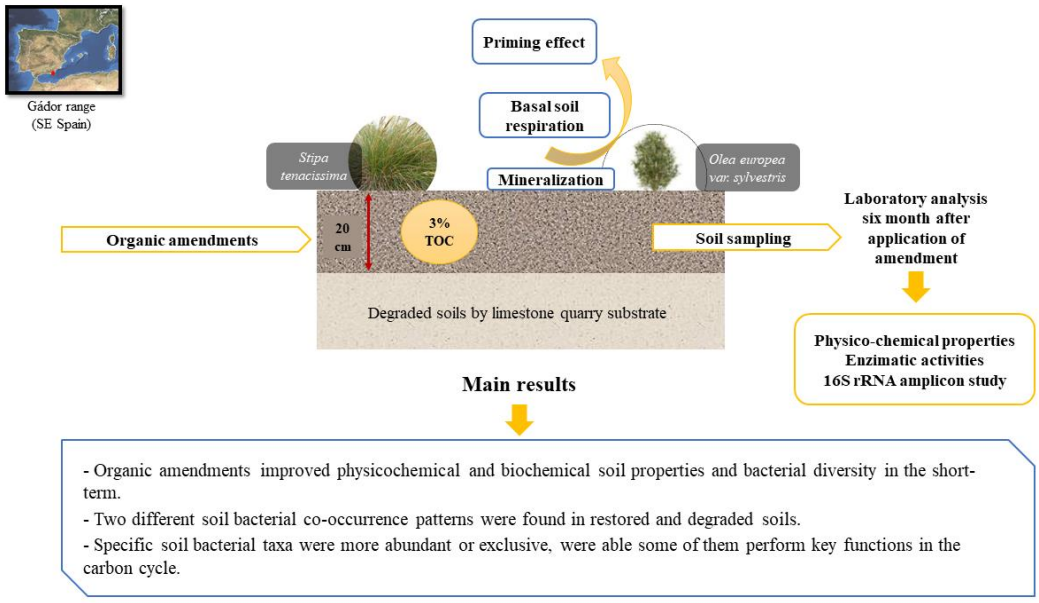
Graphical abstract:



Abstract

The selection of a suitable organic amendment for recovery of semi-arid soils degraded by mining is key to the success of an ecological restoration. The aim of this research is to study the short-term responses of physicochemical, biochemical and biological properties, as well as the changes of a soil bacterial community at the genus level after application of five types of organic amendments in a limestone quarry in Almería (SE, Spain). The relationship among bacterial taxa with biochemical and physicochemical properties and priming effect from restored soils was also analysed. Six months after the application of organic amendments, the values of different soil status, such as total organic carbon, total nitrogen, assimilable phosphorus and labile organic matter forms

(carbohydrates and polyphenols), basal respiration (BR) and enzymatic activities increased significantly with respect to unrestored soils. Similarly, a positive priming effect of soil organic matter mineralisation was produced by all organic amendments, being significantly higher ($p < 0.05$) in sewage sludge-treated soils. Bacterial diversity was higher in restored than in control soils. The restoration caused changes in soil bacterial communities' composition at the phylum and genus levels. It was observed that soil bacterial communities were significantly related to several physical, chemical and biochemical soil properties, establishing two different co-occurrence patterns between restored and unrestored soils. A first bacterial co-occurrence pattern showed significant positive correlations to pH and C/N ratio and negativity with the rest of the soil properties. The second bacterial pattern was positively correlated with carbohydrates, μg of C, priming effect, BR, β-glucosidase and phosphatase and negatively with pH and C/N ratio. It was concluded that soil bacterial communities are clearly influenced by the types of organic amendments applied. Bacterial taxa such us *Taibaiella* or *Pseudomonas* could perform key functions in the carbon cycle in restored soils.

Keywords: Organic amendments, 16S rRNA amplicon study, soil respiration, enzymatic activity, carbon cycle, soil bacterial co-occurrence patterns, drylands.

1. Introduction

In arid and semi-arid ecosystems, open mining activities are responsible for severe soil degradation owing to the elimination of organo-mineral soil horizons and vegetation cover (Kasting and Siefert, 2002; Luna et al., 2017). Moreover, the hostile environmental conditions and the low organic matter content in drylands (Bastida et al., 2007, 2016; Bukar et al., 2019; Miralles et al., 2009) severely complicate their natural restoration when mining ceases. For this reason, soil restoration using organic amendments could be a successful strategy for rehabilitation of soils in semi-arid regions (Bastida et al., 2017; Bello et al., 2020; González-Ubierna et al., 2012; Luna et al., 2017, 2016b).

Different authors have demonstrated that organic amendments improve physical, chemical and biological soil properties, contributing to plant stabilisation and organic matter transformation (Bukar et al., 2019; Luna et al., 2016b; Neilson et al., 2012). Both compost and sewage sludge amendments are characterised by high organic matter, nitrogen and phosphorus contents (Estévez-Schwarz et al., 2012), improving soil fertility and physical soil structure by reducing runoff and erosion processes as well as enhancing the biomass and activity of soil microbial communities (Saison et al., 2006). In addition to these benefits, the use of organic amendments from recycled waste could contribute to the new paradigm of the European Strategy for a Circular Economy, in which the waste

generated could be reused for the preservation of resources (Hueso-González et al., 2018). This gives rise to the development of a secondary activity for industrial waste generated in large quantities, giving it a second life, such as its use in soil restoration, simultaneously generating a new business network for management of this organic waste that implements efficient composting processes.

The success of ecological restoration, including the addition of organic amendments into degraded mine ecosystems is particularly complex thanks to the heterogeneous pattern of ecosystems functioning in drylands (Costantini et al., 2016). This heterogeneity of soils allows the hosting of enormously diverse prokaryotic communities (Fierer, 2017). These soil microbial communities are key for proper ecosystem functioning, so it is essential to understand the effect of different restoration treatments on the proliferation of microbial communities and changes in their activity, given that microbial diversity is directly involved in soil multifunctionality (Delgado-Baquerizo et al., 2016). However, to date we have little knowledge about the effect of the application of organic amendments on bacterial communities and their functionality. Soil microbial communities are clearly affected both by degradation processes such as increased aridity and/or mining practices (Luna et al., 2016b) and by restoration processes (Bastida et al., 2007, 2017; Rodríguez-Berbel et al., 2020), and they respond quickly to environmental change (Ros et al., 2003). The restoration treatments based on the application of organic amendments could influence the soil microbial communities' composition through its effect on physicochemical soil properties (Bukar et al., 2019; Lauber et al., 2009; Rodríguez-Berbel et al., 2020), but this also depends on the composition of organic matter added to the soils (Ye et al., 2019). Goldfarb et al. (2011) showed that some soil bacterial communities have a greater preference for labile carbon sources, whereas others have a greater preference for recalcitrant carbon compounds, so it is vital to understand how the chemical composition of organic amendments could influence the microbiological communities and their functions in biogeochemical cycles after the restoration. After the addition of organic amendments to the soils, there is an increase in the CO_2 emission owing to the interactions between soil microorganisms, transformation of the added organic substances and the natural soil C cycle (Kuzyakov, 2010). The chemical composition of organic amendments can have a direct impact on the magnitude of CO_2 emissions through the 'priming effect' (Blagodatskaya et al., 2014), which is defined as changed decomposition of soil organic carbon (SOC) responding to

fresh carbon inputs to the soils (Felipe Bastida et al., 2019; Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). Nevertheless, abiotic factors such as pH, temperature and, especially, soil moisture also affect the soil microbial activity and carbon dynamics (Blagodatskaya and Kuzyakov, 2008). The increased activity of microbial enzymes degrading labile and recalcitrant organic compounds have a direct impact on CO_2 emission (Blagodatskaya and Kuzyakov, 2008). However, despite the essential role of soil microorganisms in soil organic matter mineralization (Delgado-Baquerizo et al., 2016), soil respiration and concomitant CO_2 emission and carbon soil fixation (Wang et al., 2020; Whitaker et al., 2014), the impact in CO_2 release to the environment as well as in the soil microbial communities and their functions in biogeochemical cycles in mining soils restored with different organic amendments has been poorly explored.

Here, we evaluate the short-term effect of the application of different organic amendments in the soil microbial communities of degraded mining soils and in their functions in the C cycle and priming effect. For this purpose, the following partial objectives were carried out: i) to study physicochemical soil properties (organic carbon, nitrogen, phosphorus, soil pH and water retention) and biochemical soil properties (enzymatic activities involved in C, N and P cycles), soil basal respiration (BR), priming effects and glucose mineralisation in the restored and degraded soils without organic amendments; ii) to analyse the effect of each of the organic amendments on soil microbial communities at the genus and phylum level and compare them with those of degraded soils without amendments; iii) to study relationships between different soil properties and the relative abundance of bacterial taxa, diversity and bacterial taxonomic composition identified, and iv) to determine among all restoration treatments the one that most contributes to increase the diversity, abundance, activity and functionality in the biogeochemical soil cycles of the bacterial communities. We hypothesise that the type and chemical nature of organic residue determines the short-term responses of the soil microbial community in a quarry from a semi-arid region. Thus, fresh organic waste such as sludge that contains lots of labile organic compounds can stimulate soil microbial community and increase the activity and CO_2 emissions of the soil. Stabilised amendments, such as compost of vegetal origin, will also increase microbial activities, but this response is expected to be more modest than with organic sludge. Further, we hypothesise that these functional responses of soil to the addition of organic amendments will be associated with changes in bacterial community composition. In particular, we

hypothesise that the sludge addition will promote the relative abundance of copiotrophic taxa in comparison to compost, because copiotrophic bacteria can quickly grow using labile compounds (Bastida et al., 2015; Fierer et al., 2007; Miralles et al., 2020a), and that changes in bacterial community composition will be associated with changes in priming effect and enzymatic activities.

2. Material and methods

2.1. Experimental design and soil sampling

This field experiment was conducted from July 2018 to December 2018 at a limestone quarry in Gádor range in the Almería Province (SE Spain, 36°55′20″N, 2°30′29″W). This region has a semi-arid climate with an average annual rainfall of 242 mm, most of these events are in winter and autumn, and mean annual temperature is 17.6ºC, with the mean maximum temperature being 31ºC (August) and the mean minimum temperature 8ºC (January). The potential evapotranspiration is 1225 mm year $^{-1}$, and solar radiation values in the study area show values above 900 Wm^{-2} from mid-April to mid-September (Lázaro et al., 2004). The experimental area is mainly constituted of calcareous sandstones that are overlaid on partially extracted marls. In undisturbed surrounding area, soils are mainly Regosols (FAO-IUSS-ISRIC Working Group WRB, 2015). In the study area, the native vegetation is composed principally of grassland by *Stipa tenacissima* (L.) Kunth. with some perennial shrubs such as *Anthyllis terniflora* (Lag.) Pau., or *Anthyllis cytisoides* L., and other species as *Ulex parviflorus* Pourr., *Maytenus senegalensis* (Lam.) Exell., *Rhamnus lycioides* L. *and Pistacia lentiscus* L. More information about the study area is found in (Luna et al., 2016b).

The experimental plots were established in a totally exploited site without incline at 362 m.a.s.l. Using heavy machinery (i.e., mechanical excavators and bulldozers), soil homogenisation and decompaction work was done to decrease erosion by rainfall events and facilitate soil infiltration. Then, 18 experimental plots of 50 m² (10 m × 5 m) each were set up, and different treatments with organic amendments were applied (3 replicates per treatment). The treatments used were organised as: a) 100% vegetable compost derived from garden waste (CG; Total organic C: 31.4%; Total nitrogen: 1.6%; Total phosphorus: 1.8%), b) vegetable compost derived from greenhouse crop residues (CC; Total organic C: 59.1%; Total nitrogen: 1.9%; Total phosphorus: 0.8%), c) sewage sludge treated with anaerobic mesophilic digestion, dehydrated by spin and thermally dried at

70ºC (SS; Total organic C: 66.4%; Total nitrogen: 5.5%; Total phosphorus: 6.3%), d) equal mixture of CG + SS (Mix1), e) equal mixture of CC + SS (Mix2) and f) control plots without organic amendments (CON). The selection of the different organic amendments for the soil restoration treatments was based on comparing the effect of organic amendments with different chemical composition on the restored soils. Sewage sludge was used owing to the fact that it contains a greater amount of labile organic matter consisting mainly of proteins and carbohydrates to which a large amount of free or condensed lipids is incorporated (Almendros et al., 2000, 1990). The composts from different plant remains provides resilient soil organic matter with a greater contribution of lignin (Argyropoulos and Menachem, 1997) or humic-type polymers (Stevenson, 1994). Finally, mixtures of amendments were applied with the objective of obtaining a balanced chemical composition between resilient and labile forms of organic matter and to compensate for loss of nitrogen during the composting process with the extra contribution of N provided by the sewage sludge (Shou et al., 2019).

The organic amendments were exposed over the surface with a shovel backhoe (1 m^3) and mixed with the first 20 cm of degraded soils with a bulldozer; in CON soils, the same process was carried out without adding any amendment. The quantity of each organic amendment used was calculated to increase the initial soil organic matter content up to 3% in each plot. After installation of the experimental plots, two Mediterranean native species (*Stipa tenacissima* L. and *Olea europaea* L. var *sylvestris* Brot.) were manually planted from forestry pot seedlings in a relation 4:1 ratio (50 plants in total per plot) with a distance of 100 cm between plants. These species were selected according to high survival rates in previous ecological restorations carried out at the study site (Luna et al., 2017). Irrigation was carried out at the time of planting owing to the climatic conditions of the study area and the low rainfall rate because the irrigation of *Stipa tenacissima* L. seedlings during the first summer caused a high survival rate in ecological restorations previously carried out in the same quarry (Luna et al., 2017).

Composite soil samples (mixing 10 subsamples) were collected randomly up to a depth of 10 cm throughout each experimental plot after 6 months of the application of organic amendments to study short-term microbial responses in degraded open-mine areas. A total of 18 soil samples (3 replicates per treatment) were taken to the laboratory in isothermal bags. Samples were air-dried, homogenised, sieved through a 2 mm screen and preserved at 4ºC. Part of this dry soil was used to analyse different physicochemical

and biochemical soil parameters, and another part was used for DNA extraction (stored at -20ºC) and next-generation sequencing (NGS) analysis.

2.2. Physical and chemical soil parameters

pH and electrical conductivity (EC) were determined on a soil water suspension (1:2.5 soil/water ratio) (Thomas, 1996) with a pHmeter (LAQUA PH1100, HORIBA, Tokio, Japan) and a digital conductivity meter (LAQUA EC1100, ORIBA, Tokio, Japan) respectively. Total organic carbon (TOC) was determined by Walkey and Black´s method (1934) (rectified by Mingorance et al. 2007) and total nitrogen content (TN) was determined by total combustion (Vario Rapid N; Elementar, Hanau, Germany), C/N ratio was calculated from the previous two. Assimilable phosphorus (AP) was analysed using the Olsen-Watanabe method (Olsen et al. 1954) and soil water retention was measured from pF at -1500 and -33 KPa following Richards membrane method (Richards, 1941).

2.3. Biochemical properties and enzymatic activity

A cold extraction during 1h in agitation at 25ºC from 5 grams of soil with a soil-to-water ratio of 1:10 (w:v) was done. From the extract, soil carbohydrates were quantified using the anthrone–sulphuric acid method developed by Brink et al. (1960). This extract was also used to measure the polyphenols content using Folin-Ciocalteau reagent by the Folin–Denis method (Ribéreau-Gayon, 1968). Absorbance measurements were made with a spectrophotometer Spectronic Helios Gamma UV-Vis (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Soil basal respiration (herein after BR) was measured from 20 g of sample at 50% water holding capacity in 125 ml hermetically sealed vials that were incubated for 31 days at 28ºC in darkness. The CO_2 produced by microbial respiration was periodically measured (every day for the 3 first days and then each 4 days) using an infrared gas analyser (CheckmateII; PBI Dansensor, Ringsted, Denmark). Dehydrogenase activity was analysed using 1 g of sample, as the reduction of 0.2 ml of p-iodonitrotetrazolium chloride to iodonitrotetrazolium formazan (INTF) at room temperature (García et al., 1997). Urease activity was determined following the method published by Kandeler and Gerber (1988). β-glucosidase activity was analysed following the method described by Eivazi and Tabatabai (1988) and alkaline phosphatase activity according to Tabatabai and Bremner (1969).

2.4. Assay for the analyses of soil priming effect

Soil priming was analysed through microcosm incubations, as described in Bastida et al. (2019). Briefly, 20 ml glass vials with 1 g of dry soil samples were prepared. The vials were incubated at 50% of water holding capacity to re-adapt microbial communities to incubation conditions at 28 ºC in darkness for one week. Then, in one of the vial series, a dose of 100 µg C-glucose per gram of soil was dissolved in water. The other series of vials received the same amount of water without glucose. ^{13}C-glucose (99 atoms% U-13C, Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA) was used as a source of organic carbon. The vials were incubated at 28ºC for 16 days to determine basal respiration and glucose mineralisation. These would be used for priming calculations. After incubation, quantification of CO_2 and its isotopic composition were performed as described elsewhere (Felipe Bastida et al., 2019). The priming effect was defined as the increase or decrease in the soil organic matter mineralisation following substrate addition. It was calculated as the total soil respiration following glucose addition minus the amount of C respired from the added ^{13}C-substrate and from control soil without glucose (Blagodatskaya et al., 2007). It was expressed as the surplus CO_2-C (µg) released from SOC.

2.5. Soil DNA extraction, 16S rDNA gene amplification and bioinformatics

Microbial DNA was extracted from 0.3 g of soil using DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) and quantified with a spectrophotometer ND-2000 Nanodrop (Thermo Fisher Scientific, USA). The V4–V5 regions (400–500 pb) of the bacterial 16S ribosomal RNA gene were amplified *in vitro* by polymerase chain reaction (PCR) using the 515FB/926R primer pair (Walters et al., 2016) and paired-end sequenced on an Illumina MiSeq platform using v3 chemistry (2x300bp), as described in Comeau et al. (2017). To check for no contamination during DNA extraction, one blank control was done in one of the kit tubes. In turn, negative controls were analysed (1 for every 96-well plate = 4 per MiSeq run), which were verified to be clean (no bands present) and were still sequenced on the MiSeq to show no substantial reads coming through on this barcode combination.

Sequences were processed with Quantitative Insights Into Microbial Ecology 2 (QIIME2 version 19.7) software following the protocol initially established in Comeau et al. (2017), more recently updated on the Microbiome Helper website (Amplicon SOP v2

[qiime2 2018.8]; https://github.com/LangilleLab/microbiome_helper/wiki). The bacterial raw reads were trimmed of primers, quality controlled and contaminant filtered, followed by the creation of amplicon sequence variants (ASVs) using the Deblur tool. The final taxonomic identities of the ASVs were obtained using the QIIME2 feature-classifier plugin (sklearn method) against the SILVA database (version 132; trimmed to the V4V5 region of the 16S). Weighted UniFrac and other statistical measures of diversity were calculated within QIIME2 using the ASV table normalized to 10,000 sequences per sample. All these resources are available through the Microbiome Helper website (https://github.com/LangilleLab/microbiome_helper/wiki).

Alpha-diversity (intra group diversity) was also determined with QIIME2. The number of ASVs' richness and Faith's Phylogenetic Diversity index (Faith, 1992) were evenly sampled at 10,000 reads per sample. The relative abundance of soil bacterial taxa (ASVs) was calculated for taxa grouped at the genus level or the next upper taxonomic level identified in each of the 18 soil samples. For posterior statistical analyses, those bacteria that had a relative abundance greater than 0.1% in all samples were selected.

2.6. Statistical analysis

One-way Permutational Univariable Analysis of Variance (PerANOVA with 9,999 perms, $p < 0.05$) was used to analyse the differences in physical and chemical properties, carbohydrates and polyphenols content and priming effect, as well as to gauge the significant differences of diversity indices between experimental plots (restored soils [CG, CC, SS and mixes, Mix1 and Mix2] and soils without amendment [CON]) (Anderson et al., 2008). A pair-wise test comparison by permutation was used to construct a multivariate analogue of the *t* test and the probability levels of differences between groups (Eldridge et al., 2016) using Monte-Carlo test when the number of free permutations was less than 100.

The differences in relative abundance of soil bacterial genera or the next upper taxonomic level identified as greater than 0.1% in all samples (CG, CC, SS, Mix 1, Mix 2 and CON) were studied by one-way Permutational Multivariate Analysis of Variance (PerMANOVA with 9,999 perms, $p < 0.05$) (Anderson, 2005) in combination with pairwise comparisons using a Bray-Curtis distance similarity matrix to obtain the significative differences between experimental plots. A distance-based linear modelling (DISTLM) function was developed to determine the relative importance of soil properties

(physicochemical properties, carbohydrates and polyphenols content, BR and enzymatic activities) and priming effect variables and relative abundance of soil bacterial taxa. In order to know those physicochemical and biochemical of soil properties that influenced the soil bacterial community, Linear distance models (DistLM) were performed, confronting the relative abundance of soil bacterial taxa firstly with physicochemical soil variables and, secondly with biochemical soil variables analysed. For the DISTLM routine, 'marginal' tests of the relationship were developed between the response variable (relative abundance of bacterial taxa) and an individual variable (physicochemical and biochemical soil properties) to identify the independent variables that explain the variations in soil samples. Following the marginal tests, 'sequential' tests of individual variables were performed to assess whether adding an individual variable contributes significantly to the explained variation of the response variable. Finally, the distance-based redundancy analysis (dbRDA) was applied to relative abundance of bacterial taxa to build a regression model against two new response variables ('axis' 1 and 'axis' 2), built on the soil properties. The Akaike Information Criterion (AICc, Akaike, 1974) criterion was adopted to select the best model, and the step-wise procedure was followed to build the model.

PerMANOVA, PerANOVA and dbRDA analysis were performed using the statistical package PRIMER6 + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows. Pearson's correlation coefficients (r; $p < 0.05$) were obtained with Statgraphics (version 16.2.04) to evaluate the relationships between soil physicochemical variables and soil bacterial genera level or the next upper taxonomic level identified. Soil bacteria showing highly significant correlations between them ($p < 0.05$) and with the highest correlation values ($r > 0.8$) were selected as bacterial groups forming bacterial co-occurrence patterns, as described in Miralles et al. (2020b). The R Project environment (R Core Team, 2018) with 'gplot2' and 'RColorBrewer' packages was used to generate a heatmap chart to represent the relative abundance of those soil bacterial taxa selected in each soil type. Network analysis was performed to graphically represent the relationships between soil bacterial taxa that conform the co-occurrence patterns resulting from Pearson's correlations. For this purpose, the method for obtaining modularity (Blondel et al., 2008) was used together with Force Atlas 2 algorithm (Jacomy et al., 2014), based on the interactive platform Gephi 0.9.2 (Bastian et al., 2009) for visualising networks. To determine which soil bacterial taxa accounted for the differences

between two co-occurrence patterns in the different treatments and the unamended soils, a SIMPER (percentage similarity) analysis of soil bacterial taxa relative abundance (at the genus level or the next upper taxonomic level) was performed using PRIMER6 software.

3. Results

3.1. Physicochemical soil properties

In general, restored soils with organic amendments (CG, SS, CC, Mix1 and Mix2) showed significantly higher ($p < 0.05$) TOC, TN, AP, carbohydrates and polyphenols than unamended soils (CON), with SS soils, followed by Mix1 and Mix2 soils, found to be the treatments which had the highest values in the most physicochemical soil properties. In contrast, garden compost soils (CG) presented lower TOC, TN and AP values, and greenhouse compost soils (CC) showed lower carbohydrates and polyphenols with respect to the rest of the restored soils. Unamended soils (CON) showed the lowest values in such soil properties (Table 1). Nevertheless, soil pH was significantly higher ($p < 0.05$) in CON soils than in the restored soils, whereas the SS soils presented the significantly lowest pH values (Tables 1 and S2).

***Table 1**. Physicochemical soil properties of restored soils and no-amendment (mean ± SD [n = 3]).*

	pH	EC (mS/cm)	TOC (%)	TN (%)	C/N	AP (%)	pF (-1500 KPa)	pF (-33 KPa)	Carbohydrates µg g^{-1}	Polyphenols µg g^{-1}
CG	7.97 ± 0.82 ab	2.56 ± 0.63 ab	1.71 ± 0.19 a	0.32 ± 0.01 a	5.33 ± 0.40 a	0.06 ± 0.00 a	18.1 ± 1.47 ad	29.4 ± 5.59 a	487.88 ± 75.89 a	43.02 ± 17.46 abc
SS	7.45 ± 0.35 a	3.42 ± 0.49 a	2.67 ± 0.54 b	0.6 ± 0.12 b	4.46 ± 0.02 b	0.19 ± 0.09 ab	21.4 ± 1.39 b	34.3 ± 2.62 a	2315.08 ± 603.89 b	37.21 ± 8.91 a
CC	8.5 ± 0.08 b	3.30 ± 0.70 ab	2.94 ± 0.67 b	0.40 ± 0.08 ab	7.27 ± 0.15 c	0.11 ± 0.03 ab	18.5 ± 1.85 abd	30.7 ± 3.12 a	258.75 ± 14.70 c	72.75 ± 7.26 b
Mix 1	7.52 ± 0.21 a	3.25 ± 0.44 a	2.45 ± 0.18 b	0.51 ± 0.06 b	4.78 ± 0.47 ab	0.14 ± 0.04 b	19.4 ± 0.49 abc	31.6 ± 1.65 a	1363.78 ± 14.70 b	33.29 ± 7.26 a
Mix2	7.65 ± 0.06 a	3.08 ± 0.21 a	2.82 ± 0.16 b	0.51 ± 0.04 b	5.46 ± 0.19 a	0.12 ± 0.04 ab	19.4 ± 1.21 abc	32.4 ± 1.04 a	709.05 ± 125.78 a	18.02 ± 5.05 c
CON	8.59 ± 0.08 b	1.72 ± 0.71 b	0.46 ± 0.17 c	0.02 ± 0.02 c	26.0 ± 21.7 abc	0.02 ± 0.00 c	16.3 ± 1.17 d	32.3 ± 2.43 a	0 ± 0 d	3.00 ± 2.63 d

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C/N: carbon to nitrogen ratio; pF: pedo-transfer function that estimates the water content of the soil at different pressures. Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PerANOVA).

3.2. Biochemical soil properties and enzyme activities

Dehydrogenase activity was significantly higher in SS, Mix1, Mix2 and CC soils than in CON soils. SS, Mix1 and Mix2 soils showed the highest values of β-glucosidase

and phosphatase enzyme activities, whereas CON soils had the significantly lowest values (Table 2). However, CC soils showed significantly higher values ($p < 0.05$) in urease enzyme activity than the remaining restored and CON soils (Tables 2 and S2). On the contrary, CG soils showed significantly lower dehydrogenase and β-glucosidase activities and lower values in phosphatase and urease activities than the rest of amended soils (Table 2).

Table 2. *Enzymatic activity in restored soils with different organic amendments and control soils without addition of amendment (mean ± SD [n = 3]).*

	Dehydrogenase μmol INTF g^{-1} soil h^{-1}	**β-glucosidase** μmol PNF g^{-1} soil h^{-1}	**Phosphatase** μmol PNF g^{-1} soil h^{-1}	**Urease** μmol $N\text{-}NH_4^+$ g^{-1} soil h^{-1}
CG	0.15 ± 0.03 a	0.12 ± 0.02 a	2.58 ± 0.32 a	0.10 ± 0.05 a
SS	1.40 ± 0.59 b	1.26 ± 0.22 b	34.40 ± 13.41 b	0.03 ± 0.01 a
CC	0.93 ± 0.32 b	0.36 ± 0.04 c	3.09 ± 0.41 a	0.40 ± 0.19 a
Mix1	0.85 ± 0.15 b	0.64 ± 0.10 d	23.37 ± 4.44 b	0.23 ± 0.38 a
Mix2	1.19 ± 0.53 b	0.65 ± 0.14 d	12.93 ± 4.25 b	0.32 ± 0.23 a
CON	0.05 ± 0.01 c	0.02 ± 0.00 e	0.33 ± 0.15 c	0.07 ± 0.07 a

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils. Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PerANOVA).

3.3. Priming effects and mineralised glucose

In general, soils restored with organic amendments showed significantly higher values ($p < 0.05$) of priming effect and mineralised glucose, with SS and their mixtures found to be the treatments that showed the significantly highest values ($p < 0.05$) in basal soil respiration (BR), soil priming effects and mineralised glucose. In contrast, CG showed lower values than the rest of the restored soils, whereas CC soils presented values for these properties intermediate between sludge-restored soils (SS, Mix1 and Mix2) and CG soils. In contrast, unamended soils (CON) showed the significantly lowest BR, soil priming effect and mineralised glucose (Tables 3 and S2).

Table 3. *Basal respiration, soil priming effects and mineralisation rate in soils remediated with organic amendments, mixtures of organic amendments and unamended soils.*

	BR mg C-CO_2 kg^{-1} soil day	**µg of C** µg CO_2-C g^{-1}	**Priming effect** µg CO_2-C derived from SOM g^{-1}	**Mineralized glucose** %
CG	3.50 ± 0.42 a	273.36 ± 27.31 a	21.71 ± 14.51 a	17.78 ± 0.39 a
SS	46.50 ± 11.50 b	1017.84 ± 82.15 b	136.65 ± 11.77 b	24.94 ± 0.92 b
CC	9.29 ± 1.09 c	638.44 ± 154.2 c	61.01 ± 72.22 abc	21.45 ± 3.06 ab
Mix1	29.83 ± 4.48 b	975.83 ± 67.66 b	75.87 ± 9.56 c	24.76 ± 0.39 b
Mix2	32.43 ± 9.59 b	989.60 ± 21.45 b	78.96 ± 13.54 bc	23.58 ± 1.37 b
CON	0.99 ± 0.18 d	28.75 ± 8.21 d	10.22 ± 3.79 a	5.49 ± 0.84 c

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils. BR: Basal respiration. Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PerANOVA).

3.4. Bacterial diversity

Soils restored with CG, followed by Mix1 and Mix2, showed higher values ($p < 0.05$) in richness and Faith's phylogenetic indices (Fig. 1) than the rest of the soils; CON were the soils that had the lowest values in diversity indices, although they did not show significant differences with the restored soils (Table S2). Bray-Curtis and Jaccard indices showed two clusters, one grouping Mix1 and SS soil and a second group including CG and CON soils (Fig. 2 and S1). Mix1, SS and CC soils, and CG and Mix2 soils, were clustered together according to the Weighted Unifrac index (Fig. S1).

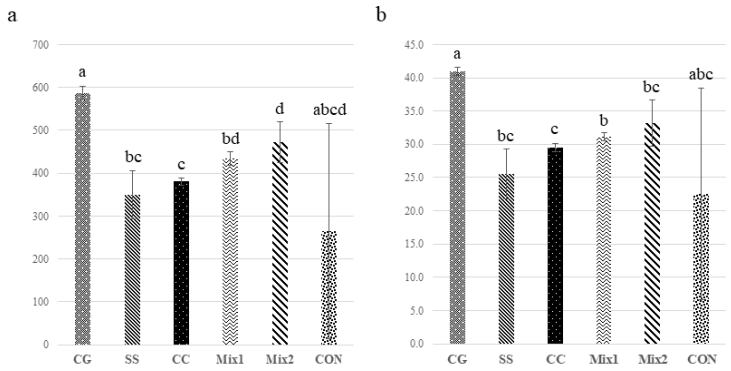


Fig. 1*. Bar charts show the microbial alpha-diversity characteristics (mean ± SD) of ASVs richness (a) and Faith's phylogenetic index (b) of different soil types (n = 3). Different letters from univariate PerANOVA test (p <0.05). Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues; SS: sewage sludge from waste water; Mix1 (CG+SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils.*

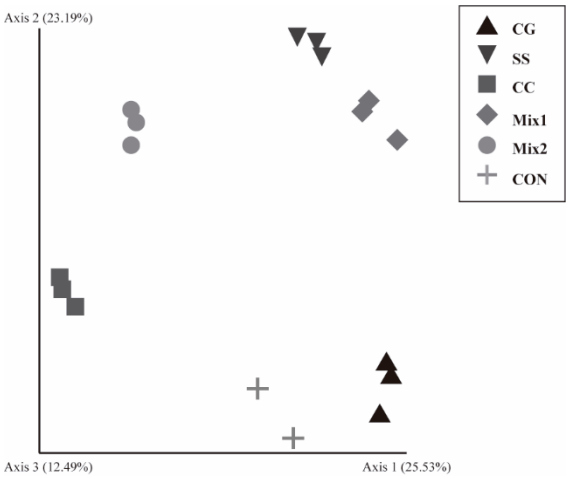


***Fig. 2**. PCoA plot of microbial community analysis by Bray-Curtis index from samples of all soil types analysed (no-amendment soils and restored soils). Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues; SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils.*

3.5. Soil bacterial communities' composition

In all, 607,712 sequences were identified as belonging to the bacteria domain (Table S5). PerMANOVA test showed that organic amendments (CG, SS, CC, Mix1 and Mix2) modified the bacterial soil community with respect to soils without amendment (CON). The restored soils showed significant differences ($p < 0.05$) from the CON soils at the phylum level (Table S2). Twelve phyla with a relative abundance above 0.1% were obtained in all samples, whereas the remaining phyla (32% of the sequences) showed relative abundances lower than 0.1% in all samples (Table S1). The most abundant phyla in all treatments were Bacterioidetes, Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes and Firmicutes, although in different proportions in each treatment (Fig. S1). Bacteroidetes was more relatively abundant in restored soils, while Proteobacteria was dominant in unamended soils (CON).

A total of 152 soil bacterial taxa were identified at the genus level or the next available higher taxonomic level, with a relative abundance of more than 0.1% in all samples. The PerMANOVA analysis reported that all soil types (CG, SS, CC, Mix1, Mix2 and CON) were significantly different ($p < 0.05$) in bacterial communities at the genus level or at the next identified higher taxonomic level (Table S1). Some of these soil bacterial taxa were more abundant or exclusive in restored soils (CG, SS, CC, Mix1 and Mix2), whereas other bacterial taxa were more abundant or almost exclusive in soils without any amendment (CON) (Fig. 3). *Verticia* and *Taibaiella* genera were exclusive

in restored soils (CG, SS, CC, Mix1 and Mix2), while *Staphylococcus* genus was exclusive to CON soils (Fig. 3).

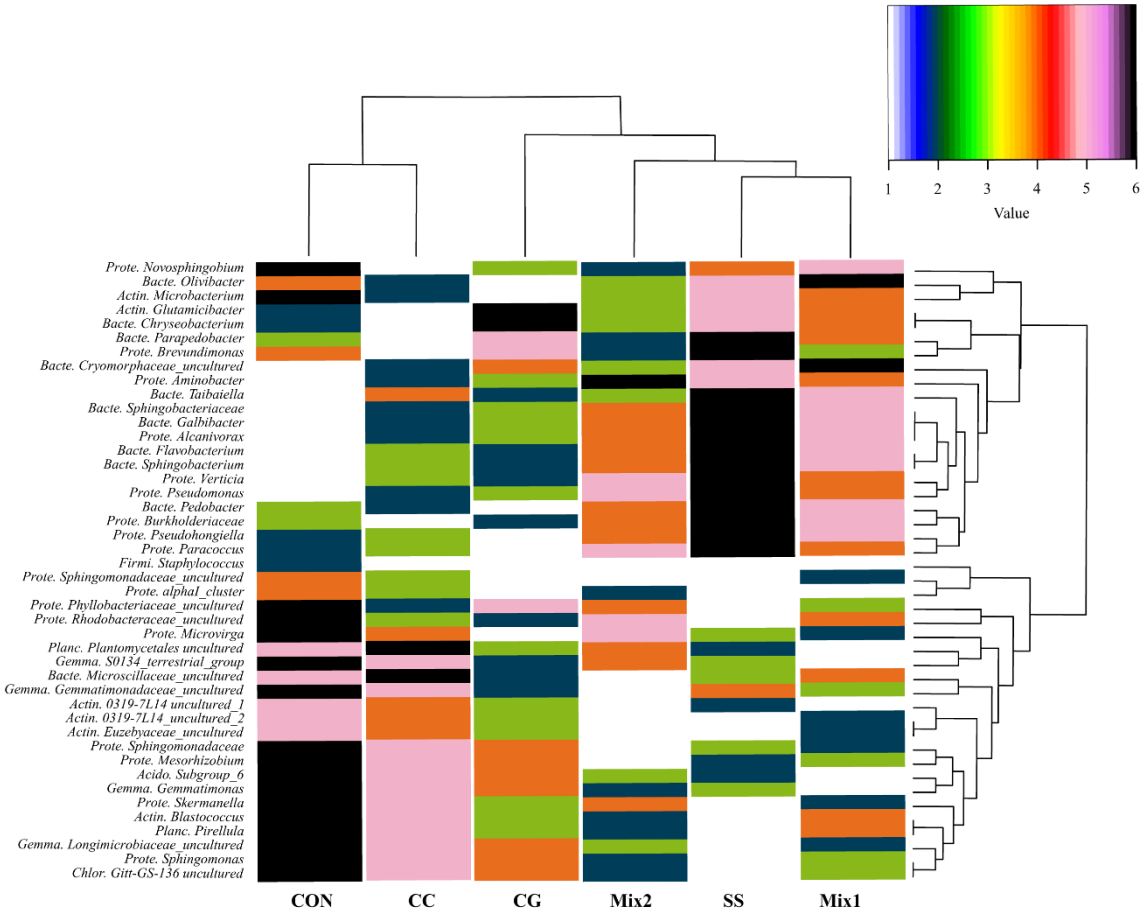


***Fig. 3**. Heatmap of relative abundance of soil bacterial taxa at the genus level or at the next identified higher taxonomic level in each soil type. Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues; SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils.*

3.6. Relationship between soil bacterial taxa and physicochemical and biochemical soil properties

DistLM analysis among physicochemical soil properties and soil bacterial taxa showed that soil pH, TOC, pF at -1500 KPa, TN, C/N ratio, AP, carbohydrates and polyphenols were significantly related to microbial soil composition (Figure 4a and Table S3). Nevertheless, EC and pF at -33 KPa did not have a statistically significant ($p < 0.05$) effect on bacterial community. According to the variations (out of the fitted model and out of the total variation) explained by dbRDA axes, the first axis (dbRDA1), applied to relative abundance of soil bacterial taxa, explained 45.7% of the adjusted model and 37% of the total variation of the variables, whereas the second axis (dbRDA2) explained 21.6% of the fitted model and 17.5% of the total variation (Fig. 4a). Sequential tests indicated

that the best linear distance model (R2 = 0.81; AICc = 135.3) to predicting relative abundance of soil bacterial taxa consisted of these nine parameters (Table S3). The dbRDA analysis clearly grouped four different groups: CON, CG and CC, mixtures (Mix1 and Mix2) and finally, SS soils (Fig. 4a). On the other hand, the second DistLM analysis, including biochemical soil properties and soil bacterial taxa, showed that the best solution for the R^2 value (0.59) explained 52.03% of the total variation (dbRDA1 35.3% and dbRDA2 16.8%) to AICc of 127.95 and identified a combination of five significant factors—dehydrogenase, β-glucosidase, urease, basal respiration and mineralized glucose—that influenced the bacterial soil composition (Fig. 4b and Table S3). In contrast, phosphatase and µg of C did not show a significant influence ($p < 0.05$) on soil bacterial microbiota. Pearson's correlations showed that numerous bacterial taxa were significantly ($p < 0.05$) correlated with each other, of which those with the highest correlation values were selected (r > 0.8; Fig. 5). It was also observed that these bacterial taxa were significantly correlated with physicochemical (Fig. S2a) and biochemical soil properties (Fig. S2b).

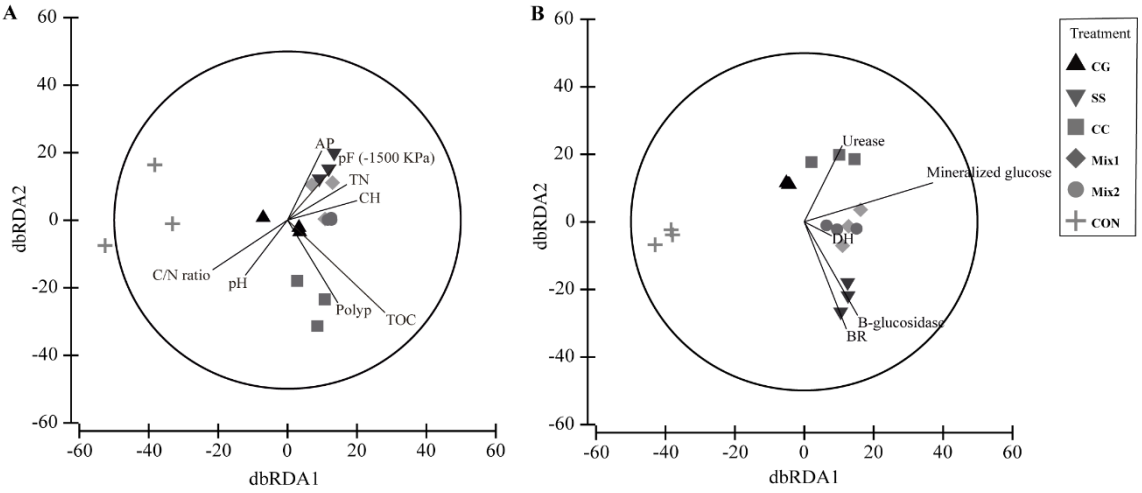


***Fig. 4**. Redundancy analysis (dbRDA) for the composition of bacterial community and (a) physicochemical and (b) biochemical soil properties (priming effects and enzymatic activity). Soil samples with compost from garden waste (CG), sewage sludge from wastewater (SS), compost from greenhouse crop residues (CC), Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge and soils without amendment (CON) are indicated by different colours and figures. Soil properties are represented by continuous lines.*

The distinction of two co-occurrence patterns was observed from relationships between the different bacterial taxa and the physical, chemical and biochemical soil properties (Figure 5). The first co-occurrence pattern (Figure 5 - grey, Table S3), represented by 25 bacterial taxa such as *Novosphingobium, uncultured* (family: Longimicrobiaceae), *uncultured* (class: 0319-7L14) or *Gemmatimonas* among others, showed a significantly high positive correlation with physicochemical parameters such

as pH (r varied between 0.67 and 0.52), high positive correlation with C/N ratio (r between 0.45 and 0.10), and negative correlations with the rest of the parameters analysed (Figure S2a). In turn, these soil bacterial taxa showed negative correlations with all biochemical soil properties and priming effect (Figure S2b).

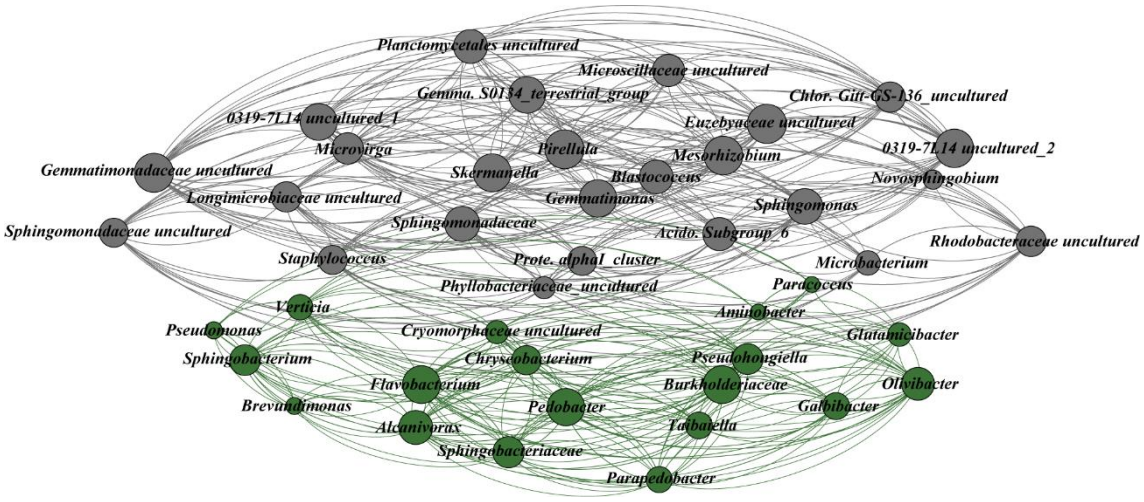


Fig. 5*. Co-occurring networks based on Pearson's correlation analysis (r > 0.8) for soil bacterial taxa shown to be present in each soil type (CG, SS, CC, mixtures [Mix1 andMix2] and CON) with the highest correlations detected. The size of each node is proportional to the number of connections, and the density of the edges indicates the intensity of the correlation. The resulting soil microbial network is made up of 45 nodes and 452 edges (average degree or node connectivity = 8.7). The clustering coefficient (how the nodes are integrated into their neighborhood, and therefore, the degree to which they tend to cluster) is 0.7, and modularity is 0.6. Two patterns are shown: co-occurrence pattern 1 (grey) and co-occurrence pattern 2 (green).*

The second pattern of concurrence (Figure 5 - green), represented by 19 bacterial taxa such as *Sphingobacterium*, *Flavobacterium*, *Chryseobacterium*, *Verticia* or *Pedobacter*, showed significantly positive correlation with physicochemical soil properties such as pF at -1500Kpa (r between 0.76 and 0.54) and carbohydrates (r between 0.93 and 0.58). In addition, some bacterial taxa belonging to this group also showed significantly positive correlations with EC, TOC, pF at -33KPa, TN and AP. Nevertheless, they showed significantly negative correlations with pH (r between -0.52 and -0.74) and negative correlations with the C/N ratio (r between -0.12 and -0.46; Figure S2a). These soil bacterial taxa also showed a significantly positive correlation with β-glucosidase (r between 0.93 and 0.62) and phosphatase (r between 0.92 and 0.52) enzymatic activities, BR (r between 0.96 and 0.56), µg of C (r between 0.85 and 0.52) and priming effect (r between 0.77 and 0.59; Figure S2b). Soil bacteria genera belonging to the first co-occurrence pattern were more abundant or almost exclusive in CON soils, whereas bacterial taxa representative of the co-occurrence pattern 2 were more abundant or almost exclusive in restored soils (CG, SS, CC and mixtures [Mix1 and Mix2]; Figure 3 and 5).

SIMPER analysis showed significant differences in soil bacterial composition between different restored and unamended soils, presenting greater similarity for the restored soils than the control soils (Table S4). In turn, the analysis showed that some bacterial taxa such as *uncultured* (family: Crymorphaceae), *Aminobacter*, *Galbibacter* and *Verticia*, among others, were clustered in restored soils. Specific soil bacterial taxa were in CG, CC and CON soils, while other bacteria (i.e., *Glutamicibacter* and *Sphingobacterium*, among others) were more abundant in sludge-treated soils.

4. Discussion

The organic amendments influenced the composition, diversity and activity of bacterial communities in restored soils in the short-term through the direct changes they produced in physical and chemical soil properties. The restored soils showed significantly higher TOC, labile organic matter content (carbohydrates and polyphenols), salinity and soil moisture content, possibly by the high TOC content improving the soil water retention (Li et al., 2018; Zancada et al., 2004), and lower soil pH, possibly by the buffering effect of organic matter (Miralles et al., 2009) than the control soil without amendment. Soil properties such as nutrients (TOC, TN, AP and others), pH, salinity and soil moisture, as well as the type of organic matter (presence of labile or resilient forms of organic matter), have been shown to be key in shaping the composition of soil bacterial communities (Goldfarb et al., 2011; Lauber et al., 2009; Miralles et al., 2020a, 2020b; Neilson et al., 2012; Rodríguez-Berbel et al., 2020; Sánchez-Marañón et al., 2017). Our statistical analysis corroborated that the effect induced by organic amendments on physicochemical soil properties (carbohydrates content, pF at -1,500kPa, AP and TN) influenced compositional changes on soil bacterial communities' in the restored soils (Figure 4a), and also influenced also their microbiological activity, by increasing the enzymatic activities (dehydrogenase, β-glucosidase and phosphatase), basal respiration and mineralised glucose, in addition to having an increased priming effect (Figure 4b).

Therefore, the improvements in physicochemical quality of restored soils could have contributed to creating a more favourable environment (i.e., higher content of available nutrients and soil moisture) for the proliferation of several microbial populations in the short-term, explaining the higher diversity (Figure 1) and activity (Tables 2 and 3) in restored than in control soils. Moreover, these changes in soil properties contribute to modify the microbial composition in restored soils with respect

to control soils, such that some bacterial taxa were very abundant in restored soils (i.e., *uncultured* (family: Crymorphaceae), *Aminobacter*, *Taibaiella*, *Sphingobacteriaceae*, *Galbibacter*, *Alcanivorax*, *Flavobacterium*, *Sphingobacterium*, *Verticia* and *Pseudomonas)*, whereas they showed very low relative abundance in CON soils (Figure 3). This could suggest that these bacteria, which were present in degraded soils, proliferated after the application of amendments due to changes in soil properties. Thus, two different patterns of bacterial co-occurrence were clearly distinguished between unamended soils (1–co-occurrence pattern) and soils restored with organic amendments (2–co-occurrence pattern). Although many researchers have evaluated the responses of microbial communities to organic restoration in terms of diversity and composition, only a few have considered biotic interactions through network analyses. The study of co-occurrence patterns has taken great prominence in recent studies of soil microbial communities (Barberán et al., 2012; Bello et al., 2020; Miralles et al., 2020b) because microbial species' interactions are crucial for understanding their structure and dynamics (Xue et al., 2017). In the study area, soil bacterial taxa associated with the 2–co-occurrence pattern (i.e., *Aminobacter*, *Taibaiella*, *Sphingobacteriaceae*, etc.) showed a preference for high nutrient content and soil moisture (Figure S2a), proliferating predominantly in restored soils. These bacterial taxa also showed high positive correlations with enzyme activities involved in C, N and P cycles (Figure S2b). The production of these soil enzymes in response to the addition of the organic amendments could be a key driver for the stabilisation of microbial communities in restored soils (Chen et al., 2020). The organic amendments could have favoured the development and homogenisation of some soil bacterial taxa into restored soils in the short term, while in control soils greater disturbances (30–40% of similarity, Table S4) were generated under more hostile conditions. These soil bacterial taxa in CON soils associated with the 1–co-occurrence pattern (i.e., *Gemmatimonas*, *Blastococcus* or *uncultured* (family: Longimicrobiaceae), among others) were correlated with high soil pH and C/N ratio and low TOC, TN, AP, etc. (Figure S2b). Some of these bacterial taxa were also frequently found in other semi-arid soils with low organic matter content and adapted to extreme conditions, such as *Blastococcus* or *uncultured* (family: Longimicrobiaceae) (Miralles et al., 2020b; Yang et al., 2019) and the genus *Staphylococcus*, which appeared exclusively in CON soils. Moreover, the soil bacterial taxa associated with the 1–co-occurrence pattern did not show positive correlations with biochemical soil properties (Figure S2a), possibly due to the low concentration of nutrients (TOC, TN, AP, carbohydrates and

polyphenols) in CON soils, suggesting that their activity in biogeochemical cycles (C, P and N) was lower than in restored soils and, therefore, that the recovery of the soil quality in degraded land affected by mining activity (CON soils) could be slower without the application of organic amendments.

Interestingly, differences in the soil bacterial composition were also found in the restored soils depending on the type of treatment or organic amendment applied to the soils. The differences in the chemical composition of the organic amendments (sludge and both compost types) could explain the changes in physicochemical and microbiological soil properties among the different restored soils. Thus, sludge-treated soils (mainly SS, followed by Mix1 and Mix2) presented higher TOC, TN, AP, labile organic fractions (carbohydrates), water retention capacity and enzymatic activities (dehydrogenase, β-glucosidase and phosphatase) than compost-restored soils (CG and CC) (Tables 1 and 2). These results suggested a higher increase of the microbiological activity in SS, Mix1 and Mix2 than in CG and CC soils as a consequence of their higher nutrient content, labile organic matter and soil moisture as well as the proliferation of some soil-specific bacterial taxa involved in the degradation of organic compounds (Figure 5 and Table S4), which would not be able to proliferate as rapidly in CC and CG soils in the short term. Thus, the increase of copiotroph communities belonging to Bacteroidetes phylum in SS soils could be explained by the greatest content of labile organic compounds (Almendros et al., 2000, 1990; González-Ubierna et al., 2012; Pascual et al., 1998), such as their high content in carbohydrates (Table 1), provided by sewage sludge. A higher percentage of Bacteroidetes was also found in biologically crusted soils from other semi-arid environments (Miralles et al., 2020a, 2020b) with high carbohydrate content (Miralles et al., 2013). In addition, Hugenholtz et al. (1998) also showed the role of Bacteroidetes in the degradation of other organic compounds such as proteins, cellulose, chitin, pectin and starch, which could favour the degradation of more complex compounds (i.e., chitin, pectin or starch) by transforming them into more bioavailable compounds for the rest of soil microbial community. However, Actinobacteria and Firmicutes phyla had higher relative abundances in soils restored with CG, characterised by their higher content of stabilised organic compounds from plant remains (i.e., lignin-derived compounds or humic-type polymers) (Argyropoulos and Menachem, 1997; Stevenson, 1994). Specifically, Firmicutes phylum increased mainly in compost-treated and in mixed soils (Mix1 and Mix2), while in SS soils they declined

to levels similar to the control soils (Table S1), which may be due to competition with other phyla and bacterial families (Bukar et al., 2019). Moreover, the differences in biochemical soil properties between SS and compost-restored soils (CC and CG; Table 2) could also be due to differences in the bacterial community composition and their functions, such that some of these bacterial communities are more efficient in the degradation of labile carbon compounds, while others specialise in degrading more recalcitrant carbon compounds (Goldfarb et al., 2011).

Specifically, sewage sludge favoured a high proliferation of some specific soil bacterial taxa such us *Pseudomonas*, *Sphingobacterium*, *Brevundimonas*, *Verticia*, *Taibaiella*, the Burkholderiaceae family and *Glutamicibacter*, among others. These taxa could play a key role in the carbon cycle, being largely involved in the mineralisation of organic matter by releasing a large amount of enzymes capable of degrading simple organic compounds such as carbohydrates (De Graaff et al., 2010). This could explain the high values in dehydrogenase and β-glucosidase activity, glucose mineralisation, respiration (BR) and/or priming effect in SS soils and their mixtures (Mix1 and Mix2), as well as the significantly highest phosphatase activity in those soils (Table 2), as well as the correlation between the microbial communities in these soils and the biochemical soil properties (Figure 4b) mentioned previously. In turn, the high values of phosphatase activity in SS soils could be due to the fact that sewage sludge is particularly rich in P (Estévez-Schwarz et al., 2012), promoting the release of this enzyme activity (Moreno et al., 1998). The presence of *Pseudomonas* in restored soils (especially abundant in SS soils) could be explained by its involvement in carbohydrate metabolism (Delmont et al., 2012), and that of *Aminobacter* by its denitrification activities (Kostrytsia et al., 2018). *Taibaiella* genus was especially abundant in Mix1 soils and could have been one of the bacterial taxa that have benefitted from organic matter input, being also previously observed during the composting of different wastes (Zhu et al., 2019). Nevertheless, the soils treated with (CG), although this was the treatment that most increased the diversity of soils, did not favour an important proliferation of specific bacterial taxa over others in the short term. The most abundant bacteria in these soils were also abundant in CC soils (i.e., *uncultured* (family: Cryomorphaceae)) and in CON soils (i.e., *uncultured* (family: Longimicrobiaceae), the Sphingomonadaceae family and *uncultured* (family: Microscillaceae), among others). This was possibly due to the resilient and hardly biodegradable character of the organic matter contributed by these types of compost,

explaining, for example, the increase in Sphingomonadaceae family, which was characterised as lignin-degrading (Goldfarb et al., 2011), as well as bacteria members of the Sphingomonadaceae family, which were also positively correlated with TOC in other semi-arid soils (Miralles et al., 2020b).

In summary, comparing the effect of the different restoration treatments on the physicochemical and microbiological soil properties, an improvement in the physicochemical quality and bacterial diversity can be observed, as well as an increase in the proliferation of bacterial communities and their activity in the biogeochemical cycles releasing essential nutrients for plants in the short-term in all restored soils, compared to control soils. However, in soils treated with sludge, the significant proliferation of copiotrophic bacteria and the rapid increase in their activity in mineralising organic matter, due to the contribution of labile organic matter from the sludge, could quickly consume the reserve of soil nutrients, generating, in turn, a greater CO_2 emission into the atmosphere (priming effect). In contrast, in soils amended with compost from vegetal remains, the contribution of a more resilient and hardly biodegradable organic matter favours the bacterial diversity of the soils and a balanced increase of essential enzymes to improve the soil fertility, but guaranteeing the reserve of long-term soil nutrients and, in parallel, less CO_2 release. However, this conclusion should be handled with caution, and long-term studies are also recommended to understand the evolution of microbial communities and their functionality after the addition of organic amendments; such studies could better analyse the changes and specific processes of the C cycle and time in which they occur. In turn, such studies could be of great help in establishing management strategies by selecting restoration treatments depending on the treatment's effect on the physical, chemical and microbiological properties of the soil.

5. Conclusions

The organic amendments from waste produced important changes in the physical, chemical and biological properties of the restored soils with respect to the control soils only six months after their application. All the organic amendments contributed to a homogenisation of the soil bacterial communities as well as an increase in the size, diversity and activity of these bacterial communities in the C, N and P cycles in the restored soils with respect to the control soils. These microbiological changes in the amended soils could be largely due to changes in the local environment of the restored

soils generated by the amendments, which increased the nutrient content such as organic matter, nitrogen and phosphorus, improved the soil moisture. Thus, two different co-occurrence patterns were found between the restored soils and the control soils related to different physical and chemical soil properties. The higher biochemical activity in soils restored with amendments from residues could guarantee the release of essential nutrients available to plants in the short term compared to soils without amendments.

Important changes were also found in the soil bacterial composition and microbial activity among the soils restored with organic amendments with different chemical composition. Soils restored with sewage sludge, with a higher content of labile organic matter, favoured the proliferation of specific copiotrophic bacterial taxa such as *Aminobacter*, *Taibaiella* or *Pseudomonas*. These taxa play an essential role in the carbon and phosphorus cycles, with SS as treatment that most contributed to the release of glucosidase, dehydrogenase and phosphatase, as well as the mineralisation of glucose, priming effect and basal soil respiration, which could contribute to a rapid consumption of soil nutrient reserves and higher CO_2 emissions in the short term. In contrast, the soils with compost from vegetal residues, with more resilient and hardly biodegradable organic matter, produced the highest microbial diversity (especially CG soils), favoured the proliferation of various microbial communities present in the control soils and produced a balanced increase in the soil microbiological activity, guaranteeing a slower release of essential nutrients for soil fertility and a lower priming effect.

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Resumen:

La aplicación de enmiendas orgánicas para recuperar las cualidades físicas, químicas y biológicas del suelo puede permitir la recuperación de suelos degradados por la minería en climas semiáridos. El objetivo de este estudio fue investigar el desarrollo y los cambios en la composición de las comunidades fúngicas en suelos restaurados con cinco tipos diferentes de enmiendas orgánicas (dos tipos de compost vegetal y compost de lodos de depuradora, y una mezcla de ambos) en comparación con suelos sin enmiendas y suelos naturales circundantes, y examinar las relaciones entre los taxones fúngicos, las nuevas propiedades fisicoquímicas y biológicas del suelo de los tecnosuelos tras 18 meses de restauración, y los suelos naturales. La restauración mejoró la calidad del suelo y la diversidad fúngica, situando estos suelos en una posición intermedia entre los suelos no restaurados (sin presencia de hongos) y los suelos de referencia no alterados, que eran los que presentaban una mayor diversidad fúngica. Los suelos tratados con aguas residuales y sus mezclas mostraron un alto contenido en nitrógeno y carbohidratos, así como una elevada respiración basal y contenido en ácidos grasos, lo que sugiere que proporcionaban materia orgánica fácilmente biodegradable. Por el contrario, los suelos tratados con compost de invernadero mostraron un alto contenido en carbono orgánico total y polifenoles, mientras que los suelos tratados con compost de jardín mostraron valores intermedios. Las propiedades biológicas del suelo mostradas por ambos compost fueron similares a las de los suelos de referencia, lo que sugiere que los compost contenían materia orgánica más resistente. Las enmiendas orgánicas de origen disímil provocaron comunidades de hongos del suelo significativamente diferentes a nivel de género entre los suelos restaurados. Los resultados indicaron que el pH del suelo, la conductividad eléctrica, el contenido total de nitrógeno, la respiración basal del suelo, la relación hongos/bacterias-PLFA y las actividades deshidrogenasa y β-glucosidasa, junto con las correlaciones de Pearson, revelaron que estas propiedades y el contenido de nutrientes (carbono orgánico total, relación C/N, carbohidratos y polifenoles) influyeron en 40 taxones fúngicos del suelo. Por tanto, las enmiendas orgánicas provocaron cambios en las propiedades del suelo que favorecieron la cubierta vegetal al promover el crecimiento de la comunidad fúngica del suelo beneficiosa para el ciclo del carbono y simbiótica con las plantas.

Palabras clave: Zona degradada; Comunidad de hongos; Cantera de piedra caliza; Actividad microbiana; Enmiendas orgánicas; Restauración del suelo.

Material suplementario disponible en ANEXO I y en https://doi.org/10.1016/j.scitotenv.2021.151226

Benefits of applying organic amendments from recycled wastes for fungal community growth in restored soils of a limestone quarry in a semiarid environment

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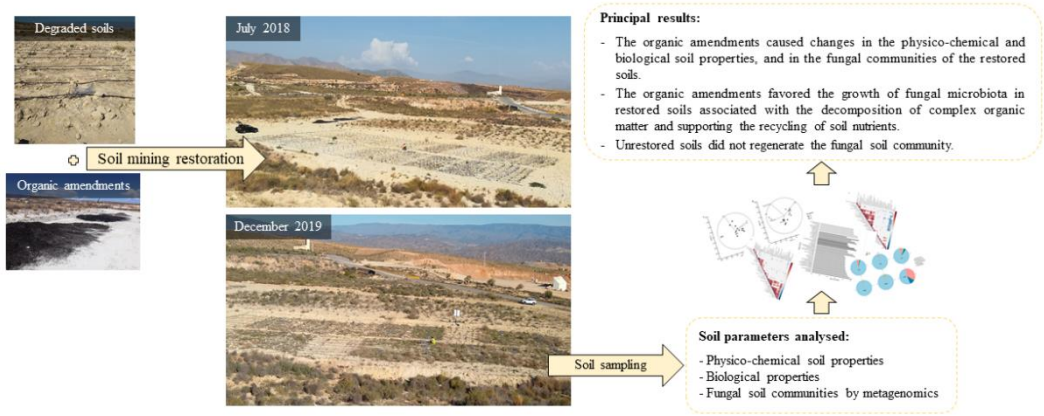
Graphical abstract:



Abstract

Applying organic amendments to recover physical, chemical, and biological qualities of soil may enable recovery of soils degraded by mining in semiarid climates. This study's aim was to investigate the development and changes in the composition of fungal communities in restored soils with five different types of organic amendments (two types of vegetable compost and sewage sludge compost, and a mixture of both) compared with unamended soils and surrounding natural soils and to examine the relationships between the fungal taxa, the new physico-chemical and biological soil properties of technosoils after 18 months of restoration, and natural soils. Restoration improved soil quality and fungal diversity, placing these soils in an intermediate position between unrestored soils (with no fungi present) and undisturbed reference soils, which were the most fungal diverse. Sewage-treated soils and their mixtures showed high nitrogen and

carbohydrate content as well as high basal respiration and fatty acid content, suggesting that they provided readily biodegradable organic matter. In contrast, greenhouse compost-treated soils showed high total organic carbon and polyphenol content, whereas garden compost-treated soils showed intermediate values. The biological soil properties of both composts showed were similar to those of the reference soils, suggesting that composts contained more resilient organic matter. Organic amendments of dissimilar origin caused significantly different fungal soil communities at the genus level among the restored soils. Results indicated that soil pH, electrical conductivity, total nitrogen content, soil basal respiration, fungi/bacteria-PLFA ratio, and dehydrogenase and β-glucosidase activities, together with Pearson's correlations, revealed that these properties and nutrient content (total organic carbon, C/N ratio, carbohydrates, and polyphenols) influenced 40 soil fungal taxa. Therefore, the organic amendments led to changes in soil properties that favoured plant cover by promoting the soil fungal community growth beneficial to the carbon cycle and symbiotic with plants.

Keywords: Degraded area, Fungal community, Limestone quarry, Microbial activity, Organic amendments, Soil restoration.

1. Introduction

Mining activity has induced the degradation of 800,000 km^2 globally (Cherlet et al., 2018), severely affecting soil quality and significantly reducing the abundance and diversity of soil biota (Ohsowski et al., 2012). In particular, opencast mining presents a major ecological threat, because it removes the soil, changing its physical, chemical, biochemical, and biological soil properties and thus altering the plant community (García-Ávalos et al., 2018; Luna et al., 2017; Song et al., 2020; Soria et al., 2021a) by directly disturbing the microbiological communities that inhabit it (Chen et al., 2020; Rodríguez-Berbel et al., 2021, 2020). Degraded ecosystems, especially those located in arid and semiarid regions, require ecological restoration for their recovery, given that the removal of the organic substrate together with adverse climatic conditions (water stress, high temperatures, erosion, etc.; Luna et al., 2016; Miralles et al., 2012) and the low organic C content that characterises these soils (Bastida et al., 2016; Pascual et al., 1997) cause changes in physical, chemical, and biological soil properties that hinder the natural regeneration of plant cover and microbiota in these soils (Ferrol et al., 2004; Ricks and Koide, 2019).

The success of a restoration depends on the integration of strategies aimed at optimising the interactions between soil components and improving soil quality (Requena et al., 2001). Many authors have proposed the potential application of organic amendments for restoring of semiarid soils because such amendments help improve physical, chemical, biochemical, and biological soil properties (Almendro-Candel et al., 2014; Luna et al., 2016b; Peñaranda Barba et al., 2020; Ros et al., 2003). Moreover, the use of these remains could provide an alternative to the accumulation and/or incineration of biodegradable waste (Almendro-Candel et al., 2014; Debosz et al., 2002), thus contributing to the circular economy through resource conservation and waste recycling (Hueso-González et al., 2018). Differences in the nature of the organic amendments provides different characteristics in the organic materials that compose them (labile or resilient C), which, together with the environmental parameters (temperature, humidity, climate, etc.), determines the availability of nutrients (C, N, and P) in the soils (Bastida et al., 2017, 2008; González-Ubierna et al., 2012; Pérez-Gimeno et al., 2019; Ros et al., 2003). The microbial community's efficiency in adapting to resource consumption (J. Guo et al., 2018) could have a differential effect on soil microbiota by influencing the microbial use of C contained in these materials (Martens, 2000), which could generate changes in the development of some microbial taxa versus others better adapted to the applied amendment (Bastida et al., 2008). However, the changes produced in soil properties (physical, chemical, nutrient content, etc.) following the application of organic amendments and their impact on soil microbiological communities have not yet been fully explored (Li and Wu, 2018; Paula et al., 2020).

Soil microbial diversity is critical for maintaining soil fertility and functionality (Xue et al., 2017), as microorganisms regulate most soil biological processes (Nannipieri et al., 2017) and biogeochemical cycles (Fierer, 2017; Li and Wu, 2018). Several authors have pointed out the soil microbial community as an important indicator of soil quality (Xue et al., 2020; Zak et al., 2003). The addition of organic amendments induces microorganisms to respond differently to changes in edaphic properties (pH, EC, nutrients, etc.) as well as in basal respiration, fatty acid profile and enzymatic activities (Bastida et al., 2016, 2008; Dick, 1997; Torres et al., 2016). Most existing studies focus on the behavioural response of bacterial communities to these perturbations (Lauber et al., 2009; Zhao et al., 2021), whereas fungal community behaviour has been poorly monitored in restored ecosystems (J. Guo et al., 2018; Hart et al., 2019). Fungi are key

links in the physical, chemical and biological soil properties (Hart et al., 2019), as they participate in nutrient cycling, organic matter (OM) decomposition and benefit the development of vegetation cover (Boer et al., 2005; Gil-Martínez et al., 2021; Yang et al., 2019). The study of the soil fungal community is of great interest, given that fungi are considered generalist organisms capable of degrading recalcitrant OM (Paula et al., 2020) participating in carbon recycling (Kabel et al., 2020) and allowing microbial succession to exist (Paterson et al., 2008). Therefore, the consideration of soil fungal communities is an important aspect for the successful restoration of a limestone extraction mine in a semiarid climate.

Previous studies performed 6 months after the application of organic amendments in the same experimental plots revealed that sewage-treated soils showed higher rates of microbial activity (priming effect (PE), basal respiration (BR), and enzymatic activity) indicating a rapid consumption of labile OM, whereas in soils treated with both vegetable compost the microbial activity was lower, indicating, through the soil microbial diversity, an OM more difficult to degrade (Rodríguez-Berbel et al., 2021; Soria et al., 2021a, 2021b, 2020). For this reason, this work describes the study of growth of soil fungal communities in restored soils using amendments from different types of organic wastes compared with surrounding undisturbed reference soils and unrestored soils after 18 months after the restoration of a limestone quarry in a semiarid climate. For this purpose, the following were studied: i) the effect of the application of organic amendments on the physico-chemical and biological soil properties of the restored, control, and natural soils; ii) diversity and composition at the lowest possible classification level of fungal communities in the different soils; iii) determination of the most influential factors in the proliferation of soil fungal taxa; iv) identification of relevant soil fungal taxa in the soils considered; and v) relationships between the physico-chemical and biological soil properties and the soil fungal taxa previously identified. We hypothesised that fungal communities at phylum and genus level would respond differently to each type of treatment applied according to changes in physico-chemical and biological soil properties and in the chemical nature of the C added. Furthermore, we hypothesised that the addition of amendments would accelerate the recovery of soil fungal communities of restored soils towards reference conditions compared with experimental plots that received any treatment. We expect that as communities progress through succession (Morriën et al.,

2017), long-term increases in soil microbial biomass and microbiological activity would occur (Debosz et al., 2002).

2. Material and methods

2.1. Study design and sampling

The analyses of this field experiment were taken in December 2019 after a year and a half of restoration (beginning July 2018) in a limestone quarry in the Sierra de Gádor in the province of Almería (SE Spain, 36°55′20″N, 2°30′29″W) in a semiarid climate. The average annual rainfall, mainly occurring in winter and autumn, is 242 mm $yr^{-1,}$ and the mean annual temperature is 17.6ºC. In areas adjacent to the study area that have not been disturbed, shallow soils overlying limestones and dolomites are found with calcareous sandstones and marly, as well as loamy marls that form Regosols (FAO-IUSS-ISRIC Working Group WRB, 2015). The substrate in the experimental study area consists predominantly of calcareous sandstones overlaid on partially extracted marls. In the study area, the native vegetation is composed principally of grassland populated by *Stipa tenacissima* (L.) Kunth., *Anthyllis terniflora* (Lag) Pau. and *A. cytisoides* L., among other species. More information about the study area is found in Luna et al. (2016) and Soria et al. (2021a).

Fifteen experimental plots of 50 m² (10 m × 5 m) each were installed on a flat site at 362 m.a.s.l. fully exploited by mining activity. Before installation, heavy machinery was used to homogenise and decompact the soil. To compare the effect of organic amendments with different chemical composition on restored soils, different organic amendments were selected and applied to these experimental plots (3 replicates per treatment) for the soil restoration process. The composts from different plant residues delivered resilient soil OM with a greater contribution of lignin (Argyropoulos and Menachem, 1997) and humic-type polymers (Stevenson, 1994). Stabilised sewage sludge was employed because it contained a higher amount of labile OM constituted principally of proteins, free carbohydrates and a large amount of condensed lipids (Almendros et al., 2000, 1990). Last, mixtures of amendments were combined with the aim to obtain a balanced chemical composition between resilient and labile of OM and to replace nitrogen losses during the composting process with the extra N supplied by the sewage sludge (Shou et al., 2019). The treatments applied were arranged as follows: a) 100% vegetable compost derived from garden waste (CG); b) vegetable compost derived from

greenhouse crop residues (CC); c) sewage sludge treated with anaerobic mesophilic digestion, dehydrated by spin and thermally dried at 70ºC (SS); d) mix equal to CG + SS (Mix1); and e) mix equal to CC + SS (Mix2). The amount of each organic amendment applied was estimated to increase the initial soil OM content to 3% in each plot. Then, the organic residues were spread over the soil surface with a shovel backhoe (1 m^3) and mixed with the first 20 cm of mining degraded soils with a bulldozer. Additionally, natural reference soils (NAT) near the experimental plots were taken as reference soils.

After installation and application of the organic amendments in the experimental plots, two Mediterranean native species (40 plants of *Stipa tenacissima* L. and 10 plants of *Olea europaea* L. var. *sylvestris* Brot.) with high survival rates in previous ecological restorations performed in the study site were selected (Luna et al., 2017). These species were planted by hand, at the distance of 100 cm between plants, from forest pot seedlings (50 plants in total per plot). At the time of planting, a stabilisation irrigation was carried out because of the climatic conditions of the study area and the low rainfall rate. This practice had been used in previous ecological restorations in the same quarry, resulting in a high survival rate of *Stipa tenacissima* L. (Luna et al., 2017). A more detailed description of the construction of experimental plots can be found in Rodríguez-Berbel et al. (2021). After 18 months of organic amendment application, composite soil samples (mixing 10 subsamples) were collected at random from each experimental plot to a depth of 10 cm to study the changes in physico-chemical and biological soil properties and soil fungal communities over the medium term in restored areas of open mines in semiarid climate. Isothermal bags were used to transport a total of 21 soil samples (3 replicates per treatment) to the laboratory. The samples were air-dried, homogenised, sieved (ø 2 mm) and stored at 4ºC to analyse different physico-chemical parameters of the soil. A portion of this dried soil was stored at -20°C for DNA extraction and next generation sequencing (NGS) analysis.

The evolution of vegetation cover in the experimental area was studied by conducting three different sampling campaigns at 6 months (November 2018; C1), 12 months (June 2019; C2) and 24 months (June 2020; C3) after restoration. The percentage of total vegetation cover area occupied by wild and spontaneous plant colonisation was recorded in each experimental plot (n = 18), as well as the identification of spontaneous vegetation.

2.2. Physical and chemical soil properties

Different physico-chemical soil parameters were studied: i) soil pH was determined on a soil water suspension (1:2.5 soil/water ratio) (Thomas, 1996) with a pHmeter (LAQUA PH1100, HORIBA, Tokio, Japan); ii) electrical conductivity (EC) was measured in an aqueous suspension 1:2.5 soil/water with a digital conductivity metre (LAQUA EC1100, ORIBA, Tokio, Japan); iii) total organic carbon (TOC) was determined by rectified method of Walkey and Black (1934) (Mingorance et al., 2007); iv) total nitrogen content (TN) was measured by total combustion (Vario Rapid N; Elementar, Hanau, Germany); v) C/N ratio was determined from TOC and TN; vi) carbohydrate content (CH) was quantified from a cold extraction (25ºC) on a soil suspension (1:10 soil/water ratio) for 1h under agitation using the anthrone–sulphuric acid method (Brink et al., 1960); and vii) polyphenol content (POL) were measured using Folin-Ciocalteau reagent by the Folin–Denis method (Ribéreau-Gayon and Gautheret, 1968). CH and POL absorbance measurements were performed with a spectrophotometer, Spectronic Helios Gamma UV-Vis (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.3. Soil basal respiration, fatty acids and enzymatic activity

The following biological soil properties were analysed: i) soil basal respiration (BR) was measured from 20 g of sample at 50% water holding capacity in 125 ml hermetically sealed vials that were incubated for 31 days (28ºC in darkness), and the CO_2 produced was periodically measured (24 h, 48 h and 72 h and then each 4 days) using an infrared gas analyser (CheckmateII; PBI Dansensor, Ringsted, Denmark) (Soria et al., 2021a); and ii) ester-linked fatty acid methyl esters (FAMEs), hereafter fatty acids, were extracted from 3 g of soil (Schutter and Dick, 2000). Fatty acids were analysed with a Trace Ultra, Thermo Scientific gas chromatograph fitted with a 60 m capillary column (SGE Analytical Science, BPX70, 60 m x 0.25 mm ID x 0.25 µm film) using helium as the carrier gas. Conditions were as follows: i) initial temperature of 120°C for 30s, increased to 140 °C with a ramp of 1 °C/min, then to 170 °C with increments of 2 °C/min, and finally to 210 °C at 2 °C/min; ii) the fatty acids i15:0, 15:0, a15:0, i16:0, i17:0, 16:1ω9, cy17:0, cy19:0, 10Me16:0, and 10Me18:0 were accounting for the bacterial biomass (B-PLFA; Dungait et al., 2011; Frostegård et al., 1993); iii) the phospholipid fatty acids 18:2ω6 were predictors of the fungal biomass (F-PLFA; Bastida et al., 2019; Rinnan and Bååth, 2009); iv) fungus/bacteria ratio calculated with the two previous ones

(F/B-PLFA ratio); v) the fatty acids 15:1, 16;1ω7, 15:1ω6, 16:1ω5, 17:1, 18:1ω9c, 18:1ω7 and 18:1ω9t represent monounsaturated fatty acid (M-PLFA); vi) the fatty acids 14:0, i15:0, a15:0, 15:0, i16:0, 16:0, i17:0, cy17:0, 17:0, 18:0, 20:0, 22:0 and 24:0 represent saturated fatty acids (S-PLFA); and, vii) The ratio of M-PLFA-to-S-PLFA is expressed as M/S-PLFA ratio.

Similarly, the enzymatic activities were studied using 1 g of sample as follows: i) Dehydrogenase activity (DHA) was analysed (García et al., 1997); ii) β-glucosidase activity and iii) urease activity were determined according to published methods (Eivazi and Tabatabai, 1988; Kandeler and Gerber, 1988).

2.4. DNA isolation, PCR amplification, sequencing and bioinformatics analysis

Microbial DNA was extracted from 0.3 g of soil sample using a PowerSoil DNA Isolation kit (QIAGEN Inc., Germany), following the manufacturer's instructions. A ND-2000 Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to quantify the DNA concentration (ng/µl). To characterise fungal community composition, fungal *ITS* genes were amplified and sequenced by polymerase chain reaction (PCR) using the primer pairs ITS86F/ITS4 (Sommermann et al., 2018). To verify no contamination throughout the DNA extraction, one blank control was added using one of the kit tubes. Also, negative controls were tested (1 for every 96-well plate = 4 per MiSeq run) and checked to be clean (no bands present) and still sequenced on the MiSeq to show that no substantial reads were generated on this barcode combination.

The final sequence files were then processed using QIIME2 software (version 19.10) (Bolyen et al., 2018) following the protocol on the Microbiome Helper website (Amplicon SOP v2 [qiime2 2019.7]; https://github.com/LangilleLab/microbiome_helper/wiki (Comeau et al., 2017)). Taxonomy assignments of fungal phylotypes were performed in reference to the UNITE database (version 7). Diversity statistical measures were calculated in QIIME2 using the ASV table normalised to 10,000 sequences per sample. This procedure provides as a result an abundance table with taxonomy information, which was then analysed and visualised using the online web tool Calypso (Zakrzewski et al., 2017). From this tool, the relative abundance of fungal taxa was calculated, and those fungi with an abundance greater than 0.1% in each type of organic amendment (n = 3) and in all samples (n = 21) were selected.

2.5. Statistical analysis

Statistical differences on physico-chemical and biological soil parameters of each experimental plot (CG, Mix1, SS, Mix2, CC and NAT) were analysed by one-way univariable and multivariate permutational analysis of variance (PERANOVA and PERMANOVA (Anderson, 2001), with 9999 perms, $p < 0.05$), using Euclidean and Bray–Curtis distance similarity matrices, respectively. A pairwise test comparison by permutation was performed to construct a multivariate analogue of the *t* test and the probability levels of differences between groups (Eldridge et al., 2016); a Monte-Carlo test was performed when the number of free permutations was less than 100.

Relationships between changes in the fungal community structure and individual soil characteristics were performed by distance-based linear modelling (DistLM; Anderson et al., 2008), confronting the relative abundance of soil fungal taxa, first, with the physico-chemical soil variables and, second, with the biological soil variables analysed. Last, distance-based redundancy analyses (dbRDA) (based on the Bray–Curtis dissimilarity) were used to estimate the relative abundance of fungal taxa caused by each of the selected soil properties, and marginal tests were performed to test the significance of each test (McArdle and Anderson, 2001). The Akaike Information Criterion (AICc) criterion was used for selecting the best model, and the stepwise approach for building the model was followed (Hirotugu Akaike, 1974). The statistical package PRIMER6 + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) was performed for PERMANOVA, PERANOVA and dbRDA analysis.

R Project environment (R Core Team, 2018) with 'corrplot' and 'RColorBrewer' packages by Pearson's correlation (r; $p < 0.05$) was used to generate a heatmap plot to assess the relationships between soil properties and identified soil fungal taxa. The linear discriminant analysis (LDA) effect size (LEfSe) method by Calypso was utilised to identify soil fungal taxa specific to each restoration treatment and natural soils.

3. Results

3.1. Physico-chemical soil parameters

Organic amendments improved the physico-chemical conditions of the restored soils (CG, SS, CC, Mix1 and Mix2), increasing nutrient content (TOC, TN, CH and POL) and salinity (EC) and decreasing soil pH compared to unrestored soils (CON; Table 1).

SS-treated soils followed by their mixtures (Mix1 and Mix2) showed significantly higher ($p < 0.05$) EC, TN and CH and lower soil pH and C/N ratio than unamended soils (CON) and reference soils (NAT; Table 1). Soils treated with greenhouse compost (CC) showed significantly higher values ($p < 0.05$) of TOC and POL than CON and reference soils while showing no significant differences in soil pH with the previous ones (Table 1). The CON and reference soils had significantly lower values of TOC, TN, CH and POL and higher C/N ratio values, while the CH and POL content in CON soils was zero (Table 1). The CG-treated soils presented intermediate values between the rest of the restored soils and the reference soils (CON and NAT) for most of the soil properties studied (Table 1).

Table 1. *Physico-chemical soil properties of restored and undisturbed natural soils (mean ± SEM [n = 3]). Different letters indicate statistical differences for each treatment (p < 0.05; one-way PERANOVA).*

	CON	CG	Mix1	SS	Mix2	CC	NAT
pH	8.67 ± 0.06 b	7.99 ± 0.09 a	8.07 ± 0.09 a	7.88 ± 0.06 a	7.97 ± 0.08 a	8.87 ± 0.16 b	8.70 ± 0.00 b
EC (mS/cm)	1.64 ± 0.35 b	2.23 ± 0.43 ab	2.75 ± 0.68 ab	3.24 ± 0.04 a	2.6 ± 0.42 ab	1.75 ± 0.35 b	0.08 ± 0.00 c
TOC (%)	0.81 ± 0.16 d	2.65 ± 0.03 a	2.46 ± 0.39 abc	3.08 ± 0.20 ab	2.90 ± 0.21 ab	3.37 ± 0.17 b	1.81 ± 0.14 c
TN (%)	0.06 ± 0.00 c	0.38 ± 0.00 a	0.42 ± 0.04 ab	0.52 ± 0.02 b	0.42 ± 0.05 ab	0.40 ± 0.01 a	0.15 ± 0.01 d
C/N ratio	12.8 ± 2.62 cd	6.97 ± 0.17 a	5.76 ± 0.69 ab	5.90 ± 0.25 b	6.87 ± 0.29 ab	8.40 ± 0.22 c	11.6 ± 0.43 d
CH (μg g^{-1})	0 ± 0 c	470.68 ± 90.7 a	566.20 ± 248. ab	770.13 ± 93.2 a	338.75 ± 115. ab	156.74 ± 20.8 b	38.12 ± 4.75 d
POL (μg g^{-1})	0 ± 0 d	20.05 ± 0.74 ac	24.42 ± 5.74 ab	26.36 ± 8.80 abc	26.98 ± 4.71 ab	35.78 ± 5.32 b	6.198 ± 3.25 c

CON: unrestored soils; CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; C/N ratio: carbon to nitrogen ratio; CH: carbohydrates content; POL: polyphenols content.

3.2. Biological soil characteristics

After 18 months of organic amendment application, the biological soil properties (BR and PLFA) and enzymatic activities (DHA, β-glucosidase and urease) of the restored soils (CG, SS, CC, Mix1 and Mix2) increased with respect to the control soils (CON), approaching the values of the reference soils (NAT; Table 2). CON soil showing significantly lower values ($p < 0.05$) for most of parameters analysed (BR, F-, B-, S- and M-PLFA, DHA and β-glucosidase activities; Table 2). BR values were significantly higher ($p < 0.05$) in SS-treated soils followed by their mixtures with compost (Mix1 and Mix2; Table 2). Soils treated with CG and CC showed no significant differences ($p < 0.05$) in BR with reference soils, showing intermediate values between CON and soils treated with SS (SS, Mix1 and Mix2; Table 2). The F-PLFA content of the SS-treated soils was similar to that shown by reference soils, while CG-treated soils exhibited

significantly lower values ($p < 0.05$) than the previous and significantly higher ($p < 0.05$) than the CON soils. B-PLFA content was significantly higher ($p < 0.05$) in SS soils, while their mixtures (Mix1 and Mix2) and CC-treated soils did not show significant differences with reference soils (Table 2). On the other hand, the F/B-PLFA ratio was higher in reference soils followed by CC soils, with the mixtures and the CON soils presenting intermediate values between the previous whereas SS and CG soils showed the lowest values (Table 2). The content of S- and M-PLFAs was significantly higher ($p < 0.05$) in SS soils than in the rest of the soils (restored, natural and unrestored soils), whereas the ratio between the above (S/M-PLFA ratio) did not show significant differences between restored, CON, and reference soils, despite showing the highest values in SS and Mix1 soils (Table 2).

As for enzyme activity (DHA, β-glucosidase and urease), restored soils showed intermediate rates between CON soils (significantly lower; $p < 0.05$) and reference soils (higher values), except for DHA activity, where CC-treated soils showed the highest significant values ($p < 0.05$), followed by reference and SS-treated soils (Table 2). β -glucosidase and urease activities rates were significantly higher ($p < 0.05$) in reference soils (NAT), followed by SS and Mix2 soils (Table 2).

3.3. Fungal soil diversity and community composition

3.3.1. Fungal taxa in organic amendments before application

From taxonomic analysis at phylum level of the organic amendments previous to their application, only Ascomycota was identified at around 10% in each amendment (CG_0, CC_0 and SS_0), whereas at the genus or the next available higher taxonomic level, seven fungal taxa were identified. In the CG amendment, *uncultured* (Class: Eurotiomycetes) and *Phialosimplex* were the most abundant fungal taxa, whereas in the SS amendment, the most abundant were *Microascus* and *uncultured* (Order: Eurotiales). Lastly, in the CC amendment, the most abundant fungal taxa were *uncultured* (Fam: Plectosphaerellaceae) and *Sodiomyces* (Table S2).

Table 2. *Soil basal respiration, fatty acids profile and enzyme activities in restored soils with organic amendments and natural reference soils (average ± SEM [n = 3]). Different letters indicate statistical differences for each treatment (p < 0.05; one-way PERANOVA).*

	CON	CG	Mix1	SS	Mix2	CC	NAT
BR (mg C-CO2 kg^{-1} soil day)	0.57 ± 0.33 a	3.61 ± 2.08 b	12.0 ± 6.95 d	17.0 ± 9.82 d	13.1 ± 7.59 d	5.93 ± 3.42 c	3.71 ± 2.14 bc
Fatty acids profile							
F-PLFA (nmol g^{-1} soil)	2.10 ± 1.21 a	10.30 ± 5.95 b	40.52 ± 23.39 c	44.48 ± 25.68 c	30.27 ± 17.47 c	27.16 ± 15.68 c	41.10 ± 23.73 c
B-PLFA (nmol g^{-1} soil)	19.14 ± 11.05 a	106.61 ± 61.55 b	340.77 ± 196.74 cd	466.54 ± 269.36 d	265.27 ± 153.15 c	175.04 ± 101.06 c	201.28 ± 116.21 c
F/B-PLFA ratio (nmol g^{-1} soil)	0.11 ± 0.06 abc	0.09 ± 0.05 ab	0.11 ± 0.06 ac	0.09 ± 0.05 b	0.11 ± 0.06 abc	0.15 ± 0.08 abc	0.20 ± 0.11 c
S-PLFA (nmol g^{-1} soil)	17.40 ± 10.05 a	120.53 ± 69.58 b	339.23 ± 195.85 cd	552.87 ± 319.20 c	272.80 ± 157.50 d	158.62 ± 91.58 b	193.16 ± 111.52 d
M-PLFA (nmol g^{-1} soil)	10.64 ± 6.14 a	54.80 ± 31.64 b	166.53 ± 96.15 bcde	206.3 ± 119.10 c	130.2 ± 75.17 de	101.6 ± 58.69 d	106.7 ± 61.65 e
S/M-PLFA ratio (nmol g^{-1} soil)	1.64 ± 0.94 a	2.18 ± 1.26 a	2.24 ± 1.29 a	2.67 ± 1.54 a	2.12 ± 1.22 a	1.68 ± 0.97 a	1.83 ± 1.06 a
Enzyme activities							
DHA (µmol INTF g^{-1} soil h^{-1})	0.04 ± 0.02 e	0.22 ± 0.13 a	0.34 ± 0.20 ad	0.65 ± 0.37 bc	0.52 ± 0.30 cd	0.94 ± 0.54 bc	0.87 ± 0.50 b
β-glucosidase (µmol PNF g^{-1} soil h^{-1})	0.02 ± 0.01 c	0.15 ± 0.08 a	0.23 ± 0.13 ab	0.35 ± 0.20 b	0.32 ± 0.18 b	0.28 ± 0.16 b	0.60 ± 0.35 d
Urease (µmol N-NH_4^+ g^{-1} soil h^{-1})	0.04 ± 0.02 a	0.24 ± 0.14 c	0.25 ± 0.14 ac	0.52 ± 0.30 abc	0.36 ± 0.21 c	0.45 ± 0.26 abc	1.34 ± 0.77 b

CON: unrestored soils; CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils. BR: basal soil respiration; F-PLFA: fatty acids of fungal biomass; B-PLFA: fatty acids of bacterial biomass; F/B ratio: ratio between fungus and bacteria fatty acids; S-PLFA: saturated fatty acids; M-PLFA: monosaturated fatty acids; S/M-PLFA ratio: ratio between saturated and monounsaturated fatty acids; DHA: dehydrogenase activity.

3.3.2. Richness and diversity indices in restored and natural soils

Restored soils (CG, SS, CC, Mix1 and Mix2) showed lower values for diversity indices with respect to reference soils (NAT; Table 3) but were higher than CON soils, given that the latter did not pass the quality parameters of the bioinformatic analysis, so they were excluded from the diversity analysis and the rest of the biological analysis (Table S1). CG-treated soils presented values significantly similar ($p < 0.05$) to those of reference soils in Pielou and Shannon indices, whereas in the richness of ASVs, they showed significantly higher values ($p < 0.05$) than the rest of the amended soils (Table 3). SS-treated soils showed lower values than the rest of the soils, being significantly lower ($p < 0.05$) for Shannon index (Table 3). The CC soils and mixtures (Mix1 and Mix2) showed intermediate values between the CG and SS soils, with Mix2 standing out in ASV richness and the CC and Mix1 soils in both diversity indices (Pielou and Shannon; Table 3).

Table 3. *Alpha-diversity analysis (average ± SEM; n = 3) of ASVs richness, Pielou, and Shannon indices of different soil types. Different letters indicate statistical differences for each treatment (p < 0.05; one-way PERANOVA).*

	CON	CG	Mix1	SS	Mix2	CC	NAT
ASVs	-	120 ± 8.95 a	81 ± 6.38 cd	75 ± 15.89 bc	83 ± 6.08 c	80 ± 12.99 bd	157 ± 7.96 e
Pielou	-	0.72 ± 0.03 abc	0.68 ± 0.02 ce	0.63 ± 0.02 d	0.63 ± 0.01 e	0.68 ± 0.02 b	0.76 ± 0.03 a
Shannon	-	5.00 ± 0.29 abc	4.32 ± 0.05 c	3.92 ± 0.32 bc	4.03 ± 0.13 b	4.31 ± 0.16 cd	5.55 ± 0.18 ad

CON: unrestored soils (no data obtained); CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

3.3.3. Fungal soil community in restored and natural soils

The soil fungal community did not show significant differences (PERMANOVA; $p < 0.05$) at phylum level between restored soils (CG, SS, CC, Mix1 and Mix2) but did differ significantly ($p < 0.05$) from reference soils (NAT; Figure S1). Four phyla (Ascomycota, Basidiomycota, Chytridiomycota and Mortierellomycota), along with one Unclassified phylum, were identified with an abundance greater than 0.1% of the total. The most abundant phylum in all soils (restored and reference soils) was Ascomycota, although it presented lower relative abundance in reference soils than in the rest of the treatments, while the Unclassified phylum was mainly recorded in reference soils (Figure S1).

Statistical analysis (PERMANOVA; $p < 0.05$) of the f soil fungal community at genus or the next available higher taxonomic level showed significant differences in

relative abundances between restored soils (CG, SS, CC, Mix1 and Mix2) and natural soils (NAT), whereas no difference was evident between SS-treated and Mix2 soils (Table S2). Fifty-seven genera were identified with a relative abundance higher than 0.1% of the total. In CG-treated soils, the most abundant soil fungal taxa were *Botryotrichum*, *uncultured* (Fam: Nectriaceae) and *Alternaria*, while in CC soils were *uncultured* (Fam: Microascaceae), *uncultured* (Fam: Plectosphaerellaceae) and *uncultured* (Fam: Nectriaceae) (Table S2). The SS soils had high relative abundances of the genera *Microascus*, *Gymnascella* and *Lophotrichus*, whereas in Mix1 the most abundant fungal genera were *Cephaliophora*, *Microascus*, *Botryotrichum* and *Acremonium*, and in the Mix2 soils the most abundant were *Microascus*, *uncultured* (Fam: Microascaceae) and *Acremonium* (Table S2). Finally, the most abundant fungal taxa in the reference soils (NAT) were Unclassified, *Exophiala* and *uncultured* (Class: Dothideomycetes) (Table S2).

3.4. Relationship between soil characteristics and fungal soil taxa

3.4.1. Fungal taxa and physico-chemical soil properties

Redundancy analysis in combination with sequential tests showed that the best linear distance model (R^2 = 0.61; AICc = 126) was explained by physico-chemical soil properties of pH, EC and TN, the crucial soil variables influencing the soil fungal community (Table S3). The first two components of the dbRDA axes explained 59.98% of the variation in the relationship between soil fungal composition and the physico-chemical parameters studied (Figure 1A). The dbRDA results clearly clustered two different groups: restored soils (CG, CC, SS, Mix1 and Mix2) and reference soils (NAT). Furthermore, it showed that the fungal community of the restored soils (CG, SS, Mix1 and Mix2) was related to TN content and salinity, whereas CC-treated soils were related to pH (Figure 1A). The community of reference soils was not related to any physico-chemical factors (Figure 1A).

3.4.2. Fungal taxa and soil basal respiration, PLFA, and enzyme activity

The relationship between soil fungal community and biological soil parameters and enzymatic activity was calculated by dbRDA analysis. Among the 10 biological variables and enzyme activities, sequential tests indicated that the best DistLM (R^2 = 0.68; AICc = 122.4) was explained by 69.52% of total variation and identified four parameters significant—BR, F/B-PLFA ratio, DHA and β-glucosidase—that were related to the soil

fungal community (Table S3 and Figure 1B). The dbRDA analysis clearly groups three different clusters: cluster 1, composed of SS, Mix1 and Mix2 samples, was related to BR, whereas cluster 2, consisting of CG and CC, was influenced by DHA; and finally, reference soils (cluster 3) were influenced by F/B-PLFA ratio and β-glucosidase activity (Figure 1B).

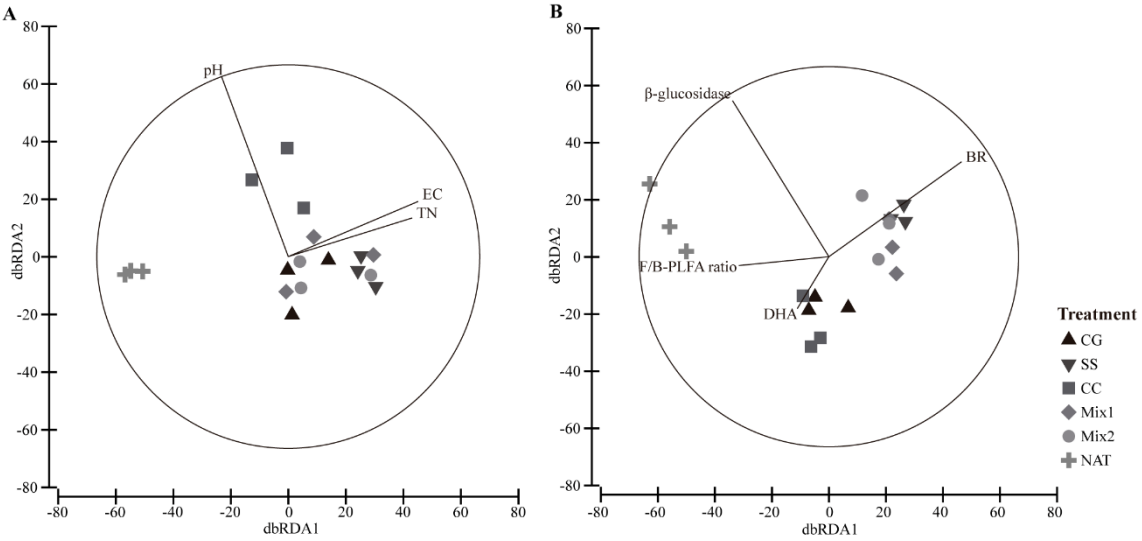


Fig. 1. *Redundancy analysis (dbRDA) showing the correlation among physico-chemical (A) and microbiological (B) soil properties (basal soil respiration, fatty acids profile and enzyme activities) and fungal soil community based on the relative abundance (%) of richness ASVs. Soil samples with compost from garden waste (CG), sewage sludge from wastewater (SS), compost from greenhouse crop residues (CC), Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge and natural reference soils (NAT) are indicated by different colours and figures.*

Footnotes: Soil characteristics are represented by continuous lines. EC: electrical conductivity; TN: total nitrogen; BR: soil basal respiration; F/B-PLFA ratio: ratio between fungi and bacteria fatty acids; DHA: dehydrogenase activity.

3.5. Identification of fungal taxa by LEfSe analysis

A linear discriminant analysis (LDA) effect size analysis (LEfSe) was performed to identify the different fungal taxa with LDA scores of > 3.5 (Figure 2). These taxa showed significant variation in their relative abundance in the soils studied (restored and natural soils). LEfSe analysis revealed that, compared with the total fungal communities (57 at genus level or the next available higher taxonomic level), 40 soil fungal taxa showed a higher sensitivity to the conditions of the six soil types analysed (CG, CC, SS, Mix1, Mix2 and NAT; Table S4). Specifically, LEfSe LDA results showed that CG-treated soils favoured the presence of *Botryotrichum*, *uncultured* (Fam: Nectriaceae), *Mycosphaerella*, *uncultured* (Fam: Chaetomiaceae), *Alfaria*, *Thermomyces* and *Neocamarosporium*, whereas CC soils favoured those of *uncultured* (Fam: Microascaceae), *uncultured* (Fam: Plectosphaerellaceae), *uncultured* (Fam:

Spizellomycetaceae), *Iodophanus* and *Stachybotrys* (Figure 2). Soils treated with sludge (SS) were associated with the genera *Gymnascella*, *Lophotrichus* and *Chrysosporium*. Mixtures of sludge and compost showed the genera *Cephaliophora* and *Arachniotus* in Mix1 (CG+SS), whereas in Mix2 (CC+SS) the taxa *Microascus, Acremonium*, *uncultured* (Fam: Gymnoascaceae), Kernia and *Arachnomyces* were present (Figure 2). The reference soils with the highest number of differentiated fungal taxa (18 out of 40 total soil fungal taxa) with LDA > 4 were *Exophiala*, *uncultured* (Class: Dothideomycetes), *Picoa*, *uncultured* (Order: Chaetothyriales), *uncultured* (Order: Sebacinales), *uncultured* (Fam: Pyronemataceae) and *uncultured* (Order: Pleosporales) with the Unclassified taxon having the highest LDA (5.3) (Figure 2).

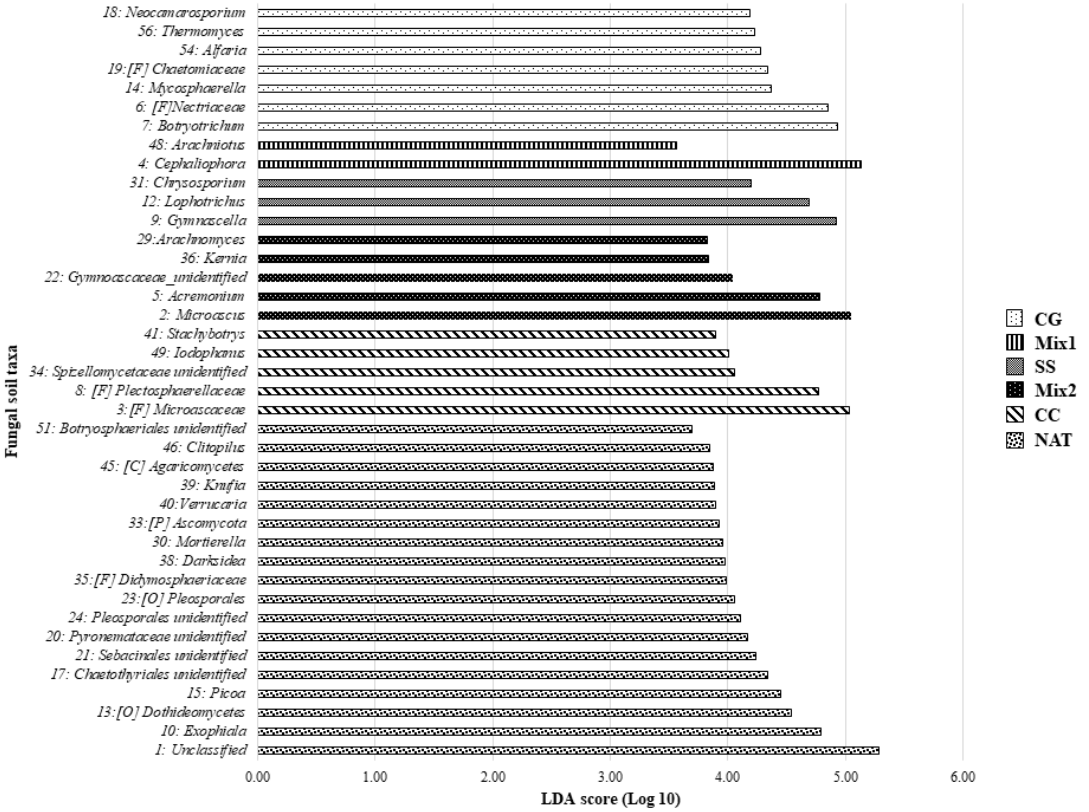


Fig. 2. *Liner discriminant analysis coupled with effect size (LEfSe) measures between different treatments (both composts, CG and CC; sewage sludge, SS; and mixtures, Mix1 and Mix2, among previous) and natural reference soils (NAT). Lineages with LDA values greater than 3.5 are shown.*

3.6. Correlations between soil parameters and fungal populations

Pearson's correlations of the 40 soil fungal taxa identified from LEfSe analysis with physico-chemical soil parameters showed that taxa influenced by soils treated with SS and Mix2 showed significantly ($p < 0.05$) positive correlations with the variables EC, TN and CH and significantly negative correlations with pH and C/N ratio (Figure 3A).

Taxa associated with CC soils showed significantly positive correlations with pH and TOC and CH content. Those associated with CG and Mix1 soils scarcely presented significant correlations with the physico-chemical soil properties. On the contrary, the soil fungal community associated with reference soils showed significantly positive ($p < 0.05$) values with C/N ratio and positive values with soil pH, and significantly negative correlations with EC properties and TOC, TN, CH and POL content (Figure 3A).

Additionally, Pearson's correlations of these soil fungal taxa and biological soil properties (BR, PLFA, and enzymatic activity) were analysed. It was observed that the taxa identified for the CG-treated soils presented negative correlations with all the parameters analysed, except for the F/B-PLFA ratio, which were positive. Soils treated with CC showed positive correlations with DHA activity and F/B-PLFA ratio and negative with the rest of the soil parameters. In contrast, the soil fungal taxa from SS-treated soils indicated significantly positive correlations ($p < 0.05$) with BR, B-, S- and M-PLFA and negative correlations with the F/B-PLFA ratio and the three enzyme activities studied. Both Mix1 and Mix2 showed positive correlations with BR, being significantly ($p < 0.05$) positive in Mix2, and with the parameters of F-, B-, S-, M- and the S/M-PLFA ratio and negative with the F/B-PLFA ratio and the enzymatic activities (DHA, β-glucosidase and urease). Finally, fungal taxa from reference soils showed significantly positive correlations with F/B-PLFA ratio and β-glucosidase and urease activities and positive correlations with fungi and DHA activity but negative correlations for all other parameters.

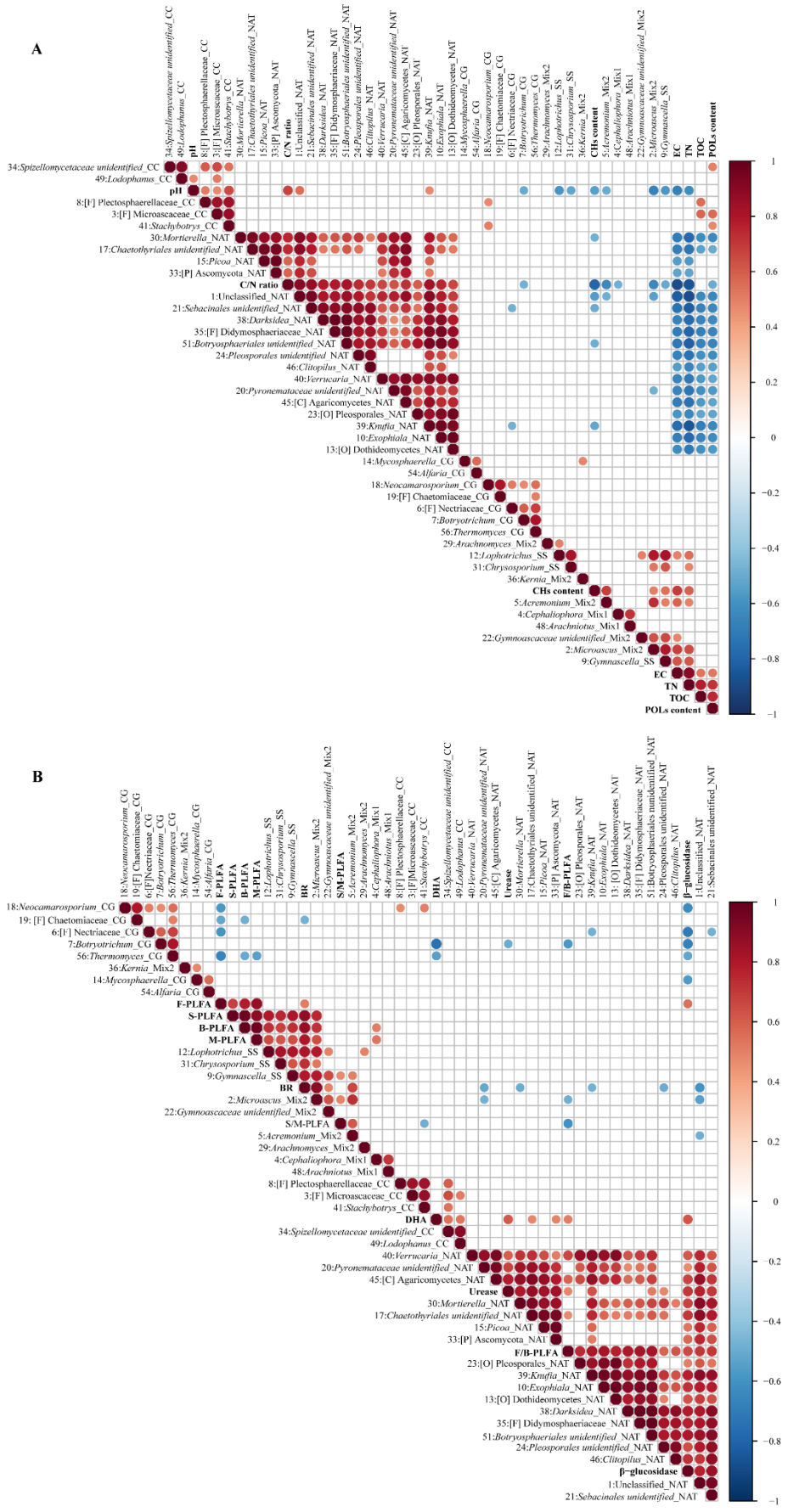


***Fig. 3**. Pearson correlation ($p < 0.05$) between physico-chemical (A) and microbiological soil characteristics (basal respiration, fatty acids and enzyme activities) (B) with 40 soil fungal taxa from LEfSe analysis.*

Footnotes: The different soil properties analysed are shown in bold. [C]: Fungal soil taxon identified to class level; [O]: Fungal soil taxon identified to order level; [F]: Fungal soil taxon identified to family level.

3.7. Plant vegetation assessment

The principal species that colonised the experimental area were *Anthyllis cytisoides* L., *Pistacia lentiscus* L., *Capparis spinosa* L., *Moricandia arvensis* L., *Atriplex halimus* L., *Limonium insigne* (Coss.) Kuntze, and *Artemisia barrelieri* Besser. Colonisation by wild plants increased considerably more over time in the restored plots than in the unrestored soils (CON; Figure S2). In C1, the percentage of total vegetation cover in the restored plots remained below 10%, except for the area with the CC-treatment, where the percentage of coverage was slightly higher. After 12 and 24 months of restoration (C2 and C3, respectively), the amended soils showed greater colonisation, with CG, CC and the mixtures (Mix1 and Mix2) standing out, while SS presented the lowest values. On the contrary, the CON soils presented similar values during the three campaigns (Figure S2).

4. Discussion

Our results demonstrated that the application of organic amendments improved the physico-chemical, biochemical and biological soil properties and allowed the recovery of plant cover in the first 18 months after the restoration. Several authors have indicated that diversity and structure of the soil microbial community are driven by the physico-chemical soil characteristics (Albornoz et al., 2016). Moreover, soil disturbances also affect the soil microbial composition (Tian et al., 2017). Results showed that the soil fungal communities were significantly different in the soils where several organic amendments with distinct origins were applied (Table S2). Physico-chemical soil properties (such as soil pH, salinity, TN content), biological parameters and enzymatic activities (such as BR, F/B-PLFA ratio, and DHA and β-glucosidase enzymatic activities) were the more important drives affecting soil fungal species (Figure 1).

Additionally, soil fungal communities were affected by the type of OM present in the amendments applied. As was previously stated by Soria et al. (2020), both vegetable composts (CG_0 and CC_0) applied in the restoration provided a more resilient OM, especially CG, which in terms of fungal diversity and BR was closest to the reference soils (Tables 2 and 3), suggesting the succession to a more mature fungal community capable of mineralising more resilient C fractions (Paterson et al., 2008). Conversely, the

SS_0 treatment provided more labile OM with high TN and CH content (Rodríguez-Berbel et al., 2021; Rocío Soria et al., 2021b), which benefited fast microbial growth (denoted by BR, B- and F-PLFA and, β-glucosidase and urease activities; Table 2) in the sludge-treated soils (SS, Mix1 and Mix2), causing a short-term PE (Soria et al., 2021).

However, the rapid mineralisation of labile C could be performed by the synergy among soil fungal and bacterial communities, as indicated by the high content of B-PLFA and the high BR (Table 2). Therefore, the chemical OM diversity provided by organic amendments from different origin could favour on one hand, fungal communities specialised in the use of specific substrates, and on the other hand, the excess multiple types of nutrients, does not promote the need for specialisation by substrate competitiveness (Allison et al., 2014; Nam et al., 2012).

The addition of organic amendments favoured the recovery of vegetation cover in the restored soils resulting in a greater proliferation of plants than in the control soils, which were practically devoid of them (Figure S2). Plants could have also favoured the proliferation and diversity of the fungal communities in the restored soils (Figure S1). For example, this proliferation could been due to the contribution of photosynthetic assimilates from vegetation to rhizospheric microorganisms (Frouz, 2021), leading to a renewal of soil biodiversity and the establishment of ecosystem functionality (Conesa et al., 2012).

Results of fungal diversity in this soils corroborated that the organic amendments allowed the specialisation and establishment of different soil fungal communities depending on the organic substrates provided to the restored soils (Soria et al., 2020) and those inherited from amendments. An example of the latter could be the incorporation into soils of fungi of the phylum Ascomycota. Many species of this phylum are involved in the decomposition of organic complex substrates (J. Guo et al., 2018; Liu et al., 2019) of plant biomass as well as in C and N cycles in arid ecosystems (Challacombe et al., 2019). This results in the increase of available recourses which can favour the emergence of other phyla, causing soil fungal taxa diversification, which can occupy more niches and reduce competition (Xiong et al., 2021).

The idea that OM decomposition from plants is taking place in these soils is reinforced, especially in CC-treated and reference soils, by the presence of the saprophytic phylum Basidiomycota (Curlevski et al., 2010), capable of metabolising plant

inputs rich in lignin (Blackwood et al., 2007). Indeed, CC-treated soils showed high values of TOC and POL, probably from decomposition of more resilient OM (Soria et al., 2020). Fungi can secrete hydrolytic enzymes capable of decomposing complex CH (Berlemont, 2017; Kabel et al., 2020), mineralising OM (Fontaine et al., 2003), and releasing nutrients (Hellequin et al., 2018), that can be used for the rest of microorganisms and for plants. The presence of more mature fungal taxa capable of degrading more resilient compounds is corroborated by the fact that after 18 months the restored soils showed values of biological soil properties such as BR, B-PLFA, F/B-PLFA ratio and DHA activity that were closer to those of the reference soils than to those described 6 months after the restoration by Rodríguez-Berbel et al. (2021).

In the restored plots, we found fungal taxa probably inherited by the initial organic amendments (CG_0, SS_0 and CC_0) and others common to the reference soils. For example, taxa such as *uncultured* (Fam: Plectosphaerellaceae) and *uncultured* (Fam: Chaetomiaceae) could be incorporated in soils from vegetable composts (CG and/or CC), whereas other taxa such as *Cephaliophora*, *Acremonium*, *Gymnascella* and *uncultured* (Fam: Microascaceae) were already present in reference soils (Table S2).

Statistical LEfSe analysis denoted indicator taxa for each treatment (Figure 2). The fungal taxon *uncultured* (Fam: Microascaceae) associated with the CC-treated soils could be indicative of an advanced stage of soil maturity because it has been observed in maturing stages of composting (Jiang et al., 2020; Klamer and Bååth, 2006). Another example of a CC-indicator was the genus *Stachybotrys*, described in arid soils (Zak and Wildman, 2004) and capable of producing cellulolytic enzymes as β-glucosidases (Amouri and Gargouri, 2006).

In relation to CG-taxa indicators, the Chaetomiaceae family was previously described as one of the main taxonomic groups that can help to differ untreated soils from organically treated soils (Banerjee et al., 2019). Thus, their presence in CG soils could indicate complex metabolisation of OM, especially in cellulose degradation (Paula et al., 2020). Interestingly, another CG-indicator was the genus *Neocamarosporium* (Figure 2), described as a leaf endophyte (Ricks and Koide, 2019) of halophytic plants (Gonçalves et al., 2019). Its presence could indicate that the spontaneous vegetation established in these plots (Figure S2) has favoured the fungal diversity of restored soils.

According to SS-indicator soil taxa, the genus *Gymnascella* was identified with significantly positive correlations with EC and TN (Figure 3a). This genus has species such as *G. dankaliensis* y *G. hyalospora* that have been identified as naturally inhabiting manure and soils (Abdel-Azeem et al., 2011), including saline and desert soils (Sybren de Hoog et al., 2005). Other species of this genus present an important biotechnological potential for obtaining pharmaceuticals (Nicoletti and Andolfi, 2018).

In soils where mixes of organic amendments were applied, the genus *Cephaliophora*, which has been described as a decomposer of cellulose (Asemaninejad et al., 2021), can be highlighted as a Mix1-indicator, whereas as a Mix2-indicator the genus *Acremonium* was described as decomposer and degrader of cellulose and xylan (Sun et al., 2016) and had significantly positive correlations with BR and CH (Figure 3b). Another Mix2-indicator taxa, the genus *Microascus*, was isolated from soils and decomposing plant material (Piñar et al., 2019). The increase in the relative abundance of fungal taxa indicators of mixed soils (Mix1 and Mix2) with respect to SS-treated soils (Table S2) could suggest that the combination of labile and recalcitrant OM from organic amendments would have benefited the development of these taxa.

Lastly, the natural soils used as reference showed the highest number of indicators fungal taxa (Figure 2). This could be explained because these ecosystems are balanced and taxa are in a steady state with a well-structured soil microbial community because soil fungi have the ability to adapt better to nutrient-poor soils by searching for heterogeneously distributed resources (Boer et al., 2005). Among NAT-indicators taxa, we found endophytic fungi such as order Botryosphaeriales and genus *Exophiala* (Singh et al., 2017; Zak and Wildman, 2004) or lichen-forming fungi such as genus *Verrucaria* (Sybren de Hoog et al., 2005). Another NAT-indicator taxon, class Agaricomycetes, characterised as saprotrophic and mycorrhizal fungi (Collins et al., 2018; Hart et al., 2019) was commonly described in semiarid soils (Bastida et al., 2013a; Tian et al., 2017).

Previous results showed that the fungal communities of the restored soils could be playing important functions, facilitating the biogeochemical cycles in these soils compared with the unrestored soils. In addition, it was also observed that the restoration favoured the transformation of OM. Interestingly, Soria et al. (2021b) noted that C losses to the atmosphere were low and that the experimental plots could act as CO_2 sinks.

5. Conclusions

Medium-term (18 months) additions of organic amendments influenced soil physico-chemical, biological and microbial variables, generating changes in soil microbial activities and nutrient use efficiency. These changes were correlated with a change in fungal community composition, which was driven principally by the chemical diversity that composes the OM. Organic amendments served mainly to support microbial growth in restored soils (e.g., *Stachybotrys* and *Acremonium* genera), which are associated with the decomposition of complex OM and soil nutrient transformations, and then enhanced soil fertility for soils degraded by mining activity. The presence of fungi in the restored plots, some common in natural soils, suggests that ecological succession has occurred in these soils approaching the quality status of natural soils in the environment, whereas in the control soils fungi have not proliferated despite the time elapsed since degradation, suggesting that these soils alone have not been able to recover in the medium term from the impact of mining. These results corroborate that restoration treatments were useful for the recovery of soil biological quality, although the lower fungal diversity in restored versus natural soils also suggests that the status of natural soils 18 months after restoration has not been achieved. However, the inherent mechanisms require better understanding through future research aimed at determining a direct link between enzymatic activities and the responsible fungal taxa in soils, and metatranscriptome/metaproteome analyses should provide promising ways to obtain direct proof of the claims presented in this study.

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3. Rodríguez-Berbel et al. 2020. Journal of Environmental Management.

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Resumen:

La aplicación de enmiendas orgánicas para mejorar las propiedades químicas y biológicas de suelos degradados de canteras calcáreas es necesaria para acelerar los procesos de restauración. El objetivo de este estudio es evaluar el éxito de diferentes tratamientos de restauración a largo plazo utilizando dos enmiendas orgánicas (lodos de depuradora de aguas residuales urbanas (SS) y compost de residuos sólidos domésticos (CW)). Las propiedades químicas y las comunidades bacterianas de los suelos restaurados se compararon con las de los suelos no enmendados (NA) y los suelos naturales circundantes (NS) de una cantera de piedra caliza en un ecosistema semiárido. Tras 10 años de la adición de enmiendas orgánicas, se analizó la abundancia de bacterias del suelo, la diversidad y la composición taxonómica a nivel de filo y género en cada tipo de suelo mediante amplificación ARNr 16S (PCR), secuenciación utilizando Illumina y comparación con la base de datos SILVA utilizando el software QIIME2. También se estudiaron las relaciones entre los taxones bacterianos del suelo y las propiedades químicas del suelo (pH, conductividad eléctrica (CE), carbono orgánico total (TOC), y contenido total de nitrógeno (TN)), así como las interrelaciones entre los taxones bacterianos del suelo a nivel de género o el siguiente nivel taxonómico superior identificado. Las enmiendas orgánicas modificaron las propiedades químicas de los suelos restaurados, influyendo en las comunidades microbianas de los mismos. El tratamiento con CW fue la enmienda orgánica que más se asemejó al NS, favoreciendo a largo plazo una mayor diversidad y proliferación de bacterias. Varias comunidades bacterianas, más abundantes en los suelos NA y CW, estaban fuertemente correlacionadas entre sí (*Craurococcus*, *Phaselicystis*, *Crossiella*, etc.), formando un patrón de co-ocurrencia bacteriana (Patrón de co-ocurrencia 1). Estas bacterias mostraron correlaciones positivas significativas con el TOC, TN y EC y correlaciones negativas con el pH del suelo. En contraste, los suelos NA presentaron otros grupos de comunidades bacterianas (Patrón de co-ocurrencia 2) representados por *Sphingomonas*, *Rubellimicrobium*, *Noviherbaspirillum*, *Psychroglaciecola* y *Caenimonas*, que mostraron altas correlaciones positivas significativas con el pH del suelo y correlaciones negativas con el TOC, TN y CE. El análisis de redundancia basado en la distancia indicó que los suelos SS permanecían en un estado intermedio de calidad química y biológica entre los suelos NS y NA. Nuestros resultados demuestran que las propiedades químicas del suelo y las comunidades bacterianas del suelo cambiaron significativamente con las enmiendas orgánicas en suelos calcáreos mediterráneos degradados por la minería.

Palabras clave: Restauración del suelo; Metagenómica; Diversidad bacteriana; Degradación del suelo; Patrones de co-ocurrencia bacteriana en el suelo; Ecosistemas semiáridos.

Material suplementario disponible en ANEXO I y en https://doi.org/10.1016/j.jenvman.2020.110920.

Long-term effects of organic amendments on bacterial communities of calcareous mediterranean soils degraded by mining

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Abstract

The application of organic amendments to improve the chemical and biological properties of degraded soils from calcareous quarries is necessary to accelerate restoration processes. The aim of this study is to assess the success of different restoration treatments in the long-term using two organic amendments (sewage sludge from urban waste water (SS) and compost from domestic solid waste (CW)). The chemical properties and bacterial communities of restored soils were compared with unamended soils (NA) and surrounding natural soils (NS) from a limestone quarry in a semi-arid ecosystem. After 10 years of the addition of organic amendments, the abundance of soil bacteria, diversity, and taxonomic composition at the phylum and genus level in each soil type was analysed by rRNA 16S amplification (PCR), sequencing using Illumina, and comparison with the SILVA database using QIIME2 software. The relationships between bacterial soil taxa and chemical soil properties (pH, electrical conductivity (EC), total organic carbon (TOC), and total nitrogen content (TN)) were also studied, as well as the interrelations between bacterial groups at the lowest classification level (subgroup to genus). The organic amendments changed the chemical properties of the restored soils, influencing the microbial communities of the restored soils. CW treatment was the organic amendment that most resembled NS, favouring in the long term a greater diversity and proliferation of bacteria. Several bacterial communities, more abundant in NA and CW soils, were strongly correlated with each other (*Craurococcus*, *Phaselicystis*, *Crossiella*, etc.), forming a bacterial consortium (Consortium 1). Those bacteria showed high significant positive correlations with TOC, TN, and EC and negative correlations with the soil pH. In contrast, NA soils presented other groups of bacterial communities (Consortium 2) represented by *Sphingomonas*, *Rubellimicrobium*, *Noviherbaspirillum*, *Psychroglaciecola*, *Caenimonas*, and *WD2101*, which showed high significant positive correlations with soil pH and negative correlations with TOC, TN, and EC. Principal component analysis indicated that SS soils remained in an intermediate stage of chemical

and biological quality between NS and NA soils. The results showed that the soil bacterial communities could be excellent biomarkers reporting the success of the restoration and the extent to which the restored soils present similar bacterial communities to the surrounding natural soils.

Keywords: Soil restoration, metagenomics, bacterial diversity, soil degradation, soil bacterial consortia, semiarid.

1. Introduction

Soil is of vital importance in the biosphere, performing multiple functions necessary for life on the planet, such as maintaining plant and animal productivity, conserving water and air quality, and improving human health (Singer and Sojka, 2002). Soil degradation caused by the indiscriminate exploitation of natural resources affects large areas of the world, leading to the loss of soil quality and the reduction of soil productivity through the loss of nutrients or unfavourable changes in physical, chemical, and biological soil properties. The problem of environmental degradation is aggravated in semi-arid climate and, in particular, in the Mediterranean ecosystems, due to scarce and torrential rainfall, high solar radiation, and low plant cover that favours the erosion processes (Bastida et al., 2007b; Bruneel et al., 2019; Hueso et al., 2011; Miralles et al., 2009). In these ecosystems the practice of mining, especially the opencast type, causes the total loss of the soil and plant cover, which disturbs the soil–plant stability (Luna et al., 2016a), producing the leaching of nutrients by erosion and reducing the fertility of the soil (Li et al., 2018). Physical and chemical limitations of the soil properties and the low microbial activity in semi-arid zones, as well as the climatic characteristics, complicate the natural restoration of the soils, slowing down the vegetal regeneration in these regions (Juwarkar and Jambhulkar, 2008). Therefore, the restoration of the quarries is necessary to accelerate the recovery of the soil and cover plants, and one way of solving these problems could be the use of organic amendments such as sewage sludge or compost from domestic organic waste (Luna et al., 2016a, 2016b; Rodríguez-Berbel et al., 2019).

Several authors have shown that the application of organic amendments is a good method of restoration, because restoring absent soil microbiota that is vital for soil structural formation can contribute to plant establishment and transformation of organic matter (Diaz et al., 1994; Zink and Allen, 1998). Moreover, organic amendments improve soil properties such as pH, humidity, organic carbon, and plant cover, which are important factors influencing the microbial diversity in semi-arid soils (Bukar et al., 2019; Neilson

et al., 2012; Peñuelas et al., 2013; Reynolds et al., 2007). Many authors have documented the beneficial effects of the use of sewage sludge or compost on fertility and soil functionality, just as on the proliferation of microbial communities with different metabolic activities (Almendro-Candel et al., 2014; Bastida et al., 2015; Luna et al., 2016a; Rico Hernández et al., 2018; Yanardağ et al., 2017). Organic amendments such as sewage sludge, urban solid waste compost, and poultry manure also influence enzymatic activities involved in the cycles of C, N, and P and biochemical soil properties such as biomass-C and basal respiration (Bastida et al., 2007; Luna et al., 2016a; Tejada et al., 2006).

Soil microbial communities have a crucial role in the functioning of ecosystems because they have a direct relationship with biogeochemical cycles (Adak and Sachan, 2009), fundamental for plant growth and survival (Bender et al., 2016). Microbial enzymatic activity plays a key role in biochemical cycles by the recycling of nutrients, making them accessible to plants and other microorganisms (Ai et al., 2015). However, most studies focusing on soil microorganisms in restored soils are based on knowledge of the size and activity of microbial communities from biochemical techniques such as soil basal respiration and soil enzymatic activity, fatty acid profile, or molecular tools such as polymerase chain reaction (PCR) combined with denaturant gradient gel electrophoresis (DGGE) (Bastida et al., 2008; Garcia and Hernandez, 1994; Luna et al., 2016b; Schmalenberger et al., 2013; Trasar-Cepeda et al., 1998; Zornoza et al., 2007). There are many studies in which organic amendments have been used to restore degraded soils from semi-arid areas, but their influence on the structure of the microbial community in the soil is still poorly explored (Yanardağ et al., 2017). Moreover, at present, studies in which the microbial communities of natural soils are compared with environments degraded by human activities are also very scarce, and there is an important gap in our knowledge about the representative bacterial taxa necessary for the ecological restoration of degraded soils to the natural state (Liddicoat et al., 2019). Recently, new methodologies based on massive sequencing have been used to amplify 16S RNA based on the Illumina MiSeq platform or shotgun metagenomic sequencing that allows the study of high-resolution soil microbial communities at the lowest classification level. Some studies have analysed microbial communities at the phylum level in restored soils with organic amendments by metaproteomic techniques. Bastida et al. (2015) deployed metaproteomics to study a restoration with organic amendments and concluded that these

affected the functionality and structure of the microbial community at the phyla level in the short and long-term. However, the same phylum includes a great diversity of bacterial genera that could perform a wide variety of functions in soils. Few studies have investigated soil microbial communities at the lowest classification level (subgroup to genus) in semi-arid ecosystems (Miralles et al., 2020a; Sánchez-Marañón et al., 2017). The study of soil microbial communities can provide important information on the specific functions they perform in soils, as well as contrasting the most abundant bacterial taxa of degraded soils, restored soils with different organic treatments, and natural soils, in order to determine the optimal treatments that allow favouring bacterial proliferation similar to natural soils considered with the highest soil quality thresholds. Breed et al. (2019) refer to the potential of genomics techniques for ecological restoration, given the need to know and better understand soil microbial communities to be successful in restoration (Garris et al., 2016). Moreover, soil microbial communities are considered better soil quality indicators than physico-chemical soil properties (Deng et al., 2019; Van der Heijden et al., 2008). On the other hand, the ecological factors influencing these communities have also been poorly studied. Thus, the microclimate (humidity and soil temperature) and especially physical and chemical soil properties could drive the soil bacterial communities (Curiel Yuste et al., 2014; Garcia and Hernandez, 1994; Trasar-Cepeda et al., 1998). Some studies on soil bacterial communities have focused on soils under different uses (Sánchez-Marañón et al., 2017), but they are of very limited relevance in restored soils with organic amendments, which can change soil microbial communities by incorporating new non-native communities to the soils (Bastida et al., 2013, 2008; Luna et al., 2016a). In this sense, the need to know the changes that occur in soil bacterial communities and the ecological factors influencing these changes are crucial for the assessment and monitoring of ecological restoration.

The aim of this study was to analyse the effect of two organic amendments (sewage sludge [SS] and compost from domestic solid organic waste [CW]) on physicochemical and microbiological soil properties in degraded limestone quarry soils after 10 years of experimentation in a semi-arid environment. In addition, natural soils (NS, soils that have not suffered any anthropic alteration since before mining activity) and degraded soils without organic amendment (NA) were selected as reference and for comparison with treated plots. We studied changes in chemical properties and the relative abundance of bacterial taxa, diversity and taxonomic composition at the phylum and

bacterial taxa at the genus level or the next upper taxonomic level identified in all plots (SS, CW, NS, NA) after 10 years. We hypothesized that the organic amendments used for soil restoration may modify soil properties, helping to recover physicochemical and microbiological soil properties in the long term.

2. Material and methods

2.1. Study area and experimental design

The study was carried out in a homogeneous hillslope of a limestone quarry located between the intermountain basin formed by Tortonian (Tertiary Upper Miocene) marls (calcitic-gypsiferous mudstones and calcareous sandstones) and the Gádor range (Cenozoic dolomites and limestones), 15 km north of Almería (SE Spain, 36°55′20″N, 2°30′29″W). The lithology of the study area is fundamentally calcareous sandstones which overlay the marls and are partly quarried. Then, most of the restoration area rests on both types of rock. In undisturbed surrounding areas, soils are mainly Calcaric Regosols (FAO-IUSS-ISRIC, 2015) over (a) calcitic-gypsiferous mudstone (marl), (b) calcareous sandstone, and (c) slope deposits mostly fed by the shallow soils over limestone and dolomite from upper reliefs, which partly contain remains of pre-erosion terra-rossa. The climate is arid/semi-arid Mediterranean with a mean annual temperature of 17.6ºC and with a minimum absolute temperature of -2.6ºC and a maximum of 42.7ºC. The potential evapotranspiration is 1,225 mm $year^{-1}$, and the mean annual precipitation is 245 mm with precipitation mainly in winter and autumn. The area is predominantly grassland that is dominated by native vegetation including *Macrochloa tenacissima* (L.) Kunth and some dwarf perennial shrubs such as *Anthyllis terniflora* (Lag) Pau; other areas constitute a mosaic formed by patches of grassland alternating with patches of dwarf shrub scrubland, where *Anthyllis cytisoides* L. and other species are found. More information about the study area is found in Luna et al. (2016b).

The installation of the experimental plots was carried out in 2008 in an area completely degraded due to mining activity where all the limestone was extracted. The experimental plots were set up at 370 m.a.s.l. in 75 m^2 surfaces (15 m × 5 m) over a hillslope with an average slope of 19%. Two organic amendments for restoration were applied in the experimental plots: (a) a filter-press dried sewage sludge from urban waste water (SS; Total organic C = 351.5 g kg^{-1} and Total nitrogen = 54.3 g kg^{-1}; (Luna et al., 2016a, 2016b)) and (b) compost from domestic solid organic waste (CW; Total organic

C = 196.5 g kg^{-1} and Total nitrogen = 20.7 g kg^{-1}; (Luna et al., 2016a, 2016b)). The crushed marly substrate without organic amendments was used as a control (NA), and undisturbed natural soils (NS) that had not suffered any anthropic alteration were found surrounding the experimental plots. These soils are shallow and stony calcaric regosols over marls and calcaric sandsones and support mostly *Anthyllis cytisoides* and *A. terniflora* among other shrubs, and *Macrochloa tenacissima*. NS were considered as reference soils because they follow the natural evolution determined by the climatic and ecological properties of the study area. The organic amendment treatments were laid on the soil surface layer and mixed (0–20 cm) with the marly substrate with a mechanical backhoe; in NA soils, the same process was performed without any amendment. The quantity of organic amendments used was determined according to their carbon content to increase the initial organic matter content up to 2% in each plot. Additionally, one-year shrubs and herbal species were directly and manually planted from forestry pot seedlings at each experimental plot the three most abundant species of native vegetation (*Macrochloa tenacissima* (L.) Kunth, *Anthyllis terniflora* (Lag) Pau, and *Anthyllis cytisoides* L.) with a separation of 1 m (75 per plot), alternating the species in the same proportion as they are present in unaltered natural soils surrounding the quarry. A more detailed description of the construction of experimental plots can be found in Luna et al. (2016a).

Ten years after the experimental plots were established, six composite soil samples (by mixing 10 subsamples) were collected randomly to a depth of 10 cm throughout each plot, as well as in soils without amendment and in reference soils. A total of 24 soil samples were taken to the laboratory in isothermal bags. Samples were air-dried, homogenized, and sieved through a 2-mm screen. Part of these dry soil samples was used for chemical soil analysis and another part for DNA extraction and next-generation sequencing (NGS) analysis.

2.2. Chemical soil analysis

Soil pH and electrical conductivity (EC) were analysed in an aqueous solution 1/1 (w/v) (Thomas, 1996) by pH meter (Crison, Carpi, Italy) and by digital conductivity meter (Crison, Carpi, Italy), respectively. Total organic carbon (TOC) was determined by Walkley and Black's method (1934) (rectified by Mingorance et al., 2007), and total nitrogen content (TN) was determined by total combustion (Vario Rapid N; Elementar, Hanau, Germany).

2.3. DNA extraction, high-throughput sequencing, and bioinformatics analysis

Microbial DNA was extracted from 0.3 g of soil using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) and quantified using an ND-2000 Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). The V4-V5 regions (400–500 pb) of bacteria 16S ribosomal RNA gene were amplified *in vitro* by PCR using 515FB/926R 16S rRNA gene primer pair (Walters et al., 2016) and paired-end sequenced on an Illumina MiSeq platform using v3 chemistry (2x300bp), as described in Comeau et al. (2017). Subsequently, sequences were processed with Quantitative Insights Into Microbial Ecology version 2 (QIIME2 version 18.8) software (Bolyen et al., 2018) following the protocol initially established in Comeau et al. (2017), more recently updated on the Microbiome Helper website (Amplicon SOP v2 [qiime2 2018.8]; https://github.com/LangilleLab/microbiome_helper/wiki). Briefly, the bacterial raw reads were trimmed of primers obtaining 643,774 sequences, quality controlled and contaminant filtered, followed by the creation of Amplicon Sequence Variants (ASVs) using the Deblur tool, resulting in a total of 531,060 sequences. The final taxonomic identities of the ASVs were obtained using the QIIME2 feature-classifier plugin (sklearn method) against the SILVA database (version 132; trimmed to the V4V5 version of the 16S). The different statistical of diversity measures were calculated within QIIME2 using the ASV table normalized to 20000 sequences per sample. All these resources are available through the Microbiome Helper website (https://github.com/LangilleLab/microbiome_helper/wiki).

To check for no contamination during ADN extraction one blank control was done in one of the kit tubes. Besides PCR negative controls were done (1 for every 96-well plate = 4 per MiSeq run) which were verified to be clean (no bands present) and still were sequenced on the MiSeq in order to show no substantial reads coming through on this barcode combination.

2.4. Statistical analyses

Alpha-diversity (intragroup diversity) was determined with QIIME2 using the number of observed ASVs in each sample. Faith's Phylogenetic Diversity (Faith, 1992), Pielou (J'), and Shannon indices were evenly sampled at 20,000 reads per sample. Principal-coordinate analysis (PCoA) was obtained from QIIME2 to study β-diversity (intergroup diversity) by Bray-Curtis and Jaccard indices. The relative abundance of soil

bacterial taxa was calculated based on the number of reads for the different phyla. The soil bacterial taxa (ASVs) were grouped in genera or the next upper taxonomic level identified in each of the 24 soil samples, and those bacterial taxa were selected that had a relative abundance greater than 0.1% in all samples. One-way permutational multivariate analysis of variance (PerMANOVA with 9,999 perms, $p < 0.05$) was used to analyse the differences between the different soil types (restored soils - CW and SS, natural soils - NS, and non-treated soils - NA). A similarity matrix used by multivariate PerMANOVA was constructed using Bray–Curtis distance for obtaining significant differences between soils according to the relative abundance of bacterial genera or the next upper taxonomic level identified greater than 0.1% in all samples. To discern between which soils there were significant differences, pairwise comparisons were made using a multivariate analogue of the *t* statistic to find the probability levels by permutation (Eldridge et al., 2016). Moreover, significant differences of each individual chemical soil property and the diversity index between soils (organic amended soils - SS and CW -, NA, and NS) were assessed using one-way permutational univariable analysis of variance (PerANOVA with 9,999 perms, $p < 0.05$) and pairwise comparisons (Anderson et al., 2008). The distance-based redundancy analysis (dbRDA) was calculated according to the relative abundance of soil bacterial taxa and chemical soil properties. The statistical package PRIMER + PERMANOVA (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows was used for PerMANOVA, PerANOVA and dbRDA analysis. A significance level of 0.05 was used, unless otherwise indicated.

The R Project environment (R Core Team, 2018) with “stats” and “ggplot2” packages was used to generate boxplot charts and to evaluate the influence of amendments on soil bacterial communities. Generalized linear models (GLMs) were applied using the “survival” library (Therneau, 2015). Pearson’s correlations (r; $p < 0.05$) were applied to determine the significant correlations between soil bacterial genera or the next upper taxonomic level identified and chemical soil parameters. Soil bacterial taxa showing highly significant correlations between them ($p < 0.05$) and with the highest correlation values ($r > 0.7$) were selected as bacterial groups forming bacterial co-occurrence patterns, as described in Xue et al. (2020) and Miralles et al. (2020b). This correlation coefficient was selected because we could clearly identify two different co-occurrence groups. Pearson’s correlations were calculated using Statgraphics (version 16.2.04 for Microsoft Windows). Network analysis was performed to graphically

represent the relationships among soil bacterial taxa that conformed to the co-occurrence patterns resulting from Pearson's correlations. Topological properties were calculated to describe the complex pattern of interrelations among nodes and to distinguish differences in taxon correlations. For this purpose, the method of obtaining modularity (Blondel et al., 2008) was used together with the Force Atlas 2 algorithm (Jacomy et al., 2014), based on the interactive platform Gephi 0.9.2 (Bastian et al., 2009) for visualizing networks.

3. Results

3.1. Chemical soil properties

The different restoration treatments applied generated changes in the soil's chemical properties. PerANOVA ($p < 0.05$) and pairwise tests showed that the chemical properties were significantly different in SS, CW, NA and NS soils 10 years after starting the restoration. Nevertheless, the treatment of CW and NS did not show significant differences between them, except in TN, which was significatively higher in CW soils (Table 1 and S1). CW and NS soil showed significantly ($p < 0.05$) higher EC and TOC and significantly ($p < 0.05$) lower pH than SS soils, for which values of such soil properties ranged between the CW and NS soils (Table 1). NS and CW soils also presented comparatively higher TOC and EC values and lower soil pH values than NA soils (Table 1).

Table 1. *Chemical soil properties of restored soils, no-amendment soils, and natural soils (mean ± SD (n = 6)).*

	pH	EC	TOC	TN
Sewage sludge	8.391 ± 0.131 a	0.213 ± 0.059 a	0.550 ± 0.137 a	0.082 ± 0.020 a
Compost	8.001 ± 0.148 b	0.388 ± 0.174 b	3.416 ± 1.519 b	0.532 ± 0.230 b
No-amendment	8.736 ± 0.088 c	0.108 ± 0.028 c	0.173 ± 0.033 c	0.028 ± 0.009 c
Natural soils	8.111 ± 0.137 b	0.391 ± 0.089 b	3.109 ± 0.542 b	0.262 ± 0.092 d

EC: Electrical Conductivity (mS cm^{-1}); TOC: Total Organic Carbon content (%); TN: Total Nitrogen content (%). Different letters indicate statistical differences for each treatment ($p < 0.05$).

3.2. Bacterial richness and α- and β-diversity

Organic amendments added to restored soils produced changes in soil's microbial properties. The restored soils (CW and SS soils) showed the significantly highest values ($p < 0.05$) of observed ASVs per soil type and Faith's phylogenetic index (Tables 2 and S2), while NA and NS soils showed the significantly lowest values in both indices. Shannon and Pielou indices presented higher values in CW and NS soils than in NA and

SS soils, NA soils being those that presented the lowest values in Shannon index and SS soils those that presented the lowest values in Pielou index (Table 2 and S2). The PCoA graphs representing β-diversity showed that SS and CW soils were similar to each other but different from NA and NS soils in Bray-Curtis and Jaccard indices (Figure 1).

Table 2. *Results of diversity index expressed as media ± SD of each soil type (n = 6). From univariate PerANOVA test (p < 0.05).*

	Observed ASVs	Faith PD	Shannon	Pielou e
Sewage sludge	963.83 ± 20.81 a	41.44 ± 0.59 a	8.41 ± 0.12 ac	0.84 ± 0.01 ab
Compost	914.66 ± 65.03 a	38.70 ± 1.59 b	8.69 ± 0.16 b	0.88 ± 0.01 c
No-amendment	798.16 ± 48.70 b	36.34 ± 1.24 c	8.36 ± 0.17 c	0.86 ± 0.01 a
Natural soils	802.00 ± 50.82 b	33.70 ± 1.30 d	8.57 ± 0.13 ab	0.88 ± 0.00 bc

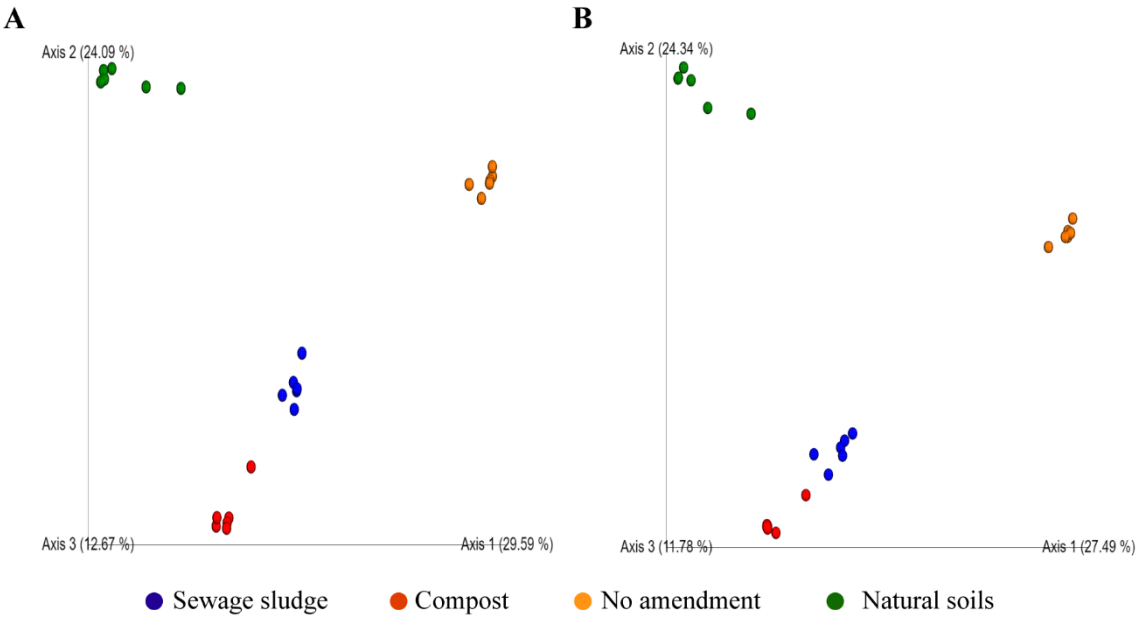

Figure 1. *PCoA plots of microbial community analysis by (a) Bray–Curtis index and (b) Jaccard index from samples of all soil types analysed (natural soils, no- amendment soils, and restored soils).*

3.3. Bacterial community composition

The number of sequences belonging to the bacteria domain was 127,348. Fourteen phyla, as well as a phylum in which unclassified sequences were grouped, showed relative frequencies greater than 0.1% in all samples (Figure S1). The most abundant phyla in all treatments were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacterioidetes* and *Planctomycetes*, although in different proportions depending on each treatment (Figure S1). The two most abundant phyla in SS and NS soils were *Proteobacteria* and

Acidobacteria; while in CW and NA soils they were *Proteobacteria* and *Actinobacteria* (Figure S1).

In the studied soils 171 bacterial taxa were identified at the genus level or the next upper taxonomic level available, with a relative abundance greater than 0.1% in all samples. The PerMANOVA test revealed that all soil types (CW, SS, NA, and NS) showed significant differences ($p < 0.05$) in bacterial communities at the genus level or the next upper taxonomic level identified (Table S3).

Some bacterial taxa were much more abundant or were associated almost exclusively with each soil type, except in SS soils, where no more abundant or exclusive bacterial taxa were found associated with this type of soil (Figure S2). The bacterial taxa (e.g., *Caenimonas, Sphingomonas, Psychroglaciecola, Blastocatella, Noviherbaspirillum* and *Rubellimicrobium*) were more abundant in NA soils (Figure S2a), whereas bacterial taxa belonging to *Xanthomonadaceae* family, *Phaselicystis,* and *Amb-16S-1323* uncultured bacteria were dominant in CW soils (Figure S2b). Other soil bacterial communities (e.g., *Craurococcus, Crossiella, uncultured* [family: Gemmataceae], and *uncultured* [order: C0119]) were more abundant or almost exclusive in NS (Figure S1c). Several bacterial taxa (*Pir4 lineage*, *Pedomicrobium, uncultured* [family: Micromonosporaceae], *Terrimonas*, *uncultured* [family: Xanthobacteraceae], *uncultured* [family: Solirubrobacterales 67-14*], Geodermatophilus*, *Singulisphaera, Chryseolinea* and *Blastococcus*) were very abundant or almost exclusive in CW and NS soils and, in contrast, present in low abundance in SS and NA soils (Figure S2d).

GLMs analyses showed that soil amendment with CW was the restoration treatment that most significantly influenced ($p < 0.05$) for the largest number of soil bacterial taxa (95 of the 171 analysed), followed by NA soils with 44 bacterial taxa, 23 bacterial taxa were influenced by NS and lastly soils amended with SS, which significantly influenced only 9 bacterial taxa (Table S4).

3.4. Relationships between bacterial community taxa and soils' chemical properties

According to the variations (out of the fitted model and out of the total variation) explained by the axes of dbRDA, axis one (dbRDA1) applied to relative abundance of bacterial taxa explained 63.7% of the fitted model and 35.9% of the total variation of the variables, whereas axis two (dbRDA2) explained 26.7% and 15% of the total variation

(Figure 2). The dbRDA analysis clearly clustered four different groups encompassing the samples of each type of soil: CW, NS, NA and SS (Figure 2).

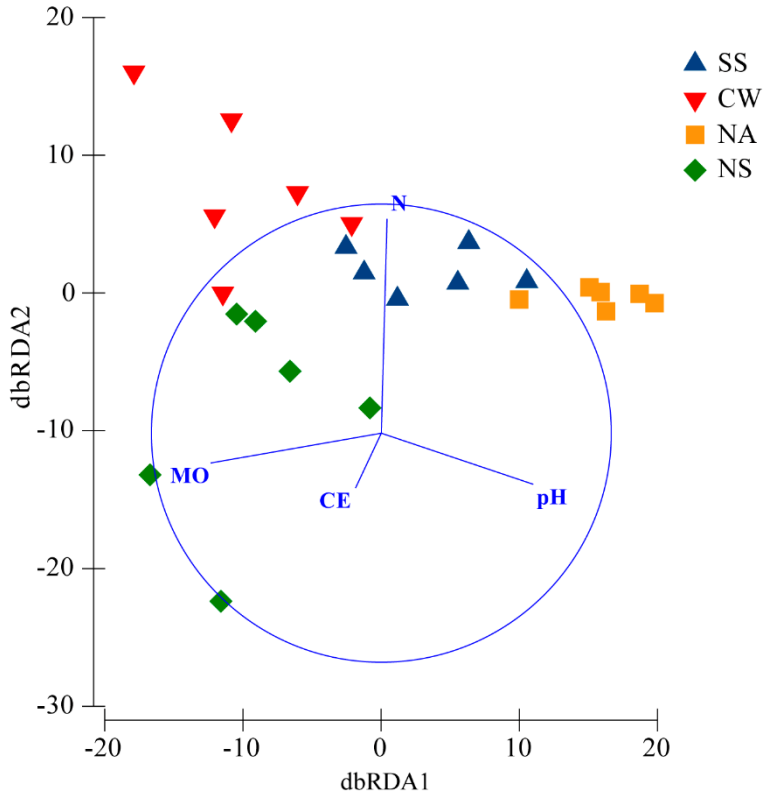

Figure 2. *Redundancy analysis for the structure of the bacterial community and chemical soil properties. Soil samples with sewage sludge (SS), compost (CW), no amendment soils (NA) and natural soils (NS) are indicated by different colours and figures. Soil properties are represented by continuous lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).*

Chemical soil parameters were significantly correlated ($p < 0.05$) with several soil bacterial taxa (Table S6). Moreover, numerous bacterial taxa were significantly correlated between them and those with the highest correlation values ($r > 0.7$) were selected (Table S7).

The relationships among the different bacterial rates and chemical soil properties allowed the differentiation of two co-occurrence parts patterns (Figure 3, Table S7). The first co-occurrence pattern (Figure 3 – green, Table S7), represented by the genera *Craurococcus*, *Phaselicystis*, and *Crossiella*, showed high positive correlation with TOC (r ranged between 0.55 and 0.32), TN (r between 0.74 and 0.66), and EC (r between 0.60 and 0.41), but had negative correlations with pH (Table S6). The second co-occurrence pattern (Figure 3 – orange, Table S7), represented by the genera *Sphingomonas*, *Rubellimicrobium*, *Noviherbaspirillum*, *Psychroglaciecola*, and *Caenimonas*, showed positive correlation with pH (r ranged between 0.86 and 0.58) and negative correlations

with TOC, TN, and EC (Table S6). The soil bacteria genera belonging to the first co-occurrence pattern were more abundant in NS and CW soils, whereas those bacteria taxa representative of the second co-occurrence pattern were more abundant in NA soils (Figure 3 and S2, Table S4). No clear tendency of soil bacterial groups was found in the SS-treated soils because different soil bacterial taxa were shared with the other types of soils (Figure S2).

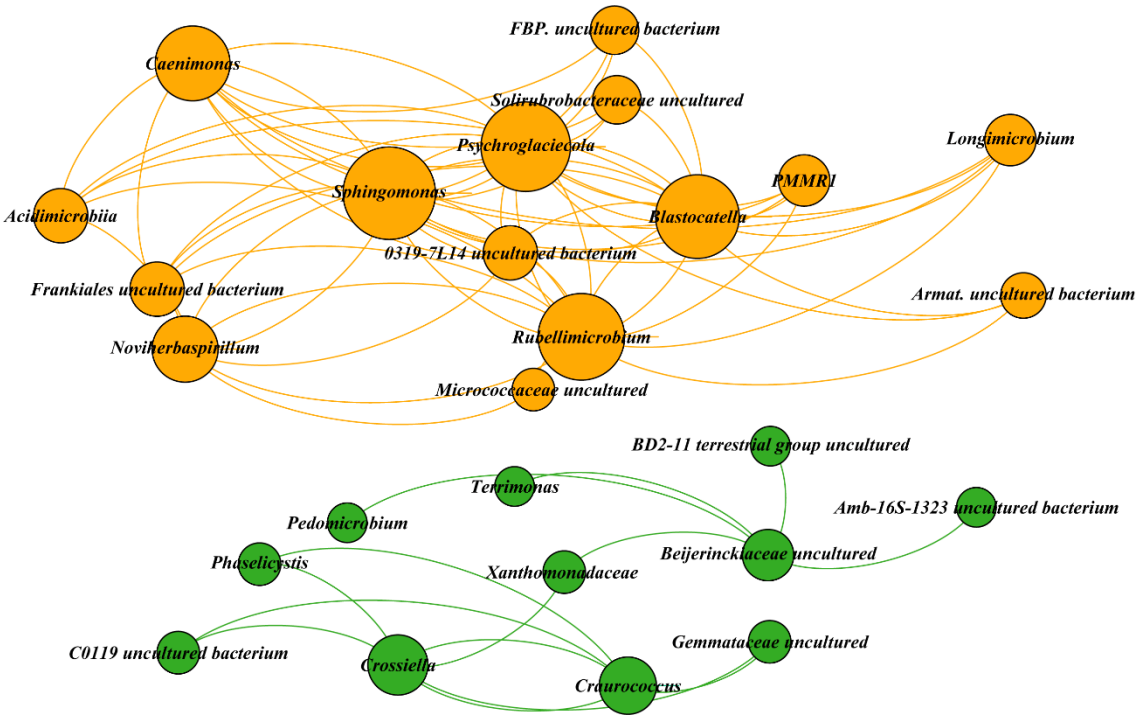

***Figure 3**. Co-occurring networks based on Pearson's correlation analysis (r > 0.7) for soil bacterial taxa shown to be present in each soil type (CW, SS, NS and NA) with the highest correlations detected. The size of each node is proportional to the number of connections, and the density of the edges indicates the intensity of the correlation. The resulting soil microbial network is made up of 26 nodes and 83 edges (average degree or node connectivity ¼ 3.19). The clustering coefficient (how the nodes are integrated into their neighbourhood, and therefore, the degree to which they tend to cluster) is 0.5, and modularity is 0.33. Two consortia are shown: Co-occurrence pattern 1 (green) and Co-occurrence pattern 2 (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).*

4. Discussion

The different soil types studied (restored soils [CW and SS], soils without organic amendment [NA] and natural soils [NS]) present the same environmental conditions (climate, slope and geological material); therefore, the significant differences among their properties are a result of the impact of mining activity and restoration treatments carried out more than 10 years ago. Both types of organic amendments significantly modified the chemical soil properties, reducing pH and increasing nutrient availability (TOC, TN) and salinity in restored soils (Table 1 and S1). CW was the type that most contributed to the increase in TOC content in the long term. This could have been because CW contains

highly resilient carbon forms (González-Ubierna et al., 2012), acting as a long-term nutrient reservoir (Table 1 and S1). However, lower TOC values in SS-treated soils (Table 1) than in CW soils suggested that SS initially presented a higher content of labile organic matter, as has also been shown by González-Ubierna et al. (2012). Possibly a large part of this labile organic matter could have been quickly depleted after the application of the amendment through processes of biodegradation (Bernal et al., 1998; Cook and Allan, 1992; Jenkins et al., 2017; Ojeda et al., 2015). The pH in CW soils decreased significantly (Table 1), as was also observed by Tian et al. (2015). Possibly the high-acid organic compounds from TOC in CW soils could have contributed to the decrease in pH. Compost treatment increases soil salinity levels in the short term, but they decrease progressively over time as has been observed by other authors (González-Ubierna et al., 2012; Luna et al., 2017). The highest TN content in CW soils could be a result of inputs from plant residues (Ros et al., 2003), given that the plant cover showed the highest growth rates in those soils (Luna et al., 2017).

The identification of the greatest diversity and major soil bacterial taxa present in restored soils has clearly shown that a change has been achieved by the restoration effort. The organic amendments contributed to increasing the soil diversity and relative abundance of several bacterial taxa in the long term (Figure 1, Table 2 and S2) as has also been shown by other authors (Bastida et al., 2008; Ros et al., 2003; Tejada et al., 2006). The dominant phyla presented different proportions depending on the soil type (Figure S1), although, in general, they were also common in other semi-arid ecosystems from North America, Kuwait desert, Brazil, China and Spain (Fernandes et al., 2018; Fierer et al., 2007; Lv et al., 2014; Miralles et al., 2020a, 2020b; Quoreshi et al., 2019).

The statistical analysis suggested that the distribution patterns of the soil microbial communities at the genus level or the next upper taxonomic level in the study soils are not random but instead could be related to changes in soil properties generated by the soil disturbance arising from quarrying activity and the type of organic amendment used in the restoration treatment (Figure 2). Several bacterial taxa present in NA soils that had previously adapted to the harsh environmental conditions of semi-arid ecosystems (nutrient shortage, water scarcity, extreme environmental conditions during the summer etc.) could have proliferated in the restored soils favoured by the local soil conditions generated by the organic amendments in the long term (Table S4 and S6). Nevertheless, the organic amendments could have influenced soil microbial communities directly by

inoculating new soil bacterial communities present in the amendments and indirectly modifying soil properties. Compost shows different associated microbial communities (Jurado et al., 2014; Kitamura et al., 2016), which are incorporated into soils when the restoration treatments are applied, also contributing to changes in soil native microbial communities. Our results lead us to think that some bacterial taxa could have been introduced with the compost and persisted in CW-restored soils for more than 10 years, given that some bacterial genera were exclusive or practically exclusive in CW but not in the other soil types (Figure S2b and S4). The bacterial taxon *uncultured* [Family: Amb-16S 1323] was also found in waste deposit and in soil landfill cover (Freitas et al., 2008; Stralis-Pavese et al., 2006). However, the changes in the restored soil properties generated by organic amendments for more than 10 years have played a key role in the diversity and composition of microbial communities in restored soils as supported by statistical analysis (Figure 2, Table S7). The chemical soil parameters studied are considered essential factors driving the composition of soil bacterial communities (Canfora et al., 2014; Goldfarb et al., 2011; Griffiths et al., 2011; Kielak et al., 2016; Lauber et al., 2009a; Liu et al., 2014; Lucas-Borja et al., 2020; Miralles et al., 2020a; Sánchez-Marañón et al., 2017). The increase in TOC and TN from organic amendments and debris from vegetation cover colonizing the restored soils for 10 years could have favoured the presence and proliferation of different bacterial taxa (Table 1, 2 and S4, Figure S2b). Bastida et al. (2013) also observed that the nutrient availability (TOC and TN) and, especially, the soil organic carbon exerts deep control over the dynamics of certain microbial groups in semi-arid soils. Moreover, the different types of organic matter from compost and sewage sludge could have contributed to the selection of soil bacterial communities because some bacterial taxa present greater preference for labile carbon sources, while others favour recalcitrant carbon compounds such as cellulose, lignin or tannin-protein (Goldfarb et al., 2011). In turn, the organic amendments generate changes in the vegetation cover, which could have caused modifications in diversity and composition of soil microbial communities essentially through plant remains and deposition of phytochemicals within the rhizosphere (Barea et al., 2002; Bastida et al., 2013a; Kramer and Gleixner, 2008; Williams et al., 2013). Guo et al. (2018) noted that revegetated soils had a high microbial diversity owing to factors such as the presence of roots, small aggregates, nutrients and seemingly governed pores that improve the distribution of bacteria in microhabitats (Nunan et al., 2003) as a result of nutrient accumulation at the root–soil interface (Kaplan et al., 2013; Nessner-Kavamura et al., 2013). The changes in pH and salinity after the

application of the organic amendments could also play a key role in soil bacterial communities (Figure 2). Canfora et al. (2014) observed that some soil bacterial taxa can adapt to changes in salinity, while others may be highly sensitive to such changes. The significantly higher diversity and presence of several bacterial communities, which are more abundant in restored soils, could be the result of high salinity, especially in CW (Table 2). Such salinity levels could have contributed to the selection of these microbial communities (Figure S1) by controlling the proliferation of soil bacterial taxa that are more sensitive to salinity and, in turn, offering a greater advantage to those bacterial communities that are better adapted to EC values (Table 1 and S6). Likewise, the microbial community in restored soils could have been influenced by soil pH, which can induce significant stress on those soil bacterial taxa that are capable of surviving in specific pH ranges (Liu et al., 2014). Soil pH also influences other soil parameters such as nutrient availability, organic carbon characteristics, soil moisture, cationic metal solubility and salinity, which could indirectly affect soil microbial composition (Lauber et al., 2009).

Interestingly, the CW-restored soils and NS soils presented several soil bacterial communities shared between them (Figure S2d), suggesting that those soil bacterial taxa could have the same preferences for soil local conditions (Figure 2 and S2, Table S6 and S7). In general, the soil bacterial taxa that are more abundant in CW and NS soils showed highly significant positive correlations with TOC, EC and TN (Table S6). Therefore, these soil bacterial taxa could have benefited from a high content of nutrients (TOC, TN; Table 1-FQ), giving them an advantage over other bacterial communities with survival strategies aimed at proliferating in more unfavourable conditions with low nutrient availability. Some of these bacteria showed lignocellulitic activity (Houfani et al., 2019); for example, *Xanthomonadaceae* and *Beijerinckiaceae* families were able to degrade lignin (Ceballos et al., 2017), while *Phaselycistis* degraded different polysaccharides as cellulose, demonstrating an important role in the soil carbon cycle (Sharma et al., 2016). On the other hand, the statistical analysis showed that SS-restored soils had an intermediate stage of development and soil functionality among NS, CW and NA soils. These soils shared different bacterial communities with the other soil types (Figure 2 and S2, Table 2), being soils that can host a greater number of taxa (Figure S2, Table 2). The evolution of the restored soils' properties for more than 10 years, approaching natural soils, could also have induced major changes in the functionality of bacterial community

in N and C cycles. Therefore, new studies focused on exploring the taxonomy–function relationships in restored soils would be needed to clarify this knowledge gap.

Curiously, the results clearly showed two bacterial co-occurrence patterns, one dominant in CW and NS soils (defined as Co-occurrence pattern 1; Figure 3-green) and the other dominant in NA soils (Co-occurrence pattern 2; Figure 3-orange). Relationships among soil microbial taxa shape the distribution of microbial communities, and therefore non-random patterns of occurrence and significant relationships among taxa in soils can be expected (Trivedi et al., 2013). This was revealed by Barberán et al. (2012), who found general non-random associations forming different patterns of co-occurrence, which included common life strategies at broad taxonomic levels and relationships among soil bacterial communities. In general, soil bacterial taxa with the highest correlations between them in the co-occurrence pattern 1 also had highly significant positive correlations with TOC, TN and EC and negative correlations with soil pH (Table S6 and S7). In contrast, the dominant bacterial taxa in co-occurrence pattern 2 showed highly significant positive correlations with pH and negative correlations with TOC, TN and EC (Table S6 and S7). These results suggest that different bacterial niches have been developed in the different soil types, depending largely on their chemical properties (TOC, TN, EC and pH), where different groups of soil bacterial communities could have stabilized (Figure 3, Table S7). These soil bacterial communities that make up each of these co-occurrence patterns could not only find chemical conditions in the soil that are more favourable for their proliferation but also could establish synergistic relationships in their functions related to the biogeochemical cycles of the soils. A more in-depth study on the relationships between soil bacteria and their functions in biogeochemical cycles in restored soils is warranted.

5. Conclusions

Soil restoration treatments based on the use of organic amendments from residues have had an important effect on chemical properties and on diversity and composition of microbial communities of restored soils in the long term. Domestic solid waste compost was the organic amendment that, in the long term, favoured greater diversity and the proliferation of several more abundant bacterial taxa in those soils. This could mainly be because the organic amendment favoured greater improvement in soil quality, creating conditions of pH, salinity and nutrients that offer a greater advantage to certain microbial

communities. On the other hand, soils with sewage sludge treatment did not regain their chemical and biological quality compared to natural soils, but rather they remained in an intermediate stage of chemical and biological quality between natural and no-amendment soils. These soils achieved a partial improvement but without reaching the development state of surrounding natural soils. Therefore, the compost amendment accelerated the soil restoration process, both at the level of bacterial communities and in chemical parameters, up to the natural soils within 10 years. On the contrary, soils without any type of organic amendment showed the lowest TOC, TN and EC, and the highest soil pH, as well as other bacterial communities typical of degraded soils or extreme environments. Thus, the use of both organic amendments could be recommended to restore soils exploited by mining in semi-arid environments, although compost amendment proved more effective in expediting soil restoration to the reference soil state in the long term. Likewise, the study of soil bacterial communities is key to understanding the evolution and naturalization of mining soils after restoration.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.110920.

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Conclusions

1. Restoration with recycled organic residues favored the improvement in the physical, chemical, biochemical and biological soil properties in the short, medium and long term, although without reaching the natural reference soils. In contrast, control soils without any treatment showed no improvement over time. These results confirm that in semi-arid soils the restoration works could accelerate their recovery.

2. The use of recycled organic matter to restore soils increased the enzymatic activity of microbial communities involved in C, N and P cycling, which could ensure the release, through the mineralization process, of available essential nutrients over time compared to unamended soils.

3. Regardless of the time elapsed since the restoration, the differences in the composition and activity of the bacterial and fungal communities between the restored soils suggest that the different origin and molecular composition of the recycled residues influenced in a different way the diversity, biological activity and structure of the edaphic microbial communities of the restored soils.

4. In abandoned agricultural soils, the greatest changes in physico-chemical properties and nutrient availability in the restored soils with respect to unamended and surrounding natural soils occurred at 3 months. These alterations could have influenced with the uneven evolution of the bacterial community, promoting a bacterial phyla succession in the different restored soils to short-medium term (between 3 and 12 months). Because a faster growth of the bacterial phyla community was observed after 3 months, which were replaced by slower growing communities after 12 months of the restoration.

5. The organic matter provided by each amendment in the restoration of abandoned agricultural soils could have favored the presence of specific bacterial taxa for each of the study soils with important implications on C, N and P cycles, as well as the establishment of bacterial genera considered PGPR. After 12 months of restoration, greenhouse compost was the treatment that showed a bacterial community and soil nutrient content closest to reference soils, suggesting that it could be an appropriate amendment for the restoration of degraded agricultural soils in semi-arid climates.

6. In the short term (6 months), the addition of labile organic matter from stabilized sewage sludge increased microbial activity and favored bacterial taxa involved in C and P cycling. Besides, labile carbon could have contributed to higher initial CO_2 emissions ("priming effect") coupled with the active consumption of nutrient reserves in these restored soils. Long-term studies are needed to analyze the evolution of the activity and composition of soil microbial communities and changes in the nutrient pool and carbon balance of restored soils.

7. The application of more resilient and hardly biodegradable organic matter from plant waste compost produced a balanced increase in microbiological activity and greater bacterial diversity in the soil in the short term (6 months). At the same time, the priming effect was lower than in soils restored with organic amendments with labile organic matter mentioned before, suggesting a slower release of essential nutrients that could guarantee soil fertility over time.

8. In the medium term (18 months), soils restored with organic amendments benefited fungal diversity as well as different fungal communities for each type of amendment, while control soils did not present fungi indicating that could not recover without amendments. Also, depending on chemical nature of organic matter provided by each restoration treatment, the growth of different taxa involved in nutrient transformation and in the decomposition of complex organic matter was observed, sharing some of them with natural soils. These results suggest that the improvement of the properties together with the type of organic C applied in the restored soils could have influenced the uneven development of the fungal community causing an ecological succession leading to a more mature community able to mineralize more resilient C fractions, resembling the quality status of the surrounding natural soils.

9. In the long term (10 years) compost from domestic solid waste drove restoration processes in mining degraded soil that which resulted development of similar to those natural in terms of chemical properties, diversity and composition of bacterial communities. Meanwhile, soils treated with sewage sludge only achieved a partial improvement in chemical and biological quality compared to the surrounding natural soils.

10. In general, the results suggest that the application of plant and domestic solid wastes could be a suitable restoration strategy to favor the recovery of physico-chemical and microbiological properties of soils degraded by agricultural and mining activities in semi-arid conditions. Although the effects on these properties may vary depending on the amendment composition as well as other factors (e.g. edaphic factors, type of use, etc.), to deepen the understanding of the effect of organic amendments as restoration treatments, the integration of future short- and long-term research aimed at determining the functional diversity of microbial communities and their involvement in C, N and P cycles would be of interest.

Conclusiones

1. La restauración con residuos orgánicos reciclados favoreció la mejora de las propiedades físicas, químicas, bioquímicas y biológicas del suelo a corto, medio y largo plazo, aunque sin alcanzar los suelos naturales de referencia. Por el contrario, los suelos control sin ningún tratamiento no mostraron mejoras a lo largo del tiempo. Estos resultados confirman que, en suelos semiáridos, los trabajos de restauración podrían acelerar su recuperación.

2. El uso de materia orgánica reciclada para restaurar los suelos aumentó la actividad enzimática de las comunidades microbianas implicadas en el ciclo del C, N y P, lo que podría asegurar la liberación, a través del proceso de mineralización, de nutrientes esenciales disponibles a lo largo del tiempo en comparación con los suelos no enmendados.

3. Independientemente del tiempo transcurrido desde la restauración, las diferencias en la composición y actividad de las comunidades bacterianas y fúngicas entre los suelos restaurados sugieren que el diferente origen y composición molecular de los residuos reciclados influyó de forma diferente en la diversidad, actividad biológica y estructura de las comunidades microbianas edáficas de los suelos restaurados.

4. En los suelos agrícolas abandonados, los mayores cambios en las propiedades físico-químicas y en la disponibilidad de nutrientes de los suelos restaurados respecto a los suelos naturales no enmendados y circundantes se produjeron a los 3 meses. Estas alteraciones podrían haber influido en la evolución desigual de la comunidad bacteriana, promoviendo una sucesión de filos bacterianos en los diferentes suelos restaurados a corto-medio plazo (entre 3 y 12 meses). Ya que se observó un crecimiento más rápido de la comunidad de filos bacterianos a los 3 meses, que fueron sustituidos por comunidades de crecimiento más lento a los 12 meses de la restauración.

5. La materia orgánica aportada por cada enmienda en la restauración de suelos agrícolas abandonados podría haber favorecido la presencia de taxones bacterianos específicos para cada uno de los suelos de estudio con importantes implicaciones en los ciclos de C, N y P, así como el establecimiento de géneros bacterianos considerados PGPR. Tras 12 meses de restauración, el compost de invernadero fue el tratamiento que

mostró una comunidad bacteriana y un contenido en nutrientes del suelo más cercano a los suelos de referencia, sugiriendo que podría ser una enmienda apropiada para la restauración de suelos agrícolas degradados en climas semiáridos.

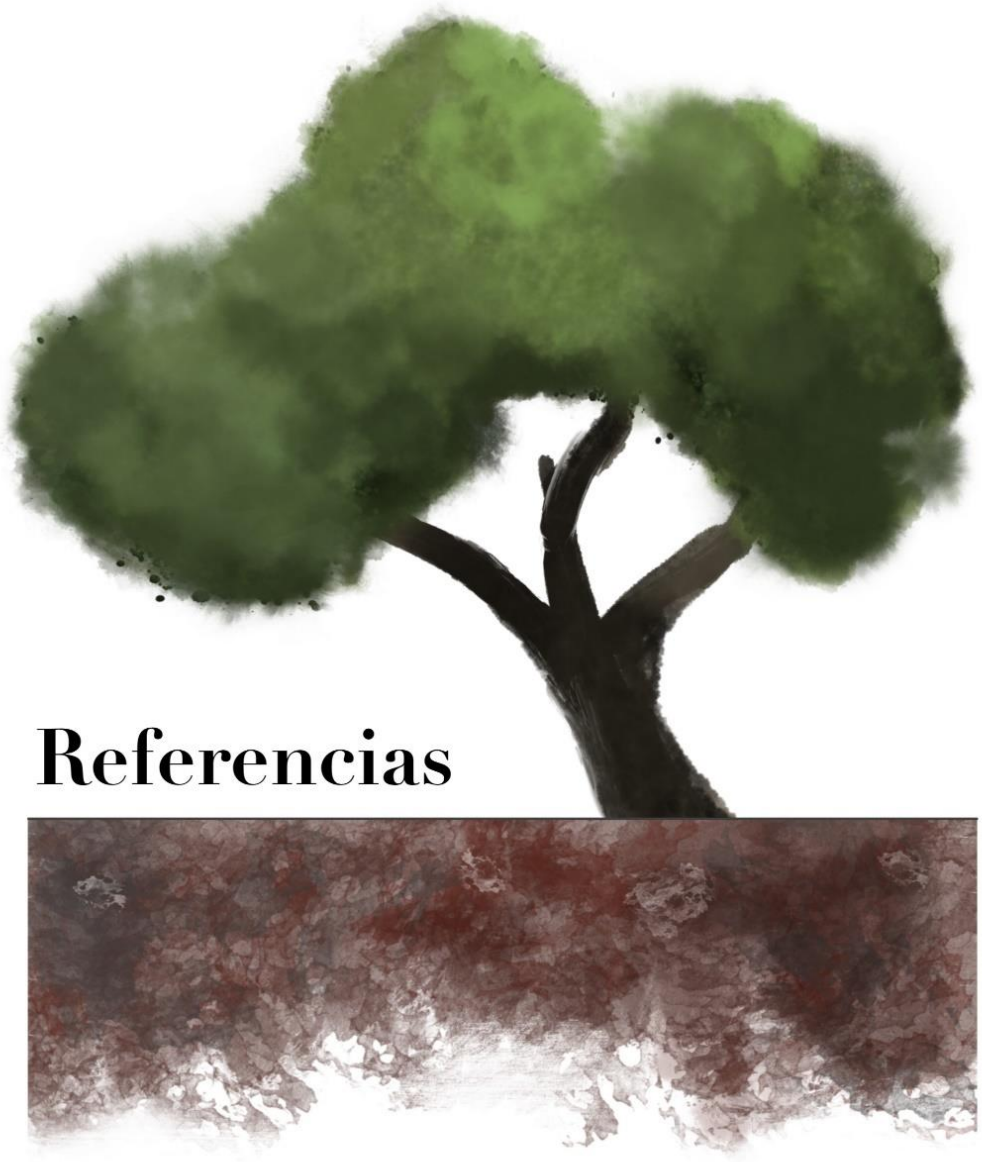
6. A corto plazo (6 meses), la adición de materia orgánica lábil procedente de lodos de depuradora estabilizados aumentó la actividad microbiana y favoreció a los taxones bacterianos implicados en el ciclo del C y el P. Además, el carbono lábil podría haber contribuido a aumentar las emisiones iniciales de CO_2 ("priming effect") junto con el consumo activo de las reservas de nutrientes en estos suelos restaurados. Se necesitan estudios a largo plazo para analizar la evolución de la actividad y composición de las comunidades microbianas del suelo y los cambios en la reserva de nutrientes y el balance de carbono de los suelos restaurados.

7. La aplicación de materia orgánica más resistente y difícilmente biodegradable procedente de compost de residuos vegetales produjo un aumento equilibrado de la actividad microbiológica y una mayor diversidad bacteriana en el suelo a corto plazo (6 meses). Al mismo tiempo, el efecto de cebado fue menor que en los suelos restaurados con las enmiendas orgánicas con materia orgánica lábil antes mencionadas, lo que sugiere una liberación más lenta de los nutrientes esenciales que podrían garantizar la fertilidad del suelo a lo largo del tiempo.

8. A medio plazo (18 meses), los suelos restaurados con enmiendas orgánicas beneficiaron a la diversidad fúngica, así como a diferentes comunidades fúngicas para cada tipo de enmienda, mientras que, los suelos control no presentaron hongos indicadores de que no pudieran recuperarse sin enmiendas. Además, dependiendo de la naturaleza química de la materia orgánica aportada por cada tratamiento de restauración, se observó el crecimiento de diferentes taxones implicados en la transformación de nutrientes y en la descomposición de materia orgánica compleja, compartiendo algunos de ellos con los suelos naturales. Estos resultados sugieren que la mejora de las propiedades junto con el tipo de C orgánico aplicado en los suelos restaurados podría haber influido en el desarrollo desigual de la comunidad fúngica provocando una sucesión ecológica que condujera a una comunidad más madura capaz de mineralizar fracciones de C más resistentes, asemejándose al estado de calidad de los suelos naturales circundantes.

9. A largo plazo (10 años), el compost procedente de residuos sólidos domésticos impulsó procesos de restauración en suelos degradados mineros que dieron como resultado el desarrollo de suelos similares a los naturales en cuanto a propiedades químicas, diversidad y composición de las comunidades bacterianas. Mientras tanto, los suelos tratados con lodos de depuradora sólo lograron una mejora parcial de la calidad química y biológica en comparación con los suelos naturales circundantes.

10. En general, los resultados sugieren que la aplicación de residuos sólidos vegetales y domésticos podría ser una estrategia de restauración adecuada para favorecer la recuperación de las propiedades físico-químicas y microbiológicas de suelos degradados por actividades agrícolas y mineras en condiciones semiáridas. Aunque los efectos sobre estas propiedades pueden variar en función de la composición de la enmienda así como de otros factores (p.e. factores edáficos, tipo de uso, etc.), para profundizar en el conocimiento del efecto de las enmiendas orgánicas como tratamientos de restauración, sería de interés la integración de futuras investigaciones a corto y largo plazo dirigidas a determinar la diversidad funcional de las comunidades microbianas y su implicación en los ciclos de C, N y P.



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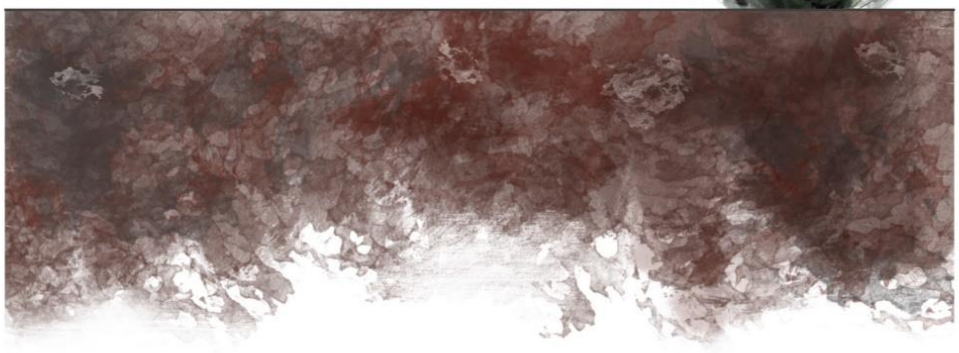

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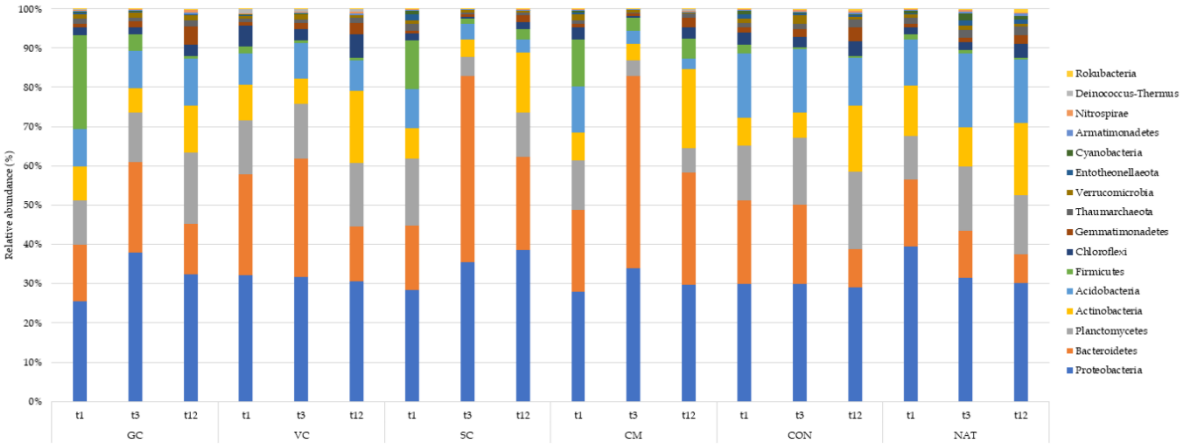
“Soy de las que piensan que la ciencia tiene una gran belleza. Un científico en su laboratorio no es sólo un simple técnico; es también un niño colocado ante fenómenos naturales que lo impresionan como si fuera un cuento de hadas.”

Marie Curie

Anexo 1

1. Supplementary material: Rodríguez-Berbel et al. 2023. Agronomy

Figure S1. Bacterial community at phylum level with a relative abundance ≥ 0.1%.



Footnotes: GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months.

Table S1. Results of different multivariate PERMANOVA analysis ($p < 0.05$; 999 perm) by the factors treatment (GC, VC, SC, CM CON and NAT soils) and time (t1, t3 and t12) for the data of soil physico-chemical properties and bacterial composition (phylum level and genus or the next upper taxonomic level identified).

a**) Physico-chemical soil properties:**

Factor	DF	SS	MS	F-Value	P-Value
Treatment	5	4.63E+05	9,26E+05	43,882	0,001
Time	2	5.91E+05	2,95E+05	13,996	0,001
Treatment x Time	10	3,02E+0	30257	1,433	0,194
Residual	36	7,60E+05	21112		
Total	53	6,29E+06			

b) Soil bacterial community at phylum level:

Factor	DF	SS	MS	F-Value	P-Value
Treatment	5	3758,1	751,62	10,432	0,001
Time	2	2030,5	1015,2	14,091	0,001
Treatment x Time	10	2309,6	230,96	3,2055	0,001
Residual	36	2593,8	72,051		
Total	53	10692			

c) Soil bacterial community at genus or the next upper taxonomic level identified:

Factor	DF	SS	MS	F-Value	P-Value
Treatment	5	9452,4	1890,5	6,1747	0,001
Time	2	5395,8	2697,9	8,8118	0,001
Treatment x Time	10	6318,4	631,84	2,0637	0,001
Residual	36	11022	306,17		
Total	53	32189			

Table S2. Component loadings on all principal-component-analysis (PCA) solution for physico-chemical soil properties and nutrient stoichiometry ratios in restored, natural and control soils.

Variable	PC1	PC2	PC3	PC4	PC5
EC	0.373	-0.154	-0.389	0.081	-0.393
pH	-0.211	0.19	0.677	0.12	-0.048
TOC	-0.082	-0.629	0.144	0.118	-0.164
TN	0.15	-0.596	-0.024	-0.005	0.101
AP	0.371	-0.185	0.252	0.389	0.653
C:N	-0.369	-0.282	0.272	0.206	-0.43
C:P	-0.459	-0.049	-0.326	0.324	0.2
N:P	-0.447	0.02	-0.354	0.341	0.212
AW	-0.322	-0.27	-0.007	-0.742	0.33

EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon to nitrogen ratio; C:P: carbon to phosphorus ratio; N:P: nitrogen to phosphorus ratio; AW: available plant water. CG: greenhouse crop residues compost; VC: vermicompost; CS: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil.

Table S3. Distance-based redundancy analysis results, explanatory variance and contribution of relative abundance of bacterial taxa and abiotic properties (physico-chemical properties and nutrient stoichiometry) of the soil.

AICc 345.14 R^2 0.37

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual	Cumulative
1	21.87	21.87
2	5.16	27.03

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
EC	-0,64	4,06
pH	-1,40	0,59
TOC	-1,33	36,82
TN	1,07	-32,13
AP	-16,90	-3,70
C:N	22,32	-19,30
C:P	-220,04	-6,19
N:P	183,50	5,25
AW	2,49	1,59

EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon to nitrogen ratio; C:P: carbon to phosphorus ratio; N:P: nitrogen to phosphorus ratio; AW: available plant water.

Table S4. Results of the analysis of indicator species at the phylum and genus or the next upper taxonomic level identified for each type of restored, control and natural reference soil.

Treatment	Phylum	Soil Bacterial taxa	Indicator value	p-value	Sig.
GC	Firmicutes	g_Staphylococcus	0.674	0.0001	***
GC	Firmicutes	f_Bacillaceae	0.506	0.0046	**
GC	Proteobacteria	f_BIrii41_g_uncultured	0.414	0.0410	*
GC	Proteobacteria	g_Halomonas	0.455	0.0185	*
GC	Proteobacteria	g_Arenimonas	0.454	0.0187	*
VC	Bacteroidetes	g_Chryseolinea	0.663	0.0001	***
VC	Bacteroidetes	g_Alistipes	0.509	0.0034	**
VC	Bacteroidetes	f_Flavobacteriaceae	0.410	0.0056	**
VC	Deinococcus-Thermus	g_Truepera	0.776	0.0001	***
VC	Chloroflexi	o_SBR1031_g_anaerobic bacterium MO-CFX2	0.834	0.0001	***
VC	Chloroflexi	o_Ardenticatenales_g_bacterium YC-LK-LKJ3	0.895	0.0001	***
VC	Proteobacteria	f_Xanthomonadaceae	0.810	0.0001	***
CM	Actinobacteria	g_Arthrobacter	0.526	0.0008	***
CM	Actinobacteria	g_Leifsonia	0.524	0.0011	**
CM	Bacteroidetes	g_Sphingobacterium	0.495	0.0050	**
CM	Bacteroidetes	g_Pontibacter	0.623	0.0003	***
CM	Bacteroidetes	g_Myroides	0.430	0.0419	*
CM	Firmicutes	g_Atopostipes	0.550	0.0010	***
CM	Firmicutes	o_Bacillales	0.627	0.0001	***
CM	Proteobacteria	g_Alcaligenes	0.579	0.0008	***
CM	Proteobacteria	g_Stenotrophomonas	0.477	0.0148	*
CM	Proteobacteria	g_Nitrosospira	0.618	0.0004	***
SC	Bacteroidetes	g_Flavobacterium	0.507	0.0054	**
SC	Proteobacteria	g_Cellvibrio	0.503	0.0054	**
SC	Proteobacteria	g_Phenylobacterium	0.401	0.0089	**
CON	Acidobacteria	c_Subgroup 6_o_unknown_f_unknown	0.500	0.0008	***
CON	Actinobacteria	p_Actinobacteria_g_uncultured	0.446	0.0164	*
CON	Bacteroidetes	f_Microscillaceae	0.491	0.0013	**
CON	Bacteroidetes	g_Parasegetibacter	0.541	0.0009	***
CON	Chloroflexi	f_Anaerolineaceae_g_UTCFX1	0.584	0.0003	***
NAT	Acidobacteria	f_Pyrinomonadaceae_g_RB41	0.890	0.0001	***
NAT	Acidobacteria	f_Blastocatellaceae	0.617	0.0001	***
NAT	Acidobacteria	o_Subgroup 7_g_uncultured	0.667	0.0001	***
NAT	Actinobacteria	o_Gaiellales_f_uncultured_g_uncultured	0.449	0.0156	*
NAT	Actinobacteria	g_Blastococcus	0.602	0.0001	***
NAT	Actinobacteria	o_Gaiellales_f_uncultured	0.841	0.0001	***
NAT	Actinobacteria	g_Solirubrobacter	0.793	0.0001	***
NAT	Actinobacteria	g_Parviterribacter	0.516	0.0020	**
NAT	Actinobacteria	g_Rubrobacteria	0.650	0.0001	***
NAT	Bacteroidetes	g_Segetibacter	0.417	0.0378	*
NAT	Chloroflexi	f_JG30-KF-CM45_g_uncultured	0.579	0.0004	***
NAT	Cyanobacteria	g_Tychonema CCAP 1459-11B	0.535	0.0020	**

NAT	Planctomycetes	g_Zavarzinella	0.672	0.0001	***
NAT	Proteobacteria	f_Beijerinckiaceae	0.922	0.0001	***
NAT	Proteobacteria	f_Archangiaceae	0.443	0.0168	*
NAT	Proteobacteria	g_Psychroglaciecola	0.811	0.0001	***
NAT	Proteobacteria	g_PMMR1	0.861	0.0001	***
NAT	Proteobacteria	g_Reyranella	0.631	0.0002	***
NAT	Proteobacteria	o_Elsterales_f_uncultured_g_uncultured	0.768	0.0001	***
NAT	Proteobacteria	g_Rhodoplanes	0.694	0.0001	***
NAT	Proteobacteria	o_Rhizobiales_f_uncultured_g_uncultured	0.813	0.0001	***
NAT	Proteobacteria	o_Betaproteobacteriales_f_TRA3-20	0.883	0.0001	***
NAT	Proteobacteria	g_Ellin6067	0.706	0.0001	***

GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

2. Supplementary material: Rodríguez-Berbel et al. 2021. Science of the Total Environment

Supplementary Table 1. Relative abundance and distribution of ASVs attributed to phylum taxonomic rank on organic amendments and in restored and control soils. The 12 most abundant phyla are shown in the legend (relative abundance > 0.1%). Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PerMANOVA).

	Organic amendments			Restored and unrestored soils						Total relative abundance (%)
	CG_0	SS_0	CC_0	CG	SS	CC	Mix 1	Mix 2	CON	
Bacteroidetes	4.55	0.05	3.40	5.06	8.68	7.98	8.88	4.53	2.06	37.19
Proteobacteria	3.86	2.06	3.03	3.78	5.73	4.59	3.73	4.63	8.93	31.40
Actinobacteria	1.34	0.81	0.39	2.82	1.32	1.77	1.90	1.96	1.88	11.64
Planctomycetes	0.14	0.00	1.40	1.21	0.41	0.37	0.52	2.02	1.12	5.65
Firmicutes	0.24	8.08	0.20	1.66	0.20	0.56	1.07	1.90	0.20	5.60
Deinococcus-Thermus	0.30	0.00	1.23	0.32	0.13	0.76	0.22	0.84	0.16	2.44
Chloroflexi	0.46	0.04	0.27	1.00	0.07	0.30	0.21	0.46	0.18	2.21
Gemmatimonadetes	0.17	0.00	0.13	0.42	0.04	0.19	0.04	0.10	1.06	1.85
Acidobacteria	0.00	0.02	0.01	0.15	0.01	0.02	0.01	0.01	0.86	1.06
BRC1	0.00	0.00	0.00	0.09	0.02	0.08	0.05	0.13	0.02	0.39
Verrucomicrobia	0.00	0.00	0.01	0.06	0.03	0.02	0.03	0.04	0.05	0.24
Cyanobacteria	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.05	0.08
				a	**b**	**b**	**b**	**a**	**c**	

Supplementary Table 2. PerANOVA and PerMANOVA analysis. Significant differences ($p < 0.05$) in physical, chemical and biological properties by soil types (CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; CON: unamended control soils.). Pairwise test comparing the different soil types.

Physico-chemical soil properties

PerANOVA

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
pH	Tt	5	3,727	0,7454	5,123	0,0126	8955
	Res	12	1,746	0,1455			
	Total	17	5,473				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	1.015	0.4982	10	0.3704
CG, CC	1.0913	0.3949	10	0.3377
CG, Mix1	0.92606	0.4941	10	0.4115
CG, Mix2	0.66867	0.7003	10	0.5453
CG, CON	1.2935	0.4004	10	0.2711
SS, CC	4.9981	0.1023	10	0.0067
SS, Mix1	0.29234	0.7992	10	0.7854
SS, Mix2	0.99458	0.4137	10	0.3781
SS, CON	5.472	0.1028	10	0.0058
CC, Mix1	7.291	0.098	9	0.0021
CC, Mix2	13.485	0.0998	10	0.0001
CC, CON	1.4184	0.2937	10	0.2324
Mix1, Mix2	1.0448	0.505	9	0.3547

Mix1, CON	8.0599	0.1032	10	0.0018	
Mix2, CON	15.477	0.0993	10	0.0001	

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
EC	Tt	5	6.2257	1.2451	3.9379	0.0227	9940
	Res	12	3.7944	0.3162			
	Total	17	10.02				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	1.8356	0.3058	10	0.1433
CG, CC	1.3401	0.3034	10	0.2556
CG, Mix1	1.5222	0.3006	10	0.2159
CG, Mix2	1.3179	0.2997	10	0.2577
CG, CON	1.5271	0.2996	10	0.2067
SS, CC	0.23487	0.7963	10	0.8232
SS, Mix1	0.44435	0.8989	10	0.6802
SS, Mix2	1.0941	0.3013	10	0.338
SS, CON	3.3913	0.0986	10	0.0268
CC, Mix1	0.11072	0.8994	10	0.9172
CC, Mix2	0.52315	0.6008	10	0.6335
CC, CON	2.7243	0.1029	10	0.0515
Mix1, Mix2	0.59415	0.6928	10	0.5876
Mix1, CON	3.145	0.0986	10	0.0362
Mix2, CON	3.1479	0.097	10	0.0348

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
TOC	Tt	5	13.482	2.6965	18.283	0.0004	9954
	Res	12	1.7698	0.14748			
	Total	17	15.252				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	2.8487	0.0968	10	0.0475
CG, CC	3.0199	0.0944	10	0.0382
CG, Mix1	4.7291	0.0974	10	0.0079
CG, Mix2	7.5535	0.1002	10	0.0024
CG, CON	8.4504	0.1002	10	0.001
SS, CC	0.53406	0.6073	10	0.6198
SS, Mix1	0.64441	0.6061	10	0.5553
SS, Mix2	0.46716	0.7963	10	0.6645
SS, CON	6.6843	0.1025	10	0.0022
CC, Mix1	1.1955	0.4002	10	0.2932
CC, Mix2	0.28336	0.8037	10	0.7927
CC, CON	6.179	0.0948	10	0.0033
Mix1, Mix2	2.5486	0.1023	10	0.0627
Mix1, CON	13.578	0.095	10	0.0003
Mix2, CON	17.286	0.1023	10	0.0001

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
TN	Tt	5	0.63409	0.12682	25.634	0.0001	9906
	Res	12	5.94E-02	4.95E-03			
	Total	17	0.69346				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	3.799	0.0965	10	0.0191
CG, CC	1.61	0.305	9	0.1788
CG, Mix1	5.2821	0.1084	10	0.0055
CG, Mix2	6.6631	0.1009	10	0.0036
CG, CON	16.961	0.1034	10	0.0001
SS, CC	2.2471	0.2005	10	0.0889
SS, Mix1	1.0388	0.4003	10	0.3578

	SS, Mix2	1.0544	0.404	10	0.3529		
	SS, CON	7.784	0.0964	10	0.0014		
	CC, Mix1	1.8716	0.1965	10	0.1364		
	CC, Mix2	2.0458	0.1942	10	0.1114		
	CC, CON	7.3609	0.1028	10	0.0023		
	Mix1, Mix2	4.52E-02	1	10	0.9663		
	Mix1, CON	13.069	0.1043	10	0.0002		
	Mix2, CON	16.258	0.0989	10	0.0001		
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
AP	Tt	5	5.62E-02	1.12E-02	4.4865	0.0094	9955
	Res	12	3.01E-02	2.51E-03			
	Total	17	8.63E-02				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms	P(MC)		
	CG, SS	2.2998	0.0961	10	0.087		
	CG, CC	2.0456	0.2027	10	0.1085		
	CG, Mix1	3.0552	0.1079	10	0.0381		
	CG, Mix2	2.2782	0.0999	10	0.0866		
	CG, CON	10.057	0.1034	10	0.0005		
	SS, CC	1.3762	0.3029	10	0.2371		
	SS, Mix1	0.70475	0.6116	10	0.5242		
	SS, Mix2	1.1256	0.4032	10	0.3261		
	SS, CON	3.1042	0.1025	10	0.0354		
	CC, Mix1	1.0995	0.487	10	0.3305		
	CC, Mix2	0.39509	0.8006	10	0.7143		
	CC, CON	4.0844	0.0997	10	0.0143		
	Mix1, Mix2	0.67315	0.597	10	0.5446		
	Mix1, CON	4.7052	0.1005	10	0.0091		
	Mix2, CON	4.0289	0.0966	10	0.0145		
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
C/N ratio	Tt	5	1076	215.2	2.7316	0.0001	9830
	Res	12	945.37	78.781			
	Total	17	2021.3				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms	P(MC)		
	CG, SS	3.6891	0.1009	10	0.0189		
	CG, CC	7.7219	0.098	10	0.0014		
	CG, Mix1	1.4938	0.1972	10	0.2125		
	CG, Mix2	0.52115	0.6994	10	0.6357		
	CG, CON	1.6529	0.0979	10	0.1752		
	SS, CC	31.499	0.1058	10	0.0001		
	SS, Mix1	1.181	0.3975	10	0.2982		
	SS, Mix2	9.033	0.1017	10	0.0009		
	SS, CON	1.7224	0.0969	10	0.1592		
	CC, Mix1	8.5528	0.0993	10	0.0008		
	CC, Mix2	12.771	0.0975	10	0.0003		
	CC, CON	1.4985	0.101	10	0.2079		
	Mix1, Mix2	2.2763	0.1999	10	0.0885		
	Mix1, CON	1.696	0.0991	10	0.1664		
	Mix2, CON	1.6423	0.1063	10	0.1805		
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
pF -1500 KPa	Tt	5	42.907	8.5814	4.8278	0.0092	9950
	Res	12	21.33	1.7775			
	Total	17	64.236				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms	P(MC)		
	CG, SS	2.8176	0.0961	10	0.0473		
	CG, CC	0.33074	0.6998	10	0.7585		

CG, Mix1	1.4679	0.1983	10	0.2102	
CG, Mix2	1.1632	0.4955	10	0.3086	
CG, CON	1.6471	0.3006	10	0.1812	
SS, CC	2.1332	0.1961	10	0.1043	
SS, Mix1	2.3241	0.1006	10	0.0832	
SS, Mix2	1.898	0.2008	10	0.1285	
SS, CON	4.8371	0.1025	10	0.0075	
CC, Mix1	0.78433	0.6014	10	0.4771	
CC, Mix2	0.65027	0.4991	10	0.5531	
CC, CON	1.7753	0.293	10	0.151	
Mix1, Mix2	4.91E-02	1	10	0.9638	
Mix1, CON	4.2303	0.0985	10	0.0164	
Mix2, CON	3.1585	0.1004	10	0.0333	

pF -33 KPa

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Tt	5	41.396	8.2792	0.85878	0.5657	9954
Res	12	115.69	9.6407			
Total	17	157.08				

Carbohydrates

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Tt	5	1.09E+07	2.18E+06	31.528	0.0001	9950
Res	12	8.30E+05	69144			
Total	17	1.17E+07				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	5.1998	0.102	10	0.007
CG, CC	5.1334	0.1029	10	0.0075
CG, Mix1	8.2102	0.0993	10	0.0018
CG, Mix2	2.6075	0.098	10	0.0616
CG, CON	11.134	0.0996	4	0.0004
SS, CC	5.8961	0.0955	10	0.0049
SS, Mix1	2.6281	0.0987	10	0.0545
SS, Mix2	4.5096	0.0899	10	0.0122
SS, CON	6.64	0.0988	4	0.0021
CC, Mix1	11.317	0.1009	10	0.0005
CC, Mix2	6.1585	0.0986	10	0.0032
CC, CON	30.484	0.1015	4	0.0001
Mix1, Mix2	5.3937	0.1017	10	0.0052
Mix1, CON	14.021	0.0977	4	0.0001
Mix2, CON	9.7634	0.0941	4	0.0006

Polyphenols

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Tt	5	8423.9	1684.8	20.86	0.0001	9956
Res	12	969.16	80.764			
Total	17	9393				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	0.51264	0.6996	10	0.6291
CG, CC	2.7222	0.1015	10	0.0506
CG, Mix1	0.94209	0.4069	10	0.3899
CG, Mix2	2.3816	0.1017	10	0.0723
CG, CON	3.9246	0.1043	10	0.0166
SS, CC	5.3485	0.094	10	0.0057
SS, Mix1	0.69941	0.7081	10	0.5229
SS, Mix2	3.2431	0.1014	10	0.0342
SS, CON	6.3719	0.0957	10	0.0035
CC, Mix1	8.3109	0.1016	10	0.0012
CC, Mix2	10.708	0.0965	10	0.0003
CC, CON	15.626	0.0981	10	0.0002
Mix1, Mix2	4.1677	0.1034	10	0.013
Mix1, CON	11.265	0.0946	10	0.0005

Enzymatic soil activity

PerANOVA

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Dehydrogenase	Tt	5	4.5427	0.90855	6.9771	0.0037	9955
	Res	12	1.5626	0.13022			
	Total	17	6.1054				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	3.6144	0.1016	10	0.0231
CG, CC	4.0484	0.0922	10	0.0117
CG, Mix1	7.7362	0.1028	10	0.0013
CG, Mix2	3.3513	0.0924	10	0.0266
CG, CON	5.3688	0.0997	10	0.0061
SS, CC	1.2103	0.3024	10	0.2891
SS, Mix1	1.5599	0.3023	10	0.1996
SS, Mix2	0.44862	0.7021	10	0.6705
SS, CON	3.9181	0.1008	10	0.02
CC, Mix1	0.37618	0.8077	10	0.7287
CC, Mix2	0.73877	0.4037	10	0.5042
CC, CON	4.6061	0.1048	10	0.0128
Mix1, Mix2	1.0778	0.3992	10	0.3399
Mix1, CON	9.0213	0.1045	10	0.0012
Mix2, CON	3.6893	0.1011	10	0.0212

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
β-glucosidase	Tt	5	3.0488	0.60976	43.362	0.0001	9958
	Res	12	0.16875	1.41E-02			
	Total	17	3.2176				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	8.8788	0.0968	10	0.0009
CG, CC	8.0419	0.0919	10	0.0014
CG, Mix1	8.1889	0.1056	10	0.0016
CG, Mix2	6.3974	0.0962	10	0.0044
CG, CON	7.2996	0.0946	10	0.0014
SS, CC	6.887	0.0977	10	0.0022
SS, Mix1	4.3356	0.1008	10	0.0116
SS, Mix2	4.0029	0.0991	10	0.0148
SS, CON	9.6511	0.1001	10	0.0009
CC, Mix1	4.1388	0.0986	10	0.0154
CC, Mix2	3.3683	0.0984	10	0.0253
CC, CON	12.108	0.1003	10	0.0002
Mix1, Mix2	8.58E-02	0.9023	10	0.9375
Mix1, CON	9.822	0.0958	10	0.0006
Mix2, CON	7.6033	0.0978	10	0.0014

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Phosphatase	Tt	5	2798	559.59	15.385	0.0002	9951
	Res	12	436.46	3.64E+01			
	Total	17	3234.4				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	4.1061	0.1047	10	0.0144
CG, CC	1.6826	0.1926	10	0.171
CG, Mix1	8.0883	0.097	10	0.0017
CG, Mix2	4.1966	0.0995	10	0.0135
CG, CON	10.836	0.0958	10	0.0009

SS, CC	4.0399	0.1056	10	0.0166	
SS, Mix1	1.3518	0.3006	10	0.2404	
SS, Mix2	2.6417	0.1024	10	0.0574	
SS, CON	4.3974	0.0992	10	0.0132	
CC, Mix1	7.8782	0.0938	10	0.0015	
CC, Mix2	3.9837	0.1034	10	0.0162	
CC, CON	10.905	0.0976	10	0.0005	
Mix1, Mix2	2.94E+00	0.1046	10	0.0466	
Mix1, CON	8.9819	0.1028	10	0.001	
Mix2, CON	5.1202	0.097	10	0.0069	

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Urease	Tt	5	0.33762	6.75E-02	1.6267	0.2116	9942
	Res	12	0.4981	4.15E-02			
	Total	17	0.83572				

Basal respiration, soil priming effects and mineralization rate

PerANOVA

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
BR	Tt	5	5100.9	1020.2	24.899	0.0001	9953
	Res	12	491.67	40.972			
	Total	17	5592.5				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	6.4695	0.1005	10	0.0029
CG, CC	8.5586	0.0985	10	0.0014
CG, Mix1	10.133	0.0999	10	0.0003
CG, Mix2	5.2189	0.0989	10	0.0066
CG, CON	9.323	0.0968	10	0.0011
SS, CC	5.5767	0.1021	10	0.0076
SS, Mix1	2.3383	0.1005	10	0.0779
SS, Mix2	1.6268	0.207	10	0.1875
SS, CON	6.8499	0.102	10	0.0033
CC, Mix1	7.7142	0.1032	10	0.0017
CC, Mix2	4.1513	0.1009	10	0.0142
CC, CON	12.989	0.1011	10	0.0002
Mix1, Mix2	0.42522	0.8958	10	0.6902
Mix1, CON	11.137	0.1024	10	0.0004
Mix2, CON	5.675	0.1	10	0.0045

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
µg of C	Tt	5	2.65E+06	5.31E+05	87.537	0.0001	9957
	Res	12	72764	6063.7			
	Total	17	2.73E+06				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	14.894	0.1032	10	0.0001
CG, CC	4.0376	0.1017	10	0.0154
CG, Mix1	16.674	0.1004	10	0.0001
CG, Mix2	35.717	0.1042	10	0.0001
CG, CON	14.854	0.0951	10	0.0002
SS, CC	3.761	0.0919	10	0.0173
SS, Mix1	0.68368	0.5074	10	0.5276
SS, Mix2	0.57602	0.6906	10	0.5962
SS, CON	20.749	0.0974	10	0.0001
CC, Mix1	3.4702	0.1012	10	0.0259
CC, Mix2	3.9066	0.0976	10	0.0172
CC, CON	6.8383	0.0975	10	0.0032
Mix1, Mix2	0.33607	0.8	10	0.7497
Mix1, CON	24.067	0.0978	10	0.0001

	Mix2, CON	72.442	0.1019	10	0.0001		

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Priming effect	Tt	5	25022	5004.4	7.2982	0.0077	9950
	Res	9	6171.3	685.71			
	Total	14	31194				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	9.2134	0.0981	10	0.0037
CG, CC	0.99299	0.3967	10	0.386
CG, Mix1	5.3961	0.0995	10	0.0073
CG, Mix2	4.4164	0.1013	10	0.0222
CG, CON	1.326	0.3039	10	0.2481
SS, CC	1.4619	0.3401	3	0.28
SS, Mix1	6.4292	0.1006	10	0.0078
SS, Mix2	4.5458	0.3335	3	0.0481
SS, CON	18.528	0.095	10	0.0006
CC, Mix1	0.3839	0.799	10	0.7142
CC, Mix2	0.34552	1	3	0.7702
CC, CON	1.3305	0.305	10	0.2756
Mix1, Mix2	0.3059	0.7952	10	0.773
Mix1, CON	11.049	0.1003	10	0.0006
Mix2, CON	8.952	0.0994	10	0.0029

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Mineralized glucose	Tt	5	830.58	166.12	75.585	0.0001	9954
	Res	12	26.373	2.1977			
	Total	17	856.95				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	12.299	0.0983	10	0.0001
CG, CC	2.0519	0.0981	10	0.1103
CG, Mix1	21.557	0.0946	10	0.0001
CG, Mix2	7.0099	0.1006	10	0.0021
CG, CON	22.734	0.1021	10	0.0001
SS, CC	1.8902	0.2051	10	0.1264
SS, Mix1	0.30866	1	10	0.7731
SS, Mix2	1.4193	0.1995	10	0.2335
SS, CON	26.832	0.0958	10	0.0001
CC, Mix1	1.8578	0.0967	10	0.1373
CC, Mix2	1.1008	0.4971	10	0.3293
CC, CON	8.6886	0.0967	10	0.0011
Mix1, Mix2	1.4273	0.2067	10	0.2272
Mix1, CON	35.684	0.0947	10	0.0001
Mix2, CON	19.386	0.0966	10	0.0001

Alpha-diversity characteristics

PerANOVA

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
ASVs richness	Tt	5	1,5899E5	31797	4,678	0,0054	9852
	Res	11	74769	6797,1			
	Total	16	2,3375E5				

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	7.0851	0,0953	10	0,0019
CG, CC	19.997	0,097	10	0,0003
CG, Mix1	11.715	0,1007	10	0,0003

CG, Mix2	3.987	0,0962	10	0,0148	
CG, CON	2.418	0,103	10	0,0883	
SS, CC	0.95456	0,4979	9	0,3887	
SS, Mix1	2.5006	0,0936	10	0,0659	
SS, Mix2	2.9186	0,1017	9	0,0458	
SS, CON	0.60944	0,7082	10	0,5805	
CC, Mix1	5.0343	0,0977	10	0,0056	
CC, Mix2	3.3329	0,1002	10	0,0326	
CC, CON	0.87208	0,7036	10	0,4504	
Mix1, Mix2	1.3609	0,406	7	0,2402	
Mix1, CON	1.2659	0,3064	10	0,3004	
Mix2, CON	1.5139	0,199	10	0,2379	

Faith's phylogenetic index	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
	Tt	5	547,03	109,41	3,9218	0,0165	9959
	Res	11	306,87	27,897			
	Total	16	853,9				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms	P(MC)		
	CG, SS	7.0947	0,0969	10	0,0021		
	CG, CC	22.068	0,1019	10	0,0001		
	CG, Mix1	18.54	0,1008	10	0,0001		
	CG, Mix2	3.7642	0,0941	10	0,0164		
	CG, CON	2.1827	0,1035	10	0,1204		
	SS, CC	1.8433	0,2014	10	0,1391		
	SS, Mix1	2.5784	0,0993	10	0,0632		
	SS, Mix2	2.6304	0,1031	10	0,0565		
	SS, CON	0.33502	0,7949	10	0,7593		
	CC, Mix1	2.9846	0,1014	10	0,0439		
	CC, Mix2	1.8193	0,3004	10	0,1434		
	CC, CON	0.82817	0,7044	10	0,4739		
	Mix1, Mix2	1.0321	0,4016	10	0,3638		
	Mix1, CON	1.0187	0,3888	10	0,3769		
	Mix2, CON	1.2153	0,2048	10	0,3134		

Bacterial community

	PerMANOVA						
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Phylum level	Tt	5	6685,1	1337	8,1772	0,0001	9922
	Res	12	1962,1	163,51			
	Total	17	8647,1				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms	P(MC)		
	CG, SS	3.4894	0,0999	10	0,0077		
	CG, CC	2.8289	0,1006	10	0,0195		
	CG, Mix1	2.2872	0,1027	10	0,0361		
	CG, Mix2	1.5161	0,0945	10	0,1232		
	CG, CON	2.4452	0,1026	10	0,0245		
	SS, CC	2.0736	0,1	10	0,056		
	SS, Mix1	1.6908	0,0995	10	0,111		
	SS, Mix2	5.0017	0,0993	10	0,0029		
	SS, CON	2.7886	0,1052	10	0,0162		
	CC, Mix1	1.4495	0,203	10	0,1859		
	CC, Mix2	4.5335	0,0997	10	0,0039		
	CC, CON	3.119	0,1004	10	0,0095		
	Mix1, Mix2	3.5309	0,1022	10	0,0057		
	Mix1, CON	3.2456	0,0984	10	0,0082		
	Mix2, CON	2.6246	0,0987	10	0,0205		

At the genus level or at the next identified	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
	Tt	5	22145	4429	5,7029	0,0001	9959

higher taxonomic level	Res	12	9319,4	776,62	
	Total	17	31464		

Pair-wise tests

Groups	t	P(perm)	Unique perms	P(MC)
CG, SS	3.2001	0,1007	10	0,0055
CG, CC	2.6481	0,1001	10	0,0118
CG, Mix1	2.3852	0,1036	10	0,0191
CG, Mix2	2.8469	0,0958	10	0,009
CG, CON	1.8597	0,1041	10	0,0485
SS, CC	3.374	0,0963	10	0,0043
SS, Mix1	1.9871	0,0998	10	0,0319
SS, Mix2	3.3765	0,0991	10	0,0049
SS, CON	2.0734	0,1002	10	0,0335
CC, Mix1	2.7904	0,0945	10	0,0091
CC, Mix2	2.6276	0,0944	10	0,0123
CC, CON	2.138	0,1016	10	0,0248
Mix1, Mix2	2.8871	0,0953	10	0,0081
Mix1, CON	2.161	0,1047	10	0,0262
Mix2, CON	2.2522	0,1054	10	0,0243

Notes: df = degree of freedom; SS = Sum of squares; MS = Mean squares; Unique perms = Number of unique values of the test statistic obtained under permutation.

Supplementary Table 3. Distance-based redundancy analysis results, explanatory variance and contribution of relative abundance of soil bacterial taxa, physicochemical and biochemical soil properties (priming effects and enzymatic activity).

Relative abundance of soil bacterial taxa and physicochemical soil properties

AICc 135.3 R^2 0.81

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual	Cumulative
1	36.97	36.97
2	17.49	54.47

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
pH	-3.56	2.35
TOC	18.83	-29.43
pF (-1500 KPa)	-0.86	2.83
TN	-10.37	22.77
C/N ratio	-4.15	-7.96
Assimilable phosphorus	1.11	4.05
Carbohydrates	6.95	1.61
Polyphenols	-1.37	-6.81

Relative abundance of soil bacterial taxa and biochemical soil properties

AICc 127.95 R^2 0.59

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual	Cumulative
1	35.27	35.27
2	16.76	52.03

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
Dehydrogenase	0.00	5.27
β-glucosidase	3.05	-6.93
Urease	2.22	5.28
Basal respiration	-4.60	-14.31
Mineralized glucose	20.03	10.19

Supplementary Table 4. Similarity percentage (SIMPER) analysis from bacterial taxa (at the genus level or the next upper taxonomic level) belonging to two co-occurrence patterns contributing to dissimilarity between different treatments and unamended soils.

Group CG	Average similarity:	76.98			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bacte. Microscillaceae uncultured	1.25	6.17	6.99	8.02	8.02
Bacte. Cryomorphaceae uncultured	1.11	5.48	4.37	7.11	15.13
Prote. Sphingomonadaceae	0.89	4.78	7.22	6.21	21.34
Gemma. Longimicrobiaceae uncultured	0.96	4.17	9.04	5.41	26.75
Planc. uncultured	0.65	2.96	5.06	3.84	30.6
Prote. Sphingomonas	0.66	2.92	9.4	3.79	34.39
Acido. Subgroup_6	0.76	2.71	4.1	3.52	37.91
Actin. Blastococcus	0.5	2.46	4.8	3.2	41.11
Prote. Pseudohongiella	0.43	2.46	9.43	3.19	44.3
Prote. Mesorhizobium	0.53	2.41	8.82	3.13	47.43
Bacte. Pedobacter	0.45	2.26	8.22	2.93	50.36
Actin. 0319-7L14 uncultured_2	0.74	2.1	8.38	2.73	53.09
Bacte. Galbibacter	0.45	2.1	9.61	2.73	55.82
Actin. Euzebyaceae uncultured	0.47	2.1	8.91	2.73	58.55
Prote. Paracoccus	0.35	1.99	9.53	2.59	61.14
Prote. Burkholderiaceae	0.33	1.9	8.63	2.47	63.61
Prote. Verticia	0.33	1.79	8.56	2.33	65.94
Prote. Aminobacter	0.3	1.66	9.22	2.16	68.1
Prote. Skermanella	0.29	1.6	9.35	2.07	70.17

Group SS	Average similarity:	84.73			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bacte. Pedobacter	3.21	10.37	13.66	12.24	12.24
Bacte. Cryomorphaceae uncultured	2.02	6.46	25.63	7.63	19.87
Bacte. Galbibacter	2.25	5.53	6.79	6.53	26.4
Bacte. Flavobacterium	1.68	5.36	7.05	6.33	32.73
Bacte. Parapedobacter	1.59	5.07	48.91	5.98	38.71
Prote. Alcanivorax	1.44	4.81	40.36	5.67	44.39
Bacte. Sphingobacteriaceae	1.55	4.62	9.34	5.45	49.84
Prote. Burkholderiaceae	1.38	4.48	17.29	5.28	55.12
Bacte. Sphingobacterium	1.52	4.41	3.2	5.21	60.33
Actin. Glutamicibacter	1.5	4.19	4.96	4.95	65.28
Prote. Verticia	1.02	3.21	9.18	3.78	69.06
Prote. Pseudomonas	1.07	3.18	9.52	3.75	72.81
Prote. Pseudohongiella	0.94	3.09	14.55	3.64	76.45
Prote. Paracoccus	0.65	2.11	17.9	2.49	78.94
Actin. Microbacterium	0.67	2.04	8.32	2.4	81.35
Prote. Brevundimonas	0.9	2	4.13	2.36	83.7
Prote. Aminobacter	0.64	1.89	23.73	2.23	85.93
Bacte. Olivibacter	0.76	1.86	2.4	2.2	88.13

Group CC	Average similarity:	74.73			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bacte. Cryomorphaceae uncultured	1.45	10.5	7.16	14.05	14.05
Gemma. Longimicrobiaceae uncultured	0.9	6.82	6.58	9.12	23.17
Bacte. Parapedobacter	1.35	5.92	2.56	7.92	31.09
Prote. Sphingomonadaceae	0.56	4.66	15.81	6.24	37.33
Bacte. Galbibacter	0.53	3.31	2.32	4.43	41.76
Prote. Brevundimonas	0.46	3.22	21.03	4.3	46.07
Prote. Burkholderiaceae	0.41	2.83	2.71	3.78	49.85
Bacte. Microscillaceae uncultured	0.45	2.71	5.45	3.63	53.48
Bacte. Pedobacter	0.35	2.57	16.6	3.44	56.92
Prote. Phyllobacteriaceae uncultured	0.37	2.35	3.18	3.15	60.07
Prote. Pseudomonas	0.4	2.33	4.59	3.12	63.19
Prote. Aminobacter	0.31	2.17	6.34	2.91	66.1
Prote. Verticia	0.29	2.13	5.6	2.85	68.94
Bacte. Sphingobacteriaceae	0.29	2.11	8.2	2.82	71.76
Prote. Skermanella	0.24	1.89	22.86	2.52	74.28
Prote. Novosphingobium	0.27	1.79	10.23	2.39	76.67

Prote. Sphingomonas	0.22	1.63	8.09	2.19	78.86
Prote. Rhodobacteraceae uncultured	0.21	1.53	4.85	2.05	80.91
Planc. uncultured	0.21	1.53	5.37	2.04	82.95

Group Mix1	Average similarity:	85.61			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bacte. Pedobacter	2.31	9.35	15.28	10.93	10.93
Bacte. Cryomorphaceae uncultured	2.15	8.36	6.59	9.77	20.69
Actin. Glutamicibacter	1.29	4.8	4.37	5.61	26.3
Bacte. Parapedobacter	1.1	4.62	19.16	5.4	31.7
Bacte. Flavobacterium	1.12	4.57	10.98	5.34	37.04
Prote. Burkholderiaceae	1.04	4.28	8.93	5.01	42.05
Bacte. Sphingobacterium	1.03	3.93	5.29	4.59	46.64
Prote. Verticia	0.87	3.42	12.51	3.99	50.63
Bacte. Galbibacter	0.99	3.28	4.97	3.83	54.46
Prote. Pseudohongiella	0.84	3.16	4.08	3.69	58.15
Bacte. Olivibacter	0.81	3.11	4.2	3.64	61.79
Actin. Microbacterium	0.57	2.34	10.83	2.74	64.53
Bacte. Sphingobacteriaceae	0.62	2.29	9.84	2.68	67.21
Bacte. Microscillaceae uncultured	0.64	2.2	5.87	2.56	69.77
Prote. Aminobacter	0.51	2.15	22.11	2.52	72.29
Prote. Alcanivorax	0.72	2.07	1.81	2.42	74.71
Prote. Sphingomonadaceae	0.45	1.87	22.24	2.19	76.89
Prote. Paracoccus	0.43	1.86	12.09	2.18	79.07
Prote. Pseudomonas	0.52	1.86	3.76	2.17	81.24
Bacte. Chryseobacterium	0.53	1.81	5.98	2.12	83.36
Bacte. Taibaiella	0.44	1.77	21.64	2.07	85.43

Group Mix2	Average similarity:	84.29			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bacte. Cryomorphaceae uncultured	1.2	6.13	34.87	7.27	7.27
Bacte. Pedobacter	1.16	6.11	18.19	7.25	14.53
Prote. Verticia	1.02	5.51	24.74	6.54	21.06
Actin. Glutamicibacter	1.15	5.44	6.26	6.46	27.52
Prote. Pseudomonas	0.89	4.5	8.35	5.34	32.86
Prote. Burkholderiaceae	0.82	4.4	9.94	5.22	38.08
Bacte. Parapedobacter	0.8	4.39	15.35	5.21	43.29
Prote. Aminobacter	0.74	4.18	18.49	4.96	48.25
Gemma. Longimicrobiaceae uncultured	0.7	3.85	30.47	4.57	52.82
Prote. Paracoccus	0.63	3.64	35.91	4.32	57.13
Bacte. Galbibacter	0.74	3.6	19.16	4.27	61.4
Prote. Pseudohongiella	0.6	2.9	6.49	3.44	64.84
Prote. Microvirga	0.47	2.38	10.51	2.83	67.67
Bacte. Sphingobacterium	0.43	2.18	5.88	2.59	70.26
Prote. Rhodobacteraceae uncultured	0.36	2.06	20.65	2.44	72.7
Bacte. Sphingobacteriaceae	0.37	1.97	5.23	2.34	75.04
Actin. Microbacterium	0.35	1.87	7.49	2.21	77.25
Prote. Sphingomonadaceae	0.33	1.73	18.21	2.05	79.3

Group CON	Average similarity:	43.97			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Prote. Sphingomonadaceae	2.47	7.81	21.92	17.76	17.76
Gemma. Longimicrobiaceae uncultured	1.73	5.57	20.35	12.68	30.43
Acido. Subgroup_6	2.01	5.11	2.86	11.63	42.06
Prote. Sphingomonas	2.16	4.77	2.5	10.85	52.91
Prote. Sphingomonadaceae uncultured	0.92	3.05	3.49	6.93	59.84
Gemma. Gemmatimonas	0.75	2.59	10.38	5.89	65.73
Gemma. Gemmatimonadaceae uncultured	0.77	2.06	5.61	4.69	70.42
Bacte. Pedobacter	0.82	1.93	3.89	4.38	74.81
Firmi. Staphylococcus	0.72	1.46	1.58	3.32	78.13
Actin. 0319-7L14 uncultured_2	1.06	1.17	0.58	2.66	80.79
Actin. Blastococcus	0.74	1.1	0.58	2.51	83.3

Groups CG & SS	Average similarity:	61.21				
	Group CG	Group SS				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	0.45	3.21	5.99	7.92	9.79	9.79
Bacte. Galbibacter	0.45	2.25	3.98	1.84	6.51	16.3
Bacte. Flavobacterium	0.25	1.68	3.11	5.13	5.09	21.38
Bacte. Parapedobacter	0.22	1.59	2.98	6.81	4.87	26.25
Bacte. Sphingobacterium	0.19	1.52	2.91	3.18	4.76	31.02
Prote. Alcanivorax	0.1	1.44	2.89	20.36	4.72	35.74
Actin. Glutamicibacter	0.17	1.5	2.87	3.92	4.68	40.42
Bacte. Sphingobacteriaceae	0.25	1.55	2.8	5.86	4.57	44.99
Prote. Burkholderiaceae	0.33	1.38	2.26	8.96	3.69	48.68
Bacte. Cryomorphaceae uncultured	1.11	2.02	1.95	3.38	3.18	51.86
Prote. Pseudomonas	0.25	1.07	1.76	4.08	2.88	54.74
Actin. 0319-7L14 uncultured_2	0.74	0	1.52	1.5	2.48	57.23
Gemma. Longimicrobiaceae uncultured	0.96	0.24	1.51	2.24	2.47	59.7
Prote. Verticia	0.33	1.02	1.51	4.9	2.46	62.16
Bacte. Microscillaceae uncultured	1.25	0.57	1.49	1.81	2.43	64.59
Prote. Brevundimonas	0.25	0.9	1.41	1.53	2.3	66.89
Bacte. Olivibacter	0.1	0.76	1.4	2.52	2.29	69.19
Acido. Subgroup_6	0.76	0.11	1.36	1.77	2.22	71.41
Prote. Sphingomonas	0.66	0.05	1.3	3.24	2.12	73.53

Groups CG & CC	Average similarity:	37.33				
	Group CG	Group CC				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Parapedobacter	0.22	1.35	3.85	1.36	10.3	10.3
Bacte. Microscillaceae uncultured	1.25	0.45	2.8	2.42	7.49	17.79
Actin. 0319-7L14 uncultured_2	0.74	0.1	2.03	1.29	5.45	23.24
Acido. Subgroup_6	0.76	0.25	1.71	1.5	4.59	27.83
Planc. uncultured	0.65	0.21	1.45	2.96	3.89	31.72
Prote. Sphingomonas	0.66	0.22	1.45	2.48	3.88	35.6
Bacte. Cryomorphaceae uncultured	1.11	1.45	1.39	1.55	3.71	39.31
Prote. Sphingomonadaceae	0.89	0.56	1.15	2.98	3.08	42.39
Actin. Blastococcus	0.5	0.18	1.13	2.2	3.02	45.41
Chlor. Gitt-GS-136 uncultured	0.52	0.19	1.11	1.07	2.98	48.39
Prote. Pseudohongiella	0.43	0.12	1.11	8.69	2.96	51.35
Actin. 0319-7L14 uncultured_1	0.39	0.05	1.1	1.31	2.94	54.29
Actin. Euzebyaceae uncultured	0.47	0.14	1.09	2.47	2.93	57.22
Planc. Pirellula	0.36	0.06	1.02	2.6	2.72	59.94
Prote. Mesorhizobium	0.53	0.22	1.01	1.75	2.71	62.65
Gemma. Longimicrobiaceae uncultured	0.96	0.9	0.95	1.61	2.55	65.2
Prote. Sphingomonadaceae uncultured	0.26	0	0.93	4.7	2.49	67.69
Prote. Paracoccus	0.35	0.11	0.83	2.5	2.22	69.91
Prote. Alcanivorax	0.1	0.31	0.82	1.04	2.2	72.11

Groups SS & CC	Average similarity:	56.45				
	Group SS	Group CC				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	3.21	0.35	6.96	10.86	12.33	12.33
Bacte. Galbibacter	2.25	0.53	4.28	1.76	7.59	19.92
Bacte. Flavobacterium	1.68	0.16	3.7	6.52	6.55	26.47
Actin. Glutamicibacter	1.5	0.02	3.6	4.5	6.38	32.85
Bacte. Sphingobacterium	1.52	0.09	3.49	3.77	6.19	39.04
Bacte. Sphingobacteriaceae	1.55	0.29	3.03	5.91	5.37	44.4
Prote. Alcanivorax	1.44	0.31	2.76	3.89	4.89	49.29
Prote. Burkholderiaceae	1.38	0.41	2.34	8.32	4.15	53.44
Prote. Pseudohongiella	0.94	0.12	2	11.29	3.55	56.99
Bacte. Parapedobacter	1.59	1.35	1.93	2.67	3.43	60.42
Bacte. Olivibacter	0.76	0	1.82	3.03	3.22	63.64
Prote. Verticia	1.02	0.29	1.77	5.61	3.13	66.77
Prote. Pseudomonas	1.07	0.4	1.62	2.77	2.87	69.64
Actin. Microbacterium	0.67	0	1.62	7.72	2.87	72.5
Gemma. Longimicrobiaceae uncultured	0.24	0.9	1.61	3.52	2.86	75.36
Bacte. Taibaiella	0.72	0.05	1.61	2.31	2.85	78.21
Bacte. Cryomorphaceae uncultured	2.02	1.45	1.38	1.82	2.45	80.67
Prote. Paracoccus	0.65	0.11	1.3	4.88	2.31	82.97

Bacte. Chryseobacterium	0.55	0.02	1.28	2.78	2.26	85.23
Prote. Brevundimonas	0.9	0.46	1.13	1.15	2	87.24

Groups CG & Mix1	Average similarity:	50.01				
	Group CG	Group Mix1				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	0.45	2.31	4.73	6.07	9.46	9.46
Actin. Glutamicibacter	0.17	1.29	2.84	4.07	5.67	15.13
Bacte. Cryomorphaceae uncultured	1.11	2.15	2.57	2.62	5.15	20.27
Bacte. Parapedobacter	0.22	1.1	2.26	5.28	4.51	24.79
Bacte. Flavobacterium	0.25	1.12	2.21	4.25	4.42	29.21
Bacte. Sphingobacterium	0.19	1.03	2.15	3.29	4.29	33.5
Bacte. Olivibacter	0.1	0.81	1.81	4.22	3.61	37.11
Prote. Burkholderiaceae	0.33	1.04	1.8	5.64	3.59	40.71
Actin. 0319-7L14 uncultured_1	0.74	0.03	1.69	1.43	3.38	44.09
Bacte. Microscillaceae uncultured	1.25	0.64	1.56	1.83	3.13	47.22
Prote. Alcanivorax	0.1	0.72	1.56	1.79	3.12	50.34
Gemma. Longimicrobiaceae uncultured	0.96	0.34	1.51	2.09	3.02	53.35
Acido. Subgroup_6	0.76	0.14	1.5	1.79	2.99	56.35
Prote. Sphingomonas	0.66	0.07	1.46	3.08	2.92	59.27
Bacte. Galbibacter	0.45	0.99	1.42	1.36	2.84	62.11
Prote. Verticia	0.33	0.87	1.39	3.52	2.78	64.89
Bacte. Chryseobacterium	0.05	0.53	1.23	2.7	2.45	67.34
Planc. uncultured	0.65	0.16	1.2	3.23	2.41	69.75
Prote. Sphingomonadaceae	0.89	0.45	1.13	3.94	2.25	72
Prote. Pseudohongiella	0.43	0.84	1.03	2.22	2.06	74.05
Actin. Euzebyaceae uncultured	0.47	0.06	1.01	2.97	2.01	76.07

Groups SS & Mix1	Average similarity:	21.95				
	Group SS	Group Mix1				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Galbibacter	2.25	0.99	2.48	1.3	11.31	11.31
Bacte. Sphingobacteriaceae	1.55	0.62	1.79	3.39	8.17	19.47
Bacte. Pedobacter	3.21	2.31	1.75	2.46	7.99	27.47
Prote. Alcanivorax	1.44	0.72	1.39	2.05	6.34	33.81
Bacte. Flavobacterium	1.68	1.12	1.1	2.7	5.01	38.82
Prote. Pseudomonas	1.07	0.52	1.07	2.31	4.89	43.71
Bacte. Sphingobacterium	1.52	1.03	1.04	1.54	4.72	48.43
Prote. Brevundimonas	0.9	0.4	0.97	1.17	4.42	52.85
Bacte. Parapedobacter	1.59	1.1	0.95	3.1	4.33	57.18
Actin. Glutamicibacter	1.5	1.29	0.76	1.4	3.47	60.65
Bacte. Cryomorphaceae uncultured	2.02	2.15	0.67	1.78	3.07	63.71
Prote. Burkholderiaceae	1.38	1.04	0.65	2.24	2.97	66.68
Bacte. Taibaiella	0.72	0.44	0.57	1.06	2.59	69.27
Bacte. Olivibacter	0.76	0.81	0.5	1.33	2.26	71.53
Bacte. Microscillaceae uncultured	0.57	0.64	0.49	1.5	2.23	73.77
Prote. Rhodobacteraceae uncultured	0.03	0.26	0.45	2.12	2.06	75.83

Groups CC & Mix1	Average similarity:	44.9				
	Group CC	Group Mix1				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	0.35	2.31	5.7	8.56	12.7	12.7
Actin. Glutamicibacter	0.02	1.29	3.71	4.83	8.27	20.96
Bacte. Flavobacterium	0.16	1.12	2.77	5.82	6.18	27.15
Bacte. Sphingobacterium	0.09	1.03	2.72	4.87	6.05	33.2
Bacte. Olivibacter	0	0.81	2.37	4.94	5.27	38.47
Prote. Pseudohongiella	0.12	0.84	2.1	4.4	4.68	43.15
Bacte. Cryomorphaceae uncultured	1.45	2.15	2.07	1.89	4.6	47.75
Bacte. Parapedobacter	1.35	1.1	1.88	1.23	4.18	51.93
Prote. Burkholderiaceae	0.41	1.04	1.83	4.59	4.07	56
Prote. Verticia	0.29	0.87	1.68	3.98	3.74	59.74
Actin. Microbacterium	0	0.57	1.66	8.46	3.69	63.43
Gemma. Longimicrobiaceae uncultured	0.9	0.34	1.64	4.04	3.64	67.07
Bacte. Chryseobacterium	0.02	0.53	1.47	3.54	3.27	70.35
Prote. Alcanivorax	0.31	0.72	1.44	1.5	3.21	73.56
Bacte. Galbibacter	0.53	0.99	1.4	1.13	3.11	76.67
Bacte. Taibaiella	0.05	0.44	1.16	4.27	2.58	79.25

Bacte. Sphingobacteriaceae	0.29	0.62	0.93	2.4	2.08	81.33
Prote. Paracoccus	0.11	0.43	0.93	3.25	2.07	83.4

Groups CG & Mix2	Average similarity:	42.83				
	Group CG	Group Mix2				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Actin. Glutamicibacter	0.17	1.15	2.89	3.04	6.76	6.76
Bacte. Microscillaceae uncultured	1.25	0.38	2.57	2.54	6.01	12.76
Bacte. Pedobacter	0.45	1.16	2.12	3.22	4.95	17.71
Prote. Verticia	0.33	1.02	2.04	5.11	4.77	22.49
Actin. 0319-7L14 uncultured_2	0.74	0	2.04	1.56	4.76	27.24
Prote. Pseudomonas	0.25	0.89	1.86	4.18	4.34	31.59
Bacte. Parapedobacter	0.22	0.8	1.73	3.65	4.05	35.64
Prote. Sphingomonas	0.66	0.06	1.72	3.45	4.01	39.65
Acido. Subgroup_6	0.76	0.18	1.65	1.54	3.85	43.49
Prote. Sphingomonadaceae	0.89	0.33	1.64	4.61	3.84	47.33
Prote. Burkholderiaceae	0.33	0.82	1.42	4.65	3.32	50.65
Actin. Euzebyaceae uncultured	0.47	0	1.34	4.17	3.14	53.79
Prote. Aminobacter	0.3	0.74	1.3	6.22	3.03	56.82
Chlor. Gitt-GS-136 uncultured	0.52	0.11	1.1	1.15	2.56	59.38
Planc. uncultured	0.65	0.26	1.1	2.18	2.56	61.94
Actin. 0319-7L14 uncultured_1	0.39	0	1.07	1.57	2.5	64.45
Prote. Alcanivorax	0.1	0.46	1.06	1.08	2.49	66.93
Prote. Mesorhizobium	0.53	0.18	0.99	1.95	2.31	69.24
Actin. Blastococcus	0.5	0.19	0.92	2.54	2.15	71.39
Bacte. Galbibacter	0.45	0.74	0.9	1.39	2.11	73.49
Planc. Pirellula	0.36	0.05	0.88	2.53	2.07	75.56

Groups SS & Mix2	Average similarity:	37.05				
	Group SS	Group Mix2				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	3.21	1.16	4.43	7.96	11.95	11.95
Bacte. Galbibacter	2.25	0.74	3.34	1.6	9	20.95
Bacte. Flavobacterium	1.68	0.32	2.94	6.45	7.95	28.9
Bacte. Sphingobacteriaceae	1.55	0.37	2.52	5.43	6.8	35.7
Bacte. Sphingobacterium	1.52	0.43	2.38	3.03	6.43	42.13
Prote. Alcanivorax	1.44	0.46	2.11	3.04	5.69	47.82
Bacte. Cryomorphaceae uncultured	2.02	1.2	1.79	3.01	4.83	52.65
Bacte. Parapedobacter	1.59	0.8	1.71	6.31	4.61	57.26
Bacte. Olivibacter	0.76	0.15	1.3	2.14	3.5	60.76
Prote. Brevundimonas	0.9	0.3	1.3	1.42	3.5	64.26
Prote. Burkholderiaceae	1.38	0.82	1.21	4.42	3.27	67.53
Bacte. Taibaiella	0.72	0.19	1.12	1.82	3.02	70.55
Gemma. Longimicrobiaceae uncultured	0.24	0.7	0.99	3.29	2.68	73.23
Actin. Glutamicibacter	1.5	1.15	0.96	1.31	2.59	75.82
Bacte. Chryseobacterium	0.55	0.19	0.78	1.76	2.09	77.91

Groups CC & Mix2	Average similarity:	37.8				
	Group CC	Group Mix2				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Actin. Glutamicibacter	0.02	1.15	3.95	3.51	10.44	10.44
Bacte. Pedobacter	0.35	1.16	2.82	4.19	7.46	17.9
Prote. Verticia	0.29	1.02	2.5	6.71	6.61	24.51
Bacte. Parapedobacter	1.35	0.8	2.44	1.13	6.46	30.97
Prote. Paracoccus	0.11	0.63	1.78	5.66	4.71	35.68
Prote. Pseudohongiella	0.12	0.6	1.68	3.45	4.46	40.13
Prote. Pseudomonas	0.4	0.89	1.66	2.36	4.4	44.53
Prote. Aminobacter	0.31	0.74	1.5	7.03	3.96	48.49
Prote. Burkholderiaceae	0.41	0.82	1.39	3.2	3.68	52.17
Prote. Microvirga	0.12	0.47	1.21	4.15	3.2	55.36
Actin. Microbacterium	0	0.35	1.2	6.72	3.17	58.54
Bacte. Cryomorphaceae uncultured	1.45	1.2	1.19	1.38	3.15	61.69
Prote. Alcanivorax	0.31	0.46	1.17	1.21	3.11	64.8
Bacte. Sphingobacterium	0.09	0.43	1.15	3.34	3.05	67.85
Bacte. Galbibacter	0.53	0.74	0.85	1.05	2.24	70.09
Prote. Sphingomonadaceae	0.56	0.33	0.78	4.2	2.07	72.15

Groups Mix1 & Mix2	Average similarity:	26.96				
	Group Mix1	Group Mix2				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	2.31	1.16	2.89	4.2	10.73	10.73
Bacte. Cryomorphaceae uncultured	2.15	1.2	2.39	2.35	8.87	19.6
Bacte. Flavobacterium	1.12	0.32	2.01	5.6	7.46	27.06
Bacte. Olivibacter	0.81	0.15	1.68	3.35	6.23	33.29
Bacte. Sphingobacterium	1.03	0.43	1.52	2.99	5.63	38.92
Prote. Alcanivorax	0.72	0.46	1.05	1.3	3.89	42.81
Prote. Pseudomonas	0.52	0.89	0.95	1.8	3.51	46.32
Gemma. Longimicrobiaceae uncultured	0.34	0.7	0.9	4.52	3.36	49.67
Bacte. Galbibacter	0.99	0.74	0.88	1.1	3.26	52.94
Actin. Glutamicibacter	1.29	1.15	0.86	1.39	3.19	56.13
Bacte. Chryseobacterium	0.53	0.19	0.86	2.06	3.19	59.32
Bacte. Parapedobacter	1.1	0.8	0.76	3.1	2.82	62.14
Bacte. Microscillaceae uncultured	0.64	0.38	0.74	1.38	2.76	64.9
Prote. Microvirga	0.19	0.47	0.71	3.4	2.65	67.54
Prote. Pseudohongiella	0.84	0.6	0.7	1.57	2.58	70.12
Bacte. Taibaiella	0.44	0.19	0.64	3.92	2.36	72.48
Bacte. Sphingobacteriaceae	0.62	0.37	0.61	1.74	2.25	74.73
Prote. Aminobacter	0.51	0.74	0.6	4.89	2.24	76.97
Prote. Burkholderiaceae	1.04	0.82	0.57	1.55	2.12	79.09
Actin. Microbacterium	0.57	0.35	0.56	3.37	2.08	81.17

Groups CG & CON	Average similarity:	53.53				
	Group CG	Group CON				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Prote. Sphingomonadaceae	0.89	2.47	3.69	2.9	6.9	6.9
Prote. Sphingomonas	0.66	2.16	3.38	1.42	6.31	13.21
Acido. Subgroup_6	0.76	2.01	3.03	1.78	5.66	18.87
Bacte. Microscillaceae uncultured	1.25	0.7	3	1.97	5.6	24.47
Actin. 0319-7L14 uncultured_2	0.74	1.06	2.19	1.63	4.09	28.56
Bacte. Cryomorphaceae uncultured	1.11	0.42	2.08	1.11	3.88	32.44
Gemma. Longimicrobiaceae uncultured	0.96	1.73	1.97	1.89	3.68	36.12
Firmi. Staphylococcus	0	0.72	1.8	1.59	3.37	39.49
Prote. Sphingomonadaceae uncultured	0.26	0.92	1.66	2.49	3.1	42.59
Planc. uncultured	0.65	0.38	1.57	2.19	2.94	45.53
Actin. Blastococcus	0.5	0.74	1.45	3.08	2.71	48.24
Actin. Euzebyaceae uncultured	0.47	0.4	1.39	2.96	2.59	50.83
Gemma. Gemmatimonadaceae uncultured	0.28	0.77	1.31	1.62	2.46	53.28
Planc. Pirellula	0.36	0.42	1.3	2.3	2.43	55.71
Actin. Microbacterium	0.21	0.48	1.27	1.22	2.37	58.08
Gemma. Gemmatimonas	0.25	0.75	1.21	4.1	2.26	60.34
Prote. Mesorhizobium	0.53	0.59	1.18	1.63	2.21	62.55
Bacte. Parapedobacter	0.22	0.54	1.18	1.04	2.2	64.75
Prote. Skermanella	0.29	0.56	1.17	1.22	2.18	66.94
Prote. alphaI_cluster	0.14	0.56	1.17	1.12	2.18	69.12
Chlor. Gitt-GS-136 uncultured	0.52	0.42	1.13	1.24	2.11	71.23
Actin. 0319-7L14 uncultured_1	0.39	0.51	1.07	1.76	2	73.22

Groups SS & CON	Average similarity:	76.85				
	Group SS	Group CON				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bacte. Pedobacter	3.21	0.82	4.74	2.51	6.16	6.16
Bacte. Galbibacter	2.25	0.11	4.26	1.86	5.55	11.71
Prote. Sphingomonas	0.05	2.16	3.72	2.08	4.84	16.55
Prote. Sphingomonadaceae	0.54	2.47	3.53	3.56	4.6	21.15
Acido. Subgroup_6	0.11	2.01	3.37	2.6	4.39	25.53
Bacte. Cryomorphaceae uncultured	2.02	0.42	3.24	2.19	4.22	29.76
Bacte. Flavobacterium	1.68	0.1	3.12	3.03	4.06	33.81
Bacte. Sphingobacterium	1.52	0.05	2.89	2.67	3.77	37.58
Gemma. Longimicrobiaceae uncultured	0.24	1.73	2.75	4.21	3.57	41.15
Bacte. Sphingobacteriaceae	1.55	0.17	2.67	3.64	3.47	44.62
Prote. Alcanivorax	1.44	0.07	2.63	6.6	3.42	48.05
Actin. Glutamicibacter	1.5	0.17	2.62	2.27	3.41	51.46
Bacte. Parapedobacter	1.59	0.54	2.19	1.33	2.85	54.31
Prote. Verticia	1.02	0	1.99	3.72	2.59	56.9

Prote. Pseudomonas	1.07	0.07	1.97	2.74	2.56	59.46
Prote. Burkholderiaceae	1.38	0.47	1.89	1.45	2.46	61.93
Prote. Sphingomonadaceae uncultured	0	0.92	1.77	3.29	2.3	64.23
Actin. 0319-7L14 uncultured_2	0	1.06	1.72	1.2	2.24	66.47
Bacte. Microscillaceae uncultured	0.57	0.7	1.61	1.97	2.09	68.56

Groups CC & CON	Average similarity: 64.78					
Species	Group CC Av.Abund	Group CON Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Prote. Sphingomonadaceae	0.56	2.47	5.24	4.77	8.08	8.08
Prote. Sphingomonas	0.22	2.16	4.92	2.04	7.59	15.67
Acido. Subgroup_6	0.25	2.01	4.54	2.64	7.01	22.69
Bacte. Cryomorphaceae uncultured	1.45	0.42	3.47	1.35	5.36	28.04
Bacte. Parapedobacter	1.35	0.54	3.42	1.14	5.28	33.32
Prote. Sphingomonadaceae uncultured	0	0.92	2.74	2.86	4.23	37.55
Actin. 0319-7L14 uncultured_2	0.1	1.06	2.4	1.39	3.7	41.25
Bacte. Microscillaceae uncultured	0.45	0.7	2.24	1.81	3.46	44.71
Gemma. Longimicrobiaceae uncultured	0.9	1.73	2.13	2.08	3.29	48
Firmi. Staphylococcus	0	0.72	2.08	1.64	3.21	51.22
Gemma. Gemmatimonas	0.13	0.75	1.74	6.69	2.69	53.9
Gemma. Gemmatimonadaceae uncultured	0.13	0.77	1.72	2	2.66	56.57
Actin. Blastococcus	0.18	0.74	1.69	2.37	2.61	59.17
Bacte. Galbibacter	0.53	0.11	1.38	1.42	2.13	61.3
Actin. 0319-7L14 uncultured_1	0.05	0.51	1.37	1.31	2.11	63.41
Prote. Mesorhizobium	0.22	0.59	1.34	2.28	2.07	65.47
Prote. alphaI_cluster	0	0.56	1.32	0.92	2.04	67.52

Groups Mix1 & CON	Average similarity: 72.07					
Species	Group Mix1 Av.Abund	Group CON Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Prote. Sphingomonadaceae	0.45	2.47	4.26	4.46	5.9	5.9
Prote. Sphingomonas	0.07	2.16	4.19	2.12	5.82	11.72
Bacte. Cryomorphaceae uncultured	2.15	0.42	4.06	1.99	5.63	17.35
Acido. Subgroup_6	0.14	2.01	3.77	2.73	5.24	22.59
Bacte. Pedobacter	2.31	0.82	3.47	1.82	4.82	27.41
Gemma. Longimicrobiaceae uncultured	0.34	1.73	2.93	4.49	4.07	31.47
Actin. Glutamicibacter	1.29	0.17	2.59	2.05	3.59	35.07
Bacte. Flavobacterium	1.12	0.1	2.35	2.5	3.25	38.32
Bacte. Sphingobacterium	1.03	0.05	2.23	2.63	3.1	41.42
Bacte. Galbibacter	0.99	0.11	2.06	1.67	2.86	44.28
Prote. Verticia	0.87	0	1.98	3.12	2.75	47.03
Prote. Sphingomonadaceae uncultured	0.04	0.92	1.96	3.08	2.72	49.75
Actin. 0319-7L14 uncultured_2	0.03	1.06	1.93	1.25	2.68	52.43
Bacte. Microscillaceae uncultured	0.64	0.7	1.92	2.64	2.67	55.1
Bacte. Parapedobacter	1.1	0.54	1.89	1.55	2.62	57.72
Bacte. Olivibacter	0.81	0.12	1.62	1.95	2.25	59.97
Firmi. Staphylococcus	0	0.72	1.57	1.57	2.18	62.15
Prote. Burkholderiaceae	1.04	0.47	1.54	1.14	2.13	64.28
Gemma. Gemmatimonas	0.05	0.75	1.51	8.55	2.1	66.38
Actin. Microbacterium	0.57	0.48	1.48	3.92	2.05	68.42

Groups Mix2 & CON	Average similarity: 68.66					
Species	Group Mix2 Av.Abund	Group CON Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Prote. Sphingomonadaceae	0.33	2.47	5.13	5.22	7.48	7.48
Prote. Sphingomonas	0.06	2.16	4.75	2.24	6.92	14.39
Acido. Subgroup_6	0.18	2.01	4.17	2.65	6.08	20.47
Prote. Verticia	1.02	0	2.66	2.99	3.88	24.35
Actin. Glutamicibacter	1.15	0.17	2.65	1.7	3.85	28.2
Gemma. Longimicrobiaceae uncultured	0.7	1.73	2.4	2.94	3.5	31.7
Prote. Sphingomonadaceae uncultured	0	0.92	2.35	3.09	3.42	35.12
Bacte. Cryomorphaceae uncultured	1.2	0.42	2.24	1.29	3.26	38.38
Prote. Pseudomonas	0.89	0.07	2.17	2.3	3.16	41.54
Actin. 0319-7L14 uncultured_2	0	1.06	2.15	1.21	3.13	44.67
Bacte. Microscillaceae uncultured	0.38	0.7	1.86	1.44	2.71	47.38
Bacte. Parapedobacter	0.8	0.54	1.84	2.12	2.68	50.06
Prote. Aminobacter	0.74	0.05	1.83	2.53	2.67	52.73

Firmi. Staphylococcus	0	0.72	1.79	1.6	2.61	55.34
Gemma. Gemmatimonas	0.04	0.75	1.75	6.15	2.54	57.89
Bacte. Galbibacter	0.74	0.11	1.7	1.75	2.48	60.37
Bacte. Pedobacter	1.16	0.82	1.62	1.85	2.36	62.73
Gemma. Gemmatimonadaceae uncultured	0.11	0.77	1.56	2.13	2.28	65.01
Actin. Blastococcus	0.19	0.74	1.49	2.47	2.16	67.17
Prote. Burkholderiaceae	0.82	0.47	1.46	1.21	2.13	69.3
Prote. Paracoccus	0.63	0.09	1.46	2.03	2.12	71.42
Actin. Microbacterium	0.35	0.48	1.43	1.98	2.08	73.5

Supplementary Table S5. Number of sequences per sample (n=18) considered in the diversity calculation and used for assignment of taxonomy from QIIME2 software.

Treatment	Sample ID	Sequence Count
CG	P1	30658
	P7	28540
	P14	43850
SS	P2	36636
	P8	43996
	P15	40699
CC	P3	25473
	P9	24357
	P13	44778
Mix1	P4	49772
	P10	38263
	P16	39475
Mix2	P5	36123
	P17	26355
	P11	36955
CON	P6	7265
	P12	53776
	P18	741

3. Supplementary material: Rodríguez-Berbel et al. 2022. Science of the Total Environment

Supplementary Table 1. Number of sequences per sample (n = 21) considered in alpha-diversity estimate and utilised for assignment of taxonomy from QIIME2 software.

	Sample ID	Feature count	Average ± SEM
CON	P6	0	-
	P12	2639	
	P18	0	
CG	P1	27373	54115 ± 16754
	P7	84972	
	P14	50002	
Mix1	P4	63081	66533 ± 3160
	P10	63675	
	P16	72845	
SS	P2	101859	100342 ± 5868
	P8	89505	
	P15	109664	
Mix2	P5	99628	87825 ± 10328
	P11	67242	
	P17	96607	
CC	P3	29982	56585 ± 14411
	P9	60281	
	P13	79494	
NAT	N1	58628	69447 ± 20831
	N2	40015	
	N3	109700	
Total Feature count		1304553	72475 ± 6098

CON: unrestored soils (these samples were removed from the analysis because they did not satisfy the sequence quality filters); CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

Supplementary Table 2. Relative abundance percentage of soil fungal taxa in organic amendments to restored and natural soils. The 13 of the 57 most abundant genera or the next available higher taxonomic level are shown in the legend (relative abundance > 0.1%). Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PERMANOVA).

	Amendments			**Restored soils**					
	CG_0	**SS_0**	**CC_0**	**CG**	**Mix1**	**SS**	**Mix2**	**CC**	**NAT**
Unclassified	0	0	0	0.2	0.0	0.0	0.1	0.2	1.2
Microascus	0.8	2.3	0.3	0.0	0.6	0.7	0.8	0.0	0.0
uncultured (Fam: Microascaceae)	0	0	0	0.2	0.1	0.1	0.4	0.9	0.0
Cephaliophora	0	0	0	0.2	1.0	0.3	0.2	0.0	0.0
Acremonium	0	0	0	0.0	0.4	0.3	0.4	0.1	0.0
uncultured (Fam: Nectriaceae)	0	0	0	0.5	0.2	0.1	0.1	0.3	0.0
Botryotrichum	0	0	0	0.6	0.4	0.1	0.1	0.1	0.0
uncultured (Fam: Plectosphaerellaceae)	0.2	0	2.5	0.0	0.0	0.0	0.1	0.5	0
Gymnascella	0	0	0	0.0	0.1	0.6	0.3	0.0	0.0
Exophiala	0	0	0	0	0	0	0.0	0	0.3
Alternaria	0	0	0	0.5	0.1	0.2	0.1	0.2	0.0
Lophotrichus	0	0	0	0.0	0.1	0.3	0.2	0.0	0.0
uncultured (Class: Dothideomycetes)	0	0	0	0.0	0	0	0	0	0.2
Sodiomyces	0.2	0	0	0.1	0.0	0.0	0.0	0.1	0.0
uncultured (Fam: Chaetomiaceae)	0	0	0.9	0	0	0	0	0	0
uncultured (Class: Eurotiomycetes)	1.3	1.0	1.0	0	0	0	0	0	0
uncultured (Order: Eurotiales)	0.4	0.8	0.7	0	0	0	0	0	0
Phialosimplex	1.1	0	0	0	0	0	0	0	0
				a	**b**	**c**	**b**	**d**	**e**

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

Supplementary Table 3. Distance-based redundancy analysis results, explanatory variance and contribution of relative abundance of soil fungal taxa. physico-chemical and biological soil characteristics.

Relative abundance of soil fungal taxa and physico-chemical soil properties

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual
1	47.57
2	12.41

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
Soil pH	-2.11	18.90
Electrical conductivity (EC)	15.33	10.81
Total nitrogen (TN)	11.51	2.57

Relative abundance of soil fungal taxa and biological soil properties

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual
1	51.05
2	18.47

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
β-glucosidase	-15.51	21.76
Basal respiration (BR)	19.65	8.10
F/B-PLFA ratio	-5.28	-2.99
Dehydrogenase (DHA)	1.45	-12.73

Supplementary Table 4. LDA bars indicate the fungal soil communities within the experimental plots with an LDA score of > 3.5.

Selected Taxa	LDA Score	Associated Group
7: *Botryotrichum*	4.93	CG
6: [F] Nectriaceae	4.85	CG
14: *Mycosphaerella*	4.36	CG
19: [F] Chaetomiaceae	4.34	CG
54: *Alfaria*	4.27	CG
56: *Thermomyces*	4.22	CG
18: *Neocamarosporium*	4.18	CG
4: *Cephaliophora*	5.13	Mix1
48: *Arachniotus*	3.56	Mix1
9: *Gymnascella*	4.92	SS
12: *Lophotrichus*	4.69	SS
31: *Chrysosporium*	4.19	SS
2: *Microascus*	5.04	Mix2
5: *Acremonium*	4.78	Mix2
22: *Gymnoascaceae unidentified*	4.04	Mix2
36: *Kernia*	3.83	Mix2
29: *Arachnomyces*	3.82	Mix2
3: [F] Microascaceae	5.03	CC
8: [F] Plectosphaerellaceae	4.77	CC
34: *Spizellomycetaceae unidentified*	4.05	CC
49: *Iodophanus*	4.00	CC
41: *Stachybotrys*	3.90	CC
1: Unclassified	5.28	NAT
10: *Exophiala*	4.79	NAT
13: [O] Dothideomycetes	4.54	NAT
15: *Picoa*	4.44	NAT
17: *Chaetothyriales unidentified*	4.34	NAT
21: *Sebacinales unidentified*	4.24	NAT
20: *Pyronemataceae unidentified*	4.17	NAT
24: *Pleosporales unidentified*	4.10	NAT
23: [O] Pleosporales	4.05	NAT
35: [F] Didymosphaeriaceae	3.98	NAT
38: *Darksidea*	3.97	NAT
30: *Mortierella*	3.95	NAT
33: [P] Ascomycota	3.92	NAT
40: *Verrucaria*	3.89	NAT
39: *Knufia*	3.89	NAT
45: [C] Agaricomycetes	3.87	NAT
46: *Clitopilus*	3.84	NAT
51: *Botryosphaeriales unidentified*	3.70	NAT

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils. [C]: Fungal soil taxon identified to class level; [O]: Fungal soil taxon identified to order level; [F]: Fungal soil taxon identified to family level.

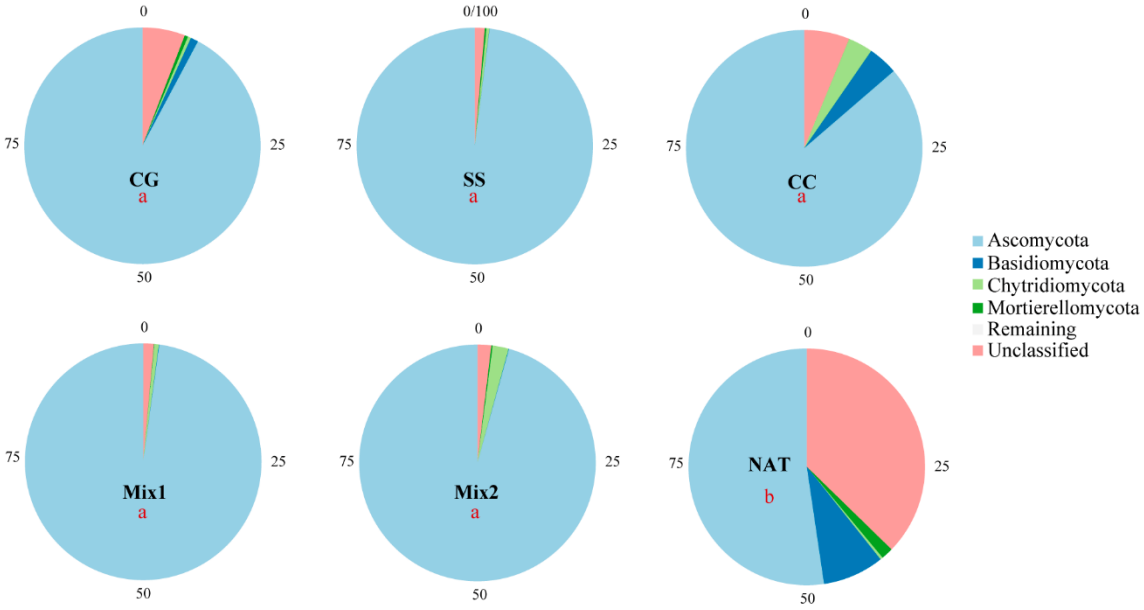


Fig. S1. Pie charts based on the relative abundance (%) of soil fungal phyla. Every pie shows the percent of the relative abundance (%) of the fungal phyla of different treatments applied. Different red letters indicate statistical differences for each treatment ($p < 0.05$; PERMANOVA).

Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

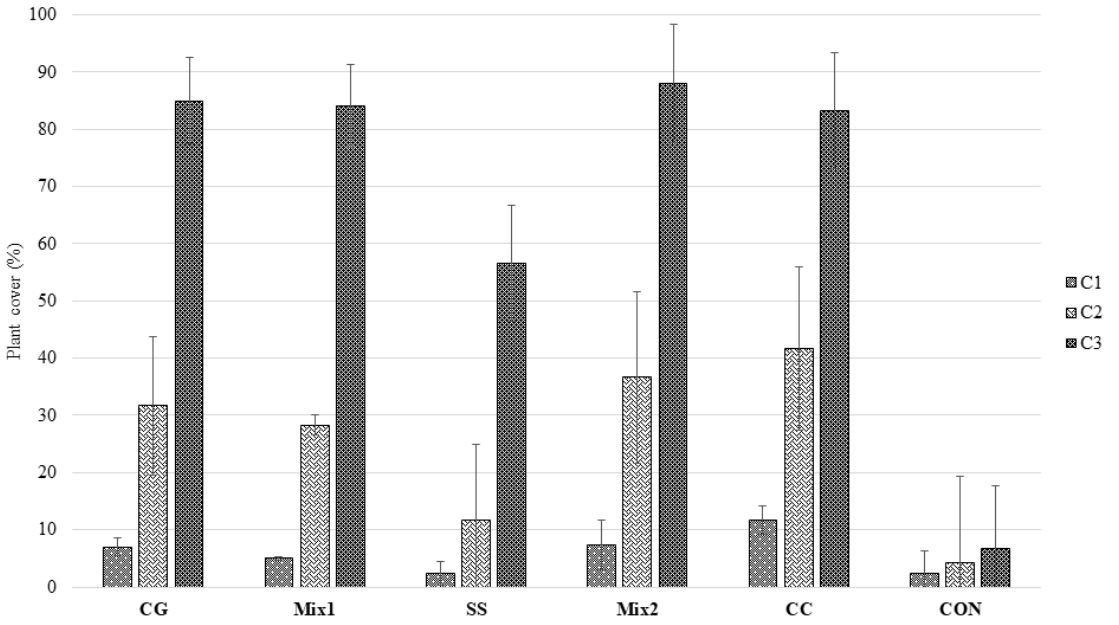


Fig. S2. Percent of the total vegetation cover on experimental plots after 6 (C1), 12 (C2) and 24 months (C3) of restoration.

Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

Restauración de suelos agrícolas abandonados

Oct 2020

Abr 2022

Restauración de suelos de cantera caliza

Dic 2018

May 2021

IN LUMINE SAPIENTIA
UNIVERSITAS ALMERIENSIS