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Study of the effects of organic amendments on soil functionality, and CO2 emission and fixation patterns in restored technosols in a limestone quarry in a semi-arid climate.

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PhD Thesis

Estudio del efecto de enmiendas orgánicas sobre la funcionalidad del suelo, y patrones de emisión y fijación de CO_2 en tecnosuelos restaurados en una cantera caliza en clima semiárido

Study of the effect of organic amendments on soil functionality, and CO_2 emission and fixation patterns in restored technosols in a limestone quarry in a semi-arid climate

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Summary

The soil is the largest terrestrial carbon reservoir, but its formation is a slow and limited process, which makes it susceptible to degradation by human activity. Soil degradation alters biochemical cycles and biodiversity, and can make ecosystems more vulnerable to global change pressure. Degraded soils lose their quality and functionality, reducing their ability to produce environmental goods and ecosystem services (e.g., carbon sequestration and water cycling). These threatens can be reversed and soil can become a source of CO_2 and other greenhouse gases. Soil restoration and conservation is especially important in the arid and semi-arid areas of the Mediterranean region, where processes leading to soil degradation due to human activities, such as open-pit mining for aggregate extraction, can cause a loss of soil quality, reducing its ability to perform its functions. Recovery of soil functions can be accelerated through the application of appropriate techniques. One key strategy for restoring degraded soils due to open-pit mining in semi-arid regions is the application of organic amendments. However, the selection of appropriate organic amendments is essential, in order to improve, in the short term, soil quality and functionality, without contributing to increase CO_2 emissions into the atmosphere.

The objective of this thesis is a better understanding of (i) the biogeochemical processes in the stabilization of exogenous organic matter applied through different types of organic amendments, (ii) their evolution in the development of technosols, and (iii) the impacts on soil functionality and quality in a current context of climate change. This research is part of a project devoted to the restoration of degraded soils by open-pit mining activities, namely the restoration of a quarry for aggregates extraction in the Sierra de Gádor (Almería), where several restoration treatments based on different organic amendments were applied. These treatments consisted of stabilized sewage sludge, vegetable compost from garden waste, vegetable compost from greenhouse crop residues, and two mixtures of sewage sludge with the different vegetable composts. Native plants were also used in the restored plots. In addition, control plots without organic amendments were established, and natural soils from the closer areas were selected as reference level.

To carry out the research, field and laboratory experiments were conducted by an integrated and functional perspective, in order to effectively provide answers to the proposed specific research questions. The accomplisment of the partial objectives also lead to the development of scientific articles, which have been included as chapters of this thesis. In Chapter 1, the short-term effects of organic amendments on different physical, chemical, and biological indicators of soil quality and functionality were examined, with a particular emphasis on organic matter mineralization and CO_2 release due to the priming effect. For this purpose, a set of traditional physical and chemical indicators of soil quality were determined, different enzymatic activity involved in the C,

N and P cycles were quantified, together with the study of soil basal respiration and fatty acid content of bacteria and fungi. Additionally, an IRMS analyzer was used to determine the δ13C isotopic signal, which allowed for the determination of the priming effect and short-term C mineralization. Chapter 2 addressed the partial objective of improving the understanding of the short- and medium-term evolution of exogenous soil organic matter applied in technosols, through the study of their structure and molecular composition, as well as their implications for CO_2 emissions. To this goal, the content of humic and fulvic acids was analyzed, and thermogravimetry (TG), differential scanning calorimetry (DSC) and analytical pyrolysis (Py-GC/MS) analyses were carried out. Chapters 3 and 4 aimed to evaluate the impact of different types of organic amendments on CO_2 emissions as well as to improve our understanding of processes associated to CO_2 exchange in soil and plants (e.g., respiration, photosynthesis). To this goal, field measurement campaigns were carried out to monitor CO_2 fluxes, using two IRGA teams (PP-Systems EGM4 with dark chamber and Licor LI-840a with transparent chamber), covering a wide range of environmental conditions.

The overall results of the study have shown that, among the tested organic amendments, vegetable compost from greenhouses and, by a lower extent, vegetable compost from gardening were more appropiate than stabilized sewage sludge and its mixtures for the restoration of a limestone quarry in a semi-arid climate, in terms of soil properties, CO_2 emission and fixation, priming effect, organic matter quality, and plant growth. Then, the first two treatments were the most favorable in terms of functional recovery in the short to medium term. In addition, the results of this thesis highlighted the importance of broading our understanding on how the restoration measures can be used as a climate change mitigation strategy in degraded semiarid areas, where extreme environmental conditions can lead soils to lose their carbon retention capacity. These findings can be useful in developing more effective and sustainable restoration treatments to promote soil recovery, establish a stable vegetation cover, increase CO_2 sequestration capacity, and improve ecosystem resilience in a changing climate scenario.

Resumen

El suelo es el mayor reservorio terrestre de carbono, pero su formación es un proceso lento y limitado, lo que hace que sea vulnerable a la degradación por la actividad humana. La degradación del suelo altera los ciclos biogeoquímicos y la biodiversidad, y puede hacer que los ecosistemas sean más vulnerables al cambio global. Los suelos degradados pierden su calidad y funcionalidad, mermando su capacidad para producir bienes y servicios ambientales, entre los que destaca el secuestro de carbono, que puede ser revertido, convirtiendo los suelos en emisores de CO_2 y otros gases de efecto invernadero. La conservación de los suelos es especialmente importante en las zonas áridas y semiáridas de la región mediterránea, donde procesos de degradación del suelo debido a actividades humanas, como por ejemplo la minería a cielo abierto para la extracción de áridos, puede causar la pérdida de la calidad del suelo, mermando la capacidad para realizar sus funciones. No obstante, la recuperación de estos suelos podría acelerarse mediante la aplicación de técnicas adecuadas de restauración, que consigan devolver al ecosistema su capacidad funcional. Una estrategia utilizada para restaurar los suelos degradados por la minería a cielo abierto en regiones semiáridas es la aplicación de enmiendas orgánicas. Sin embargo, es esencial seleccionar enmiendas orgánicas adecuadas que mejoren la calidad y funcionalidad de los suelos a corto plazo sin contribuir a incrementar las emisiones de CO_2 a la atmósfera.

En estas premisas se centra el objetivo de la presente tesis, que pretende proveer de una mayor comprensión los procesos biogeoquímicos de estabilización de la materia orgánica exógena aplicada, mediante la aplicación de enmiendas orgánicas de diferente origen, su evolución en el desarrollo de tecnosuelos y cómo pueden afectar a la funcionalidad y calidad del suelo, en el actual contexto del cambio climático. Esta investigación se enmarca en un proyecto dedicado a la restauración de suelos degradados por el impacto de la minería a cielo abierto, concretamente la restauración de una cantera caliza de extracción de áridos en la Sierra de Gádor (Almería), donde se aplicaron varios tratamientos de restauración consistentes en la aplicación de lodos de depuradora estabilizados, compost vegetal de residuos de jardinería, compost vegetal de residuos de cultivos de invernadero y dos mezclas de lodos con los diferentes compost vegetales, junto con la plantación de especies vegetales autóctonas. Además, parcelas sin enmiendas fueron establecidas como control, y suelos naturales del entorno se establecieron como niveles de referencia. Para llevar a cabo la investigación, se llevaron a cabo experimentos en campo y laboratorio, que permitieran abordar el objetivo general de esta tesis desde una perspectiva integrada y funcional, dando respuesta a los objetivos parciales planteados para su desarrollo. Los objetivos parciales en los que desemboca el objetivo principal han sido precursores de los artículos científicos que conforman los capítulos de la presente tesis. El Capítulo 1 se analizan los efectos a corto planzo de las enmiendas orgánicas en distintos indicadores físicos, químicos y biológicos de calidad y funcionalidad de los suelos, con especial énfasis en la mineralización de la materia

orgánica y la liberación de CO_2 debido al efecto de cebado (priming effect). Para ello, se analizaron un conjunto de indicadores físicos y químicos tradicionales de calidad del suelo, se midieron diferentes actividades enzimáticas implicadas en los ciclos del C, N y P, y se estudió la respiración basal del suelo y el contenido en ácidos grasos de bacterias y hongos. También, se empleó un analizador IRMS para la determinación de la señal isotópica δ^{13}C, que permitió determinar el efecto de cebado (priming effect) y la mineralización del C a corto plazo. El Capítulo 2 dio respuesta al objetivo parcial que pretendía mejorar la compresión sobre la evolución a corto y medio plazo de la materia orgánica exógena aplicada en los tecnosuelos, a través del estudio de su estructura y composición molecular, así como su implicación en las emisiones de CO_2. Para este fin, se analizó el contenido en ácidos húmicos y fúlvicos, y se realizaron análisis de termogravimetría (TG), calorimetría diferencial de barrido (DSC) y pirolisis analítica (Py-GC/MS). Los Capítulos 3 y 4 se centraron en evaluar el impacto de diferentes tipos de enmiendas orgánicas en la emisión y fijación de CO_2, así como mejorar nuestra comprensión acerca de los procesos que determinan los intercambios de CO_2 en el suelo y en las plantas (respiración, fotosíntesis). Para ello, se llevaron a cabo numerosas campañas de medición de CO_2 directamente en campo, con dos equipos IRGA (PP-Systems EGM4 con cámara oscura y Licor LI-840a con cámara transparente), cubriendo una amplia gama de condiciones ambientales.

Los resultados globales obtenidos han mostrado que, entre las enmiendas testadas en nuestra zona de estudio, los compost vegetales procedente de invernadero seguidos de los compost vegetales de jardinería fueron más apropiados que los lodos de depuradora estabilizados y sus mezclas en cuanto a las propiedades de los suelos, la emisión y fijación de CO_2, el efecto de cebado, la calidad de la materia orgánica y el efecto sobre el desarrollo de la cubierta vegetal. Así, los dos primeros tratamientos mencionados fueron los más favorables en términos de recuperación de la funcionalidad de los suelos a corto-medio plazo. Además, los resultados de esta tesis destacan la importancia de expandir nuestro conocimiento sobre como las medidas de restauración puedes ser usadas como estrategias de mitigación del cambio climático en áreas semiáridas degradadas, donde las condiciones ambientales extremas pueden provocar que los suelos puedan perder su capacidad de retener carbono. Estos resultados pueden ser útiles para desarrollar tratamientos de restauración más efectivos y sostenibles que promuevan la recuperación del suelo, establezcan una cobertura vegetal estable, aumenten la capacidad de secuestro de CO_2 y mejoren la resiliencia del ecosistema en un escenario de cambio climático.

Introducción General

El suelo es un recurso no renovable a escalas de tiempo humanas (Lal, 2015) , y un componente esencial de los ecosistemas terrestres que interviene en la interfaz de la litosfera, la biosfera, la hidrosfera y la atmósfera (Adhikari and Hartemink, 2016). Además, ha sido considerado como un capital natural que produce un flujo sostenible de bienes y servicios ecosistémicos, vinculados tanto a sus propiedades como a los procesos que en él se desarrollan (Dominati et al., 2010), jugando un papel clave en la productividad y resiliencia de los ecosistemas (Georgiou et al., 2022). Es un recurso vital para regular el agua y los nutrientes, filtrar contaminantes, mejorar la biodiversidad, regular el clima y secuestrar carbono, entre otros (Adhikari and Hartemink, 2016; Jónsson and Davídsdóttir, 2016). En este sentido, los suelos tienen una gran importancia ya que son consideradores el reservorio de carbono terrestre más grande del planeta, y se estima que el suelo alberga actualmente 2500 Pg C a una profundidad de un metro (Lal et al., 2021b). Por lo tanto, su conservación ha sido subestimada a pesar de ser el principal soporte de la vida en la Tierra (Robinson et al., 2014) y su alto potencial de secuestro de carbono, que se estima entre 2 y 5 Gt CO_2 año^{-1}(Fuss et al., 2018), convirtiéndose en un recurso que se encuentra gravemente amenazado (Kraamwinkel et al., 2021) debido principalmente a procesos de degradación por actividades humanas. Actualmente, el 33% de los suelos del planeta sufren procesos de degradación, y más del 90% podrían estar degradados en el año 2050 (FAO and ITPS, 2015). Teniendo en cuenta que el suelo es un recurso vulnerable a la degradación, la cual ha sido definida como la pérdida de capacidad de un suelo para realizar sus funciones ecosistémicas a largo plazo y como consecuencia del deterioro de su productividad (Bai et al., 2008; Nunes et al., 2020). La degradación del suelo se produce a través de un conjunto de procesos físicos, químicos, biológicos y ecológicos, que pueden ser provocados por causas naturales o antropogénicas, respondiendo esta última a la acción de impulsores directos e indirectos que habitualmente responden a motivos socioeconómicos, políticos y culturales (Lal, 2015). Entre estos procesos encontramos la erosión, la pérdida de fertilidad, la salinización, el desequilibrio de nutrientes, la pérdida de biodiversidad, la compactación o el agotamiento de la reserva de carbono del suelo (Lal, 2015, 2012; Nunes et al., 2020). Además, si el suelo se gestiona de forma incorrecta, puede convertirse en una fuente importante de emisiones de gases de efecto invernadero (Pereira et al., 2018), con la consiguiente retroalimentación negativa sobre el cambio climático (Hellebrand et al., 2003). Esto se debe principalmente al agotamiento de la reserva de materia orgánica del suelo (MOS) y su cubierta vegetal fijadora de CO_2 (Lal, 2004a; Mgalula et al., 2021).

En este contexto, los suelos de las zonas áridas y semiáridas del planeta son extremadamente frágiles y sensibles a la degradación (Lal, 2004a), siendo los ecosistemas más gravemente amenazados del planeta debido a su creciente tasa de aridez (Berdugo et al., 2020; Lal, 2019). Actualmente, ocupan el 45,36% de la superficie terrestre (Lal, 2019) y se estima que almacenan el 32% del carbono de la Tierra (Dacal et al., 2020). No obstante, los motores del cambio global como las actividades humanas (como el cambio de uso del suelo) y el cambio climático (Huang et al., 2016; Schlesinger et al., 1990) pueden acelerar la expansión de las zonas áridas y semiáridas (Huang et al., 2016; Yao et al., 2020) y aumentar los procesos de desertificación (Hueso-González et al., 2018; Lavee et al., 1998; Reynolds et al., 2007). Se prevé que podrían representar un área del 56% de la superficie terrestre en 2100 si el calentamiento global incrementa la temperatura de la Tierra hasta 3°C (Huang et al., 2017; Lal, 2019; Plaza et al., 2018), lo que consecuentemente produciría una retroalimentación positiva con graves consecuencias en el aumento de gases de efecto invernadero (GEI) en la atmósfera.

A pesar de la baja concentración de carbono orgánico del suelo en zonas áridas y semiáridas del planeta se estima que acumulan 241 Pg C a 1 metro de profundidad, sin embargo, los procesos de degradación y desertificación son habituales en estas regiones, lo que con frecuencia desencadena emisiones de CO_2 a la atmósfera (Lal, 2004a). Por otro lado, en dichos ecosistemas, las condiciones climáticas extremas no permiten el adecuado desarrollo de la cubierta vegetal dificultando la recuperación de la funcionalidad de los ecosistemas áridos y semiáridos degradado por actividades antropogénicas (Gonzalez-Dugo et al., 2005; Josa et al., 2012). Teniendo en cuenta que el clima, el tipo de suelo, las prácticas de manejo, y otros factores ambientales intervienen en la capacidad del suelo para secuestrar carbono (Lal, 2015), y que los suelos degradados pueden liberar cantidades significativas de dióxido de carbono y otros gases de efecto invernadero a la atmósfera (Smith et al., 2007), es indispensable comprender cómo los suelos degradados afectan a las emisiones de CO_2 y cómo se pueden implementar estrategias de manejo sostenibles que promuevan la acumulación de carbono orgánico y reduzcan las emisiones de carbono a la atmósfera, lo que es crucial en el actual paradigma del cambio climático (FAO, 2019; Horwath and Kuzyakov, 2018; Lal, 2004b).

Un importante impulsor antropogénico de la degradación del suelo en las zonas áridas y semiáridas es la minería. Sin embargo, es una actividad muy extendida en estas regiones debido a sus beneficios económicos y sociales (Gratzfeld, 2003), ya que proporciona materias primas necesarias para el desarrollo socioeconómico (Rahmonov et al., 2022). La elevada tasa de población ha provocado un incremento de la demanda de recursos naturales y como consecuencia la minería ha experimentado un crecimiento exponencial (Yang et al., 2016). Sin embargo, los procesos mineros generan impactos ambientales que perduran incluso tras el cese de la actividad (Gratzfeld, 2003). La extracción de materias primas como arenas, gravas, piedras, yeso y arcillas se produce en operaciones mineras a cielo abierto que movilizan un gran volumen de materiales dando lugar a la formación de canteras (Altiti et al., 2021), lo que conlleva la pérdida de suelo fértil y un severo impacto en la realización de sus funciones (Carabassa et al., 2020b). La minería a cielo abierto se encuentra entre las principales actividades antrópicas que causan severos cambios en los ecosistemas, y específicamente las canteras causan una perturbación drástica, destruyendo prácticamente todos los componentes y atributos del ecosistema original, provocando graves daños ambientales y paisajísticos (Carabassa et al., 2019; Padró et al., 2022). Entre los principales impactos causado por este tipo de actividad, destacan los procesos erosivos y deslizamientos que están estrechamente vinculados con los factores formadores del suelo, además la pérdida de flora y fauna, modificación de la hidrología, cambios en el relieve, y especialmente causan la degradación del suelo (Padró et al., 2022, 2019), cuya recuperación en la mayoría de ocasiones es irreversible (Soliveres et al., 2021), y además, pueden acentuar las emisiones de CO_2 (Shrestha and Lal, 2006). En este contexto, las políticas públicas y directrices para la protección del medio ambiente y sostenibilidad han ido en aumento desde la década de 1990, considerando imprescindibles las tareas de restauración que pretenden minimizar los impactos ambientales (Padró et al., 2022; Segura-Salazar and Tavares, 2018), y tratar de reconducir los ecosistemas naturales a los niveles de referencias (Carabassa et al., 2019).

En este sentido, diferentes orientaciones y aspectos enfocados a la sostenibilidad han sido propuestos a nivel internacional hasta la actualidad con el fin de mitigar los posibles impactos que pueda causar la minería en el medio natural (Segura-Salazar and Tavares, 2018), entre los que se encuentra la restauración de los suelos degradados. Además, organismos internacionales como la Organización de las Naciones Unidas han planteado una estrecha relación entre la salud de suelo y los Objetivos de Desarrollo Sostenible (Lal et al., 2021a), también la Organización para la Cooperación y el Desarrollo Económicos (OCDE) ha establecido directrices en este aspecto (OECD, 2020; ONU, 2020), mientras que se han desarrollado políticas a niveles más locales en diferentes países como Australia, China, Canadá, Sudáfrica, etc. y, por supuesto, en España (Padró et al., 2022). Las políticas españolas respecto a la gestión de terrenos dedicados la minería presenta diferente lineamientos que se encuentran reflejados en la Ley La Ley 22/1973, de 21 de julio y el Real Decreto 975/2009, de 12 de junio (BOE, 2009, 1973), donde se establecen directrices para la protección y rehabilitación del espacio afectado por actividades mineras, y donde se expone la obligación de las empresas minerías a constituir una garantía financiera para asegurar el cumplimiento del plan de restauración, previamente autorizado, para que la gestión y la rehabilitación del espacio natural afectado se lleve a cabo. Estas mismas directrices de gestión post-minera se indican igualmente en la mayoría de los países europeos (Domene et al., 2010; Ros et al., 2006).

En la rehabilitación de las zonas mineras durante mucho tiempo se ha reutilizado la capa superior de suelo fértil que fue retirada antes de la explotación y la posterior revegetación como medida de restauración (Ruiz et al., 2020), o simplemente se lleva cabo la plantación sin tener en cuenta el sustrato asociado (Soria et al., 2021a). Sin embargo, con estas técnicas no se logra una recuperación de la calidad del suelo tras la extracción. Más aún, si tenemos en cuenta que la restauración de zonas áridas es especialmente compleja debido a las extremas condiciones climáticas que presentan y el déficit hídrico, que dificultan el desarrollo de una cubierta vegetal estable (Gonzalez-Dugo et al., 2005; Josa et al., 2012). Por tanto, la restauración de estos frágiles ecosistemas requiere de un manejo adecuado del suelo (Lal, 2015), ya que los procesos edáficos ejercen un fuerte control sobre la productividad y composición de la vegetación

(Booker et al., 2013; Gravuer et al., 2019). Por tanto, la restauración de un suelo degradado, entendido como aquel proceso que favorece que ecosistemas degradados recuperen sus condiciones originales en términos de productividad vegetal y biodiversidad requiere de una estrategia adecuada, cuyo éxito dependerá de su capacidad para mantener las funciones del suelo (Ruiz et al., 2020). En este aspecto, la MOS es uno de los determinantes de la calidad y la salud del suelo, concepto que se considera sinónimo de la medida de la capacidad de un suelo para llevar a cabo sus funciones ecológicas (Kubiëna, 1953), así como su pérdida es la principal causa de degradación en suelos de zonas semiáridas (Hernández et al., 2015).

Se ha demostrado que la creación de suelos funcionales con adecuados niveles de materia orgánica y la reactivación del ciclo de nutrientes es indispensable en la recuperación de los ecosistemas (Moreno-de las Heras, 2009), logrando un elevado grado de éxito en el diseño y ejecución de la restauración en zonas semiáridas mediante la incorporación de enmiendas procedentes de residuos orgánicos (Bastida et al., 2008b; Carabassa et al., 2020a; Domene et al., 2010; Hueso-González et al., 2018; Luna et al., 2018, 2016a; Ros et al., 2003), que además pueden contibuir a recuperar la calidad del suelo mejorando las propiedades físicas, químicas y biológicas de los suelos, garantizando el desarrollo de la cubierta vegetal (Bastida et al., 2013a; Larney and Angers, 2012; Luna et al., 2018; Rodríguez-Berbel et al., 2021a). Ya que el incremento de MOS favorece la mejora de las propiedades físicas y químicas del suelo (Miralles et al., 2009; Soria et al., 2021a), contribuyendo a la formación de su estructura, incrementando la estabilidad estructural de los agregados y reduciendo la densidad aparente, lo que aumenta la porosidad del suelo (Caravaca et al., 2002; Luna et al., 2016a).

La aplicación de materia orgánica en suelos degradados es además una forma muy eficaz para garantizar el aumento inmediato de la capacidad de retención de agua necesaria para la supervivencia de las plantas durante los primeros meses críticos de su desarrollo (García-Ávalos et al., 2018; Luna et al., 2018; Zancada et al., 2004) y mejorar las características químicas y microbiológicas del suelo, enriqueciéndolo con compuestos similares a las sustancias húmicas y con macro y micronutrientes (Doni et al., 2014; Hernández et al., 2015; Rodríguez-Berbel et al., 2020). Al mismo tiempo, estimulan el desarrollo y la actividad de las poblaciones microbianas del suelo (Bastida et al., 2017b; Doni et al., 2014; Pajares et al., 2009; Ros et al., 2003; Tejada et al., 2006), favorecen el establecimiento de una cobertuba vegetal estable (Muñoz-Rojas et al., 2016), recupera la capacidad de secuestro de C (Ojeda et al., 2015) y mejora la resiliencia del suelo (Reddy et al., 2020). Además, la utilización de materia orgánica a partir de desechos reciclados en suelos degradados contribuye a la economía circular local (Fabbri et al., 2021; Hueso-González et al., 2018; Ruiz et al., 2020), promoviendo la recuperación de la salud del suelo y de los servicios ecosistémicos (Abhilash, 2021; Alba-Patiño et al., 2021).

En este contexto, el uso de enmiendas orgánicas permite la creación de suelos preparados artificialmente, denominados como Tecnosoles (IUSS Working Group WRB, 2015) debido a que constituyen una nueva capa de suelo cubriendo el suelo original que resulta tras la explotación minera (Asensio et al., 2013), así como su origen antropogénico. Muchos estudios han demostrado la viabilidad de los tecnosuelos construidos con residuos orgánicos en la restauración minera (Asensio et al., 2019, 2013; Carabassa et al., 2021; Hedde et al., 2019; Luna et al., 2016b; Neina et al., 2016; Rodríguez-Vila et al., 2017; Salazar et al., 2009). Y variados los materiales reciclados incorporados a partir de diferentes tipos de residuos orgánicos tales como lodos de aguas residuales municipales, compost de residuos sólidos urbanos o restos vegetales o animales y estiércoles a los substratos de restauración ha sido contemplado en los últimos años (Albaladejo et al., 2000; Jorba and Vallejo, 2008; Melgar et al., 2000; Solé-Benet et al., , 2009; Soria et al., 2021a) con el objetivo de mejorar la calidad y la fertilidad de los suelos degradados creando las condiciones edáficas que faciliten la colonización vegetal, y eliminar los desechos orgánicos de una manera racional y respetuosa del medio ambiente de acuerdo con los objetivos marcados en el contexto del desarrollo sostenible. Sin embargo, el origen de los residuos orgánicos que pueden ser empleados en el desarrollo de Tecnosoles para este fin, es diverso y variado, y su impacto en el ciclo de C puede producir cambios en las tasas de emisión y absorción de CO_2 del suelo, lo que resulta de gran importancia ante el cambiante paradigma del cambio climático (Ray et al., 2020).

Dado que existe una amplia gama de enmiendas orgánicas procedentes de actividades productivas que pueden ser potencialmente utilizadas para la creación de tecnosuelos, y que su origen y naturaleza es variada, también existe una gran variabilidad en la composición química de su materia orgánica, lo que va a afectar a su tasa de descomposición y mantenimiento a largo plazo (Larney y Angers, 2012; Carabassa et al., 2020b). Por tanto, no está claro si las enmiendas orgánicas comúnmente utilizadas pueden ser consideradas como un material adecuado para contribuir al conjunto de sustancias de tipo húmico estables en el suelo o si, por el contrario, son consumidas rápidamente por los microorganismos, ya que la estabilidad y calidad de la materia orgánica aportada dependerá de la proporción y distribución de formas lábiles y reclacitrantes (Arias et al., 2005; Soria et al., 2022). Después de la adición de enmiendas orgánicas al suelo se produce un aumento en la emisión de CO_2 debido a las interacciones entre microorganismos del suelo, transformación de las sustancias orgánicas añadidas y del ciclo natural del C del suelo (Fangueiro et al., 2007; Kuzyakov, 2010). Estas interacciones causan intensos cambios a corto plazo en el ciclo de la materia orgánica del suelo nativa que se traducen en una liberación adicional de CO_2 conocida con el término "efecto de cebado” (Priming effect) (Kuzyakov et al., 2000), con un concomitante efecto negativo en el cambio climático (Jónsson and Davídsdóttir, 2016). La dosis empleada y la composición química de las enmiendas orgánicas puede tener un impacto directo en la magnitud de emisión de CO_2 debidos al “efecto de cebado” en función de la biodisponibilidad de los compuestos orgánicos para los microorganismos edáficos (Blagodatskaya et al., 2014). La magnitud de la emisión de CO_2 tras la aplicación de las enmiendas orgánicas no sólo depende de su naturaleza, sino también de otros factores abióticos como el pH, temperatura y especialmente humedad del suelo, los cuales afectan indirectamente a la actividad microbiana del suelo (Blagodatskaya and Kuzyakov, 2008), por tanto, la descomposición de la materia orgánica aplicada al suelo y su comportamiento tendrán una retroalimentación directa con el clima (Georgiou et al., 2022). Además, la mayoría de las transformaciones de la MOS son catalizadas por enzimas de bacterias y hongos principalmente (Hernández et al., 2015). El aumento de la actividad de enzimas que degradan celulosa (celulasas, y glucosidasas) y lignina (fenol oxidasa y fenol peroxidasa) es una causa directa de la emisión de CO_2 (Blagodatskaya and Kuzyakov, 2008; Mondini et al., 2006). Sin embargo, también existen enzimas que juegan un importante papel en el ciclo de nutrientes. Concretamente, las enzimas extracelulares como las hidrolasas brindan información sobre los ciclos de C (β-glucosidasa), N (ureasa) y P (fosfatasa) en el suelo, y están involucradas en la fertilidad del suelo, poniendo a disposición los nutrientes que las plantas y los propios microorganismos del suelo necesitan para su desarrollo (García et al., 2017; Hernández et al., 2015). Por tanto, tras la aplicación de enmiendas orgánicas se favorecen los procesos biogeoquímicos del suelo que son fundamentales para el crecimiento de las plantas (Honeyman et al., 2022). Las cuales junto con otros organismos del suelo contribuyen al secuestro de carbono (Ray et al., 2020). La capacidad de secuestro de C del suelo o el intercambio neto de CO_2 del ecosistema (NEE), considerando el suelo y la vegetación, estará mediado tanto por la entrada de carbono de la fotosíntesis como por la renovación del C del suelo (He et al., 2022; Ray et al., 2020; Ruiz et al., 2020). Obtener una mayor comprensión de las retroalimentaciones planta-suelo tras la aplicación de enmiendas orgánicas, así como la evolución de su composición química y los mecanismos subyacentes mejorará nuestra capacidad para predecir las consecuencias de estas interacciones sobre la contribución al balance de C de los tecnosuelos.

Teniendo en cuenta que el suelo es un sistema complejo con múltiples interrelaciones, y que sus propiedades son el resultado del efecto neto de todas ellas, su investigación debe estar asociada a enfoques amplios e integrados que puedan evaluar con precisión la capacidad de los Tecnosoles diseñados para soportar las funciones básicas del suelo. Para ello es necesario mejorar la comprensión de los procesos resultantes tras la aplicación de las enmiendas orgánicas, que van a condicionar que el ecosistema pueda comportarse como una fuente o un sumidero neto de carbono. Por lo tanto, será indispensable evaluar la dinámica del flujo de carbono a medio-largo plazo para comprender el movimiento de carbono dentro y fuera de un ecosistema, teniendo en cuenta la composición química de las enmiendas, los cambios en el conjunto de propiedades físicas, químicas y biológicas, y variables ambientales que entran en juego y la evolución de la cobertura vegetal tras la preparación de los Tecnosoles. Esta investigación permitirá una mejor predicción y mitigación de las consecuencias de los cambios de uso del suelo inducidos por el hombre, mejorará los esfuerzos de restauración y conservación y promoverá la provisión sostenible de servicios ecosistémicos en un escenario cambiante.

Así pues, el objetivo general de la presente tesis era mejorar nuestro conocimiento actual sobre cómo la materia orgánica exógena aplicada, mediante enmiendas orgánicas de diferente origen, evoluciona en el desarrollo de tecnosuelos y como pueden afectar a la funcionalidad y calidad del suelo, procesos de mineralización, y de emisión y fijación de C. Para ello, se diseñaron diferentes experimentos de campo y de laboratorio con el fin de ensayar diferentes tratamientos de restauración mediante la incorporación de distintos tipos de enmiendas orgánicas procedentes de residuos orgánicos reciclados, que minimicen la emisión de CO_2 y faciliten la evolución de la materia orgánica del suelo, con la finalidad última de obtener un suelo viable en el menor tiempo posible.

Este objetivo general de esta tesis desemboca en los siguientes objetivos parciales, que son desarrollados en los capítulos que la conforman:

i) Estudiar los efectos de diferentes combinaciones de enmiendas orgánicas obtenidas a partir de residuos sobre las propiedades físicas, químicas y bioquímicas del suelo mediante diferentes indicadores de calidad y funcionalidad del suelo, con especial énfasis en la evaluación de la mineralización de C y liberación de CO_2 por efecto cebado.

ii) Estudiar de las tasas de respiración del suelo in-situ a corto-medio plazo tras la adición de enmiendas orgánicas generadas a partir de diferentes tipos de residuos con el fin de conocer tratamientos óptimos de restauración que favorezcan el secuestro de carbono en el suelo a medio-largo plazo y minimicen la emisión de CO_2 a la atmósfera

iii) Conocer cómo evoluciona la actividad de las comunidades microbianas durante el proceso de restauración a través del estudio de actividades enzimáticas, respiración basal del suelo y perfiles de ácidos grasos de hongos y bacterias, y su implicación en el efecto cebado.

iv) Saber cómo evoluciona la materia orgánica del suelo en los Tecnosoles y en suelos naturales del entorno a partir de indicadores tradicionales de calidad de la MOS, a partir de patrones de humificación y degradación de compuestos lábiles o resilientes de la MOS, y análisis de la estructura y composición molecular de la MOS.

v) Estudiar el efecto de los distintos tipos de enmiendas orgánicas sobre las tasas de supervivencia y crecimiento de la vegetación instaurada con el fin de seleccionar los tratamientos óptimos que favorezcan el desarrollo de una cubierta vegetal estable a corto plazo.

vi) Conocer los factores ambientales que intervienen en el intercambio de CO_2 en el suelo y en las plantas (flujo neto, fotosíntesis y respiración) tanto en las parcelas experimentales como en suelos naturales del entorno, usados como referencia.

Esta tesis está estructurada en 4 capítulos, que son presentados a continuación de la introducción y la metodología general. Los capítulos 1, 2, 3 y 4 redactados en inglés responden los contenidos principales abordados en esta tesis, correspondientes a artículos originales de investigación que se encuentran publicados en revistas que figuran en los listados de Journal Citatioin Reports (JRC), los cuales son citados al inicio de cada capítulo. Finalmente, se enumeran las conclusiones generales.

Los contenidos principales abordados en cuatro capítulos quedan resumidos en la tabla que se expone a continuación:

Capítulos		**Objetivos**	**Principales técnicas empleadas**
Capítulo 1	Role of organic amendment application on soil quality, functionality and greenhouse emission in a limestone quarry from semiarid ecosystems.	Estudiar los efectos de diferentes combinaciones de enmiendas orgánicas obtenidas a partir de residuos sobre las propiedades físicas, químicas, bioquímicas y microbianas del suelo utilizando diferentes indicadores de calidad y funcionalidad del suelo, con especial énfasis en la evaluación del CO_2 liberación por efecto cebado en suelos mineros de un ecosistema semiárido.	Determinación de actividades enzimáticas implicadas en el ciclo de C, N y P, y de respiración basal del suelo. Determinación de contenido de ácidos grasos unidos a ésteres metílicos (FAMEs) de bacterias y hongos mediante cromatografía de gases (GC). Determinación de procesos de mineralización y efecto cebado mediante análisis del isótopo δ 13C se realizó utilizando un analizador IRMS.
Capítulo 2	Effects of Technosols based on organic amendments addition for the recovery of the functionality of degraded quarry soils under semiarid Mediterranean climate: A field study.	Comprender la dinámica y composición molecular de la MO aportada por las enmiendas orgánicas proporcionaría información importante sobre el funcionamiento del suelo, la evolución de los tecnosoles y su efectividad en las acciones de restauración.	Análisis térmico mediante termogravimetría y calorimetría diferencial de barrido (TG-DSC). Pirolisis analítica mediante cromatografía de gases-pirolisis-espectrometría de masas (Py-GC/MS). Aislamiento y cuantificación de las fracciones húmicas del suelo.
Capítulo 3	Soil amendments from recycled waste differently affect CO_2 soil emissions in restored mining soils under semiarid conditions.	Evaluar el impacto de diferentes tipos de enmiendas en la emisión de CO_2 de suelos semiáridos completamente degradados en una cantera de piedra caliza restaurados con enmiendas orgánicas a partir de residuos orgánicos locales a medio plazo. Estudiar la influencia de factores ambientales y propiedades físicas y químicas del suelo en las tasas de emisión de CO_2.	Determinación de las emisiones de CO_2 en las diferentes parcelas restauradas con enmiendas orgánicas, mediante una sistema IRGA (PP-System EGM4) con cámara cerrada en oscuridad en diferentes condiciones ambientales. Determinación de los parámetros ambientales y propiedades físicas y químicas del suelo, y análisis de influencia en las tasas de emisión de CO_2 a medio plazo.
Capítulo 4	Organic amendments from recycled waste promote short-term carbon sequestration of restored soils in drylands.	Determinar qué tratamiento de restauración con enmiendas orgánicas promueve la fijación de CO_2. Identificar si los suelos restaurados con enmiendas orgánics podrían funcionar como sumidero de CO_2.	Determinación del intercambio neto de CO_2 del ecosistema (NEE), mediante una sistema IRGA (Li-840a) con cámara cerrada en estado transitorio. Mediciones realizadas en suelo y planta en diferentes condiciones climáticas.

Metodología General

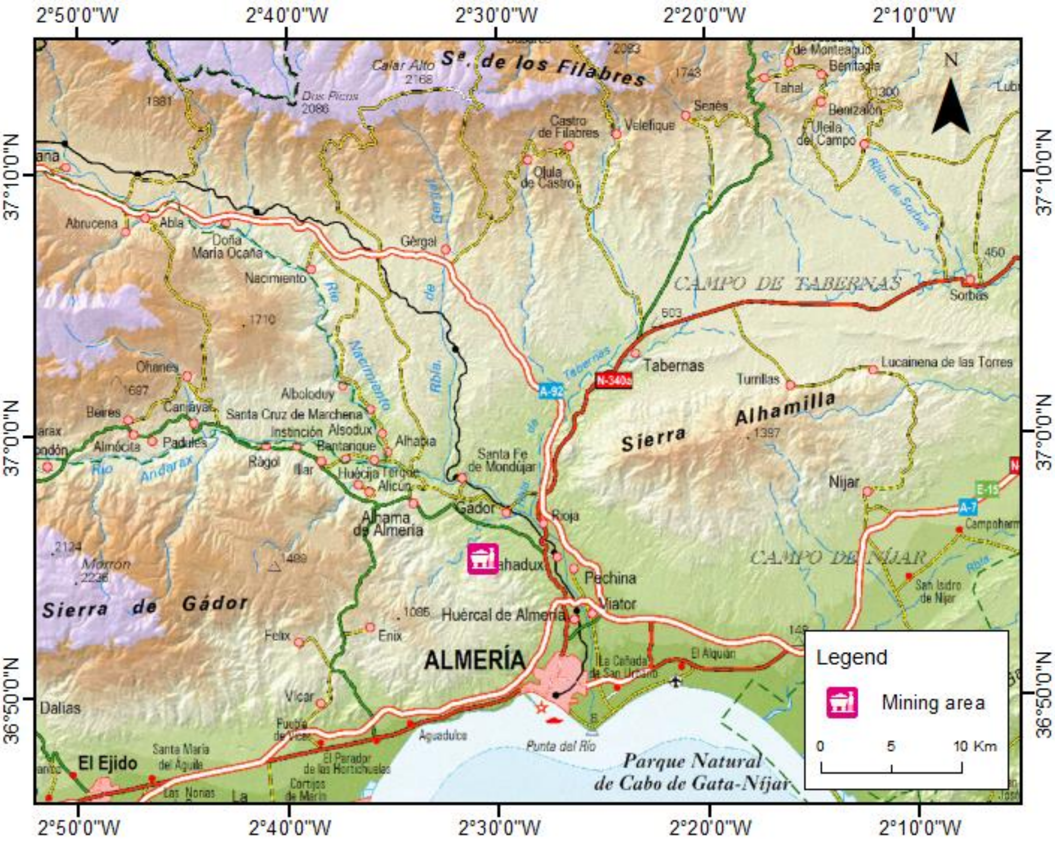
I. Zona de estudio

El estudio se llevó a cabo en una cantera de caliza perteneciente a la empresa Cemex Operaciones España, S.L.U., la cual se encuentra localizada en el borde suroriental del macizo de Gádor en el Paraje Venta de Araoz en el Término Municipal de Gádor, situado a 15 km al norte de la ciudad de Almería (36º55'18'N, 2º30'40'W) y una elevación media de 370 m.s.n.m. (Figura I). El clima del área de estudio corresponde a un clima mediterráneo semiárido con un índice de aridez relativamente bajo (Luna et al., 2018). Se caracteriza por una alta variabilidad meteorológica interanual, constituida por veranos muy secos y episodios de lluvia irregulares y escasos producidos principalmente durante los meses de otoño e invierno. La precipitación media anual de la zona es de 242 mm (datos obtenidos de la estación meteorológica de Alhama de Almería, AL003, Junta de Andalucía). La temperatura media anual es de 17,6ºC, con máximos en julio (42,7ºC) y mínimos en febrero (2,6ºC) (Luna et al., 2016b). La evapotranspiración ha sido estimada en 1.225 mm/año y, los valores de radiación solar recogidos indican máximos diarios entorno a los 1000 W/m^2 durante los meses de abril a septiembre, mientras que, en los meses de invierno, los máximos oscilan entre 500 y 600 W/m^2. El 13% de los días de cada año, se registran vientos con velocidades iguales o superiores a 55km/h (Luna et al., 2018).

El material extraído en esta cantera corresponde a rocas calcáreas para la producción de cemento pertenecientes al Mioceno superior (Tortoniense) y la cordillera de Gádor (dolomías y calizas cenozoicas) (Luna et al., 2018). Concretamente, se trata de calcarenitas que son totalmente explotadas, intercaladas con margas calcáreas que solo son aprovechadas en el caso de no contener yesos. El sustrato resultante tras la explotación corresponde con un suelo margoso y extremadamente compactado donde es difícil el desarrollo de vegetación.

En lugares adyacentes no explotados se encuentran suelos poco profundos sobre calizas y dolomitas con areniscas calcáreas y franco arcillosas o franco arenosas o limosas formando Regosoles (FAO-IUSS-ISRIC Working Group WRB, 2015). La vegetación que ocupaba la zona de estudio antes de la explotación minera, y que se encuentra en las zonas aledañas sin explotar es propia del piso bioclimático mesomediterráneo, predominando los espartales compuestos por especies adaptadas a zonas termo-mediterráneas donde es predominante la climatología seca o semiárida (Rivas Martínez, 1987). Son abundantes las especies de tipo arbustivo y herbáceo, conformando parches irregulares de pastizales dominados por *Macrochloa tenacissima* Kunth (=*Stipa tenacissima* (L.)). Otros pequeños arbustos formando mosaicos se presentaron en el área de estudio que correspondían a especies como *Anthyllis cytisoides* L. y *Ulex parviflorus* Pourr, entre otros, y algunos individuos dispersos de *Maytenus senegalensis* (Lam.) Exell, *Rhamnus lycioides* L., *Pistacia lentiscus* L., *Genista spartoides* Spach y *Thymus baeticus* Boiss., *entre otros* (Luna et al., 2016a). En cuanto a plantas anuales, eran más estacionales y son menos frecuentes, entre ellas se pueden encontrar *Avena barbata* Pott., *Launaea resedifolia* Druce., *Linum strictum* L., *Rhagadiolus stellatus* (L.) Gaertn., *Moricandia foetida* Bourg. Ex Coss, *Helianthemum ledifolium* (L.) Miller., *Stipa capensis Thunb.*, *Reichardia tingitana* (L.) Roth., (Rivas Martínez, 1987).

Figura I. Localización de la zona de estudio



II. Diseño e instalación experimental

La zona seleccionada para el estudio dentro de la cantera comenzó a ser explotada aproximadamente en 1985, tal como puede observarse en la reconstrucción cronológica de la Figura II. Las diversas tareas que se llevaron a cabo para la instalación experimental dieron comienzo en el mes de mayo de 2018. En la zona seleccionada se delimitó un área de 1000 m^2 que carecía de pendiente y donde fueron posteriormente instaladas las parcelas experimentales. El sustrato resultante tras la explotación sirvió como base para la preparación de las parcelas experimentales. Sin embargo, debido a su alto grado de compactación y dureza fue sometido previamente a labores de descompactación mediante el uso de maquinaria pesada de la propia mina (Figura III) para facilitar las posteriores tareas de restauración.

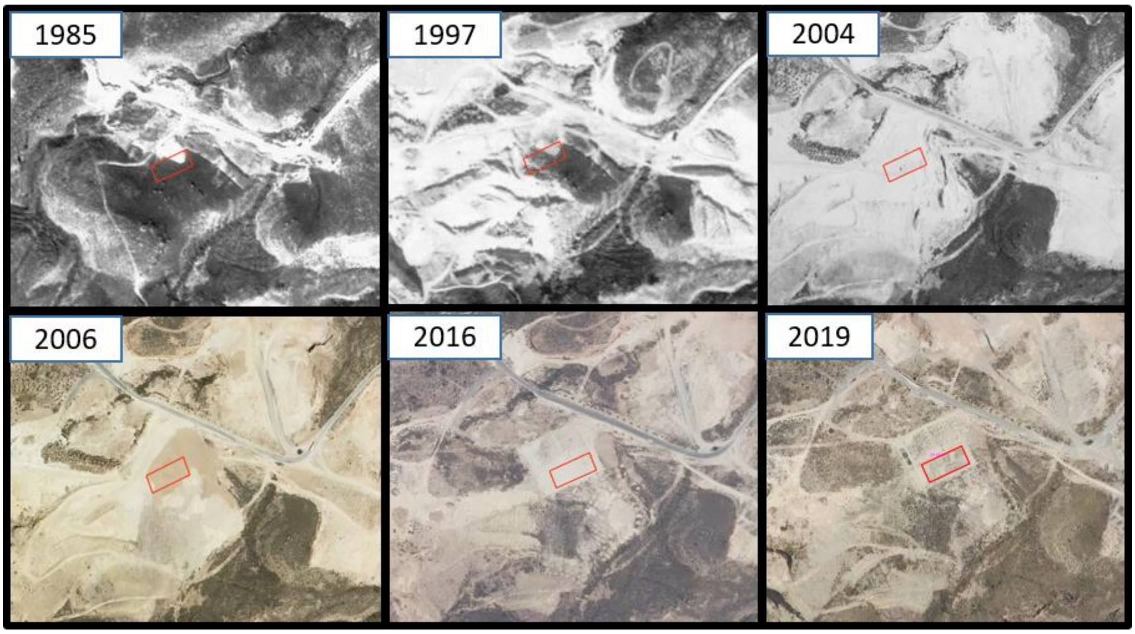


Figura II. Reconstrucción cronológica del aspecto de la zona de estudio desde el año 1985 hasta el año 2019. En rojo aparece remarcada el área donde se ubicó el experimento. Fuente: Sentinel2 (Julio-2019) y Plan Nacional de Ortofotografía Aérea de España (PNOA).

Figura III. Trabajos de descompactación y diseño de parcelas experimentales, mayo 2018.

En el área experimental, se delimitaron 18 parcelas de 50 m^2 (10 x 5m) separadas por un metro de distancia. Finalmente, se añadieron las diferentes enmiendas orgánicas seleccionadas en cada una de las parcelas experimentales para la creación de los tecnosuelos. Las enmiendas fueron mezcladas con ayuda del cazo de una retroexcavadora giratoria (Figura IV) con el substrato resultante de la extracción minera tras el cese de la actividad extractiva, procurando una buena homogenización de las mismas. La profundidad alcanzada en la mezcla de las enmiendas orgánicas con el sustrato resultante tras la explotación fue de aproximadamente 20 cm.

Figura IV. Trabajos de aplicación y homogeneización de las enmiendas orgánicas.

El experimento siguió un diseño de bloques aleatorios que se distribuyeron al azar en los que se aplicaron los diferentes tratamientos de restauración procedentes de residuos orgánicos de diferente origen, cada uno de los cuáles se dispuso en tres parcelas experimentales (3 réplicas por tratamiento). Además de las parcelas con los diferentes tratamientos añadidos, se establecieron 3 parcelas control en las que no se incorporó ningún tipo de enmienda orgánica (Figura V).

Las enmiendas orgánicas que finalmente fueron seleccionadas para construir los tecnosuelos procedían de empresas locales dedicadas a la recogida, transporte, gestión y transformación de residuos, y su origen y dosis empleadas se exponen a continuación:

i. *Compost de residuos procedentes de restos de podas y de jardines* (CG). Su origen es origen 100% vegetal, y procedía de restos de residuos generados las labores de jardinería de jardines en espacios urbanos. Las proporciones en cuanto a aportes fueron de 286,6 t ha^{-1} (40,5% de carbono orgánico en peso seco y 22,5% de humedad).

ii. *Compost vegetal procedente de la horticultura intensiva bajo plástico* (CC). Este compost compuesto fundamentalmente de frutas y hortalizas, así como restos vegetales de las plantas cultivadas. La aplicación de dicho compost se realizó a razón de 346,1 t ha^{-1} (43,33% carbono orgánico total en peso seco y una humedad del 40%).

iii. *Compost de lodo de depuradora estabilizado* (SS). Compost procedente de residuos de depuradora que fueron previamente tratados mediante digestión anaerobia, mesófila y deshidratación térmica a una temperatura de 70 ºC, con el fin de garantizar su inocuidad en el medio ambiente, así como durante la manipulación. La enmienda fue incorporada en una proporción de 147,4 t ha^{-1} (66,4% de carbono orgánico total en peso seco y 8,8% de humedad)

iv. Mezcla de *compost de residuos procedentes de restos de podas y de jardines* (CG) con *compost de lodo de depuradora estabilizado* (SS). Se preparó una una mezcla de enmiendas orgánicas CG + SS (Mix1) con una dosis de CG de 143,3 t ha$^{- 1}$ combinada con SS a una tasa de 77,3 t ha $^{-1}$.

v. Mezcla de *compost vegetal procedente de la horticultura intensiva bajo plástico* (CC) con *compost de lodo de depuradora estabilizado* (SS). Se preparó una mezcla de enmiendas orgánicas CC + SS (Mix2) a una tasa de CC de 173 t ha^{-1} combinada con SS a una tasa de 77,3 t ha^{-1}.

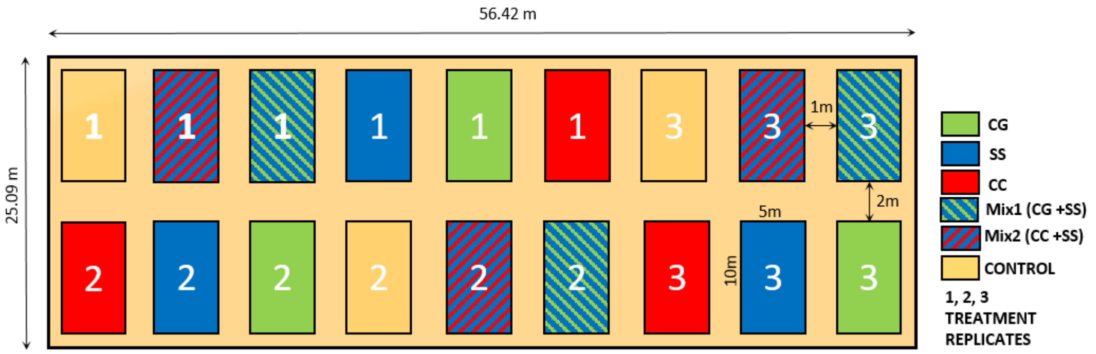


Figura V. Diagrama de la distribución de las parcelas y réplicas experimentales con los diferentes tratamientos de restauración de suelos y suelos tratados (Soria et al., 2023).

Finalmente, se procedió a la instalación de vegetación. Para ello, se seleccionaron dos especies vegetales autóctonas presentes en las zonas colindantes al área de estudio que no estaban afectadas por la explotación minera. Su grado de idoneidad fue constatado por un alto grado de supervivencia de las mismas en otros experimentos de restauración en la misma zona de estudio (García-Ávalos et al., 2018). Las especies seleccionadas fueron *Macrochloa tenacissima* Kunth (=Stipa tenacissima (L.), conocida comúnmente como esparto, de la que se plantaron 40 individuos en cada parcela experimental, así como 10 individuos de acebuche (*Olea europea* L. var. *sylvestris* Brot.). Toda la vegetación fue plantada una semana tras la aplicación de las enmiendas orgánicas, teniendo un total de 50 plantas por parcela. La plantación siguió un patrón de plantación de 5 filas compuestas por 10 plantas cada una, separadas por 1 m de distancia. Las plantas de acebuche fueron protegidas inicialmente con un protector de malla para evitar problemas de ramoneo por parte de la fauna silvestre. Se realizó un riego de establecimiento con el fin de asegurar la mayor supervivencia de la vegetación plantada (Cortina et al., 2011; Valdecantos et al., 2014; Luna et al., 2018) a través de un riego por goteo que fue instalado previamente con un caudal de 3 L h^{-1}. En el primer riego se aplicó una cantidad de agua de 1 L/planta. Posteriormente, se realizaron 5 riegos con una frecuencia quincenal durante el primer verano tras la plantación (finalizando en la última quincena del mes de agosto de 2018), los cuales fueron considerados como indispensables debido a las duras condiciones climáticas que caracterizan el periodo estival de una región semiárida mediterránea, ya que este periodo se considera el más crítico para el éxito de la reforestación (Vallejo et al., 2012). Posteriormente, no se volvieron a regar las plantas, recibiendo únicamente el agua de la lluvia.

En la figura VI se muestra un esquema que reproduce una sección del aspecto del resultado final de la restauración. La vista general de las parcelas experimentales tras su instalación en 2018 y su aspecto tres años tras la instalación de las mismas son mostradas en la figura VII.

Figura VI. Esquema de la conformación de un tecnosuelo experimental al final de la restauración.

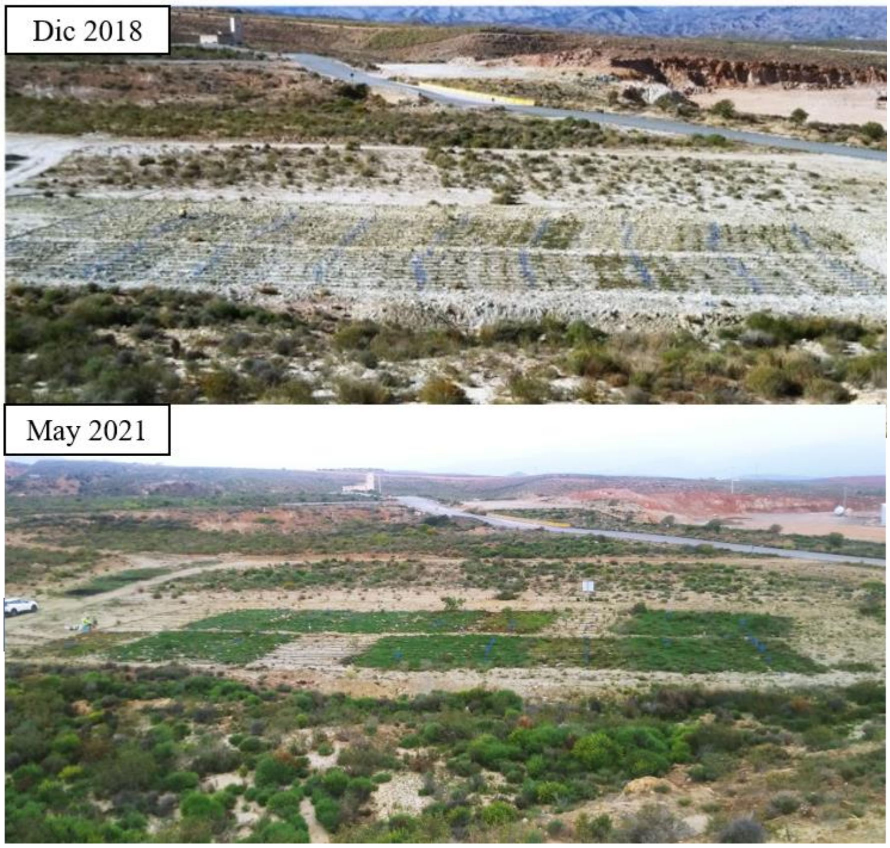


Figura VII. Vista general de las parcelas de restauración

Por último, se seleccionó una zona natural en las zonas aledañas a las parcelas experimentales (figura VIII) que históricamente no fue explotada. En ella se instalaron 3 parcelas, que consideramos como ecosistema de referencia de calidad y que nos permitió establecer un sistema de referencia (Miralles et al., 2009) para comparar con los tecnosuelos restaurados y de control.

Figura VIII. Zona natural seleccionado como referencia de calidad y detalle de vegetación.

Chapter 1

Role of organic amendment application on soil quality, functionality and greenhouse emission in a limestone quarry from semiarid ecosystems.

***Soria, R.**, Ortega, R., Bastida, F., Miralles, I., 2021b. Role of organic amendment application on soil quality, functionality and greenhouse emission in a limestone quarry from semiarid ecosystems. Applied Soil Ecology 164, 103925.*

Abstract

One strategy for restoring degraded soils by opencast mining in semiarid regions is the application of organic amendments. However, selecting an appropriate organic amendment that improves soil quality and functionality for recovery in the short-term without a high contribution to increased CO_2 *emissions is important for improving restoration strategies. Therefore, the objective of this work was to study the short and medium-term changes in physico-chemical and biological properties in restored soils with organic amendments from a limestone quarry (SE, Spain). Several restoration treatments were applied consisting stabilized sewage sludge, vegetable compost garden waste, vegetable compost from greenhouse crop residues and two mixtures thereof, then native plants were planted. After six months several soil properties were evaluated (electrical conductivity (EC), pH, total organic carbon (TOC), total nitrogen (TN), assimilable phosphorus (AP), soil water retention, carbohydrates and polyphenols content, enzymatic activities, bacteria and fungi fatty acids and soil respiration). Some physico-chemical soil properties and the survival rates and biovolume of the introduced native plants, as well as the percentage of land cover occupied by wild plants, were also analyzed 2 years after the application of the amendments. Organic amendments improved significantly all soil properties compared with unamended soils. Labile organic matter forms (carbohydrates and polyphenols) showed significant positive correlations with parameters related to microbial activity. Restored soils with sewage sludge had the highest significant labile C values, following by its mixtures. All organic amendments increased in situ* CO_2 *release, a positive priming effect and organic matter mineralization, being these properties higher in sludge-treated soils than in the rest restored and non-amendment soils. The content of TOC and TN and water retention remained similar two after the application of the amendments, but the EC decreased in restored soils. Nevertheless, despite PCA analysis clearly demonstrated that all restoration treatments had an important effect on the functionality and soil quality, vegetable compost from garden waste or from horticultural greenhouse crop waste amendments were the best restoration treatments for short and medium-term restoration of degraded quarry soils in harsh environmental and climate conditions because they also presented lower* CO_2 *emission rates to the atmosphere and the highest survival and growth rates of the introduced plants and wild cover in the experimental plots.*

1.1. Introduction

Arid and semiarid ecosystems are highly vulnerable to effects of global change (Hueso-González et al., 2018; Maestre et al., 2012; Puigdefábregas, 1998; Reynolds et al., 2007). Soil degradation in dry regions causes irreversible losses in soil productivity and quality (Dregne, 1977; Miralles et al., 2009; Zhang, 2020) as well as in environmental quality in general (D'Odorico et al., 2019). In these areas, resource extraction through mining is an activity with great potential for economic and social development, but its poor management results in impacts that remain even after the activity has ceased (Gratzfeld, 2003). In particular, opencast mining activities lead to serious problems of soil degradation (Josa et al., 2012; Luna et al., 2016a) which must be ecologically restored when the quarrying operations are completed, as is indicated by legislation in most European countries (Domene et al., 2010; Ros et al., 2006). The ecological restoration of these fragile ecosystems requires the adequate soil management, stimulating the microbial activity of the soil, favouring the soil structure, increasing carbon and nitrogen content and improving the efficiency and resilience of the ecosystems (Lal, 2015). However, long, dry summers and limitations in water availability make difficult both restoring and developing vegetation cover in semiarid Mediterranean ecosystems (Gonzalez-Dugo et al., 2005; Josa et al., 2012). Further, this situation will be even more complicated in the coming decades given the predictions of climate change and aridity extension (Huang et al., 2016; Yao et al., 2020). For a long time, efforts to restore mining areas have focused on the recovery of plant cover without taking into account the associated substrate. In restoration programs, the benefits associated with the increase in soil organic matter are improved soil water retention, fertility, structure and productivity (Bastida et al., 2008b; González-Ubierna et al., 2012; Pérez-Gimeno et al., 2019; Salazar et al., 2009). Many authors have proposed organic waste amendments as a successful restoration solution for semiarid areas (Domene et al., 2010; Donn et al., 2014; Hueso-González et al., 2018; Jordán et al., 2008; Luna et al., 2016b; Ojeda et al., 2003; Ros et al., 2003) because they improve physical, chemical and biological soil properties, encouraging plant colonization and restocking maintenance (Breton et al., 2016; Muñoz-Rojas et al., 2016), increasing microbiological soil activity (Albiach et al., 2000; Bastida et al., 2013a; Mora et al., 2005) and improving carbon sequestration (Ojeda et al., 2015; Piccolo et al., 2004). In this context, Bastida et al. (2009, 2008a) showed that sewage sludge application improves soil quality in Mediterranean semiarid environments. Luna et al. (2018, 2016b) showed that different combinations of sewage sludge, organic domestic waste and different mulches increased the soil fertility and improved hydrological soil properties 5 years after application in a quarry in a semiarid Mediterranean ecosystem. Other authors found that addition of garden waste compost increased soil organic matter and improved the establishment of native plant communities (Noyd et al., 1997) and could replace the use of chemical fertilizers on crops (Tits et al., 2014). Hueso-González et al. (2018) have also argued that combinations of different types of amendments have yielded better results in soil fertility than their individual application. However, although sewage sludge has been widely studied as an organic amendment to recover degraded mining areas in semiarid zones, little is known about how the application of other types of plant waste, such as garden waste or intensive greenhouse cultivation, could influence the soil properties in the restoration of highly degraded areas.

On the other hand, soil microbes are responsible for the mineralization of soil organic carbon (SOC). In SOC mineralization, soil priming is defined as the change in the microbial decomposition of SOC in response to fresh C inputs and is considered a key component of the global carbon cycle that can potentially affect the capacity of soils for C sequestration (Bastida et al., 2019; Guenet et al., 2018; Kuzyakov, 2010). The organic amendments have been shown to increase soil priming in semiarid soils (Bastida et al., 2017a). The magnitude of CO_2 emission after applying organic amendments may depend on amendment type because the chemical composition of the added organic matter influences soil priming (Blagodatskaya et al., 2014) and research regarding how restoration programs in abandoned mines affect this key component of soil C cycling in degraded areas is still lacking. Therefore, since the application of organic amendments modifies the above mentioned physico-chemical, biochemical and biological soil properties, assessing quality indicators to evaluate the functionality of restored soils will provide relevant information on soil condition and recovery, defining soil functionality as the soil’s ability to facilitate key processes for the functioning of terrestrial ecosystems such as nutrient recycling, vegetation development or improving biological productivity efficiency (Muñoz-Rojas et al., 2016).

Given the variety of potential organic amendments that may determine the effects of restoration programs in open-cast mines, the objective was study the effects of different organic amendment combinations obtained from wastes (sewage sludge, different composts from gardening or intensive agriculture and mixtures thereof) on the soil's physico-chemical, biochemical and microbial properties using different indicators of soil quality and functionality, with an special emphasis on evaluating CO_2 release due to the priming effect in mining soils from a semiarid ecosystem. The effect of the different organic amendments in the native and wild plant cover in the restored soils will be also evaluated. The overall purpose of this research will be to provide a scientific basis for determining the optimal restoration treatment, favouring soil fertility and increasing the biomass and activity of microbial communities, with the consequent feedback on soil quality and functionality of restored mining areas from semiarid ecosystems, minimizing CO_2 emissions with the consequent feedback in the global warming. We hypothesize that organic amendments with different chemical nature and different C, N and P content will have positive effects on soil physical and nutrient status, as well as on the biomass and activity of soil microbial communities, and that these changes will be associated with fluxes in CO_2 and vegetation cover development. We expect that these soil responses to organic amendments will differ based on the chemical composition of each amendment and thus will determine how effective the soil restoration strategy is in the short term.

1.2. Material and methods

1.2.1. Description of the study site.

The study was carried out in a limestone quarry located between the intermountain Tertiary basin formed by Tortonian loams (high Miocene) (calcareous muds and sandstones-gipsipheres) and Gádor mountain-range (dolomites and Cenozoic limestones). In adjacent untapped areas, the soils were calcaric Regosols (FAO-IUSS Working Group WRB, 2014). Specifically, the experimental area is located in a fully exploited site without slope at an altitude of 362 m. a. s. l (36º55'18'N, 2º30'40'W), situated approximately 15 km north of the city of Almeria (SE, Spain). Climate is semiarid Mediterranean with highly irregular rainfalls. Most rainfall events are in winter and autumn with a mean annual precipitation of about 242 mm year. Summers are dry and hot; the maximum temperature recorded was in August (31 ºC) and the minimum in January (8 ºC), with an annual average temperature of 17.6 ºC. This region has high evapotranspiration rates with an annual mean of about 1225 mm year^{-1} (weather data collected from the Alhama de Almería meteorological station as defined in Luna et al., (2018)). Before mining, irregular patches of grassland dominated by *Macrochloa tenacissima* (L.) Kunth *(=Stipa tenacissima* L.*)* formed the principal native vegetation communities in the study area. Perennial dwarf shrubs or mosaics formed by meadows alternating with patches of dwarf shrubs, composed of *Anthyllis cytisoides* L. and *Ulex parviflorus Pourr*, among others, and some scattered individuals of *Maytenus senegalensis* (Lam.) Exell, *Rhamnus lycioides* L. *and Pistacia lentiscus* L. were also presented in the study area (Luna et al., 2016a).

1.2.2. Experimental design and description of restoration treatments.

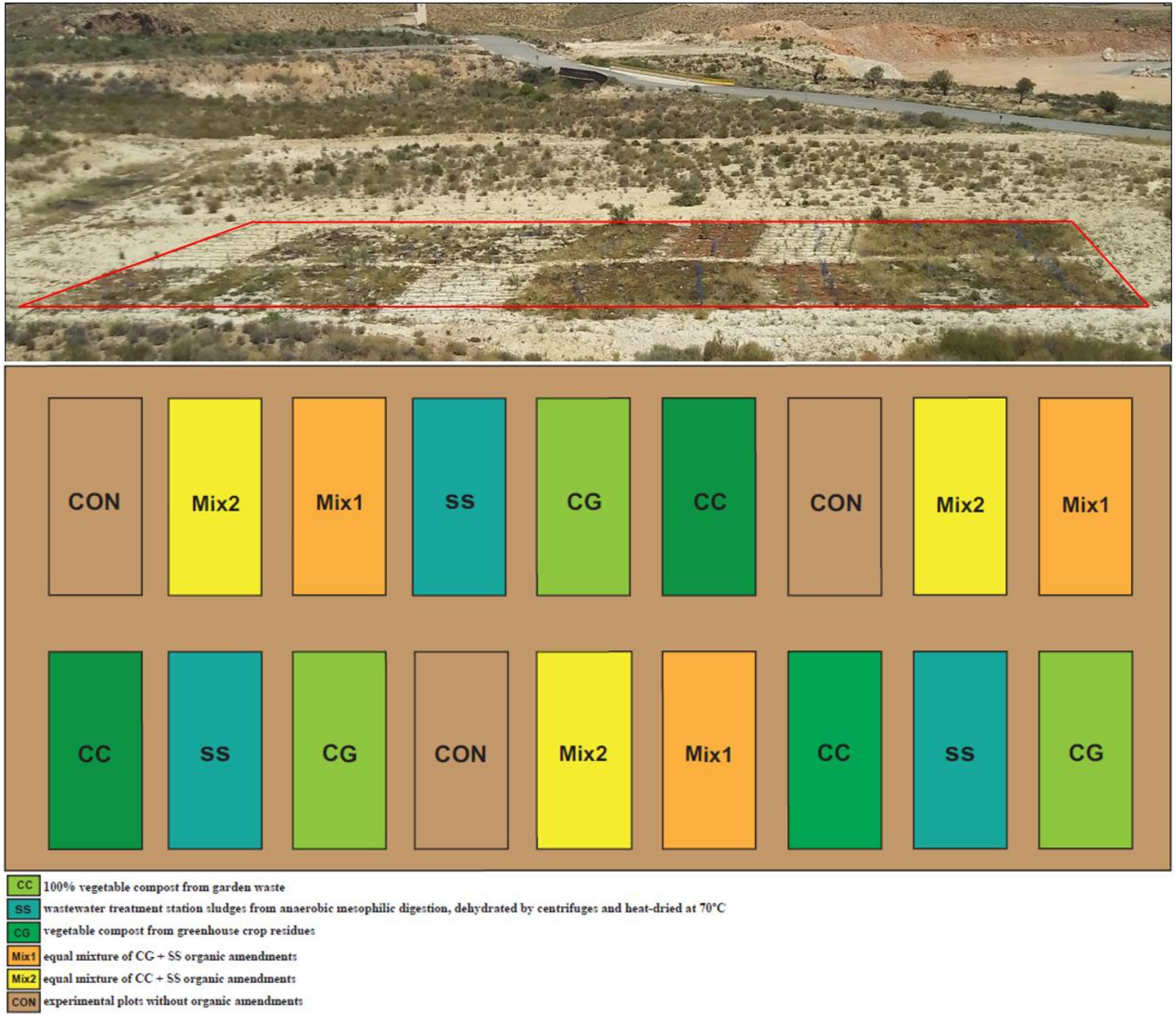
The experimental installation started in the last week of May 2018. Previous experimental plots were installed, soil homogenization and decompaction work were carried out using heavy machinery available in the mine (i.e., mechanical excavators and bulldozers) to reduce erosion and soil flow during rainfall events as well as to make conditions favourable for soil infiltration. Subsequently, 18 experimental plots 50 m^2 (10 m × 5 m) were set up. The experiment had a completely randomized block design with three replications (Figure 1) in which the following soil restoration treatments were applied in a single dose: (i) 100% vegetable compost from garden waste (CG) was used at a rate of 286.6 t/ha; (ii) vegetable compost from greenhouse crop residues (CC) was added at a rate of 346.1 t/ha; (iii) wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70ºC (SS) at a rate of 147.4 t/ha; (iv) equal mixture of CG + SS organic amendments (Mix1) was added at a CG rate of 143.3 t/ha combined with SS at a rate of 77.3 t/ha; (v) equal mixture of CC + SS organic amendments (Mix2) was added at a CC rate of 173 t/ha combined with SS at a rate of 77.3 t/ha.; and (vi) experimental plots without organic amendments, which were considered control plots (CON). The quantities of organic amendments added to soils were calculated to increase the organic matter in each plot up to a value of 3%. Amendments were applied with a shovel backhoe (1 m^3 capacity) and mixed up to the first 20 cm of the soil surface with a bulldozer. *Stipa tenacissima* L. and *Olea europaea*

L. var *sylvestris* Brot. were selected as the initial restoration plant species because they are native species present in the surrounding natural soils close to the quarry and they have demonstrated high survival rates in previous ecological restorations carried out in the study site (García-Ávalos et al., 2018; Luna et al., 2018). In each experimental plot, 40 *Stipa tenacissima* L and 10 *Olea europaea* L. var *sylvestris* Brot plants were planted in lines with a planting pattern of 100 x 100 cm. According to Vallejo et al. (2005), the first summer after planting was the most critical for the success of reforestation in the Mediterranean drylands (Jorba and Vallejo, 2008; Soliveres et al., 2012). Previous results of ecological restorations carried out in the same quarry have shown high survival rates of the *Stipa tenacissima* L. species after a first "establishment irrigation" of the seedlings during the first summer (Luna et al., 2018). Therefore, a drip irrigation system with a dripper per plant (3 L h^{-1}) was installed in the experimental plots. Two PVC water tanks of 1000 L and a portable motor pump were used to perform establishment and summer irrigations. A first irrigation of the establishment plant was carried out at the time of planting. Subsequently, the plants were watered every 2 weeks after the installation process until mid-August a total of 5 watering in which approximately 1 liter was provided per plant. Subsequently, they were not watered again at any time and only received rainwater.

The process for the complete experimental installation was carried out in one week, using 2 days for the preparation of the soil (previous homogenization, application of organic amendments, and mixing with the marl substrate). A week after the application of the treatments (June 7, 2018), the plantation was carried out, with a maximum of 1 day spent on planting the different species and 1 day to install the drip irrigation system. The survival and growth of plants were measured 2 years after planting (June 1–2, 2020) for each species planted.

The first soil sampling was carried out six months after organic amendment application (17-12-2018), one composite 0–10 cm soil sample, created from a random mixture of 10 subsamples, was collected in each experimental plot. A total of 18 soil samples (three replicates per treatment and three to untreated soils) were taken to the laboratory in isothermal bags. One portion of these soil samples was air dried, homogenized and sieved through a 2 mm sieve for analysis of physical and chemical soil properties. Another portion was also homogenized, sieved to 2 mm and preserved at 4°C, destined for biochemical analyses carried out within a maximum of 2 months after field sampling. The second soil sampling was carried out 2 years after application of the organic amendments, coinciding with the vegetation sampling (1-2 June 2020), and a specific set of physical-chemical soil properties were analyzed to estimate the soil state at that time.

Figure 1.1. Experimental design. At the top picture of the experimental plots in June 2020. Below a diagram of the distribution of the experimental plots with the different soil restoration treatments and unmodified soils is shown.



1.2.3. Analyses of physico-chemical, biochemical and microbiological properties.

1.2.3.1. Physical and chemical soil properties.

The following physico-chemical soil properties were measured 6 months after amendments application: Total organic carbon (TOC) was analyzed colorimetrically using Walkley and Black´s method (1934) (rectified by Mingorance et al., 2007). Total nitrogen content (TN) was determined by total combustion at high temperature (960º) and measurement of separate gases in a controlled heating column using a TCD detector (ELEMENTAR Rapid N; Elementar Analysensysteme GmbH, Hanau, Germany). These measurements were then used to calculate the C/N ratio. Assimilable phosphorus (AP) was analyzed according the method described by Olsen et al., (1954). Soil pH and electrical conductivity (EC) were measured in an aqueous 1:2.5 soil/water suspension by using a pHmeter (LAQUA PH1100, HORIBA, Tokio, Japan) and a digital conductivity meter (LAQUA EC1100, HORIBA, Tokio, Japan), respectively. Soil water retention measuring pF at -1500 and -33 kPa was determined by the Richards membrane method (Richards, 1941). The active lime (% "active" $CaCO_3$ equivalent) was determined using NH_4-oxalate method (Drouineau, 1942). The textural particle size distributions were analysed following Bouyoucos' method (Bouyoucos, 1962). Subsequently, a set of physico-chemical properties (TOC, TN, C/N, EC, pH and pF at -1500 and -33kPa) were repeated in soils samples collected 2 years after of the amendment's application, coinciding with the vegetation sampling.

1.2.3.2. Carbohydrates and polyphenols content.

Carbohydrate content was determined by the anthrone–sulphuric acid method of quantifying soil carbohydrates developed by Brink et al. (1960). First, a cold extraction was realized with 5 g soil samples using a soil-to-water ratio of 1:10 (w:v). After centrifugation, filtration, dilution, and addition of the anthrone–sulphuric acid reagent, absorbance was read at 625 nm to measure carbohydrates. The same extract was used to determinate polyphenols content but with the addition of Folin-Ciocalteau reagent for total polyphenolics by the Folin–Denis method (Ribéreau-Gayon, 1968); absorbance reads were obtained at 750 nm. All absorbance measurements were carried out in a spectrophotometer, Spectronic Helios Gamma UV-Vis (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

1.2.3.3. Experimental incubations to determinate glucose mineralization and priming effect.

Soil priming was evaluated through microcosm incubations, as described elsewhere (Bastida et al., 2019). In detail, the incubation consisted of preparing a pair of 20 ml glass vials with 1 g of dry soil samples. These vials were preincubated at 50% of the water holding capacity to readapt microbial communities to incubation conditions at 28 ºC in the dark for one week. After this preincubation, ^{13}C-glucose (99 atoms% U-^{13}C, Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA) was used as a source of organic carbon. A dose of 100 µg C-glucose per gram of soil was dissolved in water and added to one of the vial series. The other vials received the same amount of water without glucose. The vials were incubated for 16 days at 28ºC to determine basal respiration and glucose mineralization that would serve us for subsequent priming calculations. Thus, after the incubation, 4 ml of gas was extracted from the incubation vials and introduced in glass vacuum tubes (Labco Limited, Lampeter, United Kingdom) to determine isotopic composition and amount of released CO_2. The δ ^{13}C isotope analysis was performed using a Thermo Scientific GasBench-PreCon trace gas system coupled to a Delta V Plus IRMS (Thermo Scientific, Bremen, Germany). The isotopic ratio of CO_2 was used to calculate the percentage of CO_2-C derived from added glucose (Waldrop and Firestone, 2004). The priming effect was defined as the increase or decrease in the mineralization of soil organic matter after the substrate was added (Bastida et al., 2013b; Blagodatskaya et al., 2007). Priming effect was calculated as the change in total soil respiration following glucose addition minus the amount of carbon respired from glucose and from control soil without glucose (Bastida et al., 2013b; Blagodatskaya et al., 2007).

1.2.3.4. Biochemical soils properties, enzymatic activities and fatty acids.

Soil basal respiration (hereafter BR) was measured in 125-ml hermetically sealed vials by placing 20 g of sample at 50% of the water holding capacity. The vials were incubated for 31 days at 28º C in darkness. The CO_2 produced by microbial respiration was measured periodically (every day for the first 3 days and then every 4 days) using an infrared gas analyzer (CheckmateII; PBI Dansen- sor, Ringsted, Denmark). BR results were expressed as mg CO_2-C kg^{-1} soil day^{-1}. Dehydrogenase activity was analyzed 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) and expressed in units of micromoles of ammonium–N produced per gram of soil (dry weight) and hour (μmol NH_4^+–N g^{-1} h^{-1}). β-glucosidase and alkaline phosphatase activities were analysed following the methods described in Eivazi and Tabatabai (1988) and Tabatabai and Bremner (1969), respectively. Both activities were expressed in units of micromoles of p-nitrophenol (PNP) produced per gram of soil (dry weight) and hour (μmol PNP g^{-1} h^{-1}).

Ester-linked fatty acid methyl esters (FAMEs), hereafter fatty acids, were extracted from 3 g of soil according to (Schutter and Dick, 2000). Fatty acids were analyzed 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. The conditions were as follows: initial temperature of 120 °C for 0.5 min, 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. The fatty acids i15:0, 15:0, a15:0, i16:0, i17:0, 16:1w9, cy17:0, cy19:0, 10Me16:0, and 10Me18:0 were representative of the bacterial biomass (Dungait et al., 2011; Frostegård et al., 1993), and the fatty acids 18:2w6,9t and 18:2w6,9c were indicators of the fungal biomass (Brant et al., 2006; Rinnan and Bååth, 2009). The Gram-positive (Gram+) representative fatty acids were i15:0, a15:0, i16:0, i17:0, 10Me16:0 and 10Me18:0; and the Gram-negative (Gram-) representative fatty acids were 16:1ω9, cy17:0 and cy19:0 (Dungait et al., 2011; Frostegård et al., 1993). The actinobacterial representative fatty acids were 10Me16:0 and 10Me18:0 (Dungait et al., 2011).

1.2.4. In situ soils respiration measurements and environmental variables.

In each experimental plot, three randomly distributed PVC soil-borne collars with 5 cm in height and 10 cm in diameter were inserted in the soil, leaving 2–3 cm above ground (nine collars per treatment). Dark soil respiration (therefore *in situ* CO_2 emissions) throughout the soil column was measured *in situ* in the collars using a manual, portable soil chamber system gas analyzer (EGM-4 IRGA, PP-systems, Hitchin, UK). The measurement was time set for 90 s. The chamber's volume was 1170 cm^3 with a flat surface of 78 cm^2. Three measurement field campaigns were carried out after rainfalls, with the first taking place one month before soil sample collection (05/12/2018), the second taking place during the soil sampling (17/12/2018), and the last taking place one month after the sampling (16/01/2019). Soil moisture (M3) and temperature (T3) were measured close to each PVC soil-borne collar at a depth of 3 cm during the field campaigns with a portable handheld readout sensor (ProCheck, Decagon Devices, Inc., Pullman, WA, USA). A pluviometer installed in the experimental field area registered daily rainfalls.

1.2.5. Plant sampling and environmental variables

The survival rate of the seedlings was calculated as the proportion of individuals alive at any given time (survival rate (%) = (Number of live seedlings / Number of seedlings planted) × 100) and biovolume index (BI) was also calculated (height x average width) (Solé-Benet et al., 2009) and it was expressed as percentage biovolume per treatment with respect the total biovolume in all experimental plots for each plant type. In addition, the affections of native plant by animals browsing or digging of rooting were also studied. The surface of coverage by colonization of wild and spontaneous plants in each experimental plot was monitored. The rainfall events were monitored by a rain sensor (Rain-O-Matic Small, Pronamic ApS, Denmark) located in the experimental area and air temperature was taken from a nearby station located 4 km and at the same height above sea level (RAIFALL003, Junta de Andalucía). Both environmental variables were monitored throughout the experiment to know the local climatology influence in survival and growth rate (Supplementary Figure 1.1).

1.2.6. Statistical analysis.

Significant differences in physical, chemical, and biochemical soil properties; enzyme activities; fatty acids; *in situ* CO_2 emission; environmental variables and plant survival, growth, wild animals affection and spontaneous plants cover among the different restoration treatments and un-amendment soils were studied using one-way PERMANOVA. This statistical analysis uses permutation tests to obtain *P* values and does not rely on the assumptions of traditional parametric ANOVA (Anderson, 2001). The similarity matrix of the samples was obtained with Euclidean distances to check the effects of the soil restoration treatment factor on each individual variable. Pairwise test comparisons were made using a multivariate analogue of the t test and by finding the probability levels by permutation (Eldridge et al., 2016). The total number of permutations used was 999, and the Monte-Carlo test was used when the number of permutations found was less than 100. The differences were considered significant at the 95% probability level. Pearson's correlation coefficients (r) and principal component analysis (PCA) were used to assess the relationship among the variables (physical, chemical and biochemical soil properties, enzyme activities, fatty acids, BS, *in situ* CO_2 emission, environmental parameters, priming effect and glucose mineralization). Furthermore, PCA was used to investigate trends in variability for the different variables in accordance with the applied treatments. The PCA displayed sample scores in the space defined by the original variables on new axes represented by principal components that explain the most variability between variables. The statistical package PRIMER + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows was used for PERMANOVA analysis. Pearson correlations and PCA were performed with Statgraphic Centurion XVIII-X64 software.

1.3. Results

1.3.1. Changes in the physico-chemical soil properties after the addition of the organic amendments.

Organic amendments significantly increased TOC, TN and AP content in the short-term (six months after its application). SS soils showed the significantly highest values of TOC, TN and AP followed by Mix1, Mix2 and CC soils. The soils with garden compost (GC) presented lower TOC, TN and AP values with respect to the rest of the soils with organic amendments and non-amendments soils (CON) showed the lowest values in these soil properties (Table 1). CC soils had the significantly highest C/N ratio, whereas SS soils showed the lowest values in the C/N ratio (Table 1). In general, restored soils with organic amendments showed higher carbohydrate and polyphenol content than CON soils

(Table 2). The carbohydrate content was higher in SS soils, followed by GC and Mix1 soils, whereas the polyphenol content was higher in SS soils, followed by CC and Mix2 (Table 2). CON and CC soils showed significantly higher soil pH values ($P < 0.05$) than SS, Mix1 and Mix2 soils (Table 1). However, all experimental plots with organic amendments showed significantly higher EC values and water retention capacity at -1500 kPa than CON soils (Table 1). All restored soils presented high active lime content (around 7%), being similar to CON soils, except CC soils which showed the significantly lowest values of active lime (Table 1). The textural classification of the soils was clayey in CG, SS and CON soils, being CON the soils with the most abundant clay content (Table 1). CC, Mix1 and Mix2 soils presented a clay-loam texture, showing the CC soils the significantly lowest clay content. Silt content was higher than sand content in all soils, being the silt values similar in the restored and CON soils. Sand content was significant higher in CC, Mix1 and Mix2, followed by CG and SS and CON soils showed the lowest silt values (Table 1). Restored soils showed also significantly higher ($P < 0.05$) TOC and TN contents than CON soils two years after the application of the organic amendments (Supplementary Table 1.1). CC presented the highest C/N ratio of restored soils, followed by GC, while SS, Mix1 and Mix2, presented significantly lower TOC and TN values (Supplementary Table 1.1). The EC content decreased significantly in all the experimental plots with organic amendments two years after its application, with no significant differences between the restored soils and control soils. The same happened with water retention capacity (pF at -1500 and -33 kPa) which did not also show significant differences between restored and CON soils (Supplementary Table 1.1).

Table 1.1. Main chemical and physical characteristics of soils with restored organic amendments and control soils. Average of three replicates (average ± standard deviation).

	Treatments					
PC properties [†]	**CON[‡]**	**CG[‡]**	**SS[‡]**	**CC[‡]**	**Mix1[‡]**	**Mix2[‡]**
pH**	8.59 ± 0.08b[§]	7.97 ± 0.82ab	7.45 ± 0.35a	8.5 ± 0.08b	7.52 ± 0.21a	7.65 ± 0.06a
EC (mScm^{-1})***	1.72 ± 0.71b	2.56 ± 0.63ab	3.42 ± 0.49ab	3.30 ± 0.70a	3.25 ± 0.44a	3.08 ± 0.21ab
TOC (%)***	0.46 ± 0.17c	1.71 ± 0.19a	2.67 ± 0.54b	2.94 ± 0.67b	2.45 ± 0.18b	2.82 ± 0.16b
TN (%)***	0.02 ± 0.02d	0.32 ± 0.01a	0.60 ± 0.12b	0.40 ± 0.08ac	0.51 ± 0.06bc	0.51 ± 0.04bc
AP (%)**	0.02 ± 0.00c	0.06 ± 0.01a	0.19 ± 0.09ab	0.11 ± 0.03ab	0.14 ± 0.04b	0.12 ± 0.04ab
C:N*	23.0 ± 21.7abc	5.33 ± 0.40a	4.46 ± 0.02b	7.27 ± 0.15c	4.78 ± 0.47ab	5.46 ± 0.19a
pF -1500 kPa***	16.34 ± 1.17b	18.14 ± 1.47ab	21.45 ± 1.39a	18.59 ± 1.85ab	19.46 ± 0.49a	19.42 ± 1.21a
pF - 33 kPa***	32.36 ± 2.43a	29.47 ± 5.59a	34.34 ± 2.62a	30.70 ± 3.12a	31.61 ± 1.65a	32.40 ± 1.04a
Ac. Lime (%)***	7.56 ± 0.33a	7.31 ± 0.42ab	7.80 ± 0.31a	4.94 ± 0.17c	7.58 ± 0.41a	6.58 ± 0.32b
Clay (%)***	49.70 ± 046d	41.13 ± 1.20ab	42.73 ± 1.77a	31.37 ± 1.89c	37.83 ± 2.39b	35.37 ± 3.87bc
Silt (%)*	26.50 ± 3.03b	30.73 ± 1.16ab	30.23 ± 2.81ab	34.80 ± 3.08a	27.53 ± 2.89b	30.00 ± 2.95ab
Sand (%)**	23.80 ±3.40b	28.33 ± 2.68ab	27.01 ± 3.38ab	33.80 ± 4.20ac	34.67 ± 1.55c	34.60 ± 4.40ac
Textural class	clay	clay	clay	clay-loam	clay-loam	clay-loam

* Significant at the 0.05 probabilility level. ** Significant at the 0.01 probabilility level. *** Significant at the 0.001 probabilility level.

[†] Physicochemical properties of amended and control soils. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: ratio carbon nitrogen; pF: water retention in soil at different pressures; Ac. Lime: active lime. [‡] Types of amended and control soils. CON: unamended control soils; CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. [§]Values with the same lower case letters in a row are not significantly different at $P < 0.05$. [PERMANOVA].

1.3.2. Priming effects and glucose mineralization.

All amended soils had a positive priming effect, with SS and Mix2 soils showing the highest significant values ($P < 0.05$), followed by Mix1 and CC soils (Figure 2a). In contrast, CON and CG soils showed the lowest priming effect values. The results also showed that glucose mineralization, as an indicator of labile C mineralization, was greater in SS soils and mixtures (Mix1 and Mix2), followed by CC soils with a percentage of mineralized glucose ranging between 18% and 24%. GC soils showed values of mineralized glucose around 17–18%, and CON soils had the lowest significant values (4.5-6.2%) (Figure 2b).

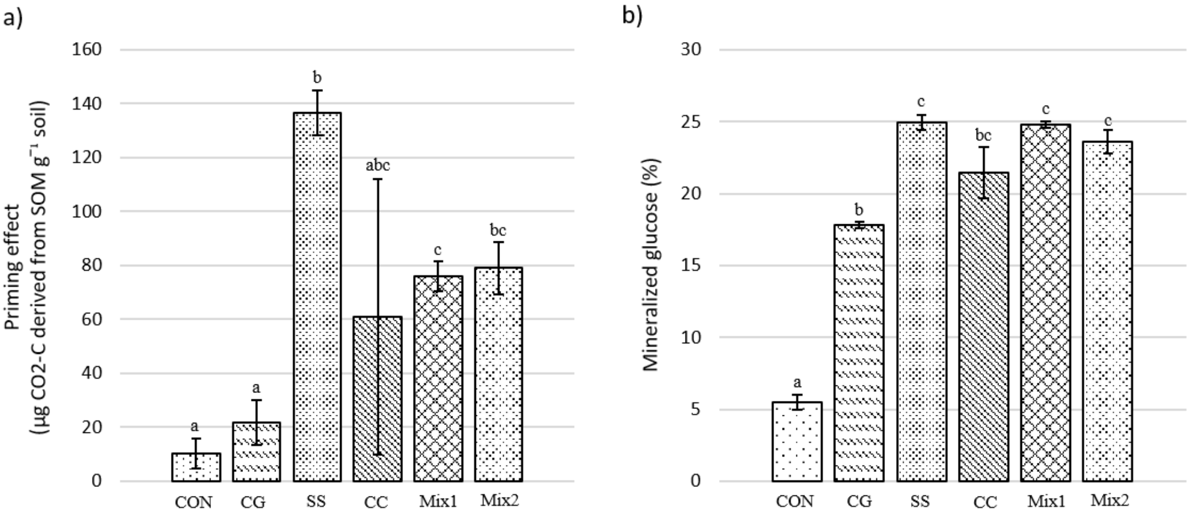


Figure 1.2. Soil priming effects (a) and mineralization rate (b) in soils remediated with organic amendments, mixtures of organic amendments and unamended soils. CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. Different letters in this panel indicate significant differences between treatments ($P < 0.05$) [PERMANOVA].

1.3.3. Biochemical properties, enzyme activities and fatty acids.

Soils restored with sewage sludge (SS) and their mixture with both compost types (Mix1 and Mix2) and compost from greenhouse crop residues (CC) showed a significantly higher ($P < 0.05$) basal respiration (BR) and dehydrogenase activity than compost from garden waste (CG) and unamended soil (CON) (Table 2). The CG soils showed significantly lower values in such properties than the other organic amendment treatments, and CON soils had the lowest significant BR and dehydrogenase activity (Table 2).

All soils treated with organic amendments showed similar values of β-glucosidase and phosphatase activities (except Mix2, with values in phosphatase activity similar to those of CON soils), with SS and Mix1 soils showing the highest values in both enzymatic activities and CON soils showing the lowest significant values (Table 2). Nevertheless, CC soils showed higher values in urease activity than all soils with organic amendments and CON soils (Table 2).

The SS soils had the highest significant ($P < 0.05$) fungal biomass, as estimated from the fungal fatty acid biomarker, followed by Mix2, while Mix1 and CON soils presented the lowest fungal fatty acid content (Table 2). The SS, Mix1 and Mix2 showed the highest values in bacterial fatty acid content while CC and CG soils had significantly lower values by comparison. CON soils showed the lowest significant bacterial fatty acid content (Table 2). SS soils presented the highest values in fungi/bacteria fatty acid ratio (F/B), followed by Mix2, whereas the rest of the restored soils showed values similar to those of CON soils, except for Mix1 soils, which had the lowest values in this ratio (Table 2). In SS, Mix1, Mix2 and CC soils, the content of fatty acids representative of Gram+ bacteria was higher than that of Gram- bacteria, whereas in CG and CON soils the content of Gram- bacterial fatty acids was higher. The Gram +/Gram- fatty acid ratio was significantly higher in SS and CC soils ($P < 0.05$), followed by Mix1 and Mix2 soils, which showed lower values by comparison, although there were no significant differences between them. The CG and CON soils showed the lowest values in the Gram+/Gram- ratio (Table 2).

Table 1.2. Biochemical properties, enzyme activities and fatty acids in soils with organic amendments, control soils. Average of three replicates (average ± standard deviation).

	Treatments					
Biological properties	**CON**	**CG**	**SS**	**CC**	**Mix 1**	**Mix2**
Biochemical						
*Carbohydrates (µg g-^{1})***	0 ± 0d	487.88 ± 75.89a	2315.08 ± 603.89b	258.75 ± 14.70c	1363.78 ± 168.47b	709.05 ± 125.78a
*Polyphenols (µg g-^{1})***	3.01± 2.63d	43.02 ± 17.46ab	37.21 ± 8.91a	72.75 ± 7.26c	33.29 ± 3.84a	18.02 ± 5.05b
*Basal Respiration (mg C-CO_2 kg^{-1} soil day)****	0.99 ± 0.18d	3.50 ± 0.42a	46.5 ± 11.5b	9.29 ± 1.09c b	29.8 ± 4.48b	32.4 ± 9.59b
Enzyme activities						
*Dehydrogenase (µmol INTF g^{-1} soil h^{-1})****	0.05 ± 0.01c	0.15 ± 0.03a	1.40 ± 0.59b	0.93 ± 0.32b	0.85 ± 0.15b	1.19 ± 0.53b
*β-Glucosidase (µmol PNF g^{-1} soil h^{-1})****	0.02 ± 0.00e	0.12 ± 0.02a	1.26 ± 0.22b	0.36 ± 0.04c	0.64 ± 0.10d	0.65 ± 0.14d
*Phosphatase (µmol PNF g^{-1} soil h^{-1})****	0.33 ± 0.15c	2.58 ± 0.32a	34.4 ± 13.4b	3.09 ± 0.41a	23.3 ± 4.44b	12.9 ± 4.25c
*Urease (µmol N-NH_4^+ g^{-1} soil h^{-1})**	0.07 ± 0.07ac	0.10 ± 0.05abc	0.03 ± 0.01c	0.40 ± 0.19b	0.10 ± 0.06abc	0.22 ± 0.16ab
Fatty acids						
*Bacteria (nmol g^{-1} soil)****	13.92 ± 6.00d	82.51 ± 22.26a	419.04 ± 107.24b	143.5 ± 15.39c	402.55 ± 89.5b	356.6 ± 108.48b
*Gram+ (nmol g^{-1} soil l)****	4.61 ± 1.75d	31.80 ± 9.14a	274.01 ± 67.77b	74.84 ± 10.61c	206.59 ± 61.32b	178.55 ± 68.01b
*Gram- (nmol g^{-1} soil)****	9.31 ± 4.52c	50.70 ± 13.42a	145.03 ± 39.64bd	68.71 ± 8.53ad	195.96 ± 45.12b	178.15 ± 48.13b
*Actinobacteria (nmol g^{-1} soil)****	0.86 ± 0.37d	4.45 ± 1.05a	16.18 ± 4.27b	7.53 ± 0.68c	16.66 ± 5.73bc	4.17 ± 2.08acd
*Fungi (nmol g^{-1} soil)****	1.50 ± 0.39c	8.36 ± 3.12a	103.34 ± 6.10b	13.99 ± 4.88a	2.11 ± 0.50c	47.33 ± 17.2d
*Gram+/Gram- ratio****	0.54 ± 0.22a	0.62 ± 0.05a	1.90 ± 0.07b	1.09 ± 0.16b	1.06 ± 0.30ab	0.99 ± 0.23ab
*Fung/Bac ratio****	0.11 ± 0.02a	0.09 ± 0.01a	0.25 ± 0.06b	0.10 ± 0.03a	0.005 ± 0.00c	0.13 ± 0.04ab

* Significant at $P < 0.05$ probabilility level. ** Significant at $P < 0.01$ probabilility level. *** Significant at $P < 0.001$ probabilility level. CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. [§]Across treatments, data with different lowercase letters are significantly different, $P < 0.05$ [PERMANOVA]

1.3.4. Relationships between in situ CO_2 emission and environmental parameters.

Significant differences in the *in situ* CO_2 emissions were also observed between the three field campaigns carried out under different environmental conditions (Table 1.3). The only rain event recorded was 13/12/2018 with 1 L/m². In general, the decrease in soil temperature progressed from the first to the third field campaign, on both restored and unamended soils (CON) (Table 1.3). In the first field campaign SS, Mix1 and Mix2 soils showed the highest significant *in situ* CO_2 emission rate and soil moisture at 3 cm depth, whereas CON soils had the lowest CO_2 emissions and soil moisture values (Table 1.3). The CO_2 emission from soils treated with both types of vegetal compost (CC and CG) did not show significant differences ($P < 0.05$) with CON soils (Table 1.3). In the second field campaign, all soils showed the same pattern as in the first field campaign, but CON soils showed significant differences ($P < 0.05$) in CO_2 emission rates with the rest soils treated with organic amendments. In the third field campaign the CO_2 emission rates of CON to that shown by the restored soils (Table 1.3).

Table 1 3. *In situ* CO_2 emissions, moisture and temperature at 3cm depth in experimental plots with organic amendments andcontrol soils (average ± standard deviation).

	Dates of measurements		
Variables	**05-12-18**	**17-12-18**	**16-01-19**
CO_2 emissions (μmol m^{-2}s^{-1})			
CON	0.23 ± 0.10aA	0.14 ± 0.02dA	0.03 ± 0.00aB
CG	0.43 ± 0.33aAB	0.55 ± 0.08aA	0.02 ± 0.00aB
SS	2.24 ± 0.43bA	2.59 ± 0.43bA	0.18 ± 0.15aB
CC	0.50 ± 0.36aAB	0.99 ± 0.23cA	0.05 ± 0.00aB
Mix1	2.97 ± 0.33bA	1.43 ± 0.50cB	0.07 ± 0.00aC
Mix[†]	2.38 ± 0.26bA	1.30 ± 0.50acB	0.06 ± 0.03aC
Moisture (%) 3 cm depth (M3)			
CON	15.3 ± 1.04a	14.81 ± 1.75a	11.62 ± 1.72a
CG	15.93 ± 0.55aA	14.76 ± 2.24aA	12.1 ± 2.37aA
SS	20.6 ± 3.57a	19.16 ± 3.14a	18.58 ± 4.20ab
CC	14.73 ± 1.30a	15.23 ± 1.82a	12.81 ± 0.31ab
Mix1	17.62 ± 2.14a	17.51 ± 1.52a	14.73 ± 1.90b
Mix2	16.56 ± 2.46a	15.48 ± 1.01a	15.43 ± 1.23ab
Temperature (°C) 3 cm depth (T3)			
CON	23.55 ± 2.52a	20.38 ± 0.95b	18.4 ± 1.84a
CG	23.55 ± 0.83a	20.33 ± 1.70ab	18.86 ± 2.31a
SS	23.73 ± 2.27a	20.1 ± 1.08b	18.6 ± 1.91a
CC	23.15 ± 2.24a	19.75 ± 1.10b	17.83 ± 1.44a
Mix1	23.22 ± 2.22a	20.27 ± 0.42b	18.18 ± 1.27a
Mix2	23.83 ± 2.72a	19.7 ± 0.61b	18.56 ± 1.37a

CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70° C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. Numbers with different lowercase letters are significantly different treatments on the same date, $P < 0.05$ (one-way analysis of variance with permutations [PERMANOVA]). Numbers with different capital letters indicate significant differences in the same treatment on different dates, $P < 0.05$ (one-way analysis of variance with permutations [PERMANOVA]).

1.3.5. Relationships between physico-chemical and microbiological soil properties, CO_2 emission rates and priming effect parameters.

Physico-chemical soil properties (TN, pF at -1500 KPa, TOC, carbohydrates content, AP and EC), microbiological soil properties (dehydrogenase, β-glucosidase and phosphatase activities, BR, Gram+ fatty acid content and Gram -, Gram+/Gram ratio, Actinobacteria, and Fungal), CO_2 emission rates, priming effect and glucose mineralization were strongly correlated (Table 4).

A PCA biplot was performed integrate all variables under study and evaluate the overall effects of restoration treatments on soil. The PCA biplot of the first two principal components calculated from the chemical and microbiological properties explained 72.87% of the total variability, with PC1 explaining 59.24% the variance and PC2 explaining 13.62% of the variance. Interestingly, chemical and microbiological eigenvectors differentiated soil samples according to the different soil restoration treatments. The CG, CC, SS and CON soils formed independent clusters in different regions of factor space. The horizontal axis (PC1) differentiated soil types from SS soils, followed by the cluster that included Mix1 and Mix2 soils (clusters to the right in the graph), the cluster including CC soils (next to ordinate axis), the cluster with soils samples without organic amendment (CON; cluster to the left) and, in an intermediate position between the CC and CON clusters, the cluster including CG soils. The vertical axis differentiates CON soils (lower left cluster) of the amendment soils CG, CC and Mix1 (upper right and left clusters; Figure 1.3). The physico-chemical and microbiological soil properties (variables) with the largest positive loadings on PC1 (EC, TOC, TN, AP, dehydrogenase, β-glucosidase and phosphatase activities, BR, carbohydrates and polyphenols content, Fungi and Bacteria fatty acids, pF at -1500 KPa, CO_2 emissions and glucose mineralization) mostly influenced the soil scores of SS, Mix1 and Mix2 soils. In contrast, the variables with negative loadings (soil pH, C/N, active lime and urease activity) mainly influenced the scores of CC, CG and CON soils (Figure 1.3). The progressive increase in the values of the variables represented on the left led to a decrease in the values of those on the right and vice versa (Supplementary Table 1.3). Likewise, the physico-chemical and microbiological soil properties with loadings with opposite signs (inverse correlation) in PC2 showed evident differences between amendments from plant waste (CG and CC) and CON soils (Figure 1.3).

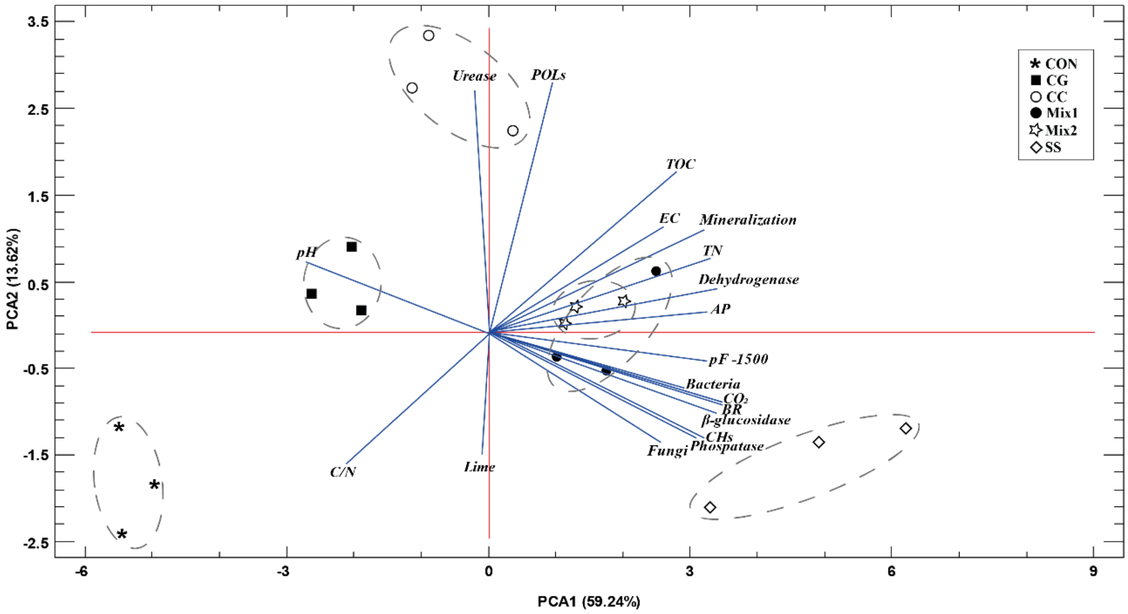
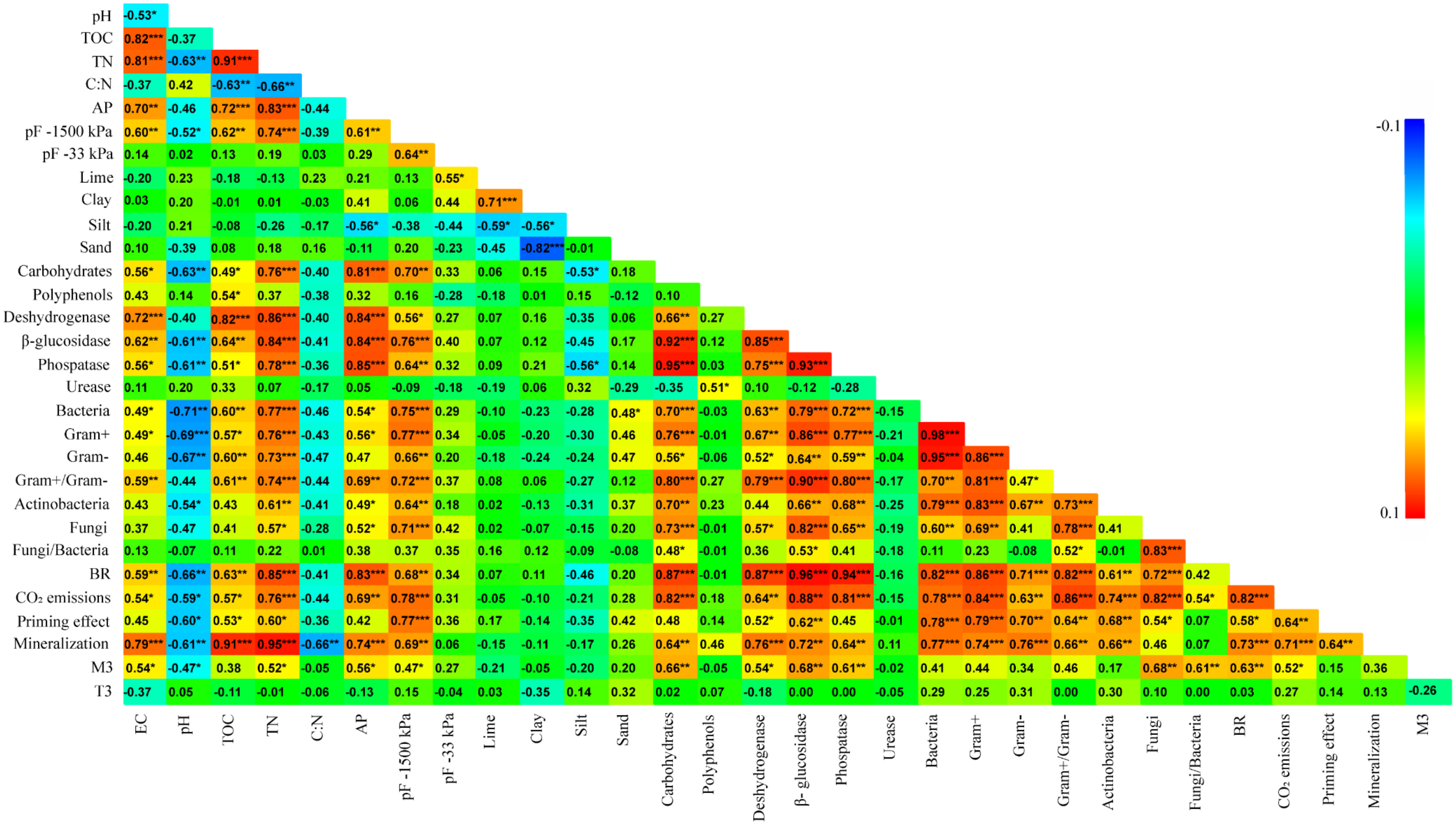


Figure 1.3. Principal component analysis ordination of the different treatments and soil chemical, biochemical and biological properties in five treatments with organic amendments and unamended soils. CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70° C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. EC: electrical conductivity; TOC: total organic carbon; pF -1500: water retention in soil at - 1500 kPa; TN: Total Nitrogen; C/N: carbon to nitrogen ratio; AP: available phosphorus; CO_2: emission of CO_2; Fungi: fungal fatty acids content; Bacteria: bacteria fatty acids content; CHs: carbohydrates; POLs: polyphenols; mineralization: glucose mineralized.

Table 1.4. Significant positive and negative Pearson correlations ($p < 0.05$) between physico-chemical soil properties and biochemical, biological and environmental parameters.



	EC	pH	TOC	TN	C:N	AP	pF -1500 kPa	pF -33 kPa	Lime	Clay	Silt	Sand	Carbohydrates	Polyphenols	Deshydrogenase	β- glucosidase	Phospatase	Urease	Bacteria	Gram+	Gram-	Gram+/Gram-	Actinobacteria	Fungi	Fungi/Bacteria	BR	CO_2 emissions	Priming effect	Mineralization	M3
pH	-0.53*																													
TOC	0.82***	-0.37																												
TN	0.81***	-0.63**	0.91***																											
C:N	-0.37	0.42	-0.63**	-0.66**																										
AP	0.70**	-0.46	0.72***	0.83***	-0.44																									
pF -1500 kPa	0.60**	-0.52*	0.62**	0.74***	-0.39	0.61**																								
pF -33 kPa	0.14	0.02	0.13	0.19	0.03	0.29	0.64**																							
Lime	-0.20	0.23	-0.18	-0.13	0.23	0.21	0.13	0.55*																						
Clay	0.03	0.20	-0.01	0.01	-0.03	0.41	0.06	0.44	0.71***																					
Silt	-0.20	0.21	-0.08	-0.26	-0.17	-0.56*	-0.38	-0.44	-0.59*	-0.56*																				
Sand	0.10	-0.39	0.08	0.18	0.16	-0.11	0.20	-0.23	-0.45	-0.82***	-0.01																			
Carbohydrates	0.56*	-0.63**	0.49*	0.76***	-0.40	0.81***	0.70**	0.33	0.06	0.15	-0.53*	0.18																		
Polyphenols	0.43	0.14	0.54*	0.37	-0.38	0.32	0.16	-0.28	-0.18	0.01	0.15	-0.12	0.10																	
Deshydrogenase	0.72***	-0.40	0.82***	0.86***	-0.40	0.84***	0.56*	0.27	0.07	0.16	-0.35	0.06	0.66**	0.27																
β-glucosidase	0.62**	-0.61**	0.64**	0.84***	-0.41	0.84***	0.76***	0.40	0.07	0.12	-0.45	0.17	0.92***	0.12	0.85***															
Phospatase	0.56*	-0.61**	0.51*	0.78***	-0.36	0.85***	0.64**	0.32	0.09	0.21	-0.56*	0.14	0.95***	0.03	0.75***	0.93***														
Urease	0.11	0.20	0.33	0.07	-0.17	0.05	-0.09	-0.18	-0.19	0.06	0.32	-0.29	-0.35	0.51*	0.10	-0.12	-0.28													
Bacteria	0.49*	-0.71**	0.60**	0.77***	-0.46	0.54*	0.75***	0.29	-0.10	-0.23	-0.28	0.48*	0.70***	-0.03	0.63**	0.79***	0.72***	-0.15												
Gram+	0.49*	-0.69***	0.57*	0.76***	-0.43	0.56*	0.77***	0.34	-0.05	-0.20	-0.30	0.46	0.76***	-0.01	0.67**	0.86***	0.77***	-0.21	0.98***											
Gram-	0.46	-0.67**	0.60**	0.73***	-0.47	0.47	0.66**	0.20	-0.18	-0.24	-0.24	0.47	0.56*	-0.06	0.52*	0.64**	0.59**	-0.04	0.95***	0.86***										
Gram+/Gram-	0.59**	-0.44	0.61**	0.74***	-0.44	0.69**	0.72***	0.37	0.08	0.06	-0.27	0.12	0.80***	0.27	0.79***	0.90***	0.80***	-0.17	0.70**	0.81***	0.47*									
Actinobacteria	0.43	-0.54*	0.43	0.61**	-0.41	0.49*	0.64**	0.18	0.02	-0.13	-0.31	0.37	0.70**	0.23	0.44	0.66**	0.68**	-0.25	0.79***	0.83***	0.67**	0.73***								
Fungi	0.37	-0.47	0.41	0.57*	-0.28	0.52*	0.71***	0.42	0.02	-0.07	-0.15	0.20	0.73***	-0.01	0.57*	0.82***	0.65**	-0.19	0.60**	0.69**	0.41	0.78***	0.41							
Fungi/Bacteria	0.13	-0.07	0.11	0.22	0.01	0.38	0.37	0.35	0.16	0.12	-0.09	-0.08	0.48*	-0.01	0.36	0.53*	0.41	-0.18	0.11	0.23	-0.08	0.52*	-0.01	0.83***						
BR	0.59**	-0.66**	0.63**	0.85***	-0.41	0.83***	0.68**	0.34	0.07	0.11	-0.46	0.20	0.87***	-0.01	0.87***	0.96***	0.94***	-0.16	0.82***	0.86***	0.71***	0.82***	0.61**	0.72***	0.42					
CO_2 emissions	0.54*	-0.59*	0.57*	0.76***	-0.44	0.69**	0.78***	0.31	-0.05	-0.10	-0.21	0.28	0.82***	0.18	0.64**	0.88**	0.81***	-0.15	0.78***	0.84***	0.63**	0.86***	0.74***	0.82***	0.54*	0.82***				
Priming effect	0.45	-0.60*	0.53*	0.60*	-0.36	0.42	0.77***	0.36	0.17	-0.14	-0.35	0.42	0.48	0.14	0.52*	0.62**	0.45	-0.01	0.78***	0.79***	0.70**	0.64**	0.68**	0.54*	0.07	0.58*	0.64**			
Mineralization	0.79***	-0.61**	0.91***	0.95***	-0.66**	0.74***	0.69**	0.06	-0.15	-0.11	-0.17	0.26	0.64**	0.46	0.76***	0.72**	0.64**	0.11	0.77***	0.74***	0.76***	0.66**	0.66**	0.46	0.07	0.73***	0.71***	0.64**		
M3	0.54*	-0.47*	0.38	0.52*	-0.05	0.56*	0.47*	0.27	-0.21	-0.05	-0.20	0.20	0.66**	-0.05	0.54*	0.68**	0.61**	-0.02	0.41	0.44	0.34	0.46	0.17	0.68**	0.61**	0.63**	0.52*	0.15	0.36	
T3	-0.37	0.05	-0.11	-0.01	-0.06	-0.13	0.15	-0.04	0.03	-0.35	0.14	0.32	0.02	0.07	-0.18	0.00	0.00	-0.05	0.29	0.25	0.31	0.00	0.30	0.10	0.00	0.03	0.27	0.14	0.13	-0.26

*Significantly different (*p* <0 .05) ; ** Significantly different *(p < 0.01); *** Significantly different (p < 0.00).* [†] Boxes without asterisks represent non-significant correlations *(p > 0.05).* [‡] EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon to nitrogen ratio; pF: water retention in soil at different pressures; Lime: active lime; BR: basal respiration from incubation laboratory experiment; CO_2 emissions: soil respiration measured *in situ* from field campaign. Bacteria, Gram +, Gram -, fungi and bacteria: fatty acids content; M3: moisture 3cm depth; T3: temperature 3cm depth.

1.3.6. Plant survival and growth.

The treatments that presented the highest survival rates were vegetable compost (GC and CC; 93.33%; Figure 1.4a), followed by Mix1. CON also presented very high survival rates, obtaining slightly lower values than GC and CC. Conversely, SS and Mix2 presented lower survival rates, although the effect of the treatment was different depending on the type of species, as the lowest survival rates were presented in *S. tenacissima* (55.83%) in SS treated soils (Figure 1.4a), while *O. europaea* had a 21% higher survival rate (76.67%) in this treatment. The same pattern occurred in soils treated with the mixtures (Mix1 and Mix2), which presented higher survival rates for *O. europaea* (Figure 1.4a). However, all the treatments significantly increased the plants' growth (biovolume), presenting higher values than in CON soils (Figure 1.4b). The highest growth rates were recorded for CC and Mix1 in both species, followed by CG. The lowest growth rates were registered in SS and Mix2 soils (Figure 1.4b), with the values lower in *O. europaea* than in *S. tenacissima*. However, the plants most affected by grazing and rooting by wild herbivores were register in *O. europaea* in SS (100%), CG (96.43%), and Mix2 (95.24 %) soils. *S. tenacissima* presented high rates of damage by this activity caused by wild animals in SS and Mix2 soils, although the affection rate did not exceed 35% (Figure 1.4c). CC and Mix1 soils had a lower affection, much higher in *O. europaea* than in *S. tenacissima*. In contrast, *S. tenacissima* did not affected by grazing and rooting, and *O. europaea* showed the lowest rates of affectation by wild animals in CON soils (Figure 1.4c). In adittion, colonization by other wild plants presented much higher coverage in restored plots compared with CON, presenting a similar area covered by plants among the different treatments around 80%, except SS soils, in which coverage was significantly lower—by approximately 20–30%. CON soils presented a significantly lower percentage—much less than 10%—of ground cover occupied by wild plants (Supplementary Figure 1.2). The main colonizing species were *Atriplex halimus* L., *Moricandia arvensis* L., *Artemisia barrelieri* Besser, *Anthyllis cytisoides* L., *Limonium insigne* **(**Coss.) Kuntze, *Pistacia lentiscus* L. and *Capparis spinosa*, L.

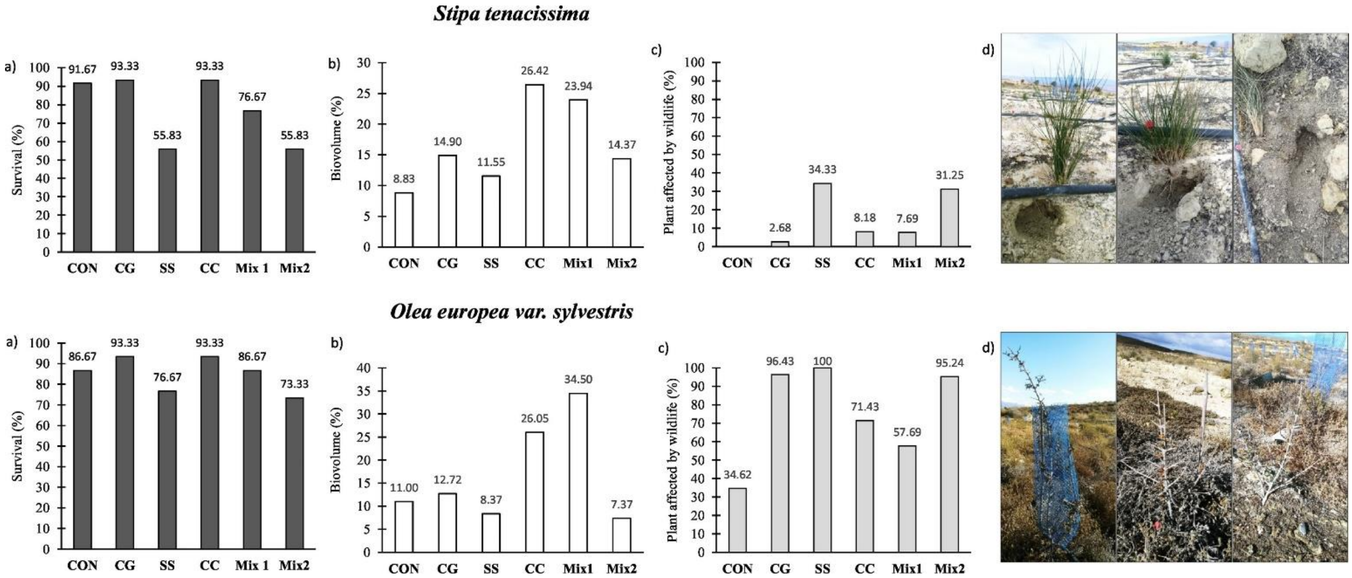


Figure 1.4. Effects of the organics amendments on survival and growth rate 2 years after organic amendment application and wildlife diseases. a) Survival rate per treatment for each species expressed as a percentage; b) Percentage of growth (biovolume) for each treatment in relation to total plant growth; c) Percentage affected by wild fauna, foraging and rooting per treatment for each species; d) Details of wildlife diseases observed for each species and treatments. CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70° C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost.

1.4. Discussion

In this study, we evaluated whether several soil restoration treatments based in different organic amendments could be beneficial in improving soil conditions in terms of changes in physico-chemical, biochemical and microbiological properties in a semiarid ecosystem degraded by mining activities.

The contribution of organic amendments had a remarkable effect on the physico-chemical soil properties as well as in the biomass and activity of soil microbial communities (Tables 1 and 2), thus improving the functionality of the restored soils just 6 months after organic amendment application respect to untreated soils and maintaining also high nutrient values (TOC and TN) and water retention capacity 2 years after the application of the amendments. Although the restored soils were initially saline (Table

1), the EC content significantly decreased 2 years after the application of the organic amendments (Supplementary Table 1.2), which could be due to rain washout or nutrient absorption by both planted and colonizing vegetation (Bastida et al., 2008b; Ortega et al., 2020; Ros et al., 2003), some of which were halophytes and could act as bioremediators (Qadir and Oster, 2002). Other authors have revealedthat organic amendment could favor the EC decline (Diacono and Montemurro, 2015; Tejada et al., 2006). In fragile ecosystems such those in arid, abandoned open mine areas, the soil functionality is a critical component of soil fertility that promotes growth and proliferation of soil microorganisms and favours reactions that release soil nutrients for vegetation development (Hannam et al., 2006). The SS, Mix1 and Mix2 soil restoration treatments most increased the soil functionality-related parameters (e.g., higher values in BR, enzyme activities, fatty acids, nutrients content), the labile organic matter content (carbohydrates and polyphenols), and the soil's water-holding capacity (e.g. pF -1500 KPa). The CC and CG soil restoration treatments showed functionality responses intermediate between CON soils and SS (Tables 1.1 and 1.2; Figure 1.3). Nevertheless, the soils without organic amendments (CON) had significantly lower values in all properties indicative of soil functionality and lacked labile organic matter (Table 1 and 2). Enzyme activities play important roles in catalysing biological reactions in soils that are strongly related to biogeochemical cycles and soil fertility (Mataix-Solera et al., 2009). In our study, ß-glucosidase evolution was related to the rate of organic matter decomposition, as indicated by its significant positive correlation with TOC and soil respiration (Table 4). In addition, our results support that β-glucosidase was responsible for the breakdown of low molecular weight carbohydrates, as suggested by the significant positive correlation between β-glucosidase and carbohydrates (Table 4), producing glucose as a product of their hydrolysis, which is an important source of resources for soil microorganisms (Eivazi and Zakaria, 1993). Nevertheless, although phosphatase activity was also positively correlated with TOC (Table 4) as observed by several authors (Antolín et al., 2005; García-Gil et al., 2004), this activity is mainly related to the high AP content in the restored soils (Table 1). This could be due to the high phosphorus concentration present in the original sludge amendments (Supplementary Table 1.1). The phosphatase activity catalyse the reaction that transforms organic and non-assimilable phosphorus into phosphate ions, which can be easily absorbed by microorganisms and plants (Eivazi and Tabatabai, 1977). In addition, SS presented a significantly higher number of fungal fatty acids (Table 2), so the increase of phosphorus could have promoted the growth of arbuscular mycorrhizal fungi (Jayachandran et al., 1992). In addition, some authors have reported that sewage sludge can be considered as a promoter of mycorrhizal colonization (Amir et al., 2019) when the dose is appropriate (Thorne et al., 1998). The increase in these enzyme activities may promote soil fertility, providing simple substrates for microbial growth and fostering soil microbial biomass, as indicated by the significant high positive correlations between fatty acid contents with β-glucosidase and phosphatase (Table 4). In turn, dehydrogenase activity, which has been used as an indicator of the overall microbial activity in semiarid soils (García et al., 1994), showed high values in restored soils. The greater values of dehydrogenase activity in SS-treated soils may be related to the higher organic C content of this material, which stimulates both microbial biomass and activity (Chakraborty et al., 2011), as suggested by the significant positive correlation between dehydrogenase activity and TOC (Table 4). In contrast, SS-restored soils showed the lowest values in the urease activity, whereas other authors found that urease activity increases with the addition of organic amendments in the soils (Bastida et al., 2008b; Pascual et al., 2002, 1998). The low urease activity in SS soils could be due to a high ammonium content in sewage sludge that inhibits the microbial synthesis of this enzyme (McCarty et al., 1992) or to the absence of specific substrates capable of activating the synthesis of urease activity (Marschner et al., 2003; Pascual et al., 1998). Furthermore, Burns (1986) reported that the low urease activity values may be related to the fact that organic matter aromatic components could inhibit the formation of urease enzyme complexes that preserve enzyme activity.

The high biomass of bacterial and fungal communities in the restored soils, as evaluated through microbial fatty acids, could be due to the labile organic matter provided by the amendments, particularly SS. Indeed, we observed significant positive correlations between the carbohydrate content and the fatty acid content of fungi, Gram+ and Gram-bacteria. In addition, F:B ratio was significantly higher in restored soils than in non-amended soils, which further suggests a change in the composition of the microbial community of the restored soils. Luna et al. (2016b) reported an increase in F:B ratio in compost-treated soils and clear differences in the structure of microbial bacterial and fungal communities in amended soils with respect to unamended soils. This could also be due to the high TOC content, including the superior content of easily decomposable carbohydrates and polyphenols in restored soils, which are mainly used by microbial soil communities. These results indicated that the high mineralization rates in soils treated with SS, Mix1 and Mix2, which have the greater labile C fractions, were due to the high levels of TOC, TN, carbohydrates and polyphenols stimulating the development and activity of the soil microbial communities. Then, treatment with SS compost content could have a negative effect, in that the organic matter and nitrogen provided in the amendment could be consumed quickly by microorganisms and not guarantee a long-term nutrient reserve in the restored soils, as well as favouring a rapid release of CO_2 into the atmosphere. Thus, within the soil C cycle, priming effect plays a key role in relation to potential CO_2 emissions from soil (Blagodatskaya et al., 2007; Bastida et al., 2019). The observed positive priming effects derives from the significant stimulation of the soil microbial community as a consequence of soil nutrient enrichment and can also be associated with changes in the composition and structure of soil microbial communities (Blagodatskaya et al., 2007; Fontaine et al., 2003; Mondini et al., 2006; Razanamalala et al., 2018) in response to organic amendments. Indeed, we found that the priming effect correlated with the ratio Gram+/Gram- but not with the fungal to bacterial ratio, which may indicate that in the studied mine system, soil priming effects can be mediated more by changes in bacterial community than by changes in the fungal community. Significant positive correlations of CO_2 emissions with other soil parameters could indicate that they are influenced by the joint effect of several biotic and abiotic factors such as TOC, TN, AP and the presence of easily biomineralizable substrates in organic amendments, as well as microclimatic variables (soil temperature and humidity), which indirectly affect soil microbial activity (Blagodatskaya and Kuzyakov, 2008). Thus, the soil moisture, which was higher in restored soils with sludge (SS, Mix1 and Mix2), favoured the metabolic activity of soil microorganisms (Bastida et al., 2017a; Feng et al., 2016; Wang et al., 2016; Tatabai and Dikc, 2002), as our correlation analyses showed (Table 4).

In contrast, the CG and CON soils showed the lowest priming effect values (Figure 2). The values of the priming effect and CO_2 emission rates in CC and CG soils could be lower than in soils restored with sludge because they had a greater content of stable organic matter with a high lignin residue content that was biodegradable with difficulty (Miralles et al., 2015). The lower microbial activity in compost garden waste amended (CG) and unamended soils (CON) could also be due to the lower labile organic matter content (i.e., carbohydrates) (Table 2) reducing the proliferation of opportunistic soil microorganisms and the CO_2 emission in the short term, even in the presence of moisture. In CON soils the low values of the priming effect and CO_2 emission rates could be due to the low TOC content and the low values in the biomass and activity of the microbial communities in those degraded soils (Tables 3 and 4).

Plants' survival rates and growth were clearly influenced by the organic treatments applied to the restored soils and the effect in each plant species depended on type of organic amendment used, as have been reported by other authors (Ortega et al., 2020). The CG and CC treatments guaranteed successful survival and, in turn, increased the growth of both types of plants with respect to CON, which also had high survival rates but no growth, with CC being much more successful in the latter aspect (Figure 1.4). However, SS showed higher mortality and lower growth, which could also be justified by a high rate of browsing and rooting suffered in the plots of this treatment that attracted more wild, herbivorous animals. Mix1 was more successful than Mix2 in plant survival and growth (Figure 1.4). The percentage of soil covered by different wild plant species was also much higher in soils with organic amendments (higher than 80% in all restored treatments except in SS, which was close to 60%), while in control soils, it was lower than 10%. The better nutrient conditions and biological activity in the C, N, and P cycles, making essential nutrients available to the plants, could have influenced the greater development of wild plants in the experimental plots.

1.5. Conclusions

All restored soils influenced physico-chemical, biochemical, and biological soils properties and the vegetation cover. The soils restored with each compost type (CC and CG) improved the soil functionality increasing TOC, TN, AP, basal respiration, and enzyme activities in the short term than control soils, and they also maintain higher TOC, TN, AP, and water retention capacity 2 years after their application than in CON soils, with a consequent positive effect on the quality and recovery of restored soils in mining areas with semiarid climates. They also showed the highest rates of survival and growth of native plants introduced and favored the highest values of colonization of other surrounding species. In addition, the mineralization rates of soils treated with compost were much lower than those of soils treated with sludge, thus initially guaranteeing the reserve of nutrients and fertility in the restored soils in the long term along with a less intense release of greenhouse gas emissions.

In contrast, although the soils restored with pretreated sludge favored chemical and microbial soil properties, including the activity and biomass of soil microbial communities and soil moisture in the short term, they produced the highest *in situ* CO_2 emission rates and an important positive priming effect with the consequent effect on global change. Another negative effect derived from the use of this amendment could be the high mineralization rates, contributing to a rapid consumption of the nutrients provided and potentially failing to guarantee a long-term nutrient reserve and soil fertility, although these soils keep higher TOC and TN content than nonamendment soils (CON) 2 years after their application. In turn, this treatment showed lower rates of survival and growth of introduced native plants and also of colonization of wild plants 2 years after its application. Therefore, the treated sludge was the least recommended amendment for the restoration of the quarry in the study area.

The mixtures of the organic amendments (pretreated sludge and compost from plant remains) presented better results than the sludge, with Mix1 being better than Mix2 as it showed higher survival and growth rates of plants and percentage of soil covered by wild plant cover and presented lower priming effect values than the soils with pretreated sludge. However, the joint analysis of the soil properties and vegetation analyzed showed worse results than soils with only compost from vegetable remains. Further, a longer-term study is recommended to examine amendment behavior to obtain more robust results and also a regular monitoring of the quality and functionality indicators used and the effect of the organic amendments on CO_2 fluxes.

Chapter 2

Effects of Technosols based on organic amendments addition for the recovery of the functionality of degraded quarry soils under semiarid Mediterranean climate: A field study.

***Soria, R.**, González-Pérez, J.A., de la Rosa, J.M., San Emeterio, L.M., Domene, M.A., Ortega, R., Miralles, I., 2022. Effects of technosols based on organic amendments addition for the recovery of the functionality of degraded quarry soils under semiarid Mediterranean climate: A field study. Science of The Total Environment 816, 15157*

Abstract

This study aims to evaluate the effects of technosols made with different organic amendments to restore degraded soils in a semiarid limestone quarry. The effects on soil quality, functionality and organic matter dynamics of the technosols amended with waste of gardening, greenhouse horticultural, stabilized sewage sludge and two mixtures of sludge with both vegetable composts were assessed. Several physical and chemical properties, humus fractions, soil respiration and molecular composition was performed after 6 and 18 months. Un-amended soils, and nearby natural undegraded soils served as reference. Amended technosols increased water retention capacity, electrical conductivity, total organic carbon and nitrogen, respect to not amended and natural soils. Humus fraction composition was not altered over time. Un-amended soils, very poor in organic matter, did not show any pyrolyzable compounds or labile soil organic matter by thermogravimetry. In contrast, the pyrochromatograms of natural soils showed lignocellulosic materials, polypeptides and a noticeable presence of alkylic compounds. In technosols with both types vegetable compost, the organic matter structure was more complex, showing compounds from lignin-derived and long-chain alkyl, polysaccharides, chlorophyll isoprenoids and nitrogen. In sludge technosol, a set of sterols was outstanding. The mixtures showed a molecular fingerprint of materials derived from the decomposition of the organic amendments that formed them. These signs of the contribution of different organic matter forms derived from the amendments were also reported by the series exothermic peaks found in the calorimetry. This short-term study indicates a clear effect of the amendments on the recovery of soil organic matter and presumably of its functionality. After the amendments application, microbial activity and soil respiration rates increased rapidly but ceased 18 months later. The molecular composition of the organic matter of the soils amended with plant compost was very similar to that of natural, non-degraded soils in nearby areas.

2.1. Introduction

Globally, it is estimated that 33% of the Earth's soil have been degraded by human activity (FAO, 2019). Among different drivers, mining produces serious soil impacts and occupies more than 1 % of the territory (Carabassa et al., 2020a; Šálek, 2012). Specifically opencast mining is a high-impact disturbance to terrestrial ecosystems (Ibarra and De Las Heras, 2005; Smirnov et al., 2021) and causes severe soil degradation, often irreversible (Larondelle and Haase, 2012; Soliveres et al., 2021). This problem is especially sensitive in arid and semi-arid lands due to extreme climatic conditions, with annual rainfall less than 300 mm, and scarcity of soil nutrients hinders the cover vegetation development after mining (Gonzalez-Dugo et al., 2005; Josa et al., 2012; Ortega et al., 2020). The resulting degraded ecosystems present severe difficulties to recover their functionality (Moreno-de las Heras, 2009) and improve the ecosystem services provision, both strongly linked to soil (Lal, 2015; Nunes et al., 2020).

As soil is a complex multi-component system with an infinite number of interrelationships (Nunes et al., 2020), its properties are the result of the net effect of all of them. In this sense, soil organic matter (SOM) is one of the determinants of soil quality and health, a concept considered synonymous with the measure of a soil's ability to carry out its ecological functions (Hoffland et al., 2020). Furthermore, because SOM responds relatively quickly to changes in both biotic and abiotic conditions, it is key in regulating and restoring the balance of environmental processes occurring in the soil, in what is defined as the "resilience" of the soil to recover from external variations (ECCE, 2005), and the integration of inputs an opportunity to improve soil resilience (Reddy et al., 2020). The development of functional soils with adequate levels of OM and nutrient cycling reactivation is a precondition for ecosystem recovery (Lal, 2015; Moreno-de las Heras, 2009). In this context, the use of organic amendments for the creation of artificially-manufactured prepared soils, called technosols or anthroposols (Larney and Angers, 2012) have been proposed as a possible solution to restore lands degraded by mining (Carabassa et al., 2018; Fabbri et al., 2021; Leguédois et al., 2016) .

Beside soil degradation, the increase of organic waste worldwide poses a global problem that requires urgent solutions (Hernández et al., 2015). The treatment of sewage sludge as well as the management of agricultural and forestry waste is still one of the main environmental concerns in developed countries, where their recycling and recovery are considered as the most economically viable sustainable options (Waste Framework Directive 2008/98/EC). Therefore, recycling and incorporation of organic waste as organic amendments for the regeneration of degraded soils, through the creation of technosols, responds to a strategy of integration into the circular economy (Fabbri et al., 2021; Hueso-González et al., 2018) while promoting to natural capital recovery (Abhilash, 2021; Alba-Patiño et al., 2021). In addition, it leads to a significant increase in OM (Bastida et al., 2008b) and contributes to improve the quality and fertility of degraded soils by creating edaphic conditions that facilitate plant colonization (Asensio et al., 2014; Hueso-González et al., 2018). Maintaining or increasing SOM levels and sustainable biomass management are probably the most feasible and effective tools to mitigate today’s most impacting environmental problems, such as global warming and desertification.

Restoration of degraded soils with organic amendments to build technosols after quarry mining is considered a sustainable soil rehabilitation technique (Carabassa et al., 2020b; Josa et al., 2012; Pérez-Gimeno et al., 2019; Ruiz et al., 2020). Numerous studies on recycling organic wastes to amend technosols in mining areas have focused on the use of organic amendments or composts from waste of different origins (Asensio et al., 2013; Watkinson et al., 2017). The application of these organic wastes improves the chemical, physical and microbiological characteristics (Breton et al., 2016; Bukar et al., 2019; Rodríguez-Berbel et al., 2021a) and as effective strategy to soil ensure an immediate OM increase, enrich the soil with humic-like compounds and macro- and micronutrients (Hernández et al., 2015). Likewise, the improvement of the above-mentioned soil properties depends mainly on OM chemical composition provided by organic amendments (Ye et al., 2019). For all these reasons, the technosols amended with local organic waste are considered a valuable source of OM to improve the quality and functionality of degraded soils. Composting has long been used to stabilise and reduce the toxicity of organic residues, and the use of composts as amendments in agricultural soils has been shown to contribute efficiently and economically to nutrient recycling and organic carbon recovery (Larney and Hao, 2007). However, it is also necessary to consider that the application of organic amendments could cause CO_2 emission, and contributing to greenhouse effect (Campos et al., 2020). This would also depends on the chemical composition of the amendments (Blagodatskaya et al., 2014; Ray et al., 2020; Soria et al., 2021b). Nevertheless, there is a great variability of the OM composition depending on its origin and nature, affecting its decomposition rate and long-term maintenance (Larney and Angers, 2012; Carabassa et al., 2020b). The SOM quality generally depends on the proportion and distribution of labile and recalcitrant forms, with the highest quality and comparatively more resilient SOM being that with a higher degree of humification, aromaticity and complexity in its molecular structure (Arias et al., 2005). Therefore, understanding the dynamics and molecular composition of OM contributed by organic amendments would provide important information on soil functioning, the evolution of technosols (Pascaud et al., 2016) and their effectiveness in the restoration actions. Taking into account that the origin of organic waste and composting processes can be diverse and condition the behaviour of OM in the soil (Garcia et al., 2017), due to the great variability of active fractions, chemical and nutritional composition that they provide when used as organic amendments. Due to the complexity of SOM, a combination of chromatographic and thermal analysis tools has been successfully employed to investigate it. Thermal analysis methods, specifically thermogravimetry-differential scanning calorimetry (TG-DSC), have been previously used with success to characterize chemical changes in SOM fractions, degraded plant tissue, and compost (De la Rosa et al., 2008; Dell'Abate et al., 2003; Lopez-Capel et al., 2006). TG-DSC has also been used to compare the proportions of reactive and more stable components in organic matter fractions under contrasting conditions (Lopez-Capel et al., 2005), and to different forms of pyrogenic carbon (Leifeld, 2007). Analytical pyrolysis is an effective tool to characterize the chemical composition, evolution and molecular markers of SOM (Derenne and Quéné, 2015). Specifically, pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS), provides not only value information about the SOM chemical structure (De la Rosa et al., 2008), but also about the origin of the different components and of a wide range of SOM products related to the origin of the analysed material (De la Rosa et al., 2012; González-Vila et al., 2009) and an opportunity to evaluate the evolution and dynamics (Jiménez-Morillo et al., 2016; Picó and Barceló, 2020).

We hypothesize that the different organic amendments applied to the soil severely degraded by the mining activities and subjected to the extreme climatic conditions typical of semi-arid soils will result in a different SOM composition. This, in turn would exert a different evolution in the technosols, conferring distinct physical and chemical properties to the restored soils. Therefore, the main objective of this work is to investigate SOM evolution and its chemical composition at the molecular level, as well as the physical and chemical soil properties in the restored technosols. In pursuing this, we analysed soil organic carbon, soil respiration, and organic carbon fractions (soluble, labile and recalcitrant), using advanced techniques of molecular analysis, as well as other classical techniques that will allow us to compare treatments and to select the most appropriate one according to the quality of the OM present in the restored soils.

2.2. Materials and methods

2.2.1. Study site

The area of study is located in a limestone quarry used for the extraction of aggregates in an exhausted mine area with severely degraded soils sited about 15 km from Almería city (SE, Spain). Geographically is located at the position 36 55’ 18’’ N, 2º 30’ 40’’ W between Sierra de Gádor (dolomites and Cenozoic limestones) and intermountain Tertiary basin formed by Tortonian loams (high Miocene) composed mainly by calcareous sandstones and calcitic-gypsiferous mudstones (Luna et al., 2016a). The dominant climate of this area is semi-arid Mediterranean, with irregular temperatures and rainfall. Summers are hot and dry, with maximum temperatures recorded in August of 31 ºC and minimum temperatures of approximately 8 °C in January, and high evapotranspiration rates reaching 1225 mm year^{-1} (data recorded at Alhama de Almería weather station located at 4 km of distance from the study area). The average annual rainfall is 242 mm distributed mainly in autumn and winter. The experimental site was located on an area completely exploited by mining activity and unsloped terrain at an altitude of 362 m. a. s. l. devoid of natural vegetation cover. The resulting post-mining substrate consisted of a mixture of calcareous sandstones rock fragments and loams with clayey texture (Soria et al., 2021a), compacted and with high resistance to plants development. In adjacent unexploited locations shallow soils are found over limestones and dolomites with calcareous sandstones and loamy loams or sandy or silty loams forming Regosols (IUSS Working Group WRB, 2015). Native vegetation corresponds to *Macrochloa tenacissima* (L.) Kunth *(=Stipa tenacissima* L.*)* as main species, accompanied by small shrubs such as *Ulex parviflorus* Pourr and *Anthyllis cytisoides* L. among others, as well as dispersed individuals of *Maytenus senegalensis* (Lam.) Exell, *Pistacia lentiscus* L. and *Rhamnus lycioides* L. (Luna et al., 2016b).

2.2.2. Experimental Design

A total of 18 experimental plots (10 m × 5 m each one) were established in May 2018 on a disturbed site using a random block design. Thus, three experimental plots for each treatment (3 replicates) with five different organic amendments treatments were settled and monitored. First, the topsoil (0-20 cm) of the plots was de-compacted and homogenized using machinery supplied by the mining company CEMEX-Spain. Then, the organic amendments were added in a single dose and the amounts used to prepare technosols were calculated to increase organic matter content by 3% in each plot. These organic amendments consisted of: i) compost from green garden waste (CG) applied with a dose of 28.66 kg m^{-2} (total organic C on dry weight = 40.5 % and moisture = 22.5 %); ii) Sewage sludge waste treated by mesophilic digestion and thermal dehydration at 70ºC (SS) was added at a rate of 14.74 kg m^{-2} (total organic C on dry weight = 66.4 % and moisture = 8.8 %); iii) Vegetable compost from greenhouse vegetables and fruits crop waste (CC) applied with a rate of 34.61 kg m^{-2} (total organic C on dry weight = 43.33 % and moisture = 40 %). In addition, two treatments were designed with mixtures of the above treatments, consisting of: iv) CG + SS (called Mix1) composed of a mixture of CG at a rate of 14.33 kg m^{-2} combined with SS at a rate of 7.73 kg m^{-2}; and v) CC + SS (called Mix2) with a CC amount of 17.30 kg m^{-2} mixed with SS at a rate of 7.73 kg m^{-2}. Untreated plots were used as control (CON) and natural soils taken from nearby area unaffected by mining were chosen as reference ecosystem (NAT) and as a model of the objective to be achieved in this restoration work. 40 plants of *Macrochloa* L. and 10 plants of *Olea europaea* L. var *sylvestris* Brot. were seeded in each experimental plot using a planting pattern of 1 m. These plant species were selected for their high survival rates in previous restorations plans in the same area (García-Ávalos et al., 2018). An initial drip irrigation after planting was carried out in order to ensure the plants survival because in semiarid

Mediterranean ecosystem during the first summer (Ramón-Vallejo et al., 2012; Sánchez et al., 2004). Initially, 3 L per plant were administered, and then 1 L per plant irrigated every two weeks after installation until the end of August, a total of 5 irrigations, thereafter they only received rainwater.

For each experimental plot composited samples made of 10 random subsamples taken from the topsoil layer (0-10 cm) were collected 6 months (T6) and 18 months (T18) after the addition of the organic amendments (17th December 2018 and 24th November 2019 respectively). At the same time complete set of samples were also taken from the nearby reference soils (NAT) for comparison purposes. A total of 21 samples in each of the sampling campaigns (3 per treatment, 3 control and 3 of natural soil samples) were collected and taken to the laboratory. All the samples were immediately air-dried at 40 ºC, homogenized and sieved to fine earth (2 mm) A subsample was used for physical and chemical analysis and humic substances insolation and another for thermal and analytical pyrolysis analysis. For the last, an aliquot was taken from each of the three replicates and combined in composite samples representative of each sample and sampling time.

2.2.3. Elemental composition, physical and chemical properties of restored technosols

Soil pH and conductivity were determined in distilled water at a soil/solution ratio of 1:2.5 using a water quality meter instrument (LAQUA PH1100, HORIBA, Tokio, Japan). Total organic carbon (TOC) was determined by wet oxidation using the as modified by (Mingorance, 2007). Total nitrogen content (TN) was determined using an elemental analyser TCD detector (ELEMENTAR Rapid N; Elementar Analysensysteme GmbH, Hanau, Germany). C:N ratio was estimated as the ratio of TOC to TN. Soil water retention was determined to pF at -1500 KPa and -33 KPa by the Richards membrane method (Richards, 1941). Carbohydrate content (CH) was determined by cold extraction of 5 g in a soil-to-water ratio of 1:10 (w:v) using the anthrone–sulphuric acid method for soil CH quantifyication developed by Brink, et al. (1960). The same extract was used to determine the polyphenol content (POL) by the Folin–Denis method (Ribéreau-Gayon, 1968). The absorbance to determine both compounds, CH (625 nm) and POL (750 nm) were measured in a UV-Vis spectrophotometer, (Spectronic Helios Gamma, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.2.4. Isolation and quantification of soil humic fractions

The methods reported by Duchaufour et al. (1975) and Dabin (1963) were used for the isolation and subsequent quantitative determination of SOM fractions. The organic carbon content in different fractions collected was determined by wet oxidation (Walkey and Black, 1934). A first physical separation of the light soil fraction consisting of organic particles not yet transformed into humic substances was performed by flotation using 5 g soil samples suspended in 2 mol L^{-1} H_3PO_4 (with rotary shaking for 1 minute). The light soil fraction or *free organic matter* was isolated by centrifuging the suspension and filtering, washed with distilled water and analyzed their C content. The resulting soil extraction residue was successively extracted with 0.1 mol L^{-1} $Na_4P_2O_7$ (horizontal movement with mechanical shaking for 3 h) and centrifugation of 4,500 rpm during 30 minutes with a centrifuge (Digicen 21, Ortoalresa, Spain). This treatment was repeated three times followed by two additional extractions with 0.1 mol L^{-1} NaOH; the successively obtained dark brown extracts (corresponding to the total humic extract (THA: Humic Acid (HA) + Fulvic Acid (FA)) were added. Two aliquots of this extract were taken and precipitated with H_2SO_4 (1:1 by volume) and used for quantitative determination of the amounts of acid soluble FA and the precipitated HA acid-insoluble fraction. The results obtained from the isolation and quantification of HAs and FAs were used to compare the grade of humification between the different modified technosols 6 and 18 months after the application of the organic amendments.

2.2.5. Thermal analysis

Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC) analyses were carried out simultaneously in a Discovery SDT 650 - Simultaneous TG-DSC (TA Instruments, New Castle, Delaware, USA). Briefly, for each sample approximately 20 mg of dry grounded material were placed in previously tared open alumina crucibles under a He flux (flow rate, 50 mL min^{-1}; 10 mL min^{-1} at the micro-furnace) heated and scanned at a rate of 20 K min^{-1} from 50 to 900 ºC. The heat of combustion (Q, in J g−1) and loss-on-ignition in TG_{tot} (%) were determined by integrating the DSC and TG curves (in Wg^{-1}) respectively over the region (50–850 ºC). The area under the TG curves was sub-divided into four sections representing different degrees of resistance to thermal oxidation (De la Rosa et al., 2008): (i) 50–200 ºC, (ii) 200–400 ºC; (iii) 400–600 ºC; and (iv) 600–850ºC. The resulting partial weights are designated as W1–W4, respectively.

2.2.6. Soil respiration and climatic variables

Soil respiration (SR) was measured in-situ the same days of the soil sample collection (T6 and T18). A portable infrared environmental gas analyzer system with a soil respiration chamber (IRGA) (EGM-4, PP-systems, Hitchin, UK) was used for SR analysis. The chamber had a volume of 1170 cm^3 and a flat surface of 78 cm^2. Measurement time was 90 s to ensure reliability. The CO_2 effluent (SR) measurements were carried out on radometrically located PVC collars in the soil (3 per experimental plot and 3 inserted in natural reference soil) with a diameter of 10 cm and 5 cm high, which were inserted in the soil at a height of 2 to 3 cm above the ground. A total of two soil respiration measurement field campaigns (T6 and T18) were carried out. At the same time, soil moisture (M) and soil temperature (T) were measured at 3 cm depth with a handheld readout sensor ProCheck and GS3 Greenhouse Sensor (Decagon Devices, Inc., Pullman, WA, USA). A pluviometer Rain-O-Matic Small (Pronamic ApS, Denmark) connected to a data logger and placed in the middle of the experimental area recorded rainfall events every 20 minutes for the entire duration of the experiment.

2.2.7. Analytical pyrolysis

Analytical pyrolysis (Py-GC/MS) was performed in duplicate using 15 mg of finely grounded composite samples representing each treatment.at 400 °C (1 min) in a micro furnace pyrolysis system (Frontier Lab. model 2020i, Fukushima, Japan) as described elsewhere (Jiménez-Morillo et al., 2020). In short, the pyrolizer was coupled with a GC/MS system (Agilent 6890) equipped with a low polar-fused silica capillary column

(Hp 5MS-UI; 30 m x 250 µm x 0.25 µm). The GC was fitted with a mass selective detector (Agilent 5973 MSD) and mass spectra acquired at 70 eV. The following chromatographic conditions were used: the carrier gas was He (flow rate 1 ml min^{-1}), the oven was preheated to 50 °C for 1 min and then increased to 100 ºC at 30 ºC min^{-1}, from 100 to 300 ºC at 10 ºC min^{-1}, and then constant at 300 ºC for the last 10 min. The identification of compounds was achieved by single-ion monitoring (SIM) and by comparison with mass spectra libraries (NIST11 and Wiley7) and published spectra databases.

2.2.8. Statistical analyses

Significant differences in physical and chemical soil properties, OM fractions and soil respiration (SR) among the different restoration treatments, un-amendment and natural reference soils were studied using two way PERMANOVA analysis ($P >0.05$), using permutation tests to obtain P values and does not rely on the assumptions of traditional parametric ANOVA (Anderson, 2001). Euclidean distances was used to obtain the samples similarity matrix with to check the effects of the soil restoration treatment and sampling date (T6 and T18) factor on each individual variable. Pairwise test comparisons were made using a multivariate analogue of the t test and by finding the probability levels by permutation (Eldridge et al., 2016) and results with $P < 0.05$ were reported by significant. The total number of permutations used was 999, and the Monte-Carlo test was used when the number of permutations found was less than 100. The statistical package PRIMER + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows was used for PERMANOVA (Anderson et al., 2008).

2.3. Results and discussion

2.3.1 Effect of organic amendments on physical and chemical properties of technosols

In general, the organic amendments changed the physical and chemical properties in restored technosols respect to CON and NAT soils (Table 2.1). Two-way PERMANOVA analysis showed significant differences ($P < 0.05$) in the physical and chemical soil parameters attending to soil treatment (SS, CG, CC, Mix1, Mix2, CON and NAT) and date of field campaign, but not between the factor interactions (treatment x date) (Supplementary Table 2.1).

Table 2.1.Values (mean and standard deviation, in parentheses) for each physical and chemical properties and fraction of humic acids content of technosols amended with compost from organics wastes, control soils and natural reference soils. Different lowercase letters indicate differences ($P < 0.05$) among the treatments in the same sampling date according to PERMANOVA pair-wise test comparisons. Continued on next page.

Variables (T6)	**CG-6**	**CC-6**	**SS-6**	**Mix1-6**	**Mix2-6**	**CON-6**	**NAT-6**
pH	7.97 ± 0.20ab	8.5 ± 0.12b	7.45 ± 0.04a	7.52 ± 0.03a	7.65 ± 0.04a	8.59 ± 0.06b	8.64 ± 0.06b
EC (mS/cm)	2.56 ± 0.28ab	3.30 ± 0.25a	3.42 ± 0.40ab	3.25 ± 0.12a	3.08 ± 0.41ab	1.72 ± 0.00b	0.07 ± 0.00c
pF -1500 Kpa	18.14 ± 0.80ab	18.59 ± 0.28ab	21.45 ± 1.06a	19.46 ± 0.69a	19.42 ± 0.67a	16.34 ± 0.14bc	14.91 ± 0.14c
pF -33 KPa	29.47 ± 1.51a	30.70 ± 0.95a	34.34 ± 1.80a	31.61 ± 0.60a	32.40 ± 1.40a	32.36 ± 0.62a	36.32 ± 0.62a
AW (%)	11.32 ± 0.83ab	12.11 ± 0.71a	12.88 ± 0.78ab	12.15 ± 0.71a	12.98 ± 0.91ab	16.02 ± 0.61b	21.41 ± 0.61c
TOC (%)	1.71 ± 0.31a	2.94 ± 0.10b	2.67 ± 0.38b	2.45 ± 0.09b	2.82 ± 0.09b	0.46 ± 0.08c	1.31 ± 0.08d
TN (%)	0.32 ± 0.07a	0.40 ± 0.03ab	0.60 ± 0.12b	0.51 ± 0.02b	0.51 ± 0.01b	0.02 ± 0.00c	0.11 ± 0.00d
C/N	5.33 ± 0.01a	7.27 ± 0.27c	4.46 ± 0.08b	4.78 ± 0.11ab	5.46 ± 12.5a	23.0 ± 1.40abcd	11.4 ± 1.40d
CH (µg g^{-1} soil)	487.88 ± 43.82a	258.75 ± 8.49c	2315.08 ± 348.66b	1363.78 ± 97.27b	709.05 ± 72.62a	0 ± 0d	36.14 ± 5.38d
POL (µg g^{-1} soil)	43.02 ± 10.08b	72.75 ± 4.20c	37.21 ± 5.15a	33.29 ± 2.22a	18.02 ± 2.92b	3.01 ± 1.52d	7.09 ± 3.44d
THA (%)	0.55 ± 0.12a	0.67 ± 0.12a	0.6 ± 0.09a	0.63 ± 0.12aA	0.62 ± 0.11aA	0 ± 0b	0.62 ± 0.08a
HAs (%)	0.16 ± 0.02a	0.2 ± 0.04a	0.1 ± 0.05ab	0.18 ± 0.04a	0.16 ± 0.04a	0 ± 0b	0.18 ± 0.02a
FAs (%)	0.44 ± 0.11a	0.47 ± 0.09a	0.5 ± 0.05a	0.46 ± 0.08a	0.46 ± 0.09a	0 ± 0b	0.44 ± 0.06a
HAs/FAs	0.31 ± 0.1a	0.46 ± 0.09a	0.19 ± 0.1a	0.38 ± 0.02a	0.36 ± 0.1a	0 ± 0a	0.41 ± 0.03a

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. EC: electrical conductivity; pF: water retention in soil at different pressures; AW: available plant water; TOC: total organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio. CH: carbohydrates content; POL: polyphenols content; THA: total humic acids; HA: humic acid content; FA: fulvic acid content; HA/FA: humification index. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019). Number 6 after the name of the technosols corresponds to the T6 campaign and number 18 to the T18 campaign.

Table 2.1.(continua) Values (mean and standard deviation, in parentheses) for each physical and chemical properties and fraction of humic acids content of technosols amended with compost from organics wastes, control soils and natural reference soils. Different lowercase letters indicate differences ($P < 0.05$) among the treatments in the same sampling date according to PERMANOVA pair-wise test comparisons.

Variables (T18)	**CG-18**	**CC-18**	**SS-18**	**Mix1-18**	**Mix2-18**	**CON-18**	**NAT-18**
pH	7.99 ± 0.09a	8.87 ± 0.16b	7.88 ± 0.06a	8.07 ± 0.09a	7.97 ± 0.08a	8.67 ± 0.06b	8.70 ± 0.01c
EC (mS/cm)	2.23 ± 0.43ab	1.75 ± 0.35b	3.24 ± 0.04a	2.75 ± 0.68ab	2.6 ± 0.42ab	1.64 ± 0.35b	0.08 ± 0.01c
pF -1500 Kpa	18.21 ± 2.13a	16.40 ± 1.09a	17.38 ± 0.51a	16.41 ± 1.13a	16.78 ± 0.69a	14.89 ± 0.99a	13.21 ± 0.84a
pF -33 KPa	35.78 ± 0.94a	36.45 ± 2.10a	36.02 ± 1.52a	34.65 ± 0.94a	43.92 ± 6.80a	33.40 ± 1.22a	33.76 ± 1.24a
AW (%)	17.56 ± 2.69a	20.05 ± 1.33a	18.64 ± 1.99a	18.24 ± 0.83a	27.13 ± 7.44a	18.50 ± 0.77a	20.54 ± 0.53a
TOC (%)	2.65 ± 0.03a	3.37 ± 0.17b	3.08 ± 0.20ab	2.46 ± 0.39abc	2.90 ± 0.21ab	0.81 ± 0.16d	1.81 ± 0.14c
TN (%)	0.38 ± 0.00a	0.40 ± 0.01a	0.60 ± 0.12b	0.42 ± 0.04ab	0.42 ± 0.05ab	0.06 ± 0.00c	0.15 ± 0.01d
C/N	7.51 ± 0.18a	8.92 ± 0.21c	6.30 ± 0.26b	6.25 ± 0.68ab	12.3 2± 5.13ab	16.1 0± 2.68abcd	13.03 ± 0.53d
CH (µg g^{-1} soil)	470.68 ± 128.48a	156.75 ± 160.36b	770.13 ± 160.36a	566.21 ± 248.98ab	338.76 ± 115.23ab	0 ± 0c	38.13 ± 4.76d
POL (µg g^{-1} soil)	20.06 ± 7.64ac	35.78 ± 8.39b	26.36 ± 5.79abc	24.43 ± 5.74ab	26.99 ± 4.71ab	0 ± 0c	6.19 ± 3.25d
THA (%)	0.78 ± 0.03a	0.75 ± 0.08a	0.48 ± 0.24a	0.69 ± 0.12a	0.62 ± 0.17a	0.1 ± 0.1a	0.43 ± 0.09a
HAs (%)	0.24 ± 0.13a	0.27 ± 0.04a	0.10 ± 0.05a	0.23 ± 0.07a	0.14 ± 0.05a	0 ± 0a	0.12 ± 0.03a
FAs (%)	0.53 ± 0.11a	0.48 ± 0.12a	0.39 ± 0.19a	0.46 ± 0.05a	0.49 ± 0.12a	0.1 ± 0.1a	0.32 ± 0.06a
HAs/FAs	0.66 ± 0.48a	0.74 ± 0.36a	0.17 ± 0.10a	0.48 ± 0.14a	0.25 ± 0.06a	0 ± 0a	0.36 ± 0.05a

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. EC: electrical conductivity; pF: water retention in soil at different pressures; AW: available plant water; TOC: total organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio. CH: carbohydrates content; POL: polyphenols content; THA: total humic acids; HA: humic acid content; FA: fulvic acid content; HA/FA: humification index. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019). Number 6 after the name of the technosols corresponds to the T6 campaign and number 18 to the T18 campaign.

As expected, the application of organic amendments clearly increased SOM contents in technosols that were maintained during the experiment, which could initially indicate an improvement in soil quality and functionality in technosols in the short term. Higher significant values of TOC and TN content than CON and NAT soils were found for both field sampling periods (T6 and T18). SS, Mix1, Mix2 and CC showed the highest significant ($P<0.05$) TOC and TN results, followed by CG, while for T18 CG showed similar values than the rest of amendments. Probably, there was no significant decrease in TOC and TN due to the continuous input of OM that the soils had from both planted and colonizing annual vegetation (Soria et al., 2021a) and by root exudates (Bastida et al., 2006). Other authors such as Carabassa et al. (2018) have also considered that this underestimation of organic carbon could be attributed to biomass inputs from both vegetation. Likewise, CH and POL content were significant higher in the restored soils (Table 2.1). SS, Mix1 and Mix2 gave rise to the highest values and CC the lowest, followed the same pattern to both sampling campaign, indicating a greater amount of labile OM (Rodríguez-Berbel et al., 2021a). Interestingly, CG showed no change in CH content over time. Nevertheless, CH content decreased notably in the second sampling (T18) respect the first campaign (T6), especially in SS, Mix1 and Mix2 that showed a decrease about 67 % to SS of the initial content and approximately a 55 % for mixtures (Table 2.1). This suggests a depletion of labile forms of carbon, which is in contrast to the behavior of labile OM in CG and CC, whose contents were lower. On the contrary, NAT showed no change in CH content and were non-existent in CON. This is important, considering that the soils of arid and semi-arid zones are poor in OM but with high resistance to biodegradation and high stability (Miralles et al., 2015) and also OM losses aggravated by degradation caused by mining as observed in CON soils where there were no traces of labile forms of OM (Table 2.1). It should be noted that a slight TOC content was quantified in CON, which could be due to the high active lime in the original soils (Soria et al., 2021a) or to the organic amendment carried by the wind to the other experimental plots. In lesser proportion, a detriment of POL was also observed in all restored soils between T6 and T18, however CG content decreased by half, CC had the largest decrease in POL content, while SS and its mixtures had smaller changes.

Technosols restored with organic amendments showed an increase of EC and pH values significantly lower than unamended technosols (CON) and natural reference soils (NAT). Results corresponding to EC data showed a more notable reduction in CC and mixtures (Table 2.1) in T18 respect to T6 that could be attributed to absorption by colonizing halophytic vegetation described in Soria et al. (2021a) or rain washing (Ortega et al., 2020; Ros et al., 2003). SS maintained high EC levels for T18 probably due to the domestic origin of wastes (Domene and Saurí, 2007). The organic amendments also favoured water retention (pF) and water available for plants (Table 2.1). Zancada et al., (2004) reported an improvement in the water retention capacity of soils after organic amendment application that could be related with a improve a soil structure, stable aggregates and soil porosity related with the increased of TOC (Miralles et al., 2009).

2.3.1.1. Effect on the addition of organic amendments on the abundance of soil humic fractions

In general, the application of organic amendments was a source of humic substances to soil, such as has been reported by other authors (Albiach et al., 2001; Rodríguez-Vila et al., 2016). The total humic acid content (THA) was similar in restored technosols than natural reference soils (NAT) in both field samplings (T6 and T18) (Table 2.1). However, the un-amended technosols (CON) did not show the presence of humic substances with exception of a residual FA amount at T18 (Table 2.1). The content of FAs were higher than HA in all technosols and NAT soils (Table 2.1), which indicated a low humification rate (Guimarães et al., 2013) in both T6 and T18 field samplings. Although no significant differences were found between organic matter fractions to the different amended technosols (Table 2.1), CG and CC technosols showed a slight increase in HA and FA from T6 to T18, even comparatively higher than NAT in T18 (Table 2.1). It is well known that plant residues are mainly formed of substances difficult to degradation, such as lignin or cellulose, with high resistance to biodegradation (Hayes and Swift, 2020). However, some authors consider them as a dominant source of soil humic substances that contributing to improve the structure and composition of OM (Yang et al., 2019). Therefore, plant residue amendments could be favouring humification in CG and CC technosols, indicating a trend towards stabilization of SOM that has been confirmed by other studies of quarry restored technosols (Ojeda et al., 2015). On the contrary, SS not only showed a lower content of humic substances but also losses of FA from T6 at T18, and presented the lower humification degree compared to the rest of technosols (Table 2.1). The latter suggests that SOM of SS contained greater non-humic substances and materials with lower decomposition resistance than vegetable residues amended technosols (CG and CC) (Asensio et al., 2014; Hayes and Swift, 2020). The addition of the mixtures (Mix1 and Mix2) showed an intermediate degree of humification between SS and vegetable compost-amended technosols (CG and CC) probably due to the OM provided by both types of amendments, one with more recalcitrant compounds and the other more labile. Although increases in humic acids following the application of composted organic amendments have been widely reported in the literature, comparisons of the performance of different residues used are always difficult due to the different characteristics and application rates of the organic amendments (Albiach et al., 2001), especially in the initial stages after their application in restoration processes.

2.3.2. Effect of organic amendments on soil thermal properties

The results of the thermal analyses are shown in Table 2.2 and in Figure 2.1. The addition of the organic amendments increased the total weight loss for all the cases in comparison with the soils from the control plots (28.1–29.4 % Vs. 26.5–26.9 %). This increase corresponds to a greater relative and absolute abundance of very labile OM, intermediate OM and recalcitrant OM (W1, W2 and W3 respectively), at expenses of the reduction in the abundance of mineral-dominated fraction (W4). It is remarkable that the presence of the intermediate and recalcitrant pools of soil OM were considerably multiplied by a factor of 3 to 6 times as a result of the addition of the amendments. Regarding the changes observed between the first and second sampling (T6 and T18) for the amended technosols, the abundance of the most labile and intermediate fraction (W1-W2) were reduced, with the exception of the CG plots, whereas the relative abundance of the most stable fractions increased when comparing similar treatments. Commonly, the low-temperature part of the thermograms (W1) has been linked to the burning of carbohydrates and other aliphatic compounds as simple lipids and amino acids, and consequently associated with the most easily-degradable fraction of the soil OM. Meanwhile, the high-temperature parts (W3 and W4) have been generally attributed to reaction of aromatic compounds or other polyphenols, distinctive of a more humified and stable fraction of the OM (De la Rosa et al., 2008; Fernández et al., 2012).

The DSC curves show some differences in the biomass that forms CG and CC amendments. CG is dominated by biomass decomposed over 360 ºC followed by a exothermic shoulder at 530 ºC, which could be typically attributed to cellulose and lignin respectively. The absence of those signals at the DSC of CON samples indicate the lack of plant remains in those plots. The shift of the exothermic signal from 360–370 to 385 ºC for the NAT samples suggests a greater thermal stability of the native soil OM conforming the remaining cellulose than of the used amendments. However, the signal corresponding to highly recalcitrant OM is negligible, which would indicate in general a rapid and complete removal of humified OM in native soils. The greatest weight loss corresponded in all cases to the mineral fraction (included in W4), especially for NAT and CON samples (raging between 86 and 90 % of the total loss). The DSC endothermic peak present over 740–750 ºC would indicate the abundance of dolomites.

Table 2.2. Comparative Comparative thermogravimetry (TG) and differential scanning calorimetry (DSC) parameters in samples summarizing: Total weight loss for the temperature interval 50¬–850 ºC (% ± 1%), weight losses and relative weight losses for the temperature intervals, 50–200 ºC, 200–400 ºC, 400–600 ºC, 600–850 ºC (Exotot), and temperature of the exothermic peaks. Continue on next page.

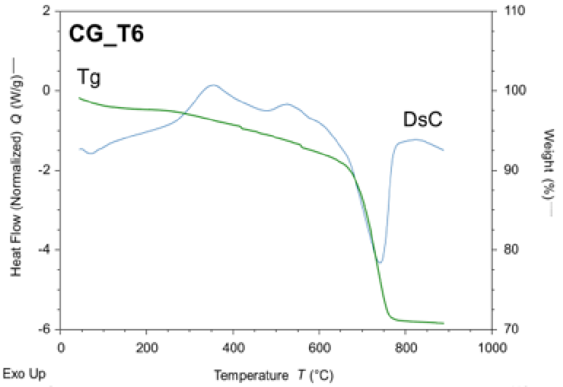
		CG_6	**CG_18**	**CC_6**	**CC_18**	**SS_6**	**SS_18**	**Mix1_6**	**Mix1_18**	**Mix2_6**	**Mix2_18**	**CON_6**	**CON_18**	**NAT_6**	**NAT_18**
Total Weight Loss (%)	**50-850 ºC**	28.1	29.4	29.6	29.8	29.8	29.0	29.1	28.5	29.4	29.2	26.5	26.9	34.9	34.4
Moisture and very labile OM-W1	**50-200 ºC**	1.3	1.5	1.8	1.5	1.2	1.0	1.5	1.4	1.3	1.1	0.8	0.8	0.8	0.8
Int OM-W2	**200-400 ºC**	1.9	2.7	3.3	2.6	2.8	2.4	2.5	2.4	2.7	2.2	0.4	0.4	1.6	1.5
Recalcitrant OM-W3	**400-600 ºC**	3.5	3.9	3.3	3.6	5.4	4.2	3.1	3.1	4.1	3.9	1.7	1.6	2.4	2.0
Minerals + stable OM-W4	**600-850 ºC**	21.3	21.4	21.2	22.1	20.4	21.4	22.0	21.6	21.3	22.0	23.6	24.1	30.1	30.1
Relative Weight Loss (%)															
Moisture and very labile OM-W1	**50-200 ºC**	4.7	5.2	6.2	5.0	4.0	3.3	5.1	4.8	4.3	3.9	3.2	2.8	2.2	2.4
Int OM-W2	**200-400 ºC**	6.8	9.0	11.0	8.9	9.5	8.2	8.7	8.3	9.1	7.5	1.7	1.4	4.7	4.4
Recalcitrant OM-W3	**400-600 ºC**	12.5	13.1	11.3	12.0	18.2	14.6	10.7	10.8	14.0	13.3	6.3	6.0	6.8	5.7
Stable OM+Minerals-W4	**600-850 ºC**	76.0	72.6	71.6	74.2	68.4	73.9	75.5	76.0	72.5	75.4	88.8	89.7	86.4	87.4

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019). Number 6 after the name of the technosols corresponds to the T6 campaign and number 18 to the T18 campaign.

Table 2.2. (Continuation). Comparative thermogravimetry (TG) and differential scanning calorimetry (DSC) parameters in samples summarizing: Total weight loss for the temperature interval 50¬–850 ºC (% ± 1%), weight losses and relative weight losses for the temperature intervals, 50–200 ºC, 200–400 ºC, 400–600 ºC, 600–850 ºC (Exotot), and temperature of the exothermic peaks.

		CG_6	**CG_18**	**CC_6**	**CC_18**	**SS_6**	**SS_18**	**Mix1_6**	**Mix1_18**	**Mix2_6**	**Mix2_18**	**CON_6**	**CON_18**	**NAT_6**	**NAT_18**
Total Weight Loss (%)	**50-850 ºC**	28.1	29.4	29.6	29.8	29.8	29.0	29.1	28.5	29.4	29.2	26.5	26.9	34.9	34.4
DSC															
Main Exo peak (ºC)		358	365	549	547	548	535	553	537	530	535	n.d.	n.d.	385	385
2nd Exo peak (ºC)		527	536	375 (sh)	375 (sh)	366	364	370	371	361	364				
Q released (Exo)	**[J g^{-1}]**	1920	2211	1892	1799	2669	2283	1706	1756	1851	1917			1935	1869
Q'	**[J g^{-1} OM]**	99	100	85	86	97	100	88	92	80	93			169	185
Endothermic peaks (ºC)		742	738	742	749	749	748	743	753	734	755	751	746	755	770
Q endothermic peak	**[J g^{-1}]**	406	383	365	416	316	412	475	405	464	574	607	640	664	505
Q'		5.3	5.3	5.1	5.6	4.6	5.6	6.3	5.3	6.4	7.6	6.8	7.1	7.7	5.8

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019). Number 6 after the name of the technosols corresponds to the T6 campaign and number 18 to the T18 campaign.


CG_T6
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
2
0
-2
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-6
110
100
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80
70
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400
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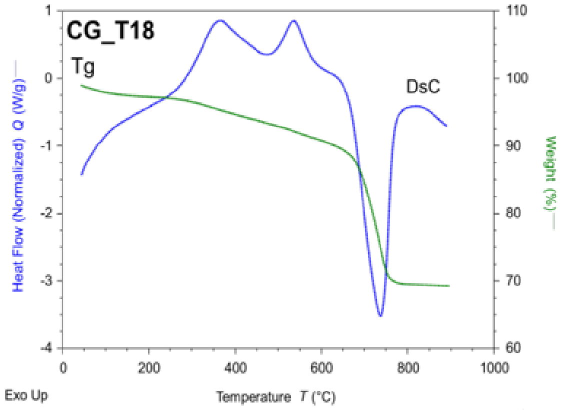
CG_T18
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
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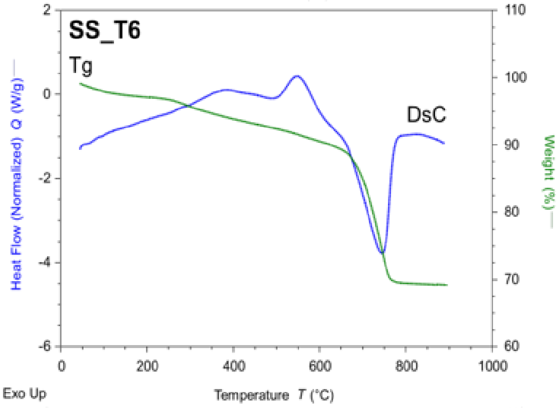
SS_T6
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
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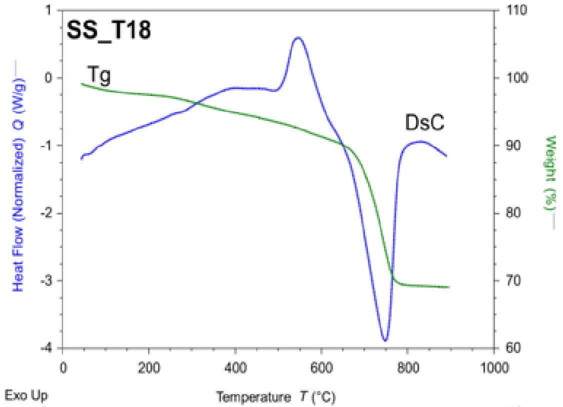
SS_T18
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
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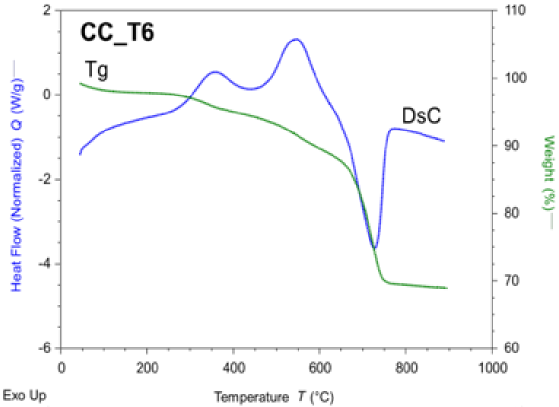
CC_T6
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
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-2
-4
-6
110
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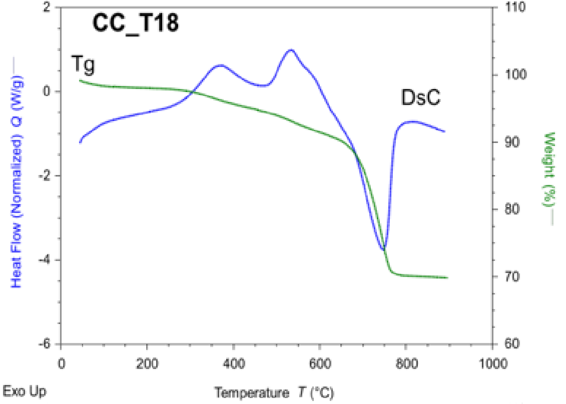
CC_T18
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
2
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-2
-4
-6
110
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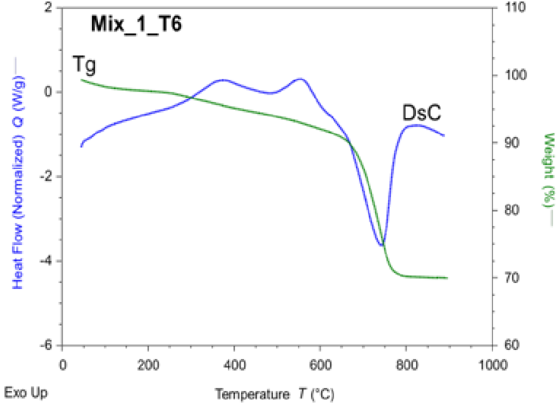
Mix_1_T6
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
2
0
-2
-4
-6
110
100
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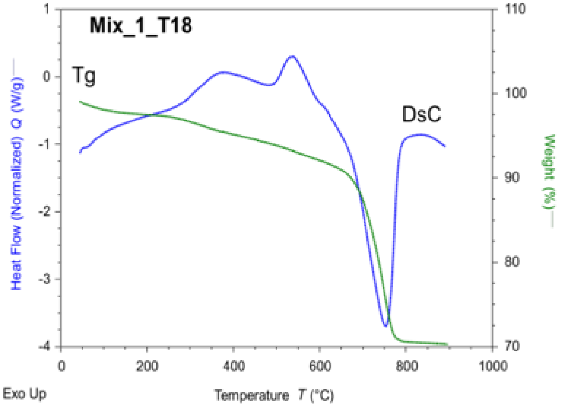
Mix_1_T18
Tg
DsC
Heat Flow (Normalized) Q (W/g)
Weight (%)
Temperature T (°C)
Exo Up
1
0
-1
-2
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110
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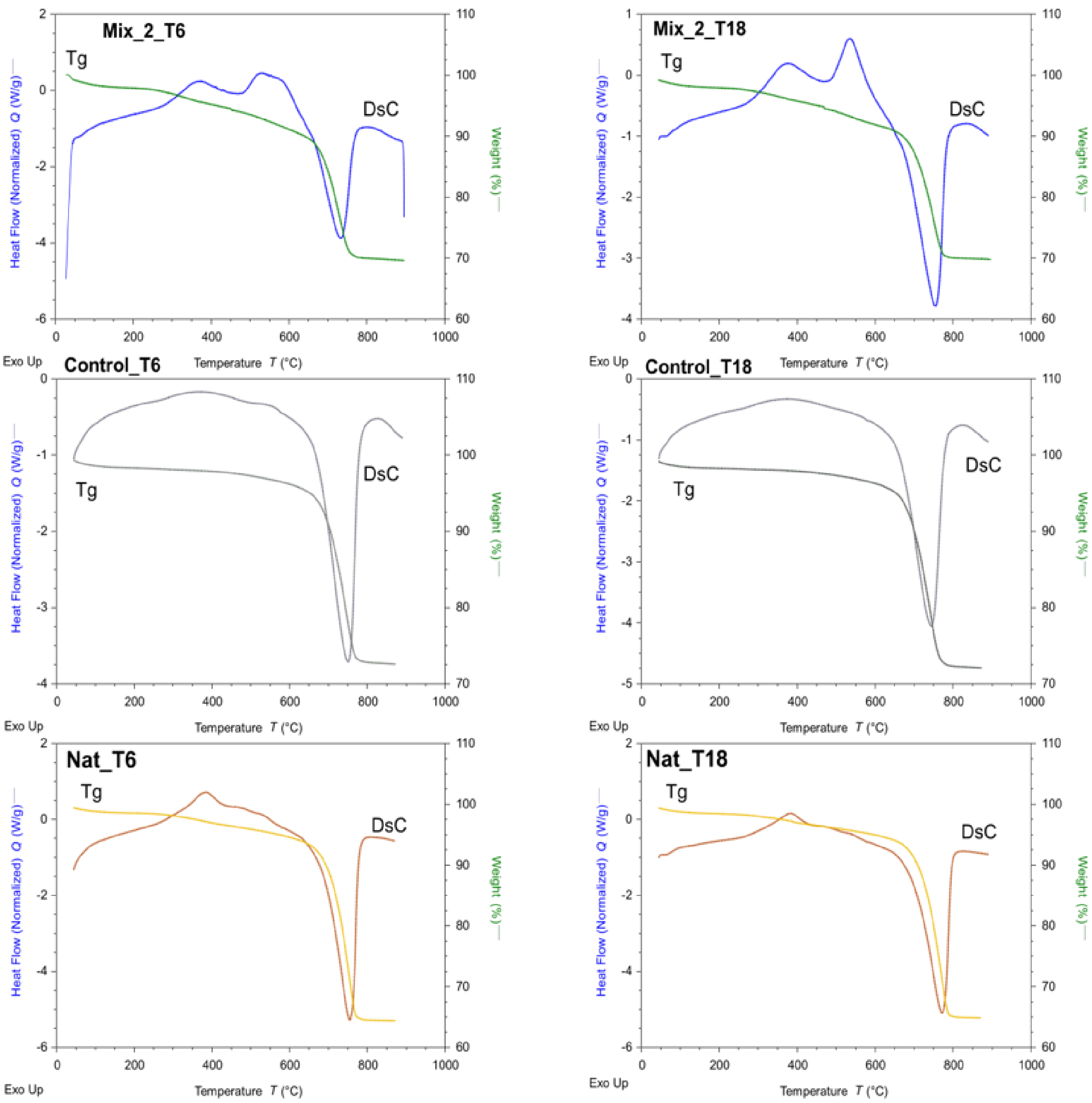


Figure 2.1. Thermograms and Differential scanning Calorimetry for the temperature interval 50-850 ºC of soil samples taken 6 months (T6) and 18 months (T18) after the amendment. Exothermic peaks are presented upward. CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019).

2.3.3 Effects on soil respiration rates

Soil respiration (SR) measured in the field showed higher rates for all technosols amended and natural reference soils (NAT) in the first campaign 6 months after application (T6), while in the second campaign measured 18 months later (T18) it presented a significant reduction resembling CON and NAT soils (Figure 2.2). Un-amended soils (CON) showed significantly lower SR rate in both measurement campaigns (Figure 2.2) due to its lack of OM. Soil temperature and humidity were similar at both times (Figure 2.2), as expected taking into account that both field measurements were carried out under similar environmental conditions, without rainfall events in the previous 5 days, and similar average daily temperatures (11.1 °C for T6 and 12.2 °C for T18; data from RAIFALL003, Junta de Andalucía). Therefore, the different results for SR measurements could be attributed to different chemical composition of technosols and depending on the type of organic amendment applied (Ray et al., 2020). For the first campaign (T6), SS presented the higher significant ($P < 0.05$) SR, followed by Mix1, Mix2 and CC that presented similar values than NAT, while CG had the lowest significant values. The high SR rates could be due to the high initial CH content observed in T6, especially in SS (Table 2.1), which presented to the high mineralization rates, as well as a priming effect that has previously been discussed in Soria et al., (2021a). This initial SOM mineralization and decomposition could be caused by stimulating microbial activity where there were dormant microbial communities that could have responded with a rapid growth of their populations in response to exogenous of labile C input (Kuzyakov et al., 2009), or by microorganisms provided by organic amendments (García et al., 2017; Rodríguez-Berbel et al., 2021a), that would have consumed the most labile fractions in T6. This decreased of microbial activity "hot spot" (Kuzyakov, 2010) in T18 could suggested a possible MOS stabilization and an increased presence of recalcitrant fractions after depletion of easily decomposable OM (Bastida et al., 2013b). Although SS had a TOC content similar to CC, Mix1 and Mix2, the high CH content could have favored mineralization and slowed down humification processes since it presented a comparatively lower HA/FA ratio than the others (Table 2.1). On the contrary, the low SR rates in CG could be due to the presence of a more recalcitrant OM, less available for the soil microbiota. However, in the second measurement campaign (T18), the RS rate was low and similar to that of NAT, probably due to the depletion of the more labile fractions. Interestingly, NAT showed a high SR rate in T6, probably caused by a contribution of root exudates or plant biomass provided by the abundant natural plants.

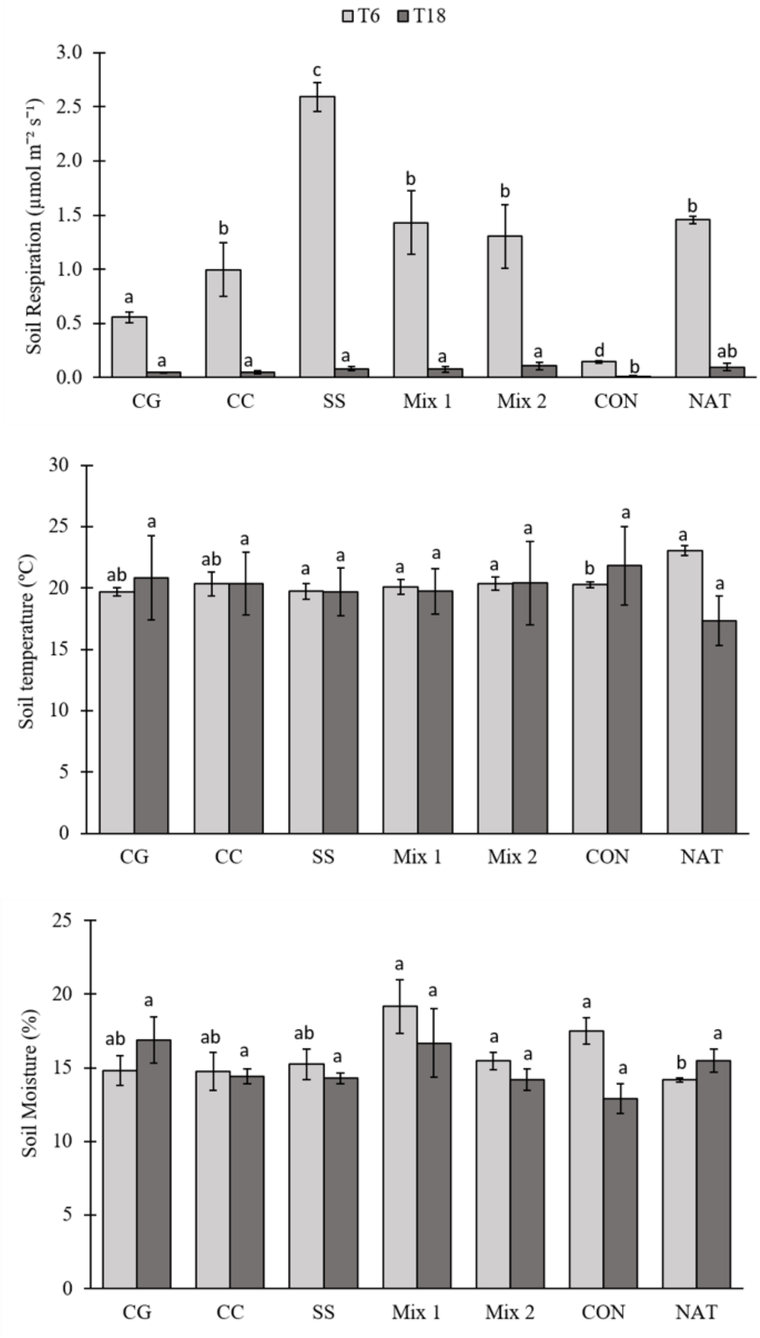


Figure 2.2. Soil respiration rate, soil moisture and soil temperature to different amended technosols, control soil and natural reference soil taken 6 months (T6) and 18 months (T18) field measurement. CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019). Different letters in this panel indicate significant differences between treatments (*P < 0.05*) [PERMANOVA] in the same date.

2.3.4. Effect of organic amendments addition on the molecular composition of soil organic constituents by analytical pyrolysis

The labelled pyrograms obtained from the soil samples in the two sampling periods are depicted in Figure 2.3. The relative distribution of the main groups of compounds identified for each sample are shown in Figure 2.4. The pyrolysis performed directly on the control degraded soils (CON) did not produced any appreciable pyrolysis compound, probably due to the extremely low OC content. However, by pyrolysis the natural soil of the area (NAT) produced a complex and varied molecular assemblage with 61 compounds identified and a clear lignocellulose signature that include guaiacyl (G) units (5.3 %) and polysaccharide (PS) derived compounds (25.2 %), mainly furan derivatives that may came from the cellulose but also from the microbial activity. A well resolved alkyl series from C14 to C30 (ALK; 9.9 %) derived mainly from epicuticular plant waxes (Eglinton et al., 1962) and nitrogen compounds (N; 23.7 %) from peptides and proteins were also found. A conspicuous high proportion of aromatic compounds with unknown origin (ARO; 35.1 %) are also part of the NAT pyrolysate. This, together with the relative low content of lignin methoxyphenols and of SOM in general, may point to accelerated humification processes with active ligninolytic activities yielding a wide variety of aromatic and polycyclic aromatic compounds (phenols, benzenes, indenes and naphthalenes) (Ceccanti et al., 2007). This NAT SOM structure remained mostly unchanged during the two sampling periods (Figure 2.3).

The technosols amended with vegetable compost from green house (CC) showed a pyrolysate with a remarkable high N compounds (26.0 %) and lignocellulose with compounds derived from lignin guaiacyl (G) and syringyl (S) structures (19.8 %) and cellulose (PS) derived compounds (c. 8.9 %), including anhydrosugars, furan and cyclopentane derivatives. In addition, high content of chlorophyll derived ISO were found (15.1 %) whereas the amount of aromatic compounds was intermediate (ARO: 14.2 %) and less than a half that in the NAT soil (Figure 2.4f). The substrate amended with the green garden waste (CG) is characterized by a high relative content of ARO (47.1 %) and N (20.4 %) compounds (Figure 2.4a). The pyrolizate produced by the technosol with sewage sludge waste (SS) produced high amounts of N (30.6 %) and ARO (29.2 %) compounds, but also included high relative content of alkyl material (ALK: 15.8 %), esterols (EST: 12.0 %) including faecal sterols (coprostanols) and fatty acids (FA: 1.1 %), reflecting the origin of the amendment (Kruge et al., 2010) (Fig. 4c).The pyrolysis of the technosols amended with mixtures of SS and CG (MIX 1) and CC (MIX2) showed the main chemical features of the biomass added. Both mixtures showed EST and FA as markers from the SS waste and the general plant biomass imprint that included lignin methoxyphenols (G and S), PS, ALK and ISO (Figure 2.4d and 2.4e). After amending the CON, unrestored, soil with the three materials and mixtures, the OM structure of the resulting technosols resembled that from the NAT soil. The general chemical structure is preserved in the timeframe of this experiment with no appreciable major changes seen by analytical pyrolysis.

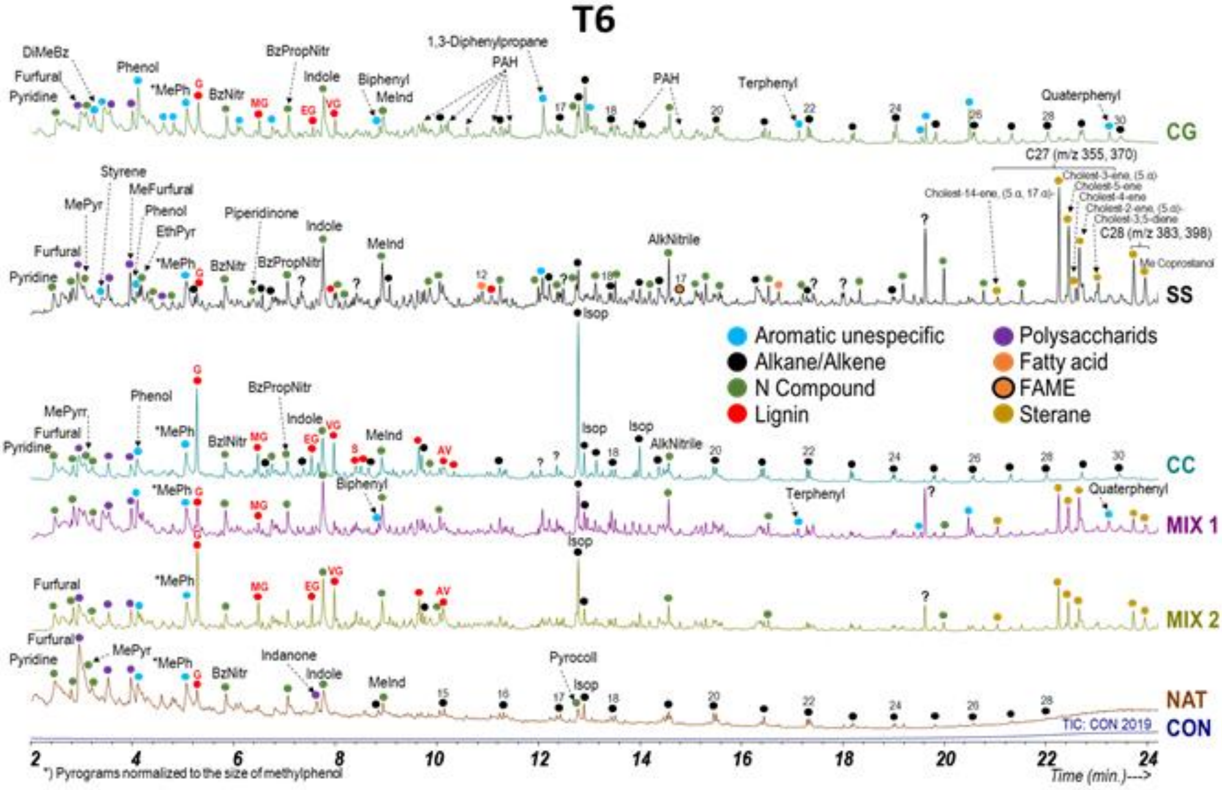


Figure 2.3. Pyrograms obtained at 400 ºC on whole soils labelled with the main compounds identified. For better readibility and comparison, the pyrograms are normalized to the high of the methylphenol peak. G: guaiacol; MG: methylguaiacol; EG; ethylguaiacol; VG: vinylguaiacol; S: Syringol; MS: methylsyringol; ES: ethylsyringol; AV: acetovanillone. Numbers on the peak indicate carbon number in the alkyl chain of the molecule. CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019).

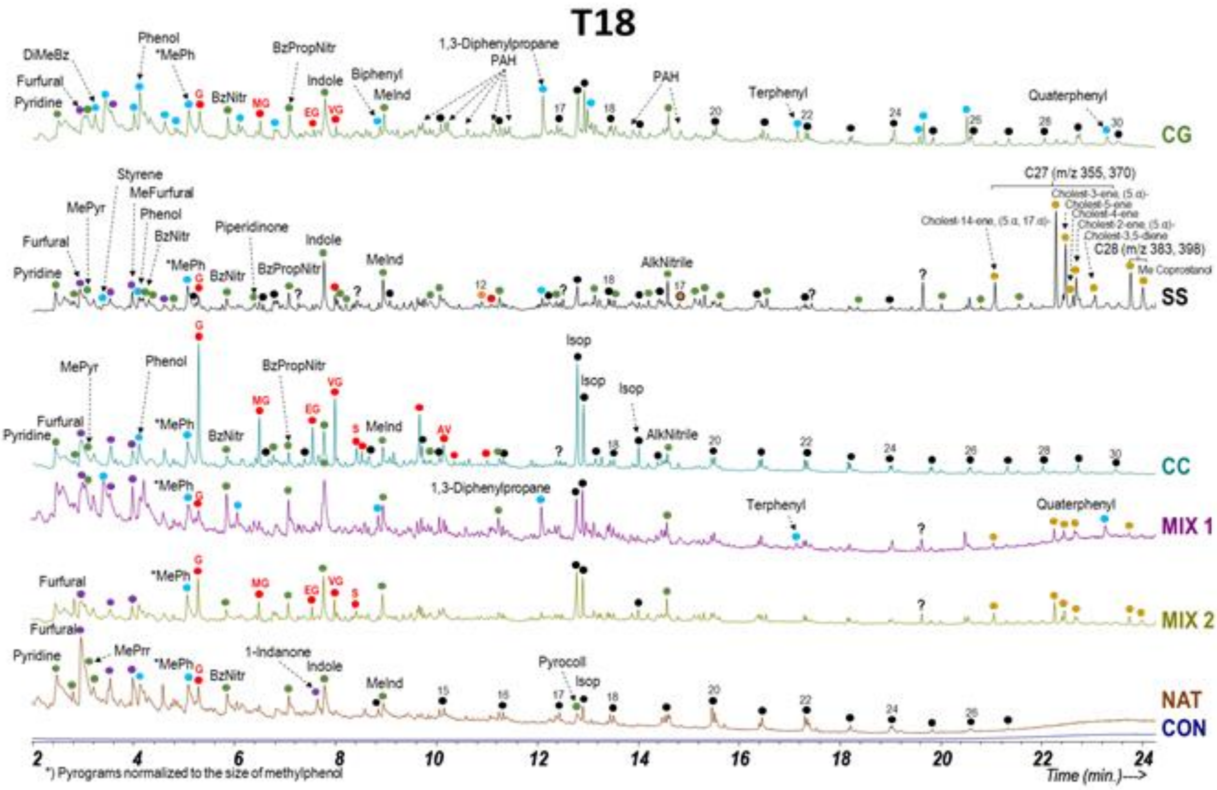


Figure 2.4. (Continuation). Pyrograms obtained at 400 ºC on whole soils labelled with the main compounds identified. For better readibility and comparison, the pyrograms are normalized to the high of the methylphenol peak. G: guaiacol; MG: methylguaiacol; EG; ethylguaiacol; VG: vinylguaiacol; S: Syringol; MS: methylsyringol; ES: ethylsyringol; AV: acetovanillone. Numbers on the peak indicate carbon number in the alkyl chain of the molecule. CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019).

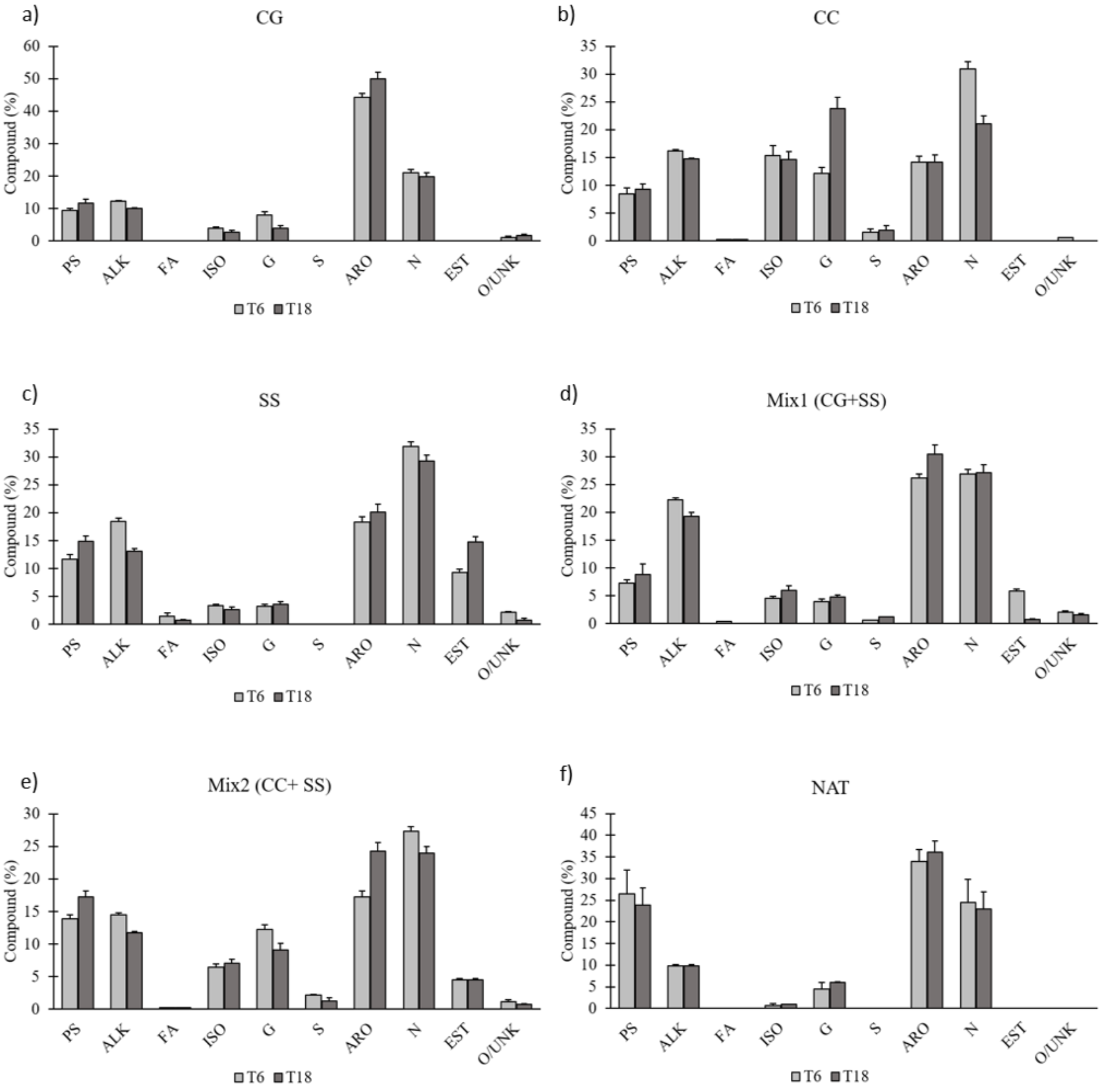


Figure 2.5. Relative distribution of the main groups of compounds identified by analytical pyrolysis performed at 400 ºC on whole soils. PS: polysaccharides; ALK: alkane alkene pairs; FA: fatty acids and FA methyl esters; ISO: isoprenoids; G: lignin guaiacyl units; S: lignin syringyl units; ARO: aromatic compounds; N: nitrogen compounds; EST: esteranes; O/UNK: other or unknown compounds. Error bars represent STD from the mean for each compound group. CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. T6: soil sampling December-2018 (date 17-12-2018); T18: soil sampling November-2019 (date 25-11-2019).

2.4. Conclusions

The application of recycled organic waste composts as amendment on degraded soils from quarrying in a semi-arid climate to form technosols rapidly improved the physical, chemical and microbiological properties and increased SOM levels in the formed technosols. All technosols amended resulted in improved soil functionality and soil quality in the short term compared to un-amended plots. Nevertheless, the SOM composition from vegetable compost amended plots was the most similar to non-degraded natural plots in only 6 months after the amendment. The studied parameters indicated a rapid recovery of microbiological activity and SOM diversity, which were maintained for at least 18 months and therefore this practice could be a valid solution to accelerate the processes of restoration and recovery of quarry mine soils in semi-arid climates, as well as improve their resilience.

Technosols amended with compost from plant residues showed a more recalcitrant SOM, lower soil respiration rates and a higher degree of humification than the sludge-amended technosols, indicating that the nutrient reserve and fertility of the restored soils could be guaranteed in the longer term with plant residues compost. In contrast, the sludge-amended technosols produced a higher respiration rate in the study at 6 months after application associated with their high labile OM content and a rapid initial consumption of nutrients. However, the soil respiration rates sharply decreased 18 months after the application of the composts, which suggested that despite the easily decomposable OM is consumed the presence of recalcitrant OM is maintained, being a long-term reserve of OC and N. In addition, sludge technosols showed the lowest degree of humification in both periods. The mixtures showed intermediate properties of both types of amendments (sludge and vegetal composts), but they showed the highest decomposition of the more labile fractions at the beginning of the experiment. The labile fractions present at the sludge composts would have favored microbiological activity at the short term, but the mixtures maintained intermediate OM contents 18 months later because of the presence of more resilient compounds from the plant composts. These findings were confirmed by thermogavimetric and analytical pyrolysis studies. These analyses showed that 6 months after the amendment the restored technosols with plant compost were composed by a combination of labile and recalcitrant OM similar to natural (undisturbed) soils, which was maintained one year later. In conclusion, the application of vegetable compost from greenhouse crop residues and garden pruning waste were the most suitable for restoring the functionality of degraded soils from quarrying in a semi-arid climate, similar to undisturbed soils (native) by mining activity. Therefore, these findings are still preliminary and further studies are needed to confirm long-term developments.

Chapter 3

Soil amendments from recycled waste differently affect CO_2 soil emissions in restored mining soils under semiarid conditions.

Soria, R., *Rodríguez-Berbel, N., Ortega, R., Lucas-Borja, M.E., Miralles, I., 2021. Soil amendments from recycled waste differently affect CO_2 soil emissions in restored mining soils under semiarid conditions. J. Environ. Manage. 294, 112894. https://doi.org/10.1016/j.jenvman.2021.112894*

Drylands affected by serious disturbances such as mining activities lose their vegetation cover and organic soil horizons, becoming CO_2 *emissions sources. Applications of organic amendments could be a good restoration solution that favours vegetation establishment and soil carbon sequestration; however, they are also associated with* CO_2 *emissions. Experimental plots with different organic amendments (sewage sludge, garden and greenhouse vegetable composts, and mixtures of both) and unamended soils were installed in a quarry in southeast Spain. The aim of this study was: i) to evaluate the magnitude and changes of in situ* CO_2 *emission from each experimental plot during a year and a half, and ii) to assess the effects of several physical–chemical (total organic carbon, total nitrogen, water retention, pH and electrical conductivity) and environmental parameters (moisture and temperature) in* CO_2 *emissions. The results showed an initial* CO_2 *emission (priming effect), produced from all restored plots just after the application of the organic amendment, which was significantly higher (*$P < 0.05$*) in soils with sewage sludge and their mixtures in comparison to vegetable compost. Garden compost had low emission rates, similar to soils without amendment and showed lower* CO_2 *emission rates than the rest of the restoration treatments. Nevertheless,* CO_2 *emissions decreased in each field campaign over time, showing that all restored soils had lower emissions than natural soils at the end of the sampled period. The different composition of organic amendments had a different effect on soil* CO_2 *emissions. DistLM analysis showed that soil properties such as total organic carbon, total nitrogen, pH and soil moisture, associated with rainfall periods, strongly influenced* CO_2 *emissions, whereas temperature did not affect the* CO_2 *flow. In conclusion, the compost from plant remains could serve better as treatment to restore degraded soils in drylands than sewage sludge because of its lower* CO_2 *emissions and concomitant effect on climate warming and carbon balance.*

3.1. Introduction

Arid and semiarid areas are characterized by extreme climatic conditions, with high temperatures, low rainfall, high evaporation rates and strong winds (Luna, et al., 2016a). Currently, they occupy 45.36% of the Earth's surface (Lal, 2019) and are estimated as storing 32% of the earth's carbon (Dacal et al., 2020). Drylands are the most severely threatened ecosystems on the planet because of their increasing aridity (Berdugo et al., 2020; Lal, 2019). Drivers of global change such as human activities (such as land use change) and climate change (Huang et al., 2016; Schlesinger et al., 1990) can accelerate the expansion of arid and semiarid areas (Huang et al., 2016; Yao et al., 2020) and increase desertification processes (Hueso-González et al., 2018; Lavee et al., 1998; Reynolds et al., 2007).

Mining is a widespread activity in arid and semiarid areas because of its economic and social benefits (Gratzfeld, 2003), but it has serious consequences for soil degradation (Moreno-de las Heras, 2009). Soil processes are closely linked to temperature and precipitation patterns, which exert strong control over vegetation productivity and composition (Booker et al., 2013; Gravuer et al., 2019), making it difficult to restore ecosystems degraded by mining in arid and semiarid environments (Josa et al., 2012; Rodríguez-Berbel et al., 2021a). Despite difficulties in achieving success in restoration, it is important to choose an appropriate strategy to restore these fragile degraded environments to recover their functionality, improve, such as their capacity for carbon sequestration (Lal, 2009; Lal, 2015) and reduce CO_2 emissions. Soil management practices can influence the carbon cycle by affecting the soil's CO_2 emissions (Ray et al., 2020).

In this context, the incorporation of organic waste has shown improvements in physical, chemical and biological soil properties (Abdelhafez et al., 2018; Breton et al., 2016; Luna et al., 2016b; Ros et al., 2003) and consequently improves the soil functionality (Mondini et al., 2018; Soria et al., 2021a). Organic amendments derived from different types of waste can improve degraded soils, and their correct selection could provide a good opportunity to successfully restore mined areas (Hernández et al., 2016; Jordán et al., 2008; Ros et al., 2003). In addition, soil loss causes a depletion of organic carbon that negatively affects the nutrient cycle and soil biological activity, whereas increasing the carbon input of biomass through the addition of organic amendments can increase soil organic carbon sequestration and nutrient cycling (Ghimire and Khanal, 2020). All in all, soil amendment is considered a good strategy for recovering soil functions related to carbon sequestration (Coyne et al., 1998; Diacono and Montemurro, 2011; Lal, 2019; Montiel-Rozas et al., 2016; Paustian et al., 2007, 2000; Tits et al., 2014; Vinson et al., 1999). Moreover organic amendments also favours development of a stable plant cover that contributes to the fixation of CO_2 through photosynthesis (Shrestha and Lal, 2006) and soil microorganisms (Rossi et al., 2015) with consequent positive feedback on global change.

Several authors have researched the effect of organic amendments in soil CO_2 emission rates (Chen et al., 2015; Li et al., 2013; Ray et al., 2020; Rochette and Gregorich, 2011). Despite all the benefits that organic amendments contribute to soil restoration, the variability of organic amendment types resulting from the different origins of organic waste, the variability of their organic matter composition, which can generate different organic matter decomposition rates (Hueso-González et al., 2018) and their long-term maintenance (Larney and Angers, 2012), makes it difficult to select the type of amendment. Thus, Albiach et al. (2000) reported that organic amendments of different plant and sheep compositions, anaerobic digestion sludge, and vermicompost significantly increased CO_2 emissions with respect to commercial amendments. Li et al. (2013), noted the impact on CO_2 emissions was greater in applying organic amendments derived from pig manure than in residue amendments from corn cultivation in Molisols in North-East China. Chaker et al. (2019) observed that in arid soils the application of amendments derived from olive pruning and palm leaves did not impact soil respiration, whereas amendments from oil mill waste water and fermented sheep manure increased the emission rate to 7.6 g CO_2 m^{-2}. Quemada and Menacho (2001) observed an increase in CO_2 fluxes with application of sewage sludge versus unamended soils one year after implementation.

Because arid and semiarid ecosystems cover a significant area of the planet and are expected to increase in the future, the projected increase in aridity as a result of climate change therefore makes restoring these fragile ecosystems a major challenge and selecting appropriate methodologies that are both successful and an adequate response to CO_2 emissions and climate change mitigation is of crucial importance. Therefore, improving our understanding of the use of organic amendments for the ecological restoration of mining areas, as well as understanding their impact on CO_2 emissions and in turn knowing the environmental and soil factors influencing CO_2 emissions from restored soils, is of crucial importance for selecting appropriate soil management techniques. This research's main objective was to evaluate the impact of different types of amendments on the CO_2 emission of fully degraded semiarid soils in a limestone quarry restored with organic amendments from local organic waste over a year and a half from their application, comparing these emission rates with degraded soils without organic amendments from the quarry and surrounding natural soils. The influence of environmental factors (soil temperature, soil moisture and precipitation) and physical and chemical soil properties on the CO_2 emission rates from different types of soils (restored, degraded and natural) in the medium term was also analysed. We hypothesised that organic amendments would increase CO_2 emissions, but these rates would differ depending on the chemical composition of the amendments applied to soils. Moreover, environmental factors and physical and chemical soil properties would also influence the magnitude and trends of CO_2 emissions from restored soils.

3.2. Material and methods

3.2.1. Study site

The study zone was located in completely degraded soils from a limestone quarry located in the Gádor mountain-range in Almería (SE, Spain) (N 36° 55′ 18″, 02° 30′ 40″ W). The experimental area was in a flat completely exploited site, at an elevation of 362 m.a.s.l. The initial substrate was formed by a mixture of fragments of calcareous rock and loams derived from mining activity. In adjacent, unexploited locations, shallow soils are found over limestones and dolomites with calcareous sandstones and marly and loamy marls forming Regosols (IUSS Working Group WRB, 2014). Irregular temperatures and rainfall are characteristic of the dominant semiarid Mediterranean climate, where the average annual rainfall is 242 mm in the autumn and winter seasons. Summers are hot and dry with maximum temperatures recorded in August of 31ºC and minimum temperatures of approximately 8ºC in January and high rates of evapotranspiration that reach 1225 mm year^{-1} (data recorded at the weather station from Alhama of Almería, a city near the study area, as described in Luna et al. (2018). Native vegetation corresponds to *Macrochloa tenacissima* (L.) Kunth *(=Stipa tenacissima* L.*)* as main species, accompanied by small shrubs such as *Ulex parviflorus* Pourr. and *Anthyllis cytisoides* L., as well as dispersed individuals of *Maytenus senegalensis* (Lam.) Exell., *Pistacia lentiscus* L. and *Rhamnus lycioides* L. (Luna et al., 2016b). Also, the presence of other species such as *Olea europaea* L., *Genista umbellata* (L'Hér.) Poir. or *Ephedra fragilis* Desf. has been verified, among others, in the thermomediterranean belt at an altitude between 200 and 800 m.a.s.l. (Carrión et al., 2003).

3.2.2. Experimental design

A total of 18 experimental plots with dimensions of 50 m^2 (10 m x 5 m) were installed in a selected flat degraded area in the quarry. A first mechanical pre-treatment to homogenization and decompaction of the marl substrate was carried out using the machinery available at the mine, such as mechanical excavators and bulldozers. Subsequently, five restoration treatments consisting of different organic amendments from wastes of different origin and chemical composition were applied in the experimental plots, increasing the organic matter content in each plot by 3% in the first 20 cm depth. The five restoration treatments used were: i) organic amendment from a 100% vegetable compost obtained from garden waste (CG), ii) organic amendment consisting in a vegetable compost from greenhouse crop waste (CC), iii) organic amendments from sewage sludge waste treated by mesophilic digestion, thermal dehydration at 70ºC, and centrifugation (SS), iv) organic amendments made from the mixture CG + SS (Mix1), and v) organic amendments made from the mixture CC + SS (Mix2). In addition, unamended experimental plots were used as control experimental plots (CON).

Three experimental plots per each restoration treatment and control soils were randomly applied in the study area using a mechanical backhoe available in the quarry facilities (3 replicates of experimental plots per each treatment x 5 restoration treatments = 15 experimental plots plus 3 replicates of control plots = 18 experimental plots). Figure 3.1 shows a diagram of all the experimental plots and the treatments applied in each plot. Moreover, surrounding natural soils close to the experimental plots not affected by mining activities were chosen as quality reference (NAT). This experimental condition allowed us to establish a reference system for comparison among soil types (restoration treatments and control soils) (Miralles et al., 2009).

Figure 3.1. Location study site and experimental design. On the right, a diagram of the experimental plots distribution with the different soil restoration treatments and unmodified soils is shown, as well as location of natural reference soil plots. Font photography: Sentinel2 (July-2019) and Spain's National Aerial Orthophotography Plan (PNOA).

Two species of native plants in the study area were selected for the restoration, planting 40 plants of *Macrochloa tenacissima* L. Kunth and 10 plants of *Olea europaea* L. var *sylvestris* Brot. in each experimental plot (restored and control soils), spaced 1 m. apart. An establishment irrigation after planting was done to ensure the vegetation's survival in the first summer because of the harsh climatic conditions typical of the Mediterranean semiarid areas, which include long summer droughts and high temperatures (Luna et al., 2018; Sánchez et al., 2004).

Six field samplings by season, from the end of summer 2018 to autumn 2019, were taken to collect soil samples to analyze for their physical-chemical properties. The first soil sampling was approximately four months after the application of the organic amendments in late summer 2018 (15-10-2018; FieldS1). The following field samplings took place in late autumn 2018 (17-12-2018; FieldS2), winter 2018 (18-03-2018; FieldS3), spring 2019 (02-07-2019; FieldS4), summer 2019 (19-09-2019; FieldS5) and autumn 2019 (25-11-2019; FieldS6). In each field sampling, one composite soil sample from mixing 10 randomly subsamples were collected from the soil surface (0–10 cm) in each experimental plot (3 replicates of soil treated samples x 5 restoration treatments = 15 soil samples) plus 3 soil samples from unamended control experimental plots and 3 soil samples from surrounding natural soils, for a total of 21 soil samples in each field sampling. Thus, 6 field sampling x 21 total soil samples per sampling = 126 soil samples which were analyzed in the laboratory.

Rainfall was continuously monitored with a pluviometry sensor located at the experiment site and also the ambient temperature was taken daily from a nearby station (distance of 4 km) and at the same height above sea level (RAIFALL003, Junta de Andalucía) at least for the duration of the experiment to obtain the local environmental climate context (Figure 3.2).

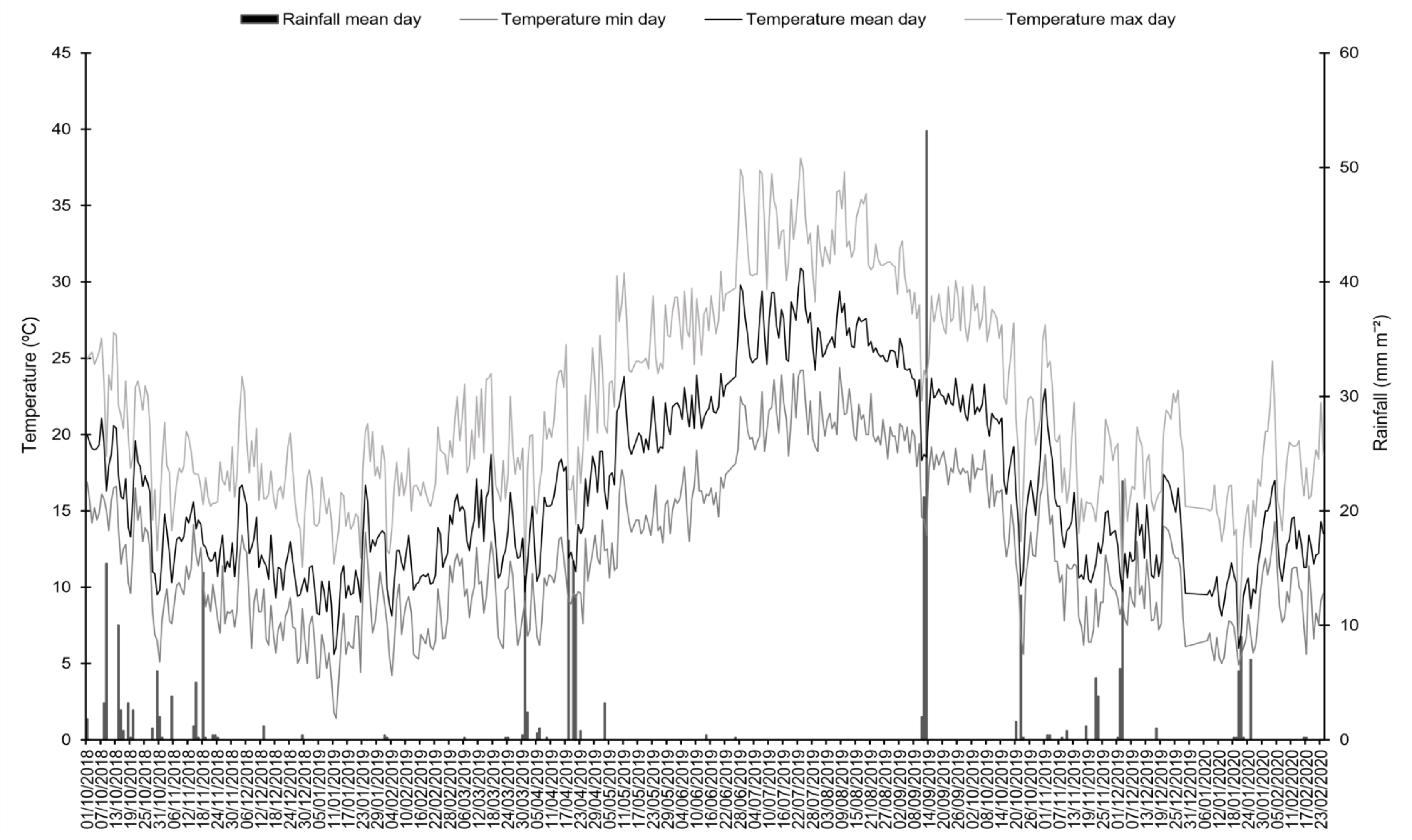


Figure 3.2. Local climate diagram of the experiment site during the experiment. Precipitation (rainfall) monitored by a rain sensor (Rain-O-Matic Small, Pronamic ApS, Denmark) located in the experimental area; temperature mean daily, maximum and minimum from weather station RAIFALL003 of Junta de Andalucía.

3.2.3. Physical-chemical properties of restored soils

Soil samples were air-dried and sieved to a 2 mm separating fine soil fraction and were used to, the following physical and chemical properties were analyzed: (1) soil pH was measured in a distilled water solution with a ratio of 1:2.5 w/v as measured with a pHmeter (LAQUA PH1100, Horiba, Tokyo, Japan); (2) electrical conductivity (EC) was measured in an aqueous suspension 1:2.5 soil/water suspensions with a digital conductivity meter (LAQUA EC1100, Horiba, Tokyo, Japan); (3) total organic carbon (TOC) was determined by wet oxidation with dichromate according to Walkley and Black's method (1934) (rectified by Mingorance, 2007); (4) total nitrogen content (TN) was analysed with an elemental analyser TCD detector (ELEMENTAR Rapid N; Elemental Analysen systems GmbH, Hanau, Germany); and (5) water retention was determined at -33 and -1500 KPa using the Richards membrane method (Richards, 1941).

3.2.4. In situ field campaigns of CO_2 emission and monitoring of climatic variables.

In situ measurements of dark respiration (CO_2 emission) were taken using a handheld, portable dark chamber connected to an infrared gas analyzer system PP-systems (EGM-4, IRGA, Hitchin, UK) with the measurement time set for 90 s. The chamber had a volume of 1170 cm^3 and a flat surface of 78 cm^2. A total of 18 field measurement campaigns were conducted for each experimental plot [soils restored with amendments (CG, CC, SS, Mix1 and Mix2) and un-amendments control plots (CON) and surrounding natural soils (NAT)]. Three randomly distributed replicas of PVC soil-borne collars (5 cm high by 10 cm diameter) were inserted into the soil one month before field measurements began, leaving 2–3 cm above ground in each experimental plot and in NAT soils, where they remained for the duration of the experiment. The inside of each soil-borne collar was kept free from vegetation to eliminate the effect of plant or root respiration on CO_2 emission measurements at each experimental plot. The field campaigns were conducted monthly from autumn 2018 to winter 2020 in the different seasons under different environmental conditions. Supplementary Table 3.1 provides detailed description of the environmental conditions in which the field measurement campaigns were done.

Soil moisture (M) and temperature (T) were measured at a depth of 3 cm next to each soil-borne collar with a handheld readout sensor (ProCheck, Decagon Devices, Inc., Pullman, WA, USA) during each field CO_2 measurement campaign. Rainfall events were registered daily every 20 minutes by a pluviometer with rain sensor with tipping bucket technology Rain-O-Matic Small (Pronamic ApS, Denmark) connected to a data logger in the middle of experimental area.

3.2.5. Statistical analyses

First, significant differences in physical, chemical, environmental and emission CO_2 were analyzed for all restoration treatments and control and natural reference soils using a two-way multivariate permutational analysis of variance (hereinafter PERMANOVA) (Anderson, 2001) that included two factors: date of field sampling and soil treatment. In cases where PERMANOVA detected a significant effect of the organic amendment treatments ($P < 0.05$), the source of the differences was evaluated by comparing the treatment pairs with the PERMANOVA posttest pairs, and the results with $P < 0.05$ were reported as significant. Pearson's correlation coefficients (r) were used to assess the relationship among physical and chemical properties and environmental parameters between different treatment and dates of field sampling. To observe the different trends in the evolution of CO_2 emissions over time, a Mann-Kendall (MK) linear regression trend test was applied (MK) (Kendall, 1948; Mann, 1945).

Physical, chemical and environmental variables with the highest importance in CO_2 emission correlation were identified using step-wise analysis DistLM (Distance-based Linear Models). For the DistLM routine we developed a "marginal" test for the relationship between variable response (CO_2 emissions) and an individual variable (EC, pH, TOC, TN and water retention at -1500 and -33 KPa) to identify independent variables that explain the variations in soil samples both by treatment and over time. Subsequently, the Akaike Information Criterion (AICc) (Akaike, 1974) was established to select the best model, and the step-by-step procedure to build the model was followed. Finally, a "sequential" test of the individual variables was performed to assess whether adding an individual variable contributes significantly to the explanation. Ordination and visualisation of the model was performed in distance-based redundancy analysis (dbRDA). The statistical package PRIMER + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows was used for PERMANOVA, DistLM and dbRDA analysis (Anderson et al., 2008), as well as for AICc analysis. The trends had been performed using TREND V1.0.2. software (Cooperative Research Centre for Catchment Hydrology, Australia). Pearson's correlations were performed with Statgraphic Centurion XVIII-X64 software.

3.3. Results

3.3.1. Changes in physical and chemical soil properties in the medium-term. Differences between restored, natural and degraded soils.

In general, the results showed progressive changes in physical and chemical properties (TOC, TN, EC, pF at -1500 KPa, pF at -33 KPa) in all restored soils throughout the field sampling campaigns carried out from application of organic amendments until the end of sampled period. Nevertheless, the values of these physical and chemical soil properties hardly changed in un-amendments control soils (CON) and natural soils (NAT) during the sampled period (Table 3.1).

Two-way PERMANOVA analysis showed significant differences ($P < 0.05$) in the physical and chemical soil parameters attending to date of field campaign (in different environmental conditions), soil treatment (SS, CG, CC, Mix1, Mix2, CON and NAT) and their interaction (Supplementary Table 3.2). Amended soils showed significantly higher TOC and TN contents than CON and NAT soils in all campaigns, being those soil properties significantly higher in FieldS1, just after the application of the organic amendments (Table 3.1). The highest values of TOC and TN were reached in SS, Mix1, Mix2 and CC soils followed by CG, whereas CON soils presented the significantly lowest values of TOC and TN in all field campaigns (Table 3.1). However, TOC and TN in all restored soils with organic amendments decreased progressively in successive field campaigns, with their values approaching those of NAT soils somewhat more closely in the last field campaign (FieldS6; Table 3.1). The organic amendments also significantly increased EC content with respect to the CON and NAT soils, although EC values decreased rapidly throughout the sampled period, approaching EC values that were more similar to those of CON soils. On the contrary, NAT soils showed the significantly lowest values of EC in all the sampled campaigns (Table 3.1). Restored soil showed a significantly lower pH than that in NAT and CON soil, especially in FieldS1 and FieldS2, then increased in subsequent measurement campaigns, minimising their differences with control and reference soils in the last sampling campaign (FieldS6) (Table 3.1). Soils with organic amendments showed higher water retention at pF -1500 KPa than CON and NAT soils only in the FieldS1 and FieldS2 campaigns (Table 3.1).

Table 3.1. Main chemical and physical characteristics of soils with restored organic amendments, control soils without addition of amendments and natural reference soils on six dates distributed along the studied chronological sequence. Average of three replicated samples (mean ± standard error). Continued on next page.

	EC (mS/cm)	**pH**	**TOC (%)**	**TN (%)**	***pF -1500 KPa***	***pF -33 KPa***
FieldS1 15/10/2018						
CG	3.46 ± 0.27a	7.58 ± 0.06a	3.43 ± 0.52a	0.57 ± 0.05a	17.05 ± 0.21ab	36.55 ± 1.93a
SS	3.76 ± 1.35abc	7.21 ± 0.04b	6.51 ± 1.07ab	0.89 ± 0.09b	25.92 ± 3.57ac	39.58 ± 3.67a
CC	5.46 ± 0.32abc	8.57 ± 0.04c	4.77 ± 0.81ab	0.62 ± 0.01ab	19.18 ± 0.44c	33.67 ± 2.52a
Mix1	5.18 ± 0.93a	7.61 ± 0.09a	6.23 ± 1.27ab	0.68 ± 0.05ab	18.57 ± 1.57abc	35.41 ± 0.95a
Mix2	4.15 ± 0.13b	8.06 ± 0.07d	5.56 ± 0.31b	0.62 ± 0.02ab	20.36 ± 1.70abc	33.61 ± 0.93a
CON	1.84 ± 0.33c	8.25 ± 0.08d	0.34 ± 0.12c	0.05 ± 0.01c	15.64 ± 0.89b	32.59 ± 2.99a
NAT	0.07 ± 0.00d	8.54 ± 0.05c	1.37 ± 0.04d	0.20 ± 0.00d	15.73 ± 0.44ab	33.09 ± 0.93a
FieldS2 17/12/2018						
CG	2.56 ± 0.28ab	7.97 ± 0.20ab	1.71 ± 0.31a	0.32 ± 0.07a	18.14 ± 0.80ab	29.47 ± 1.51a
SS	3.42 ± 0.40ab	7.45 ± 0.04a	2.67 ± 0.38b	0.60 ± 0.12b	21.45 ± 1.06a	34.34 ± 1.80a
CC	3.30 ± 0.25a	8.5 ± 0.12b	2.94 ± 0.10b	0.40 ± 0.03ab	18.59 ± 0.28ab	30.70 ± 0.95a
Mix1	3.25 ± 0.12a	7.52 ± 0.03a	2.45 ± 0.09b	0.51 ± 0.02b	19.46 ± 0.69a	31.61 ± 0.60a
Mix2	3.08 ± 0.41ab	7.65 ± 0.04a	2.82 ± 0.09b	0.51 ± 0.01b	19.42 ± 0.67a	32.40 ± 1.40a
CON	1.72 ± 0.00b	8.59 ± 0.06b	0.46 ± 0.08c	0.02 ± 0.00c	16.34 ± 0.14bc	32.36 ± 0.62a
NAT	0.07 ± 0.00c	8.64 ± 0.06b	1.31 ± 0.08d	0.11 ± 0.00d	14.91 ± 0.14c	36.32 ± 0.62a

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendment of different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio; pF: water retention determined at -33 and -1500 KPa. Across treatments, data with different lowercase letters are significantly different, $P < 0.05$ [PERMANOVA].

Table 3.1. (Continuation). Main chemical and physical characteristics of soils with restored organic amendments, control soils without addition of amendments and natural reference soils on six dates distributed along the studied chronological sequence. Average of three replicated samples (mean ± standard error).

	EC (mS/cm)	pH	TOC (%)	TN (%)	*pF -1500 KPa*	*pF -33 KPa*
FieldS3						
14/03/2019						
CG	3.72 ± 0.39ab	8.06 ± 0.52abcd	2.62 ± 0.26a	0.44 ± 0.06a	15.16 ± 0.58a	34.21 ± 1.04a
SS	3.79 ± 0.17a	7.91 ± 0.10a	2.55 ± 0.34a	0.63 ± 0.05ab	19.22 ± 6.00a	33.08 ± 0.87a
CC	2.8 ± 0.09b	8.81 ± 0.04a	4.40 ± 0.54b	0.56 ± 0.03a	12.80 ± 2.60a	30.71 ± 0.98a
Mix1	3.77 ± 0.30a	8.01 ± 0.00b	3.62 ± 0.49ab	0.66 ± 0.06ab	19.76 ± 0.37a	33.08 ± 1.31a
Mix2	2.47 ± 0.46ab	8.28 ± 0.16ac	4.42 ± 0.55b	0.71 ± 0.00b	19.01 ± 1.02a	33.47 ± 1.30a
CON	3.54 ± 0.55ab	9.19 ± 0.08d	0.28 ± 0.03c	0.06 ± 0.00c	13.83 ± 0.62a	31.96 ± 1.95a
NAT	0.10 ± 0.01c	8.62 ± 0.07bc	1.34 ± 0.01d	0.15 ± 0.01d	13.22 ± 0.34a	35.28 ± 1.18a
FieldS4						
02/07/2019						
CG	2.31 ± 0.28a	7.88 ± 0.05a	3.11 ± 0.46a	0.39 ± 0.09a	19.61 ± 1.73a	35.99 ± 1.83a
SS	3.06 ± 0.53a	8.12 ± 0.03b	3.59 ± 0.46a	0.60 ± 0.12b	21.56 ± 1.08a	36.78 ± 1.90a
CC	2.04 ± 0.33a	8.75 ± 0.05c	4.21 ± 0.17a	0.50 ± 0.05ab	19.23 ± 0.50a	34.19 ± 0.96a
Mix1	2.93 ± 0.58a	7.48 ± 0.09d	3.24 ± 1.87a	0.57 ± 0.02ab	19.36 ± 0.75a	35.34 ± 1.17a
Mix2	3.34 ± 0.52a	7.71 ± 0.02ad	5.64 ± 0.06abc	0.59 ± 0.00b	19.95 ± 0.47a	36.37 ± 1.00a
CON	3.57 ± 0.00a	7.62 ± 0.08d	0.79 ± 0.12b	0.04 ± 0.00c	17.53 ± 1.18a	33.65 ± 1.80a
NAT	0.08 ± 0.00b	8.36 ± 0.08b	1.64 ± 0.12c	0.15 ± 0.00d	18.78 ± 1.18a	33.77 ± 1.80a

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendment of different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio; pF: water retention determined at -33 and -1500 KPa. Across treatments, data with different lowercase letters are significantly different, $P < 0.05$ [PERMANOVA].

Table 3.1. (Continuation) Main chemical and physical characteristics of soils with restored organic amendments, control soils without addition of amendments and natural reference soils on six dates distributed along the studied chronological sequence. Average of three replicated samples (mean ± standard error).

	EC (mS/cm)	**pH**	**TOC (%)**	**TN (%)**	***pF -1500 KPa***	***pF -33 KPa***
FieldS5						
19/09/2019						
CG	2.25 ± 0.03a	7.89 ± 0.11a	2.29 ± 0.19a	0.28 ± 0.03a	18.78 ± 1.27ab	32.26 ± 1.34a
SS	2.55 ± 0.12a	7.79 ± 0.06a	3.41 ± 0.69ba	0.60 ± 0.12b	19.59 ± 0.26a	34.50 ± 2.82a
CC	1.72 ± 0.05ab	8.38 ± 0.22b	3.03 ± 1.16abc	0.35 ± 0.18abcd	18.35 ± 1.85abc	33.47 ± 4.87a
Mix1	3.12 ± 0.09a	7.83 ± 0.09a	3.02 ± 0.70abc	0.48 ± 0.13ab	16.82 ± 1.37bcd	31.59 ± 1.65a
Mix2	2.46 ± 0.03a	7.86 ± 0.16ac	3.27 ± 0.53b	0.48 ± 0.07b	18.12 ± 0.45b	33.92 ± 2.28a
CON	1.96 ± 0.06ab	8.9 ± 0.06d	0.75 ± 0.13d	0.05 ± 0.01c	14.95 ± 0.89c	33.29 ± 0.47a
NAT	0.06 ± 0.06b	8.17 ± 0.11bc	1.83 ± 0.19c	0.16 ± 0.02d	15.83 ± 0.74cd	31.06 ± 1.26a
FieldS6						
25/11/2019						
CG	2.23 ± 0.43ab	7.99 ± 0.09a	2.65 ± 0.03a	0.38 ± 0.00a	18.21 ± 2.13a	35.78 ± 0.94a
SS	3.24 ± 0.04a	7.88 ± 0.06a	3.08 ± 0.20ab	0.60 ± 0.12b	17.38 ± 0.51a	36.02 ± 1.52a
CC	1.75 ± 0.35b	8.87 ± 0.16b	3.37 ± 0.17b	0.40 ± 0.01a	16.40 ± 1.09a	36.45 ± 2.10a
Mix1	2.75 ± 0.68ab	8.07 ± 0.09a	2.46 ± 0.39abc	0.42 ± 0.04ab	16.41 ± 1.13a	34.65 ± 0.94a
Mix2	2.6 ± 0.42ab	7.97 ± 0.08a	42.90 ± 0.21ab	0.42 ± 0.05ab	16.78 ± 0.69a	43.92 ± 6.80a
CON	1.64 ± 0.35b	8.67 ± 0.06b	10.81 ± 0.16d	0.06 ± 0.00c	14.89 ± 0.99a	33.40 ± 1.22a
NAT	0.08 ± 0.01c	8.70 ± 0.01c	21.81 ± 0.14c	0.15 ± 0.01d	13.21 ± 0.84a	33.76 ± 1.24a

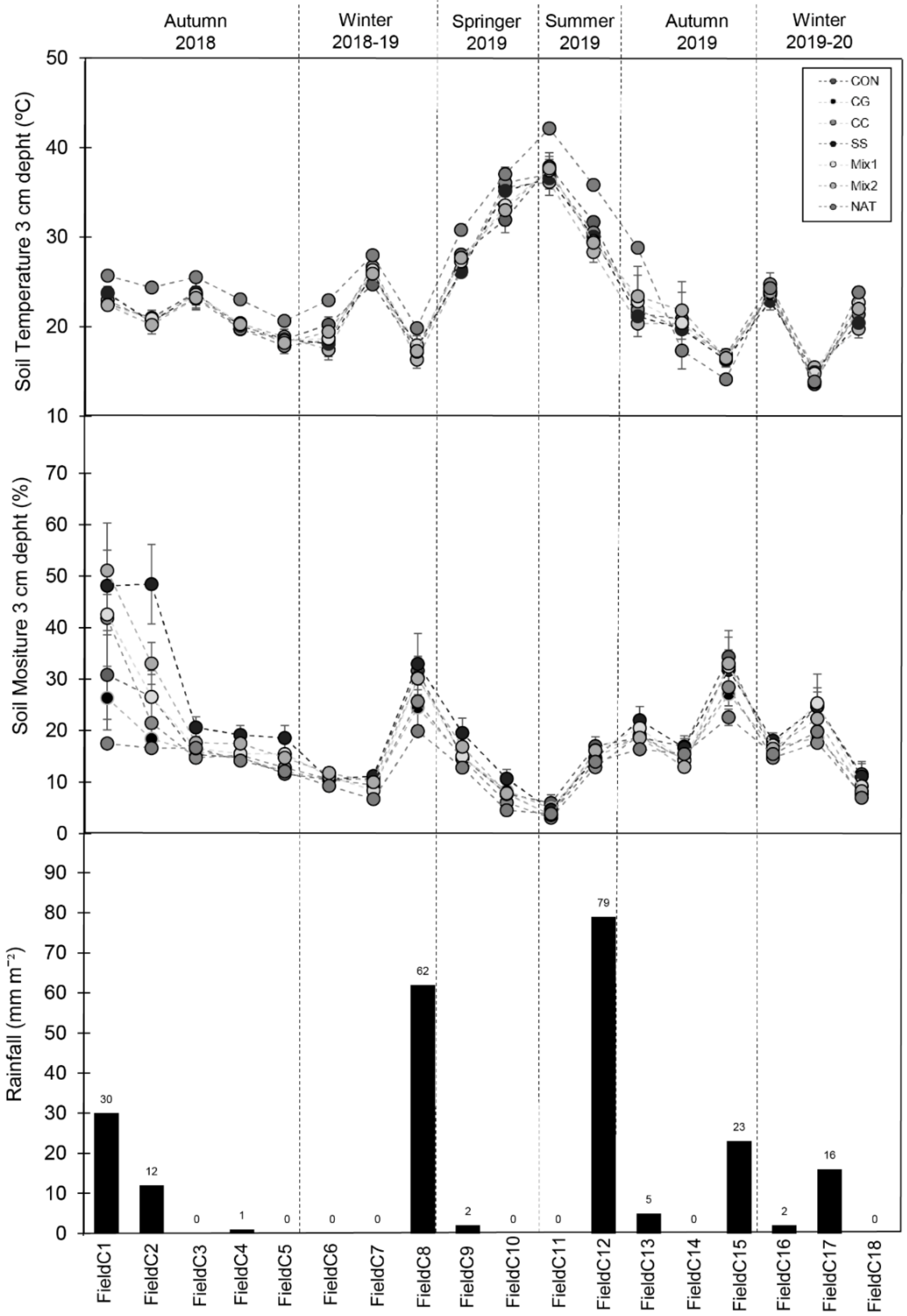
CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendment of different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soil. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio; pF: water retention determined at -33 and -1500 KPa. Across treatments, data with different lowercase letters are significantly different, $P < 0.05$ [PERMANOVA].

3.3.2. Environmental conditions and microclimatic parameters in field CO_2 measurement campaigns.

The main precipitation events were recorded in autumn and spring months throughout the experimental period (Figure 3.2), although the total accumulated rains were very scarce (173.4 mm). In these seasons, temperatures were generally mild (average temperatures ranged between 19 and 24ºC), reaching maximums of about 28–30ºC in autumn and minimums of about 8 ºC in spring (Figure 3.2). Winter was cold, with low rainfall (24.2 mm) and minimum average temperatures of about 5ºC and maximum average temperatures of 18ºC. The summer period was generally very dry, registering some light rain and occasional rainstorms at the end of the season (Figure 3.2). The long summer period coincided with mild temperatures in spring and high temperatures in the summer seasons (maximum of 38ºC and minimums of 14ºC; Figure 3.2).

During the period under study, the FieldC1, FieldC2, FieldC8, FieldC13, FieldC15 and FieldC17 campaigns took place after rain events (accumulated precipitation 10 days before the campaign) and generally mild temperatures. Supplementary Table 3.1 provides a detailed description of the environmental conditions of each field campaign. In these field campaigns, the soils restored with organic amendments presented higher soil moisture than natural (NAT) and control (CON) soils. Among all restored soils with organic amendments, SS, Mix1, and Mix2 presented higher soil moisture contents than CC and CG soils (Figure 3.3).

In FieldC8, FieldC9, and Field10 campaigns, which took place in spring, the soil moisture content decreased progressively in all restored, control, and natural soils, as temperatures increased progressively (average temperatures of about 18°C and maximum of about 27°C); and only one major rain event was recorded in the first measurement campaign (Supplementary Table 3.1). However, the soil moisture content in the restored soils was still comparatively higher than in the NAT soils (Figure 3.3). During the summer campaigns, FieldC11 and FieldC12, the soil samples were dry at first, but soil moisture increased during the second campaign from a rainfall event recorded at the end of the season coinciding with that campaign. Nevertheless, there were almost no differences in soil moisture content between the restored, control and natural soils (Figure 3.3). On the contrary, temperatures were higher in the control (about 38°C) and natural (about 42°C) soils than in the restored soils with organic amendments



(about 36°C; Figure 3.3).

Figure 3.3. Temporal distribution of rainfall, soil moisture and soil temperature during

the experiment in different field campaigns and different seasons.

3.3.3. In situ CO_2 emission pattern and relationship between CO_2 emission, environmental parameters, physical and chemical soils properties.

The MK statistical test showed that the CO_2 emission trend decreased significantly during the experimental period (from FieldC1 to FieldC18) (Table 3.2) in all restored soils with organic amendments. Two-way PERMANOVA analysis showed significant differences ($P < 0.05$) in CO_2 rates between the different field campaigns on different dates with organic amendment treatment (CC, CG, SS, Mix1 and Mix2) (Supplementary Table 3.2).

Table 3.2. Soil CO_2 emissions (soil respiration) trends shown by different organic amendments treatments, control soils and natural reference soil (Mann-Kendall test).

Treatment	**Trend**	**Z-statistic**	**Critical value (a=0.01)**	**Result**
CG	Decreasing	-1.87	0.64	Signifincant
SS	Decreasing	-2.25	0.64	Signifincant
CC	Decreasing	-2.25	0.64	Signifincant
Mix-1	Decreasing	-2.25	0.64	Signifincant
Mix-2	Decreasing	-2.25	0.64	Signifincant
Control	Decreasing	-2.25	0.64	Signifincant
NAT	Decreasing	-2.25	0.64	Signifincant

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70° C; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures amendments of different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soils.

The CO_2 emission pattern spiked after the organic amendment treatment was applied and gradually decreased over time, although CO_2 emission changed little for NAT and CON soils in the different field campaigns (Figure 3.4). At the beginning of the field experiment, the first campaign (FieldC1), conducted in the fall (15-10-2018) **13** and coinciding with the first rain event after a long dry period in summer, showed a higher soil moisture and CO_2 emission than the rest of the field campaigns until the end of the period under study (Supplementary Figure 3.1). Specifically, in FieldC1 the soils SS, Mix1 and Mix2 had the significantly highest CO_2 emission rates ($P < 0.05$) (average values of CO_2 emissions of about 40 µmol m^{-2} s^{-1}) (Table 3.3; Figure 3.4). In contrast, CC soils had significantly lower CO_2 emissions than the experimental plots with sludge although both had a similar soil moisture, whereas these CO_2 emissions were similar to those of NAT soils with a soil moisture around 25% lower than CC (Supplementary Figure 3.1). CG soils and CON soils showed the significantly lowest CO_2 emission values (Supplementary Table 3.2; Figure 3.4). The CO_2 emission rate from the restored soils, especially SS, Mix1 and Mix2, beginning with the second measurement campaign (FieldC2), also conducted in the same season, was significantly lower than that produced in FieldC1. Interestingly, there was a slight difference in the CO_2 emissions from NAT and CON soils between FieldC1 and FieldC2 that did not recur throughout the entire period studied.

In the following field campaign (FieldC3) conducted in a period without rain and with low temperatures in the winter, the CO_2 emission rates from all soil types (restored, CON and NAT soils) were lower than in previous campaigns. However, in the following spring and late summer field campaigns after the rain events (between FieldC8 and FieldC12), although the CO_2 emissions were significantly lower than in FieldC1, there were slight peaks in CO_2 emissions, especially from SS (Table 3.3; Figure 3.4). In those field campaigns, CO_2 emissions from the restored soils did not differ significantly from NAT soils in general and were only slightly higher than those from CON soils at the end of the study period (FieldC11) coinciding with the lowest values of soil moisture (Supplementary Figure 3.1). CO_2 emissions were low; and, in general, there were no significant differences between all soil types (restored, natural and control) in the field campaigns conducted in dry periods of winter and summer with low and very high temperatures, respectively (Figure 3.3 and Figure 3.4).

The CO_2 emission showed significant positive correlations ($p < 0.05$) with TOC, TN, pF -1500, EC, and soil moisture (M), which showed the highest significant correlations ($r = 0.74$), whereas pH had negative significant correlation with CO_2 emission (Table 3.4).

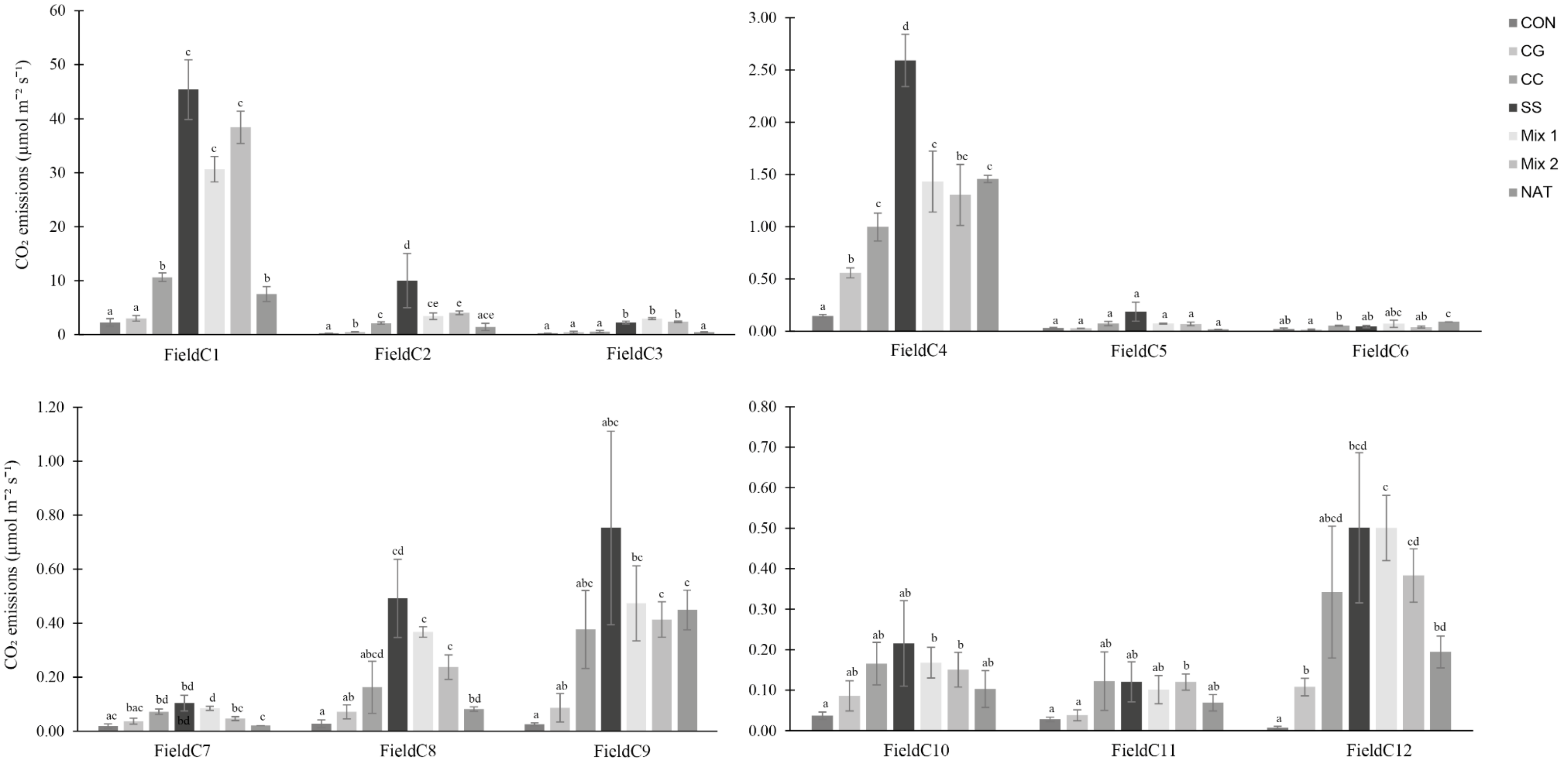


Figure 3.4. Comparison of soil CO_2 emissions to differently treated soils, control soil, and natural reference soil during the experiment (x-axis field measurement campaign) to evaluate the impact of organic amendments after application (continue on next page).

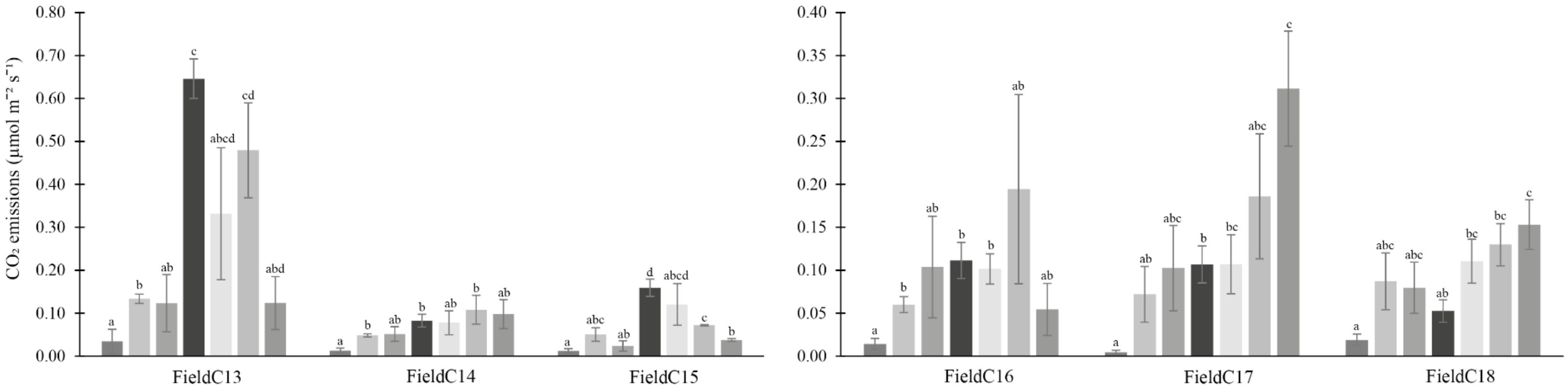


Figure 3.4. (Continuation) Comparison of soil CO_2 emissions to differently treated soils, control soil, and natural reference soil during the experiment (x-axis field measurement campaign) to evaluate the impact of organic amendments after application.

Table 3.3. Measurements of CO_2 emissions, in experimental plots with organic amendments, control and natural reference soils (average standar error). Continued on next page.

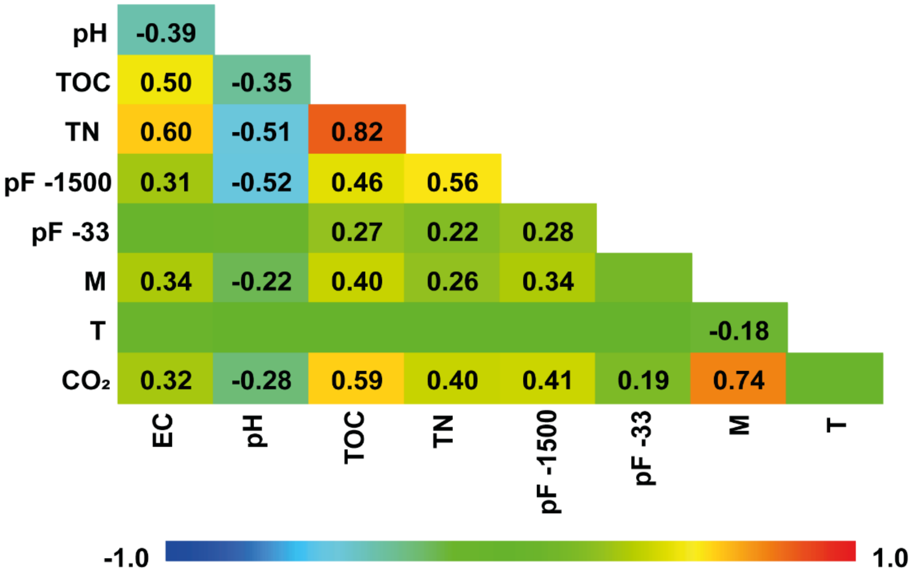
Date	CON	CG	CC	SS	Mix1	Mix2	NAT
15/10/2018	2.25 ± 0.69a	3.02 ± 0.52a	10.65 ± 0.81b	45.39 ± 5.52c	30.65 ± 2.36c	38.41 ± 0.98c	7.52 ± 1.36b
29/10/2018	0.25 ± 0.04a	0.52 ± 0.05b	2.14 ± 0.22c	14.12 ± 0.96d	3.42 ± 0.61ce	4.05 ± 0.33e	1.42 ± 0.67abc
05/12/2018	0.23 ± 0.06a	0.43 ± 0.19a	0.50 ± 0.22a	2.24 ± 0.25b	2.97 ± 0.19b	2.38 ± 0.15b	0.38 ± 0.05a
17/12/2018	0.14 ± 0.01a	0.55 ± 0.05b	0.99 ± 0.13c	2.59 ± 0.25d	1.43 ± 0.29c	1.30 ± 0.29bc	1.37 ± 0.03c
16/01/2019	0.03 ± 0.01a	0.03 ± 0.00a	0.07 ± 0.01a	0.18 ± 0.08a	0.07 ± 0.00a	0.07 ± 0.02a	0.01 ± 0.00a
18/02/2019	0.02 ± 0.01ab	0.01 ± 0.01a	0.05 ± 0.00b	0.04 ± 0.01ab	0.07 ± 0.03abc	0.04 ± 0.01ab	0.09 ± 0.00c
14/03/2019	0.02 ± 0.01ac	0.03 ± 0.01abc	0.07 ± 0.01bd	0.10 ± 0.03bd	0.08 ± 0.01d	0.05 ± 0.01bc	0.02 ± 0.00c
23/04/2019	0.3 ± 0.01a	0.07 ± 0.03ab	0.16 ± 0.10abcd	0.49 ± 0.15cd	0.37 ± 0.02c	0.24 ± 0.05c	0.08 ± 0.01bd
02/05/2019	0.03 ± 0.00a	0.09 ± 0.05ab	0.38 ± 0.14abc	0.75 ± 0.36abc	0.47 ± 0.14bc	0.41 ± 0.07c	0.45 ± 0.07c
11/06/2019	0.04 ± 0.01a	0.09 ± 0.04ab	0.17 ± 0.05ab	0.22 ± 0.11ab	0.17 ± 0.04b	0.15 ± 0.04b	0.10 ± 0.05ab
23/07/2019	0.03 ± 0.01a	0.04 ± 0.01a	0.12 ± 0.07ab	0.12 ± 0.05ab	0.10 ± 0.04ab	0.12 ± 0.02b	0.07 ± 0.01ab

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments of different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soils. Number with different lowercase letters are significantly different treatments in the same date, $P < 0.05$ (one-way analysis of variance with permutations [PERMANOVA].

Table 3.3. (Continuation). Measurements of CO_2 emissions, in experimental plots with organic amendments, control and natural reference soils (average ± standard error).

Date	CON	CG	CC	SS	Mix1	Mix2	NAT
19/09/2019	0.01 ± 0.00a	0.11 ± 0.02b	0.34 ± 0.16abcd	0.50 ± 0.19bcd	0.50 ± 0.08c	0.38 ± 0.07cd	0.19 ± 0.04bd
25/10/2019	0.03 ± 0.03a	0.13 ± 0.01b	0.12 ± 0.07ab	0.65 ± 0.05c	0.33 ± 0.15abcd	0.48 ± 0.11cd	0.12 ± 0.06abc
24/11/2019	0.01 ± 0.01a	0.05 ± 0.00b	0.05 ± 0.02ab	0.08 ± 0.02b	0.08 ± 0.03ab	0.11 ± 0.03b	0.10 ± 0.03ab
04/12/2019	0.01 ± 0.00a	0.05 ± 0.02abc	0.02 ± 0.01ab	0.16 ± 0.02d	0.12 ± 0.05abcd	0.07 ± 0.00c	0.04 ± 0.00b
23/12/2019	0.01 ± 0.01a	0.06 ± 0.01b	0.10 ± 0.06ab	0.11 ± 0.02b	0.10 ± 0.02b	0.19 ± 0.11ab	0.05 ± 0.03ab
24/01/2020	0.01 ± 0.00a	0.07 ± 0.03ab	0.10 ± 0.05abc	0.11 ± 0.03b	0.11 ± 0.03bc	0.19 ± 0.07abc	0.31 ± 0.07c
24/02/2020	0.02 ± 0.01a	0.09 ± 0.03abc	0.08 ± 0.03abc	0.05 ± 0.01ab	0.11 ± 0.03bc	0.13 ± 0.02bc	0.15 ± 0.03c

CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments of different vegetal compost and sludge compost; CON: unamended control soils; NAT: natural reference soils. Number with different lowercase letters are significantly different treatments in the same date, $P < 0.05$ (one-way analysis of variance with permutations [PERMANOVA].



	EC	pH	TOC	TN	pF -1500	pF -33	M	T
pH	-0.39							
TOC	0.50	-0.35						
TN	0.60	-0.51	0.82					
pF -1500	0.31	-0.52	0.46	0.56				
pF -33			0.27	0.22	0.28			
M	0.34	-0.22	0.40	0.26	0.34			
T							-0.18	
CO_2	0.32	-0.28	0.59	0.40	0.41	0.19	0.74	

Table 3.4. Significant positive and negative Pearson correlations ($p < 0.05$) between physical and chemical soils properties, environmental parameters and CO_2 emissions. Empty boxes obtain non-significant correlations ($p > 0.05$). EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; pF: soil water content at different pressures; M: moisture 3cm depth; T: Temperature 3cm depth; CO_2: emission of carbon dioxide.

DistLM analysis selected of all the environmental variables and soil properties variables measured the most important influencing CO_2 emission were M (59.27%), TOC (9.02%), pH (8.7%) and TN (8.09%), while the significant predictor with the lower impact on CO_2 emission were water retention at pF -1500 KPa (5.9%). Nevertheless, pF -33 KPa, EC and T did not have a statistically significant ($P < 0.05$) effect on CO_2 emission in the different field measurement campaigns under different environmental conditions (Table 3.5). A global model with the four predictor variables of CO_2 emission, which solved best for R^2 value, explained the 40.52% of the total variation to AICc of 872.68 and identified a combination of 5 significant factors M, TOC, pF-33, pH and EC (Table 3.5). According to the variations (outside the adjusted model and outside the total variation) explained by the dbRDA graphs, applied to the CO_2 emissions from soils as a function of the treatment applied (Figure 3.5a) and as a function of the sampling date (different environmental conditions) (Figure 3.5b) for restored soils, untreated soils and natural reference soils explained 96.35% of the adjusted model accumulated in the first two axes (dbRDA1 87.98% and dbRDA2 8.37%) of the total variation of the physical, chemical and environmental variables analysed. The dbRDA analysis results showed that M, TOC, pH, pF -33 and EC best explained CO_2 emissions both by dates of campaigns under different environmental conditions and restoration treatment types (Figure 3.5a and 3.5b) and thus explained a large part of the variation.

Table 3.5. Results of distLM analysis showing the physical, chemical and environmental variables that describe significant and independent proportions of the variation in CO_2 emissions between sampling date in restored soil with organic amendment.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P	Prop.
EC	4272.30	2.78	0.063	0.02
pH	12912.00	8.79	0.001	0.07
TOC	13233.00	9.02	0.001	0.07
pF - 1500	8861.30	5.90	0.004	0.05
pF -33	1952.50	1.25	0.284	0.01
TN	11940.00	8.09	0.002	0.06
M	63080.00	59.27	0.001	0.32
T	4288.80	2.79	0.064	0.02

SEQUENTIAL TESTS

Variable	AICc	SS(trace)	Pseudo-F	P	Prop.	Cumul.
M	880.31	63080	59.27	0.001	0.32	0.32
M +TOC	877.32	5219.4	5.06	0.005	0.03	0.35
M +TOC + pF -33	875.43	3987.8	3.96	0.012	0.02	0.37
M +TOC + pF -33 + pH	873.99	3469.5	3.52	0.023	0.02	0.39
M +TOC + pF -33 + pH + EC	872.68	3279.8	3.39	0.039	0.02	0.41

Best solution

AICc	R^2	RSS	Variable Selections
872.68	0.41	116020.00	5: M, TOC, pF -33, EC, pH

Percentage of variation explained by individual axes RDA

	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
1	87.98	87.98	35.65	35.65
2	8.37	96.35	3.39	39.04
3	3.54	99.89	1.43	40.48
4	0.08	99.97	0.03	40.51
5	0.03	100	0.01	40.52

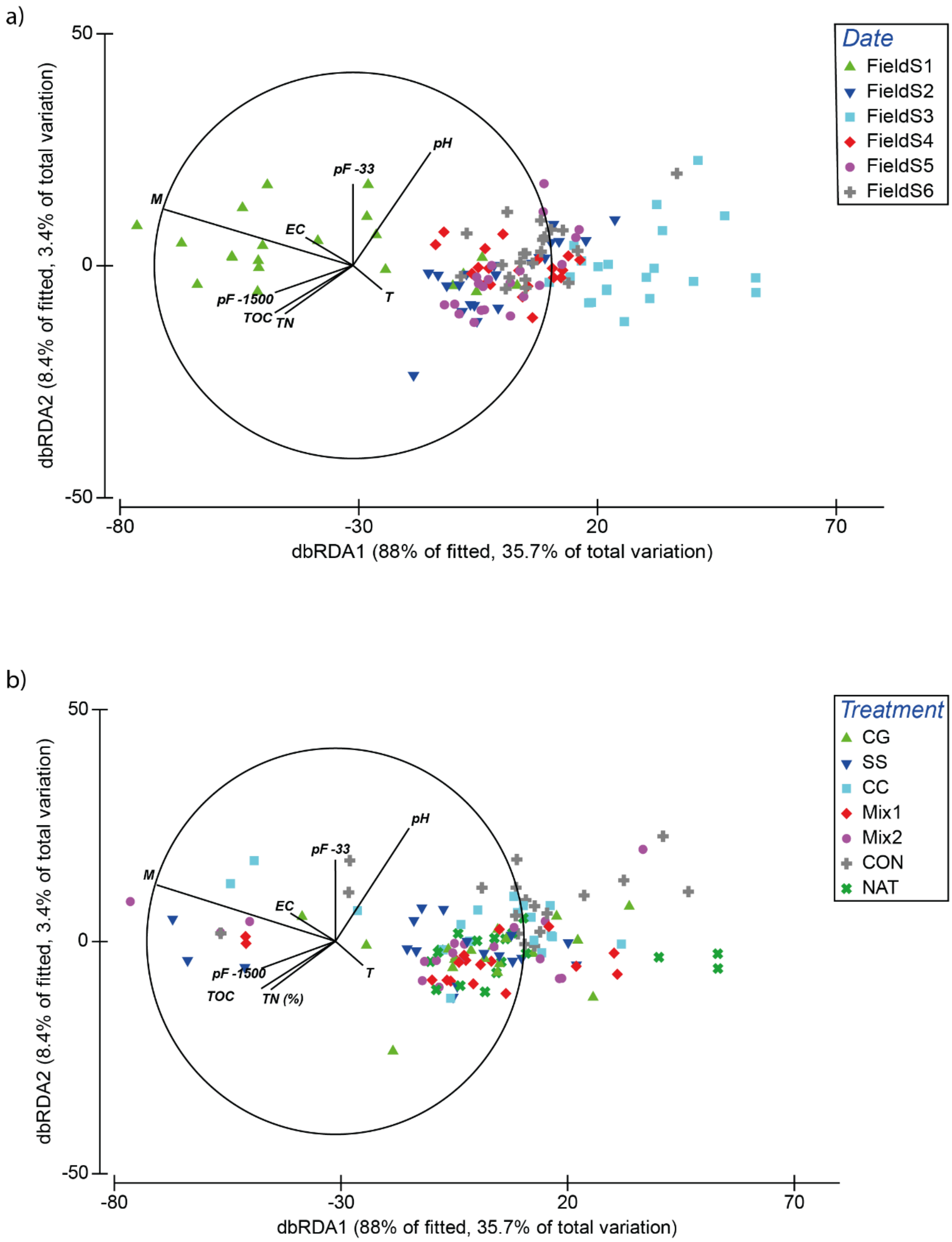


Figure 3.5. Distance-based RDA bi-plot of different soil treated, control soil and natural reference soil, showing projections of samples from different soil sampling along the experiment with various significant chemical properties as explanatory variables as a date sample function (a) and treatment function (b). CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70° C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. NAT: natural reference soils. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; pF: water retention determined at -33 and -1500 KPa.

3.4. Discussion

In this work, the effect of five restoration treatments from organic wastes on CO_2 emission compared with CO_2 emission from un-amended soils from a quarry under semiarid conditions and surrounding natural soils was monitored for a one and a half years after amendments were applied. All the restoration treatments with the different types of organic amendments produced CO_2 emissions just after their soil application, which was especially high from soils treated with sewage sludge (Figure 3.4). This CO_2 emission from the restored soil could have been due to interactions between soil microorganisms, transformation of added organic substances, and the natural soil C-cycle (Fangueiro et al., 2007; Kuzyakov, 2010). These interactions cause intense short-term changes in the native soil organic matter cycle, resulting in an additional release of CO_2 known as a "priming effect" (Kuzyakov et al., 2000). The positive priming effect is normal in soils with low organic matter content after receiving inputs (Zimmerman et al., 2011). Other authors have already pointed out that the type of amendment applied conditions soil respiration, causing CO_2 output flows (Ray et al., 2020).

The CO_2 emission from the restored experimental plots was due to complex interactions between physical, chemical and biological soil properties and environmental factors conditioning the soil climate, as was supported by our statistical analysis. DistLM analysis indicated that soil moisture, TOC, pH and TN were the most influential factors in the CO_2 emissions (Table 3.5). The high positive correlations between CO_2 emissions and TOC, TN, soil moisture (M) and pF -1500 and negative correlation with pH (Table 3.4) corroborated the key importance of these factors in the dynamics of CO_2 fluxes in the restored and control soils in the quarry and in surrounding natural soil. Soil moisture and these physical and chemical properties are considered key drivers for the soil bacterial communities (Miralles et al., 2020; Miralles et al., 2020; Rodríguez-Berbel et al., 2020; Sánchez-Marañón et al., 2017); and therefore their effect on CO_2 emission after the application of amendments is due to the priming effect. Nevertheless, our results also showed interesting differences in the magnitude of CO_2 emission depending on the restoration treatment used in comparison with natural soils (NAT) and unamended control soils (CON), especially in the first months of monitoring. In FieldC1, restored soils with sludge (SS, Mix1 and Mix2) emitted CO_2 rates approximately 80% higher than in NAT and CC soils and 90% higher than in GC and CON soils (Figure 3.4). Given that the magnitude of microbial activity is associated with TOC content and the bioavailability of organic compounds, there will be an increase in soil CO_2 emissions in soils with easily mineralizable organic matter (González-Ubierna et al., 2012), causing the priming effect. Therefore, our results showed that differences in the CO_2 emission magnitude between the restoration treatments could have been due in large part to the chemical composition of the organic amendment rather than to the amount of organic matter, given that there were no significant differences between all restored soils in the TOC content (Table 3.1).

The soils restored with sludge (SS, Mix1, and Mix2) contributed a high amount of labile organic matter to the soils, with significantly higher carbohydrate content than in the other types of restored, control and natural soils (Soria et al., 2021a). The contribution of easily biodegradable organic matter favours the proliferation of soil bacteria capable of mineralizing the excess of labile organic matter applied to the soil by increasing the production of C-cycle enzymes, a direct cause of CO_2 emission (Bastida et al., 2008b; Blagodatskaya and Kuzyakov, 2008; Mondini et al., 2006). In addition to stimulating soil microbial communities, organic amendments contribute to increasing the growth of soil microbial biomass by providing new bacterial communities associated with soil amendments (Bastida et al., 2008a; García-Gil et al., 2000; García et al., 1998; Rodríguez-Berbel et al., 2020). Therefore, the changes in the composition and structure of soil bacteria communities could be associated with the high increase in soil respiration and CO_2 production (Blagodatskaya et al., 2007; Fontaine et al., 2003; Razanamalala et al., 2018) just after the application of organic amendments in the restored soils. In this sense, most of the CO_2 derived from heterotopic respiration could be exhaled by soil microorganisms (Li et al., 2013), producing the above-mentioned priming effect (Kuzyakov, 2006). This could also explain the high mineralization rate found by Soria et al., (2021a) in a previous study in the same experimental plots amendment with sludge. On the other hand, traditionally, it has been observed that organic amendments from sewage sludge enrich the soil in nitrogen compounds, especially when thermally dried sludge is used (Smith and Durham, 2002; Tarrasón et al., 2008). This could explain that the soils restored initially with sludge (SS, Mix1 and Mix2) in the quarry had also higher TN content than the other restored, natural and control soils (Table 3.1). Nitrogen has a strong influence on the soil carbon cycle and has been described as a priming effect modulator that also contributes to activating the microbiota in decomposing organic matter (Chen et al., 2014) and favouring CO_2 emission to the atmosphere. Given that much of TOC in sewage sludge consists of labile organic compounds such as carbohydrates and fatty acids, the high rates of mineralization previously found in the experimental plots with sludge (Soria et al., 2021a) could rapidly release CO_2 into the atmosphere (Figure 3.4; Table 3.2) and, with the concomitant effect of climate change, could rapidly reduce the carbon stock applied by this amendment in restored soils.

However, interestingly, monitoring CO_2 emissions for a year and a half showed that these emissions decreased extraordinarily sharply since the first field measurement campaign (FieldC1), especially from soils amended with sludge (Table 3.3). Furthermore, CO_2 emissions from all amended soils continued to decline sharply in successive field campaigns; and, curiously, at the end of the studied period, all restored soils reached generally similar CO_2 emission rates that were lower than those in the natural soils (NAT) (Figure 3.4). These conclusions are supported by the absence of significant differences in TOC content between all restored soils, ranging the TOC from 3.08 ± 0.20 in SS soils to 2.65 ± 0.03 in CG and 3.37 ± 0.17 in CC soils, one year and a half after the amendments were applied. These results suggest a very efficient biomineralization of organic matter in the functioning of the carbon cycle in arid and semiarid Mediterranean ecosystems and a short-term stabilization of resilient organic matter provided by organic amendments in all restored soils.

Nevertheless, CO_2 emission from soils restored just after the application of both compost types (CG and CC) presented a different behaviour from that in the soils restored with sludge (SS) or their mixtures (Mix1 and Mix2). Curiously, the CG-restored soils showed the lowest rate of CO_2 emissions just after the amendment applications, comparable to that of the CO_2 emission from unamended control soils (CON), although CG soils had a slight upturn in CO_2 emission after rainfall over the last monitored month (Figure 3.4, Table 3.3). This result was very striking because the low CO_2 emission rates from CON soils could be explained by the extremely low content of TOC and TN (Table 3.1), but the CG- restored soils presented a high TOC that did not differ significantly from that of the SS-restored soils, although there were significant differences throughout the sampling period in the TN content (Table 3.1). The small peak of CO_2 emission from

CON soils could be due to the high content of carbonates and bicarbonates typical of arid and semiarid carbonate soils, which, when dissolved, could cause some CO_2 emission (Shrestha and Lal, 2006). Nevertheless, the extremely low priming effect in CG restored soils could be due to the composition of the composts applied to the soils. CG compost was made from the remains of pruning waste and plant remains, so it could be rich in lignin compounds, which are hardly biodegradable, and the microorganisms would need a period of adaptation by the bacteria to proceed with their decomposition (Kuzyakov et al., 2000). The contribution of these recalcitrant organic compounds provided by this fertilizer could limit the access of microorganisms to decomposition to organic inputs (Shrestha and Lal, 2006) and favour slow-growing organisms over fast-growing bacteria because of competition or succession processes (Leon et al., 2006); thus, the microbial activity would be lower and consequently their respiration rates, generating lower CO_2 emissions. These results corroborate the lower mineralization and enzymatic activities involved in the C cycle in CG restored soils (Soria et al., 2021a). Therefore, the low CO_2 emission pattern in CG soils throughout the sampled period suggests that in these restored soils, organic matter mineralizes very slowly, gradually providing nutrients for plants (Soria et al., 2021a) but guaranteeing at the same time a carbon reserve in the medium or long term.

A similar pattern was observed in CC restored soils with the other type of compost from plant debris from greenhouses. Although these soils presented a high content of TOC and TN in FieldS1, like the soils with sludge (Table 3.1), the CO_2 emission rates were significantly lower than in the SS, Mix1 and Mix2 soils (Figure 3.4; Table 3.3). However, they presented higher emission rates than CG and CON soils. This behaviour could also be explained by the contribution made by the organic amendment's molecular composition to the soil. Compost made from greenhouse remains could provide compounds derived from lignin such as leaves and stems, which are difficult for soil microorganisms to biodegrade but could provide more complex carbon compounds such as lignin, cellulose and hemicellulose (Cooperband, 2002). However, it could also provide other easily biodegradable organic compounds such as simple carbohydrates (polysaccharides and starches) from fruits (Cooperband, 2002). A balanced composition with labile and resilient organic compounds could explain a mineralization rate and concomitant CO_2 emission intermediate between the SS and CG treatments. Curiously, surrounding natural soils (NAT) showed CO_2 emission rates similar to those of CC restored soils, although their TOC and NT content was significantly lower (Table 3.1). This results could suggest that the CO_2 emissions from NAT soils could be attributed in part to the positive priming effect of plant inputs to substrate and root respiration (Crow and Wieder, 2005; Kuzyakov, 2006; Li et al., 2013) and the rhizosphere of readily available C supplies, known as the “rhizosphere priming effect” (Kuzyakov, 2002, 2006), which could change rhizosphere microbial activity.

Our results showed that, during a year and a half of monitoring CO_2 fluxes between soil-atmosphere, all soil types produced peaks of carbon dioxide emissions after rain events (Figure 3.3). Our statistical analyses corroborated the importance of soil moisture (M) in the CO_2 fluxes in the quarry, in addition to the soil properties, as mentioned above (Table 3.5). Soil moisture and temperature have been described as important modulators of CO_2 emissions (Kuzyakov, 2006; Miralles et al., 2018; Oyonarte et al., 2012; Ray et al., 2020). The rainfall was not constant in the study area (Figure 3.3) but responded to the pulses characteristic of arid and semiarid zones (Huxman et al., 2004a; López-Ballesteros et al., 2016; Oyonarte et al., 2012; Unger et al., , 2010). Then, the CO_2 emissions increased in relation to these observed rainfall pulses and increases in soil moisture, consequently, during the wet season compared with dry periods (Supplementary Figure 3.1). Moreover, these CO_2 emission peaks were more pronounced in soils with higher TOC and TN contents (restored soils and NAT) and especially in soils with higher contents of labile organic matter (SS, Mix1 and Mix2) and higher soil. In the contrary, the restored soils hardly showed differences in the CO_2 emission rates in dry periods, highlighting the important role that soil moisture plays in the CO_2 emission patterns in soils restored with organic amendments, as well as it was also confirmed by the significantly high positive correlation between soil moisture and CO_2 emission ($r = 0.74$). Nevertheless, curiously, as time elapses and the organic matter content decreased in the experimental plots, possibly as a result of the initial mineralization of labile organic compounds, it is also observed that the abovementioned pattern dissipated and the peaks CO_2 emission become milder, even in wet periods (Figure 3.4 and Supplementary Figure 3.1). This trend was clearly observed in the experimental plots with sludge and their mixtures (SS, Mix1 and Mix2), followed by compost CC. The influence of the wetting-drying pattern was difficult to observe in the soils restored with CG, although it is worthy to note that the soil moisture was lower in CG compared to previous amendments (Supplementary Figure 3.1). However, throughout the entire sampled period, CO_2 emission was practically non-existent in CON soils with low organic matter content even in rainy periods. The synergistic effect of easily biodegradable organic matter, nitrogen content, and soil moisture activate soil microbial communities, thus beginning to mineralize efficiently organic compounds and emitting CO_2, which is released from soil microbial respiration as mentioned above. Application of organic amendments guarantees the immediate increase of the soil water retention capacity (Zancada et al., 2004), as was supported by our statistical analysis (Table 3.1; Figure 3.3), since the increase of TOC improves the formation of soil structure and stable aggregates and increases the soil porosity favouring water infiltration (Miralles et al., 2009). This contributes to increasing the moisture content (M) in restored soils with respect to control soils (CON), especially after rain events (Table 3.1; Figure 3.3). In dry periods, CO_2 emissions decreased considerably (Supplementary Table 3.1; Table 3.4) suggesting that soil moisture could be considered as an essential limiting factor in CO_2 fluxes, although it depended also on the type of restoration treatment and the organic matter composition. Soil moisture plays a key role in microbial communities (Moyano et al., 2013) because microorganisms depend on water to support their normal cell activity (Angel and Conrad, 2013). Nevertheless, curiously, although temperature is also considered an important factor time-variant soil respiration and CO_2 emission (Buchmann, 2000; Conant et al., 2004; Li et al., 2013), our statistical results did not show that this parameter exerted a significant role in CO_2 emission from the analysed soils (Table 3.5), as even the negative correlation between T and CO_2 emission suggested (Table 3.4). This could be because the temperatures were similar in all rainy periods with a range of mild temperatures during rain events (Figure 3.3). Others authors have also described that there may be thermal acclimatization of soil microorganisms in arid and semiarid areas (Bradford et al., 2019; Dacal et al., 2019 -; Luo et al., 2001). Dacal et al. (2019) found this adaptation to temperature fluctuation in drylands around the world, and Zogg et al. (1997) reported that bacterial communities could change their function or composition by adapting to seasonal variations in temperature.

3.5. Conclusions

Compost from plant residues could be more suitable for application in the restoration of soils degraded by open-cast mining in limestone quarries, at least in terms of improving the physical and chemical soil properties in the short-term, with the consequent positive feedback in the recovery of soil quality and in turn, the soils restored with compost from gardening and from horticultural greenhouse crop, presented the lowest CO_2 emission rates, similar to unamended soils and to natural soils respectively. On contrast, the sludge amendments showed higher initial CO_2 emissions rates than in the rest restoration treatments, suggesting rapid mineralization of labile organic matter and the consequent feedback for global warming. Restoration treatments with mixtures from sludge and compost, could also be a good solution, since the CO_2 emission rates are higher at the beginning but their stabilisation is faster and therefore, this could mean that the contribution of sludge could give a first boost by providing organic matter that would stimulate the proliferation of soil microorganisms, while the plant remains would provide more stable organic matter over time.

Environmental factors and physical and chemical soil properties influencing the soil biological activity played a key role in the CO_2 emission from all restored soils and natural soils, with the soil moisture as the factor with the greatest weight in CO_2 emission rates followed by TOC, pH and TN. Nevertheless, the type of organic amendment and its chemical composition with labile or resilient organic compounds facilitating or hindering soil mineralization processes also played an important role in CO_2 emission from soils restored with organic amendments from residues recycled. These results could suggest a very efficient biomineralization of organic matter and stabilization of organic matter provided by organic amendments in the short term in a quarry from arid and semiarid Mediterranean ecosystems.

Overall, addressing this recovery through the application of organic amendments is appropriate, because increased the organic matter content, improve the water retention and stimulate the soil microorganisms compared with unamended soils. Long-term studies are necessary to obtain information on the dynamics of carbon cycle in highly degraded ecosystems from arid and semiarid zones, restored with organic residues recycled with different chemical composition.

Chapter 4

Organic amendments from recycled waste promote short-term carbon sequestration of restored soils in drylands

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Abstract

Soils are considered as a major reservoir for terrestrial carbon and it can act as a source or sink depending upon the land management activities. In semi-arid areas, the natural recovery of soils degraded by mining activities is complicated. A possible solution to recover soil quality and functionality, plant cover and carbon sequestration capacity could be the application of organic amendments. This work focuses on a restoration carried out in 2018 by applying with differentcomposted organic amendments (stabilized sludge, gardening and greenhouse waste) in a limestone quarry under semi-arid climate (SE Spain). The objective was to evaluate the effects of different organic amendments on net CO_2 *exchange in two microcosms: soil-Stipa tenacissima and soil- spontaneous vegetation. Soil physical and chemical properties, environmental and ecological variables and their interrelationship were studied in amended and unamended soils. The results obtained under soil-forming factors in the study area showed an increase in soil organic carbon and nitrogen content, improved moisture and plant growth, and plant canopy development in amended soils. Soil moisture, soil temperature and plant cover significantly influenced net* CO_2 *exchange. In general, microcosms with S. tenacissima showed higher carbon sequestration rates than soils with only spontaneous plant cover. Soils treated with a vegetable-only amendments showed higher plant cover and* CO_2 *fixation rates after significant rainfall. On the other hand, the plots treated with sludge compost presented more soil respiration than photosynthesis, especially in the wet seasons. Soils with sludge and greenhouse compost mixed had higher* CO_2 *fixation rates than soils restored with a mixture of sludge and garden compost. Soils with greenhouse waste compost showed* CO_2 *fixation in the microcosm with plants in all campaigns, being the best treatment to promote atmospheric* CO_2 *sequestration in soil restoration.*

4.1. Introduction

Land surface together with the vegetation hosted by them play a vital role in the global carbon (C) cycle (Ray et al., 2020). Particularly, the large area occupied by semi-arid regions combined with their vulnerability to degradation (Prăvălie, 2016) cause these areas to be considered critical environments of great importance for integrating new climate change mitigation and adaptation strategies. The situation of drylands is aggravated by various anthropogenic disturbances, such as open-pit mining. Degraded soils resulting from this type of human activities lose the capacity to sequester organic C (Miralles et al., 2007), due to the great impact of removing vegetation cover and the most fertile edaphic horizons (Frouz, 2021; Luna et al., 2016b; Moreno-de las Heras, 2009), negatively impacting climate change as sources of CO_2 emissions to the atmosphere (Oertel et al., 2016).

Hence, the recovery of degraded soils in arid and semi-arid zones is necessary to restore soil quality and its functionality as a C sink to these soils. The most common restoration practices carried out have included topographic modeling, the extension of a soil layer and the subsequent establishment of vegetation (Carabassa et al., 2020a), traditionally revegetating without much attention to adverse conditions resulting from mining (Asensio et al., 2014). In this sense, the improvement of biological, physical and chemical properties as well as the soil functionality could be achieved through incorporation of organic matter from organic amendments (Abdelhafez et al., 2018). Specially, amendments increase the activity of soil microorganisms (Bastida et al., 2013b) and favor the growth and maintenance of plant cover (Muñoz-Rojas et al., 2016). As a consequence, these soils restored with organic amendments can improve the soil capacity to act as a C sink (Ojeda et al., 2015). Furthermore, the utilization of organic matter from recycled waste to degraded soils contributes the local circular economy (Wainaina et al., 2020), promoting improved soil health and ecosystem services (Williams et al., 2020).

Soil can behave as a source or sink of CO_2 and contribute to carbon sequestration as well as plants and other organisms living in the soil (Ray et al., 2020). Thus, soil C sequestration capacity or net ecosystem CO_2 exchange (NEE), considering soil and vegetation, will be mediated by both carbon input from photosynthesis (Yue et al., 2021) and soil C turnover (Ray et al., 2020). As well, the decomposition and sequestration of soil organic matter (SOM) and CO_2 emission depends on environmental (soil moisture, temperature, soil type, etc.) and ecological factors, especially vegetation cover and soil microbial communities activity (He et al., 2022; Miralles et al., 2018; Niu et al., 2020; Rodríguez-Berbel et al., 2022). In addition, the chemical composition of the organic matter will be determined by its origin, also influencing its decomposition (Hueso-González et al., 2018) and regulation of microbial metabolism (Li et al., 2021). In turn, plant photosynthesis and transpiration directly affect soil water and nutrient status, and concomitantly CO_2 fluxes (Yue et al., 2021), mainly referring to total ecosystem respiration, including vegetation-associated autotrophic and heterotrophic respiration linked to soil fauna and microbiota (Huang et al., 2018). Therefore, the difference between carbon inputs (C assimilation) and carbon outputs (C emission) informs the NEE (Azeem et al., 2019). Different authors agree that the addition of organic amendments in degraded soils has an impact on soil properties and soil CO_2 fluxes in semiarid ecosystems (Lamptey et al., 2019; Marín-Martínez et al., 2021; Soria et al., 2021b), enhancing biological activity, improving the functioning of biogeochemical cycles and fertility (Bastida et al., 2008b; Miralles et al., 2021a; Rodríguez-Berbel et al., 2021b; Rodríguez-Berbel et al., 2022) as well as accelerating the regeneration of plant communities and C sequestration through photosynthesis (Caravaca et al., 2002; Soliveres et al., 2012). However, studies analyzing the NEE in soils restored with organic amendments of residues are really scarce (Soria et al., 2021b; Yue et al., 2021).

Our hypothesis is that soils restored with different organic amendments colonized by a wild vegetation cover and with plants introduced in the restoration, such as *Stipa tenacissima*, could favor CO_2 fixation. The aim of this work was to determine which restoration treatment with organic amendments derived from different recycled local organic waste is more sustainable to increase CO_2 fixation. Furthermore, to identify whether soils degraded by mining in a semi-arid climate could function as potential C sinks by applying an appropriate management practice. For this purpose, two and a half years after the restoration in a limestone quarry by application of organic amendments, the net ecosystem CO_2 exchange (NEE) was estimated using a closed transient-state chamber that enable measurements at soil and whole-plant level. Nineteen NEE measurement campaigns were conducted under different climatic conditions and changes in different properties of the amended soils were monitored at different seasons during the study period.

4.2. Material and methods

4.2.1. Overview of study site

The experimental area is located in the Gádor mountain-range (Almería, SE Spain; 36°55'20"N, 2°30'29"W). The soil resulting from mining activity of the study area is formed by a mixture of calcareous rock and loams derived from open-cast mining activity. The rock extracted from the quarry is mainly calcareous sandstones overlain by marls (Luna et al., 2018). The resulting substrate after mining corresponds to a loamy soil where vegetation development is difficult. In surrounding unspoiled area, soils are mainly Regosols (FAO-IUSS-ISRIC Working Group WRB, 2015). The study area presents a semi-arid Mediterranean climate with a relatively low aridity index (Luna et al., 2018). It is characterized by a high interannual meteorological variability, consisting of very dry summers and irregular and scarce rainfall episodes produced mainly during the autumn and winter months. The mean annual precipitation in the area is 242 mm (data obtained from AL003 weather station, Junta de Andalucía). The average annual temperature is 17.6 ºC, with maximums in July (31 ºC) and minimums in February (8 ºC) (Luna et al., 2016b). Evapotranspiration has been estimated at 1,225 mm year^{-1} and, the solar radiation has daily maximums around 1000 W m^{-2} during the months of April to September, while, in the winter months, the maximums range between 500 and 600 W m^{-2} (Luna et al., 2018). Prior to mining, the experimental area was dominated by a grassland formed by native vegetation whose most abundant species was *Stipa tenacissima* L., *Anthyllis terniflora* (Lag.) Pau., *A. cytisoides* L. and *Olea europea* L., among others (Luna et al., 2016b).

4.2.2. Experimental design and sampling

The total restored area for the experimental design occupied about 1,415 m^2 where eighteen plots of 50 m^2 (10 m x 5 m) were delimited on totally degraded soils without slope of a limestone quarry at an altitude of 362 m above sea level. Previously, the substrate has been decompacted and homogenized using heavy machinery. Each plot was separated from the others by a corridor (1 m wide) to facilitate the treatment application by machinery (Figure 4.1). The experimental area was homogeneous from the physiographic point of view (original soil, microclimate, slope and orientation). Therefore, the differences or similarities between the soil samples should not be attributed to their spatial distribution, but to the treatments applied, which are described below. Five treatments of organic residues of different origin and chemical composition were randomly applied and homogenized with the substrate by backhoe in the first 20 cm of soil surface in each experimental plot (3 replicates per treatment; Figure 4.1). The organic amendments used to soil restoration were used to increase the organic matter up to 3% and their application doses were calculated from the dry organic matter content of each amendments (Table 4.1): i) vegetable compost derived from garden waste (CG), ii) vegetable compost from greenhouse crop waste (CC), iii) sewage sludge waste treated by mesophilic digestion, thermal dehydration at 70 ºC, and centrifugation (SS), iv) the mixture CG + SS (Mix1), and v) the mixture CC + SS (Mix2). Three plots without organic amendments, considered as control plots, were also installed. All organic amendments came from local companies or companies close to the study area and were dedicated to the collection, transportation, management and transformation processes of waste. More information about the study area is found in Soria et al. (2021).

Table 4.1. Main chemical characteristics of organic amendments used (A) and compost application rate (B).

A)	**CG**	**SS**	**CC**	**CON**
pH	8.6	7.19	8.02	8.25
Electrical conductivity ($mScm^{-1}$)	4.28	3.41	6.37	1.85
Total organic carbon (%)	31.4	66.4	59.15	0.2
Total nitrogen (%)	1.64	5.49	1.87	0.03
C:N ratio	14.3	7.01	18.34	6.66

B)	**Total organic C on dry weight (%)**	**Moisture (%)**	**Dosage (kg m^{-2})**
CG	40.5	22.5	28.66
SS	66.4	8.8	14.17
CC	43.33	40	34.61
Mix1	(CG +SS)		14.33 + 7.73
Mix2	(CC+SS)		17.30 + 7.73

CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; CON original substrate without amendments. Mix1 = CG + SS and Mix2 = CC + SS.

The revegetation of the plots was carried out by planting two native plant species: *Stipa tenacissima* L. Kunth. (40 plants per plot) and *Olea europea* L. var. *sylvestris* Brot. (10 individuals per plot) making a total of 50 plants per plot. An establishment irrigation was performed in order to ensure the highest survival of the planted vegetation (Cortina et al., 2011; Valdecantos et al., 2014). Subsequently, the plants were not watered again, receiving only rainwater. The frequency and volume of rainfall were monitored by recording every 20 minutes using a tipping bucket rain gauge (Pronamic ApS, Denmark) installed in the study area. The ambient pressure, maximum, minimum and average daily temperature was obtained from the station number ESAND0400000004410A (https://www.meteoclimatic.net).

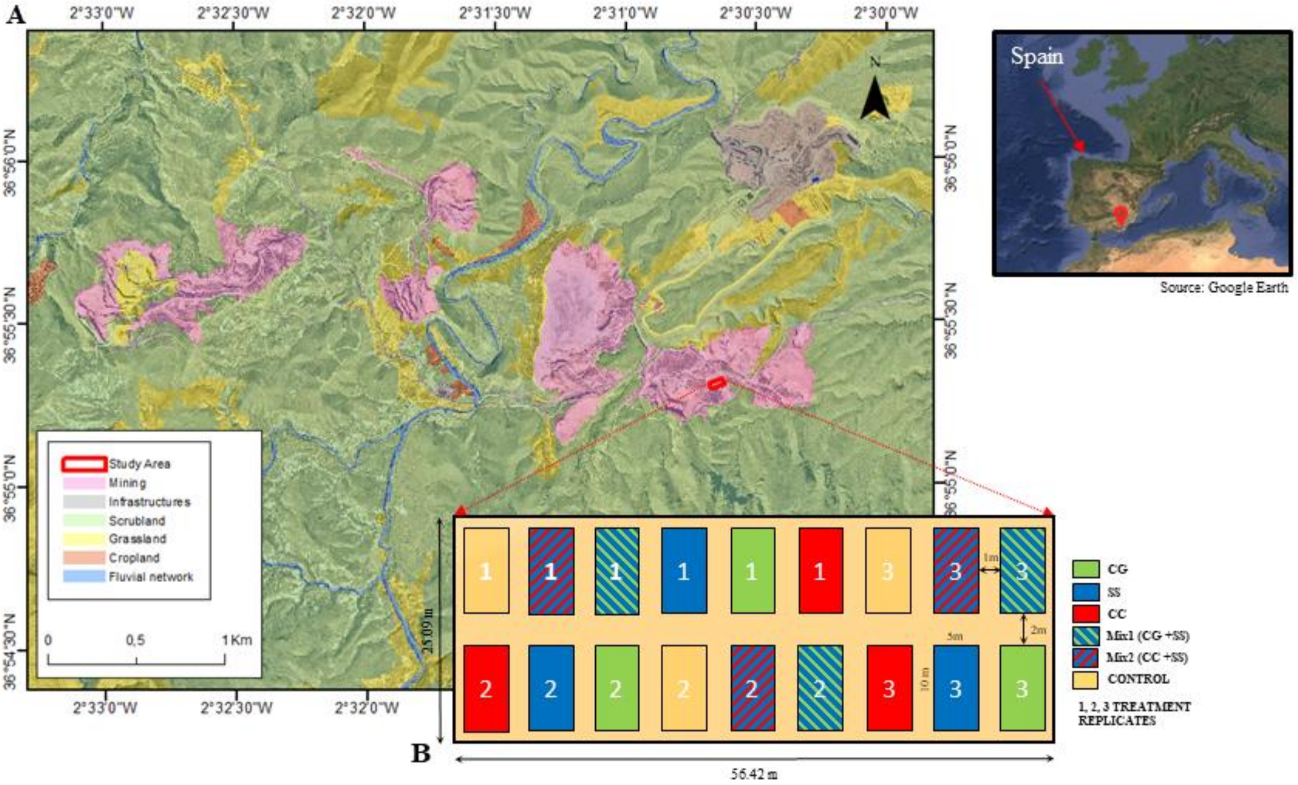


Figure 4.1. Location of the experimental site (A) and experimental layout with plots (B). Data source: Spanish Government National Aerial Orthophotography Plan (PNOA), 2021. CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; Mix1: SS + CG; Mix2: SS + CG; CON: no-amendment soils.

4.2.3. Soil property analysis

Three soil sampling campaigns were conducted for the analysis of soil physical and chemical properties at the end of each season: i) autumn (C1), ii) winter (C2) and iii) spring (C3) seasons. For the collection of soil samples, 10 samples were taken randomly from the first 20 cm of topsoil to form a composite sample in each of the restored plots. Subsequently, the samples were air-dried and sieved through a 2 mm screen. The following soil properties were analyzed in each of these soil sampling campaigns (C1, C2 and C3): i) soil pH was measured on a soil water suspension (1:2.5 soil/water ratio) using a pH-meter (LAQUA PH1100, HORIBA, Tokio, Japan); ii) electrical conductivity (EC) was determined in an aqueous suspension 1:5 soil/water using a digital conductivity meter (LAQUA EC1100, ORIBA, Tokio, Japan); iii) available water content (AW) was determined from measurements of pF at −1500 and −33 kPa by the Richards membrane method (Richards, 1941); iv) soil organic carbon (SOC) was calculated by rectified method of Walkey and Black (1934) (Mingorance et al., 2007); iv) total nitrogen content (TN) was obtained by total combustion (Vario Rapid N; Elementar, Hanau, Germany); and v) C/N ratio was calculated from SOC and TN values.

4.2.3. CO_2 exchange measurements

Nineteen measurement campaigns of net ecosystem CO_2 exchange (NEE) for each plot containing soil and plants were performed during sunny days from November 2020 to June 2021 (Figure 4.2 and Supplementary Table 4.1 on annexes). NEE measurement campaigns were carried out with a maximum interval of 1.5 months and with campaigns closer in time when there were important rainfall events. Measurements were made from 9 a.m. to 14 p.m. on different but consecutive days in order to cover all the treatments. To minimize the effect of temperature and radiation on NEE, treatment replicates were sampled in a sequential order, sampling a first replicate of each treatment, then the second replicate of treatments, and finally the third replicate. Parallel campaigns of NEE were carried out on separate but consecutive days differentiating two groups: i) soils treated with CG, CC and SS and on soils without amendments (T-CON; 10 campaigns, "T" in Supplementary Table 4.1) and ii) 9 campaigns on soils treated with mixtures (Mix1 and Mix2) and M-CON soils ("M" in Supplementary Table 4.1; Figure 4.2). The plots of soils without added amendments used as controls were always the same, named as T-CON in the amendment campaigns (T) and as M-CON in the mixture measurement campaigns (M). In each of the campaigns, the NEE was studied in two types of microcosms: i) *Stipa tenacissima* individuals (St); and ii) soil (Sc), including wild weeds cover if present. The microcosms were randomly selected in each plot of each treatment as well as in the control plots.

A transparent chamber (50 x 50 x 60 cm) coupled to an infrared CO_2 gas analyzer (Li-840a, Li-Cor, Lincoln, NE, USA), two small fans, a thermocouple (PT100), and a data logger (CR1000, Campbell Sci., Logan, UT, USA) was used to obtain NEE for each plot containing soil and plants (López-Ballesteros et al., 2016; Salazar-Tortosa et al., 2018). Before each measurement the chamber was opened and ventilated so that initial air composition and temperature represent natural atmospheric conditions. Once the chamber was placed on the ground, it was surrounded at the bottom by a non-breathable textile to ensure sealing and minimize any air ingress or leakage. All the variables were recorded at 1 Hz for 5 minutes, so a total of 300 data were collected for each microcosm. The T4 campaign was not considered in the statistical analysis due to sensor malfunction. We estimated NEE by measuring changes in CO_2, H_2O and air temperature through time using the slopes of a linear regression (López-Ballesteros et al., 2016; Pérez-Priego et al., 2015). Negative NEE data indicate net CO_2 fixation, while positive data show net CO_2 emission to the atmosphere. The vapor pressure deficit (VPD) was determined from the quotient of the pressures saturating water vapor pressure and water vapor pressure, which was considered as an index of water stress within the ecosystem (Ramírez et al., 2006).

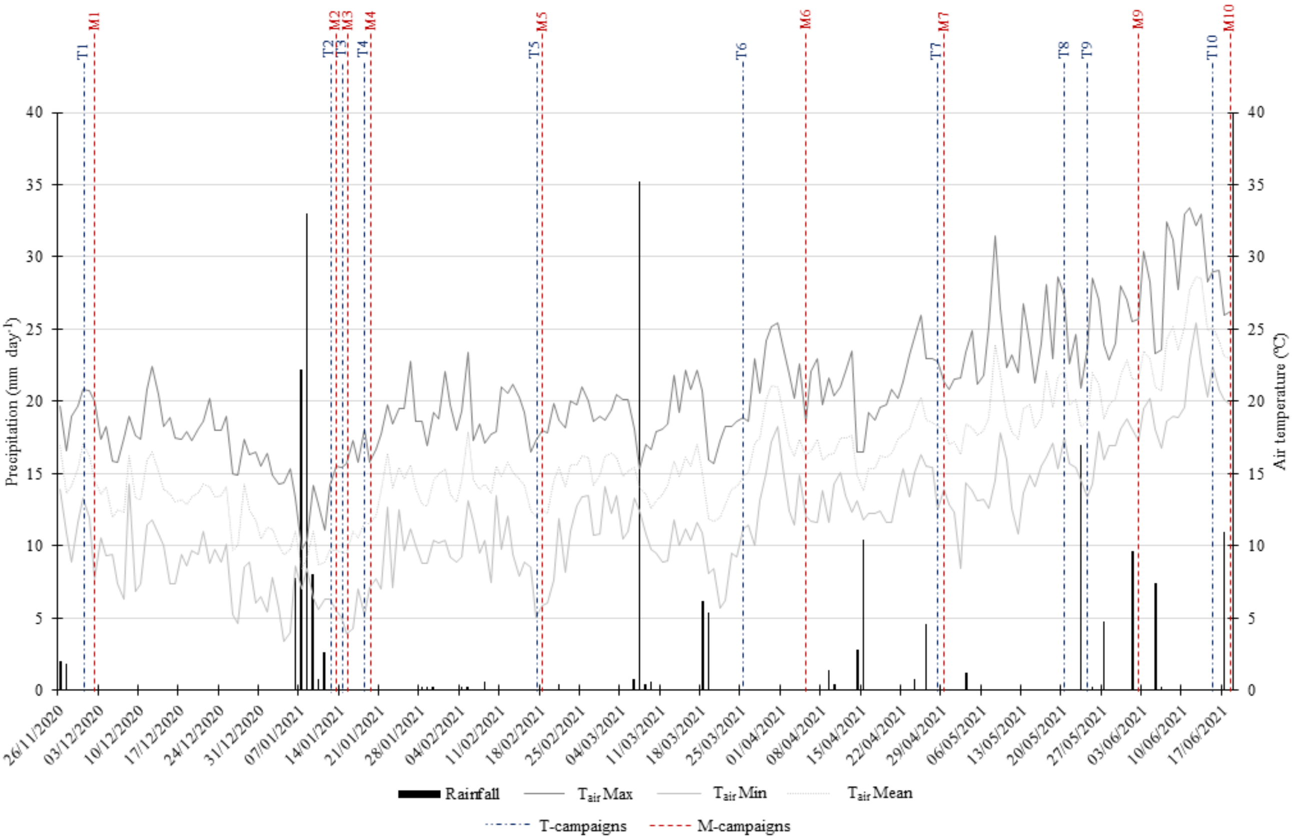


Figure 4.2. Time series of daily precipitation, maximum, minimum and mean air temperature (Tair). TairMax: Maximum Tair; TairMin: Minimum Tair; TairMean: mean Tair. The dotted blue lines show each of the T-campaigns in which the treatments (CG, SS and CC) and the control soils (T-CON) were measured, while the dotted red lines show the M-campaigns in which both mixtures of the treatments and the control soils (M-CON) were measured. CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; Mix1: SS + CG; Mix2: SS + CG; CON: no-amendment soils.

4.2.4. Environmental and ecological variables

In addition to the data collected by the gas analyzer in each microcosm (*Stipa tenacissima*: St and Soil: Sc), data were taken for: i) soil moisture (θ) at a depth of 3 cm measured with a portable handheld readout sensor (ProCheck, Decagon Devices, Inc., Pullman, WA, USA); ii) soil temperature (T_{soil}) at a depth of 3 cm determined with the same sensor to measure θ; and, iii) plant cover was determined by a square plot of 50 x 50 cm (0.25 m^2) and expressed as the percentage of soil occupied by wild plants and/or *S. tenacissima*, meaning that all vegetation within the space enclosed by the measuring chamber when placed on the ground was considered. Moreover, the biovolume of *S. tenacissima* plants analyzed in each St-microcosm was calculated from the height and maximum and minimum width as height multiplied by mean width (Solé-Benet et al., 2009).

4.2.5. Statistical analyses

To determine significant differences in all variables (physical and chemical soil properties, NEE, VPD, environmental and ecological parameters) between restored and control soils, an analysis of variance (one way ANOVA, $p < 0.05$) was used together with a post hoc analysis by a multiple range test using Fisher's LSD method at a probability level of 95% ($p < 0.05$). Principal component analysis (PCA) to assess the grouping of samples according to the physical and-chemical soil conditions by organic treatments and soils without any amendment. These analyses were obtained from Statgraphics (version 16.2.04). The graphical visualizations of ecological and environmental variables together with the cumulative NEE per treatment obtained from the different measurement campaigns (T and M campaigns) were made with Microsoft Excel 2010. The NEE field measurements were always analyzed and shown in the results by differentiating one group with the amendments (CG, SS and CG and T-CON) and another with the mixtures (Mix1, Mix2 and M-CON) separately due to the difference between the days of measurement. The cumulative NEE was considered as the sum of all negative (C sequestration) and positive (C emission) data for each treatment for T-Campaigns and M-campaigns. The overall NEE balance for each treatment was estimated as the sum of the positive and negative values obtained in the cumulative NEE calculation for individual treatment.

A liner model Redundancy Analysis (RDA) was performed to graphically visualize the relationship of the NEE and VPD with ecological variables measured (θ, T_{soil}, plant cover and biovolume) in each type of microcosms (St and Sc). To study those environmental and ecological variables that had the greatest influence on NEE and VPD a DistLM (Distance-based Linear Model) was done using best selection process and the Akaike Information Criterion (AICc) (Akaike, 1974), in order to determine the best model to explain the behavior of NEE and VPD. Moreover, to identify the influence of the independent variables that explain the variations in the samples of the different restored soils and microcosms (St and Sc) in each field campaigns (C1, C2 and C3), a "marginal" test was performed to show the relationship between the response of the NEE and VPD variables and the individual variables (θ, T_{soil}, plant cover and biovolume). The statistical package PRIMER+PERMANOVA for windows (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) was used to perform these analyses. From Statgraphics (version 16.2.04), Pearson's correlations (r) were also used to evaluate the relationship between physical and chemical soil properties as well as environmental and ecological parameters between different treatments and microcosms in the field sampling campaigns.

4.3. Results

4.3.1. Physical and chemical properties of restored and control soils

In general, the addition of organic amendment caused changes in the physical and chemical soil properties (pH, EC, AW SOC, TN and C:N ratio) in restored soils between field sampling campaigns (C1, C2 and C3), while the untreated soils (CON) barely presented change throughout the sampling time (Table 4.2). Restored soils clearly increased EC, SOC and TN, while slight differences in pH and AW were observed.

Amendments and untreated soils showed an alkaline pH in all campaigns. Although the pH in the restored soils pH was significantly lower ($p < 0.05$) than in CON soils, with exception of CC soils which were similar to CON values (Table 4.2). SS showed a slight decrease in pH in C1 and C3 with respect to the rest of the amendment and untreated soils (Table 4.2). All restored soils presented a high EC in the C1 campaign, which decreased with time. CG, SS, Mix1 and Mix2 showed the highest EC values and similar to CON, while CC obtained the lowest values compared to the rest of the treatments and CON soils in all campaigns (Table 4.2). Both, restored and CON soils hardly showed any differences in available water (AW) in the different field sampling campaigns. However, in C2 soil sampling campaign, there was more AW for vegetation than in the rest of field sampling (Table 4.2).

Application of organic amendments significantly increased ($p < 0.05$) SOC and TN contents with respect to CON in all soil sampling campaigns (Table 4.2). The highest SOC and TN values were reported in SS, CC, Mix1 and Mix2 treated soils followed by CG, whereas CON soils presented the significantly lowest values of SOC and TN contents in all field campaigns (Table 4.2). The restored soils also showed a significantly lower C:N ratio than CON soils during the whole period, especially in C1. Moreover, the increase of C:N ratio in subsequent measurements field sampling campaigns, but always significantly lower than non-restored plots (CON). SS soils and their mixtures (Mix1 and Mix2) were similar in C1, showing significantly lower values than soils amended with plant wastes (CG and CC) although these differences were minimized in C2 and C3 campaigns (Table 4.2).

Table 4.2. Physical and chemical soil properties of restored and unrestored soils (mean ± SEM [n=3]) in each field sampling campaing.

Campaing	Treatment	pH	EC (mS/cm)	AW (%)	SOC (%)	TN (%)	C/N
C1	**CG**	8.28 ± 0.04 C b	4.22 ± 0.51 DFGH a	16.7 ± 0.64 ABCD a	2.29 ± 0.11 BC b	0.28 ± 0.01 BC b	8.15 ± 0.44 ABCD b
	SS	7.92 ± 0.02 AB a	6.02 ± 0.30 H a	16.4 ± 1.04 ABC a	2.81 ± 0.12 CDEF c	0.47 ± 0.01 FGH d	5.96 ± 0.22 AB a
	CC	9.04 ± 0.10 DE c	4.14 ± 0.76 EFG a	15.3 ± 0.51 A a	3.43 ± 0.08 FGHI e	0.40 ± 0.02 DEFG c	8.53 ± 0.46 ABCD b
	Mix1	8.01 ± 0.05 ABC a	5.95 ± 0.43 GH a	14.8 ± 1.41 A a	3.23 ± 0.08 EFGH de	0.50 ± 0.01 H d	6.43 ± 0.28 AB a
	Mix2	8.24 ± 0.08 BC b	4.41 ± 0.48 FGH a	16.1 ± 1.75 ABC a	2.86 ± 0.21 CDEF cd	0.49 ± 0.01 GH d	5.72 ± 0.27 A a
	CON	8.95 ± 0.04 DE c	4.36 ± 1.20 FGH a	17.1 ± 2.66 ABCDE a	0.53 ± 0.03 A a	0.05 ± 0.00 A a	10.5 ± 0.56 DE c
C2	**CG**	8.24 ± 0.10 BC a	4.17 ± 0.55 DFGH d	20.9 ± 0.76 DE a	2.68 ± 0.09 CDE b	0.27 ± 0.01 BC b	9.73 ± 0.21 CD a
	SS	8.12 ± 0.01 BC a	2.85 ± 0.52 ABCDEF bc	20.0 ± 0.84 BCDE a	3.21 ± 0.28 EFGH bc	0.41 ± 0.05 DEFGH bc	7.97 ± 0.51 ABCD a
	CC	9.83 ± 0.13 F b	1.06 ± 0.20 A a	21.1 ± 2.60 E a	3.76 ± 0.68 GHI c	0.38 ± 0.06 DEF bc	9.93 ± 0.49 CD a
	Mix1	8.21 ± 0.18 BC a	3.64 ± 0.75 CDEF d	20.2 ± 0.66 CDE a	3.23 ± 0.18 EFGH bc	0.36 ± 0.04 CDE bc	9.09 ± 1.40 BCD a
	Mix2	8.26 ± 0.14 C a	1.97 ± 0.47 ABC ab	20.6 ± 0.92 DE a	3.88 ± 0.15 HI c	0.47 ± 0.02 FGH c	8.18 ± 0.19 ABCD a
	CON	9.09 ± 0.11 E b	1.61 ± 0.35 AB ab	20.8 ± 0.79 DE a	1 ± 0.04 A a	0.05 ± 0.01 A a	18.5 ± 3.78 F b
C3	**CG**	8.29 ± 0.14 C bc	3.42 ± 1.08 BCDEF ab	17.3 ± 1.53 ABCDE ab	1.92 ± 0.19 B b	0.21 ± 0.02 B b	9.02 ± 0.72 BCD a
	SS	7.75 ± 0.12 A a	3.81 ± 0.89 CDEF ab	16.8 ± 1.27 ABCD ab	3.12 ± 0.18 DEFG c	0.47 ± 0.06 FGH e	6.69 ± 0.73 ABC a
	CC	8.72 ± 0.20 D bc	1.70 ± 0.26 AB a	16.0 ± 1.86 AB ab	2.44 ± 0.34 BCD bc	0.27 ± 0.03 BC bc	8.74 ± 0.35 ABCD a
	Mix1	8.09 ± 0.10 ABC ab	3.98 ± 0.63 DEF b	15.2 ± 0.60 A a	2.97 ± 0.42 CDEF c	0.35 ± 0.04 CD cd	8.44 ± 0.55 ABCD a
	Mix2	8.17 ± 0.07 BC ab	2.16 ± 0.21 ABCD ab	17.5 ± 0.23 ABCDE ab	4.02 ± 0.22 I d	0.45 ± 0.02 EFGH de	8.87 ± 0.11 ABCD a
	CON	8.85 ± 0.16 DE c	2.45 ± 0.87 ABCDE ab	20.2 ± 2.48 CDE b	0.51 ± 0.05 A a	0.03 ± 0.00 A a	13.3 ± 1.99 E b

CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; Mix1: SS + CG; Mix2: SS + CG; CON: no-amendment soils. Season campaigns: C1: autumn; C2: winter; C3: spring. The capital letters indicate the significant differences obtained by ANOVAS ($p < 0.05$) between all the data collected (treatments and field sampling campaign), and the lower-case letters show the differences between treatments by field sampling campaign. EC: electrical conductivity; AW: available water; SOC: soil organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio.

Principal component analysis showed a cumulative variability percentage for the first and second components (hereafter PC1 and PC2, respectively) of 72%, describing most of the variation between the physical and chemical soil properties (EC, pH, AW, SOC and TN) in the different sampling campaigns (C1, C2 and C3). PC1 explained 50.8% and PC2 21.2% of the values variability (Figure 4.3; Supplementary Table 4.2). Structure present along the first two principal components was clearly determined by sampling campaign and organic amendment applicate type. PC1 clearly differentiated the sewage sludge treated soils (SS, Mix1 and Mix2) in the three sampling campaigns (left side in bigraph) from the control soils (CON) (right side), while soils treated with vegetable compost (CG and CC) were located in an intermediate position (Figure 4.3). On the other side, PC2 separated C1 and C2 campaigns of the soils analyzed, except for the soils treated with CC which took a higher position in the positive values of the axis. Chemical properties (SOC, TN and EC) showed the negative loadings in PC1 and mainly influenced in sludge soils amended (SS, Mix1 and Mix2). On the contrary, variables with positive loadings in PC1 (AW, pH and C/N) had a greater influence on CON soils in all campaigns (Figure 4.3).

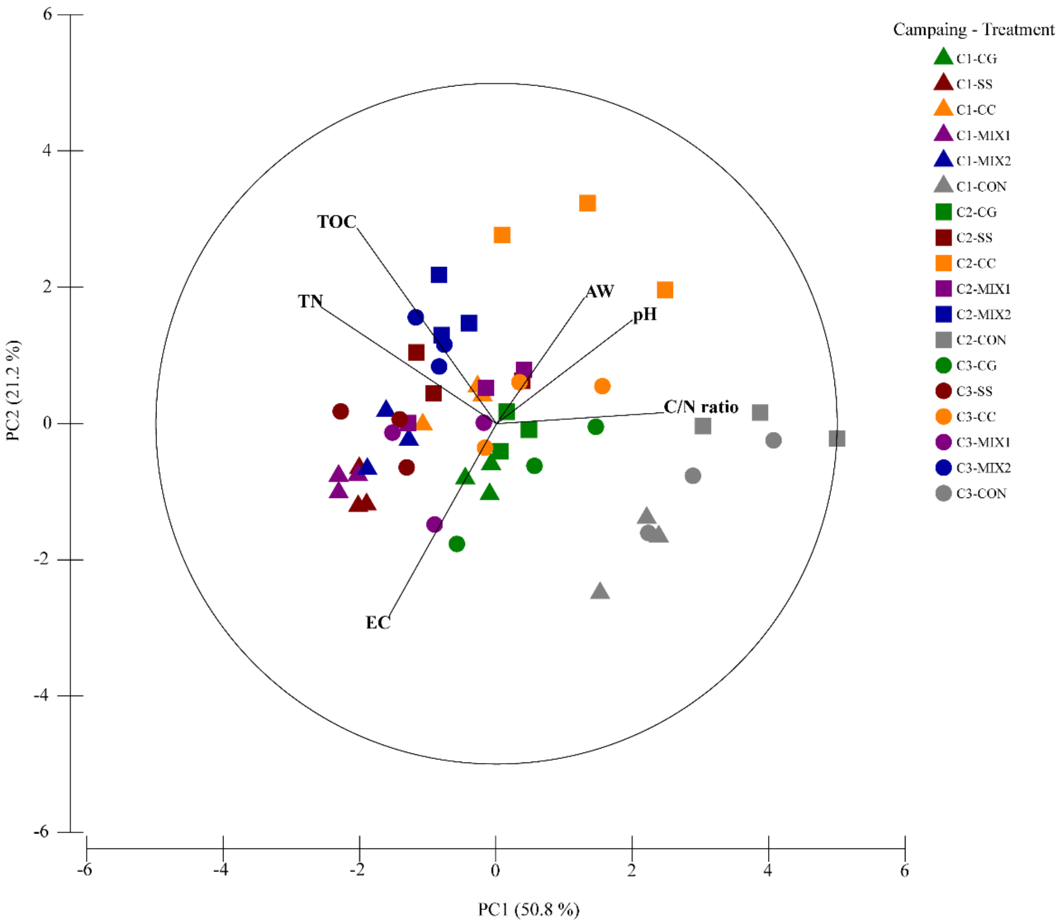


Figure 4.3. Principal component analysis. Biplot for the different restored and unrestored soils and physical and chemical soil properties per field sampling campaign. Campaigns: C1: autumn; C2: winter; C3: spring. CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; Mix1: SS + CG; Mix2: SS + CG; CON: no-amendment soils. EC: electrical conductivity; AW: available water; SOC: soil organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio.

4.3.2. Effect of organic amendments application on NEE exchange rate in reclaimed soils and different microcosm type.

The NEE measurement campaigns of both types of microcosms (S. tenacissima: St and Soil: Sc) were divided into two groups: treatments (T; CG, SS and CC) and mixtures (M; Mix1 and Mix2) compared to control soils named T-CON and M-CON, respectively, depending on the campaign to which they are compared (Figure 4.4). In general, soils with a single amendment (CG, SS, CC) and T-CON had higher cumulative CO_2 fixation rates (negative NEE) in St than Sc (Figure 4.4A). CC presented the higher carbon sequestration (negative NEE) in St-microcosm (average value (a.v): - 1.51 µmol CO_2 m$^-$

^{2} s^{-1}) between all treated soils, followed of SS (a.v.: -0.49 µmol CO_2 m^{-2} s^{-1}) and CG (a.v.: -0.43 µmol CO_2 m^{-2} s^{-1}). However, SS in turn showed the highest positive NEE values comparatively higher than the rest of treated and T-CON soils (Figure 4.3A). In Sc-microcosm, CG showed comparatively higher CO_2 emission rates followed by SS and CC (Figure 4.4A).

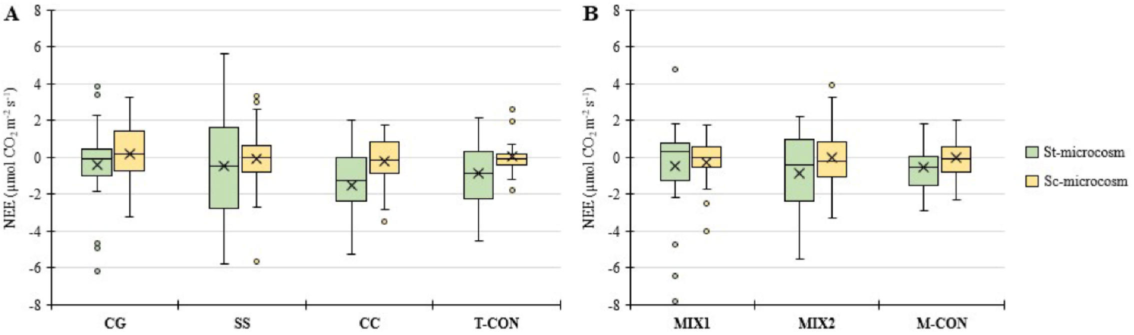


Figure 4.4. Box-and-whisker plots of the cumulative net ecosystem CO_2 exchange (NEE) in all field measurement campaigns and in the organic amendment treatments: A) soils measured in T-campaigns (CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost and T-CON: no-amendment soils); and B) soils measured in M-campaigns (Mix1: SS + CG; Mix2: SS + CG and M-CON: no-amendment soils). Each box represents the cumulative NEE balance per treatment over the entire measurement period. The top and bottom lines correspond to the first and third quartiles (interquartile range), while the middle line and the cross show the median and mean, respectively. The whiskers include values that deviate up to a maximum distance of 1.5 times the interquartile range. Values with a deviation greater than 1.5 times are represented as points.

Likewise, soils treated with the amendment mixtures (Mix1 and Mix2) and control soils (M-CON) showed negative NEE values in St-microcosm and higher fixation values than in Sc-microcosms (Figure 4.4B). Mix2 showed higher cumulative negative NEE (a.v: -0.89 µmol CO_2 m^{-2} s^{-1}) than the M-CON (a.v.: - 0.57 µmol CO_2 m^{-2} s^{-1}) and Mix1 (a.v.: - 0.44 µmol CO_2 m^{-2} ground area s^{-1}) in St-microcosm, the global NEE balance were lower to Mix1 compared to Mix2 and M-CON. Nevertheless, Mix2 presented higher CO_2 emission rate followed of Mix1, while it was very slight for M-COM (Figure 4.4B). In contrast, measurements for microcosm Sc showed an average value of -0.25 µmol CO_2 m^{-2} s^{-1} in Mix1, while Mix2 and M-CON presented a practically neutral balance (Figure 4.4B).

4.3.3. Study of environmental and ecological variables in the different NEE field campaigns.

The environmental variables (air temperature and precipitation) can be found in Figure 4.2. T1 was measured after the first rains in late autumn with air temperature between 15-20°C. The winter period (T2 to T4 and M2 to M4) presented intense rains during the month of January, coinciding with the lowest temperature values recorded, with mean values between 9 and 11.6 ºC (Figure 4.2). The rest of field campaigns registered sporadic light rain and occasional together with temperatures that increased as time progressed reaching mean values of 16 to 26.5ºC, especially in the last few campaigns during spring (T6 to T10 and M6 to M10) (Figure 4.2). Maximum temperatures above 30ºC were recorded in June, matching in M9 and T9 campaigns.

Soil temperature (T_{soil}) and soil moisture (θ) varied markedly with environmental changes. In all experimental plots, T_{soil} increased as the field campaigns progressed, while the highest θ values were recorded after rainfall (Figure 4.5). These same trends were observed for T_{soil} regardless of treatment and microcosm (St and Sc). Subsequently, T_{soil} increased progressively reaching 35-40ºC until the end of study period (Figure 4.5). It was observed that the precipitation amount prior to the measurement significantly affected θ (Supplementary Table 4.1). The greatest increase in θ was recorded after strong rainfall events in soils treated with SS during the winter and spring (T2, T3 and T9 respectively), while CG and CC showed lower θ percentage and similar to T-CON (Figure 4.5A and 4.5B, Supplementary Table 4.1). On the other hand, soils treated with organic amendment mixtures (Mix1 and Mix2) and control soils (M-CON) had a similar θ values to soil amended without mixtures (CG, SS and CC) during winter (M2 and M3), although the values were hardly increased in the M9 spring campaign (Figure 4.5C and 4.5D; , Supplementary Table 4.1).

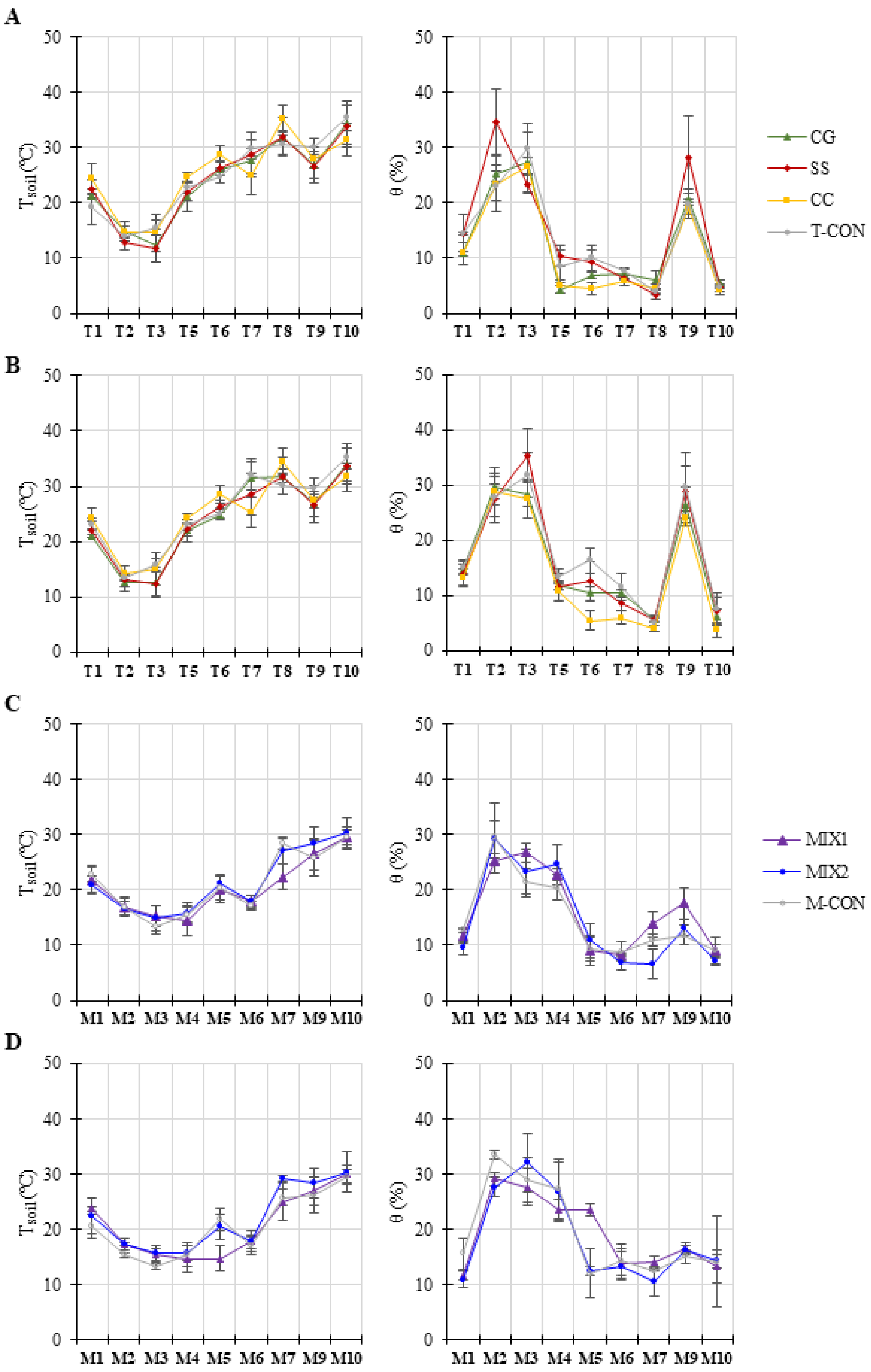


Figure 4.5. Average soil moisture (left) and temperature (right) per microcosm and field campaign. *Stipa tenacissima* (St) and Soil control (Sc). A) St-microcosms data in the different T-campaigns; B) Sc-microcosms data in the different T-campaigns; C) St-microcosms data in the different M-campaigns; D) Sc-microcosms data in the different M-campaigns. T-campaigns include: CG: soils restored with garden compost; SS: soils

restored with sewage sludge; CC: soils restored with greenhouse crop compost and T-CON: no-amendment soils. M- campaigns include: Mix1: SS + CG; Mix2: SS + CG and M-CON: no-amendment soils measured.

Plant cover development was also subject to weather conditions, significantly increasing the colonized area by wild plants as the study period progressed (Figure 4.6). All restored soils (CG, SS, CC, Mix1 and Mix2) showed significantly more vegetation cover development than control soils (T-CON and M-CON) in all field sampling campaigns, mainly from T7 and M6, respectively (Figure 4.6; Table S4). Amended plots reached 85-100% vegetative cover by the end of the study period (June 2021), while CON did not exceed 10% at best. The main wild plants that colonized the ground were *Moricandia arvensis* L., *Artemisia barrelieri* Besser., *Anthyllis cytisoides* L., *Limonium insigne* (Coss.) Kuntze., *Pistacia lentiscus* L. and *Capparis spinosa* L., among others. The highest plant covers were found mainly on St-microcosm soils (Figure 4.6A and 6B) above the Sc-microcosm (Figure 4.6C and 4.6D), as *S. tenacissima* represents a part of this plant cover. Regarding the biovolume of *S. tenacissima* in the St-microcosm (randomly chosen for each measurement campaign), soils treated with organic amendments usually had significantly higher plants biovolume than control soils (T-CON and M-CON; Figure 4.6A and 4.6B; Supplementary Table 4.4). SS showed the highest plant growth during the study, followed by CC and CG, showing no significant differences between them. Nevertheless, plants measured in the plots with mixture amendments presented a higher biovolume than the rest of the treatments (Figure 4.6B).

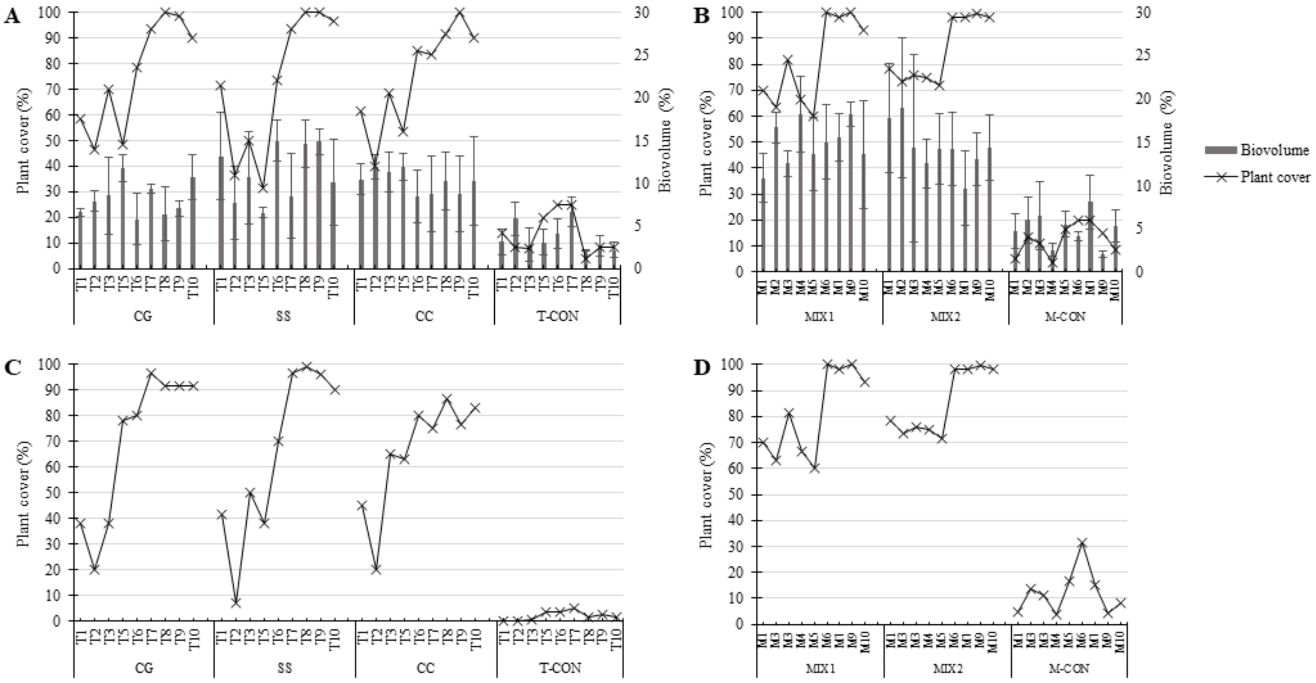


Figure 4.6. Mean biovolume of *Stipa tenacissima* and mean plant cover expressed as percentage recorded in the St-microcosms of the field measuring campaigns in soils treated with pure amendments (A) and mixtures of amendments (B) together with the corresponding control soils (T-CON and M-CON, respectively). Average plant cover in the Sc-microcosms in each field campaigns of treatments (C) and in soils with mixtures (D) with control plots. CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; Mix1: SS + CG; Mix2: SS + CG; T-CON and M-CON: no-amendment soils measured in the same campaign types to be taken as control versus restored soils with single treatments and mixtures, respectively.

4.3.4. Patterns of NEE and water stress index.

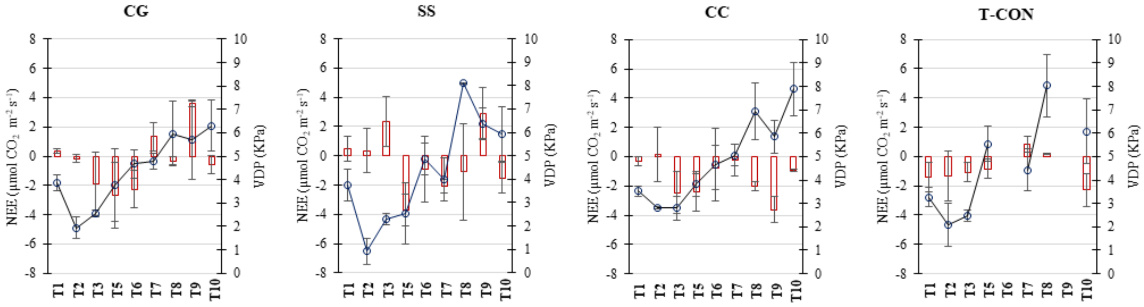
NEE showed high variability throughout in field measurements obtained under different environmental conditions, both for the different treatments and for both microcosms considered (Figure 4.7). In general, the highest CO_2 fixation rates (negative NEE) were determined in the St-microcosms, while in the Sc-microcosms negative NEE values were less common (Figure 4.7; Supplementary Table 4.5). Different trends in NEE were also found between restored and control soils and between different measurement campaigns. Specifically, in St-microcosm CC showed negative NEE in all measurement campaigns. SS amendment soils increased the CO_2 emissions in measurement campaigns carried out after rainfall showing positive NEE in T1, T2, T3 and T9 campaigns and negative NEE in dry campaigns (Figure 4.7A: Supplementary Table 4.5). Also, negative NEE were observed in CG during all the campaigns except to T7 and T9, where the NEE values were positive showing higher emissions than CC and similar to SS. T-CON presented the same trend as CC showing indicative of CO_2 fixation but with lowers values in all campaigns except T8 (Figure 4.7A). In Sc-microcosm, NEE values showed a higher variability alternating lower CO_2 fixation and emission values than in St-microcosm during all period (Figure 4.7B).

On the other hand, the patterns of NEE were clearer in soils with mixture amendments (Figure 4.7C and 4.7D; Supplementary Table 4.5). Mix1 had positive NEE values in the rainiest campaigns to M1, M2 and M3 regardless of microcosm and campaigns with light rainfall and high temperatures at the end of the study period (M7, M9 and M10), while M4, M5 and M6 showed negative NEE, being significantly greater to St-microcosm (Figure 4.7C and 4.7D). Mix2 had the highest CO_2 fixation values in St-microcosm except in M1 and M4, while weak emission values in the campaigns after heavy rainfall (M1, M2, M3 and M4) being higher in M6 after a weaker rainfall, predominated in Sc-microcosm (Figure 4.7D). As for M-CON, negative NEE values were observed throughout the period except for M1 and M9 for St and for M5, M6 and M10 for Sc-microcosm showing little difference between campaigns (Figure 4.7C and 4.7D).

Also, vegetation was subjected to water stress (VPD) in the measurement campaigns carried out in dry periods and this increased as time progressed, being comparatively higher from campaigns T6 and M7 at the end of chronosequence and coinciding with an increase of temperatures in mid-spring. Periods of lower water stress coincided with higher CO_2 fixation rates regardless of the campaign and treatment (Figure 4.7). However, the St- and Sc-microcosms of unmixed amendments had a comparatively higher VPD than the soils restored with mixed amendments in both microcosms (Figure 4.7C and 4.7D).

The results of ANOVA test applied at treatment level in both microcosms, only showed significant differences ($p < 0.05$) T1 (treatments group measurements) and M1, M2, M6 and M9 (Supplementary Table 4.5).

A) St-microcosms



B) Sc-microcosms

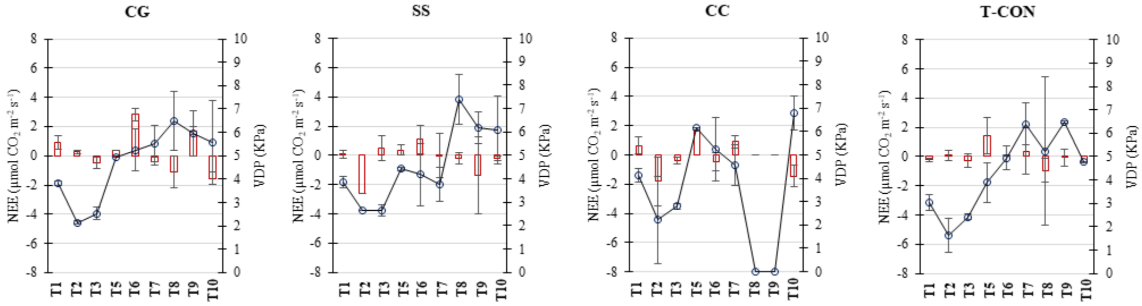
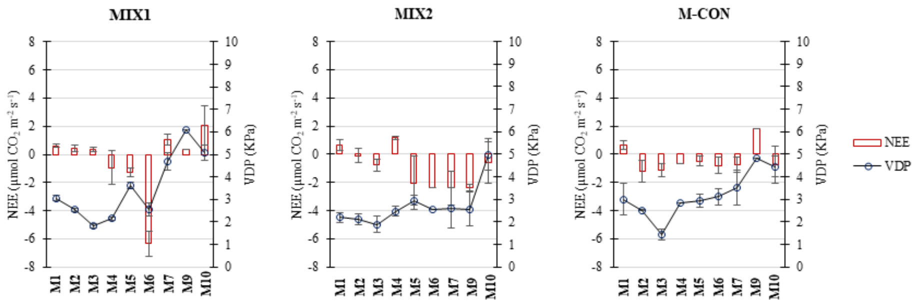


Figure 4.7. Net ecosystem CO_2 exchange (NEE) and vapor pressure deficit (VDP) registered in the St-microcosms of the field measuring campaigns in soils treated with treatments (A) and Sc-microcosm (B) with the corresponding control plot (T-CON). CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost.

C) St-microcosms



D) Sc-microcosms

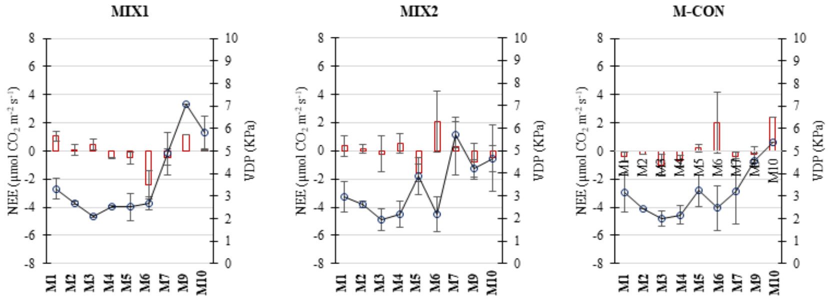


Figure 4.7C and 7D. Net ecosystem CO_2 exchange (NEE) and vapor pressure deficit (VDP) registered in the St-microcosms of the field measuring campaigns in soils treated with treatments St-microcosms in each field campaigns of amendment mixtures (C) and in Sc-microcosm treated with mixtures (D) with their control plots (M-CON). Mix1: SS + CG; Mix2: SS + CG; T-CON and M-CON: no-amendment soils measured in the same campaign types to be taken as control versus restored soils with single treatments and mixtures, respectively.

4.3.5. Interrelationships between environmental and ecological drivers affecting NEE in experimental soils.

The results of Pearson analysis showed positive significant correlations ($p < 0.05$) between NEE and soil moisture (θ) to organic amendments treatment measurement T-campaigns (Table 4.3). Nevertheless, NEE did not show significant correlations with any parameter in M-campaigns carried out for the organic amendment mixture treatments (Table 4.3B).

Chemical and ecological variables showed the same trend in their interrelationships in both types of campaigns (T- and M-campaigns). As for soil chemical properties such as SOC and TN, showed a strong significant positive correlation ($p < 0.05$) with each other. Similarly, biovolume and plant cover were also strongly correlated with one another (Table 4.3). Furthermore, chemical parameters (SOC and TN) were significantly positively correlated with *S. tenacissima* biovolume and plant cover in both field campaigns (T- and M-; Table 4.3). On the other hand, environmental variables such as soil temperature (T_{soil}) had a strong significant positive correlation ($p < 0.05$) with hydric stress (VPD), while it was negative with θ. Available water (AW) only had significant positive correlations with θ and significant negative correlations with VPD, T_{soil}, biovolume and plant cover (Table 4.3).

__T-campaigns (A)__	NEE	VPD	θ	T_{soil}	BV	PC	pH	EC	AW	SOC	TN
NEE											
VPD											
θ	__0.21*__	_-0.52***_									
T_{soil}		__0.77***__	_-0.66***_								
BV		__0.24*__		__0.21*__							
PC		__0.31**__	_-0.21*_	__0.33**__	__0.62***__						
pH				_-0.21*_	_-0.24*_	_-0.44***_					
EC							_-0.60***_				
AW		_-0.33**_	__0.26*__	_-0.40***_	_-0.36***_	_-0.55***_	__0.31**__				
SOC					__0.43***__	__0.49***__	_-0.27**_				
TN					__0.48***__	__0.52***__	_-0.46***_	__0.35***__	_-0.25*_	__0.92***__	
C/N				_-0.23*_	_-0.41***_	_-0.57***_	__0.46***__	_-0.37***_	__0.39***__	_-0.60***_	_-0.72***_
__M-campaigns (B)__	__NEE__	VPD	θ	_Tsoil_	_BV_	_PC_	__pH__	_EC_	__AW__	_SOC_	_TN_
NEE											
VPD											
θ		-0.43***									
Tsoil		0.86***	-0.52***								
BV											
PC					_0.58***_						
pH					_-0.50***_	_-0.80***_					
EC							__-0.52***__				
AW		-0.34**	0.36**	_-0.27*_	_-0.27*_	_-0.39***_	__0.28__				
SOC					_0.47***_	_0.82***_	__-0.75***__				
TN					_0.51***_	_0.82***_	__-0.82***__		__-0.24***__	_0.95***_	
C/N					_-0.42***_	_-0.64***_	__0.71***__	_-0.39***_	__0.42***__	_-0.61***_	_-0.74***_

Table 4.3. Significant Pearson correlations ($p < 0.05$) between T-campaigns (A) and M-campaigns (B) with net ecosystem CO2 exchange (NEE) and vapor pressure deficit (VPD), environmental, ecological physical and chemical properties analyzed. Positive correlations are in bold and negative correlations are in italics. For two different types of campaigns: "T-campaigns" includes measurements in experimental plots: CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost and T-CON: unamended soils. Campaigns "M-campaigns" includes measurements in experimental plots: Mix1: SS + CG; Mix2: SS + CG; M-CON: no-amendment soils. NEE: net ecosystem CO_2 exchange; VPD: vapor pressure deficit; θ: soil moisture; T_{soil}: soil temperature; BV: *S. tenacissima* biovolume; PC: plant cover; EC: electrical conductivity; AW: available water; SOC: soil organic carbon; TN: total nitrogen; C/N: carbon to nitrogen ratio. *Show statistical significance at the 0.05 level; ** Show statistical significance at the 0.01 level; *** Show statistical significance at the 0.001 level.

The RDA analysis of the different campaigns, treatments and microcosm types was performed two and a half years after the application of the different restoration treatments with organic amendments in order to represent the environmental (T_{soil} and θ) and ecological (biovolume and vegetation cover) variables that influenced the behavior shown by the net ecosystem CO_2 exchange (NEE) and vapor pressure deficit (VPD) variables (Figure 4.8). In general, RDA figure showed that NEE and VPD clustered depending on temperature, moisture and plant cover of the soil to each field campaign regardless of amendment type applied (Figure4. 8). For the field measurement campaigns performed on the treatments (CG, SS and CC) and T-CON, the first axis (RDA1) of the measurements taken in St-microcosms comprised 30% of the total variation (Figure 4.8A), while in the case of Sc-microcosms it was 26% (Figure 4.8B), grouping in both cases the campaigns in relation to the moisture and temperature of the soil (θ and ST). The second axis (dbRDA2) comprised 3.1% of the total variation in St-microcosms and in Sc-microcosms it was 0.1% of the total variation (Figure 4.7A and 4.7B, respectively). The second axis showed that in the case of St-microcosms the separation of the campaigns depended on the soil moisture (θ) and plant cover (Figure 4.8A). In contrast, the separation of the points in RDA was almost non-existent. The field measurement campaigns carried out on the mixtures restored soils (Mix1 and Mix2) and M-CON, in St-microcosms comprised 38% of the total variation in RDA1 again grouping both types of microcosms according to soil moisture and temperature (Figure 4.8C). While RDA2 comprised 3.7% of the total variation in St-microcosms grouping the field campaigns in relation to plant biovolume and θ (Figure 4.8C). Sc-microcosm RDA1 explained 31.7% of the total variation by differentiating campaigns according to T_{soil} and θ (Figure 4.8D). RDA2 explained 1.7% of the total variation in Sc-microcosms, separating plant cover campaigns and restored soils from unrestored soils (M-CON).

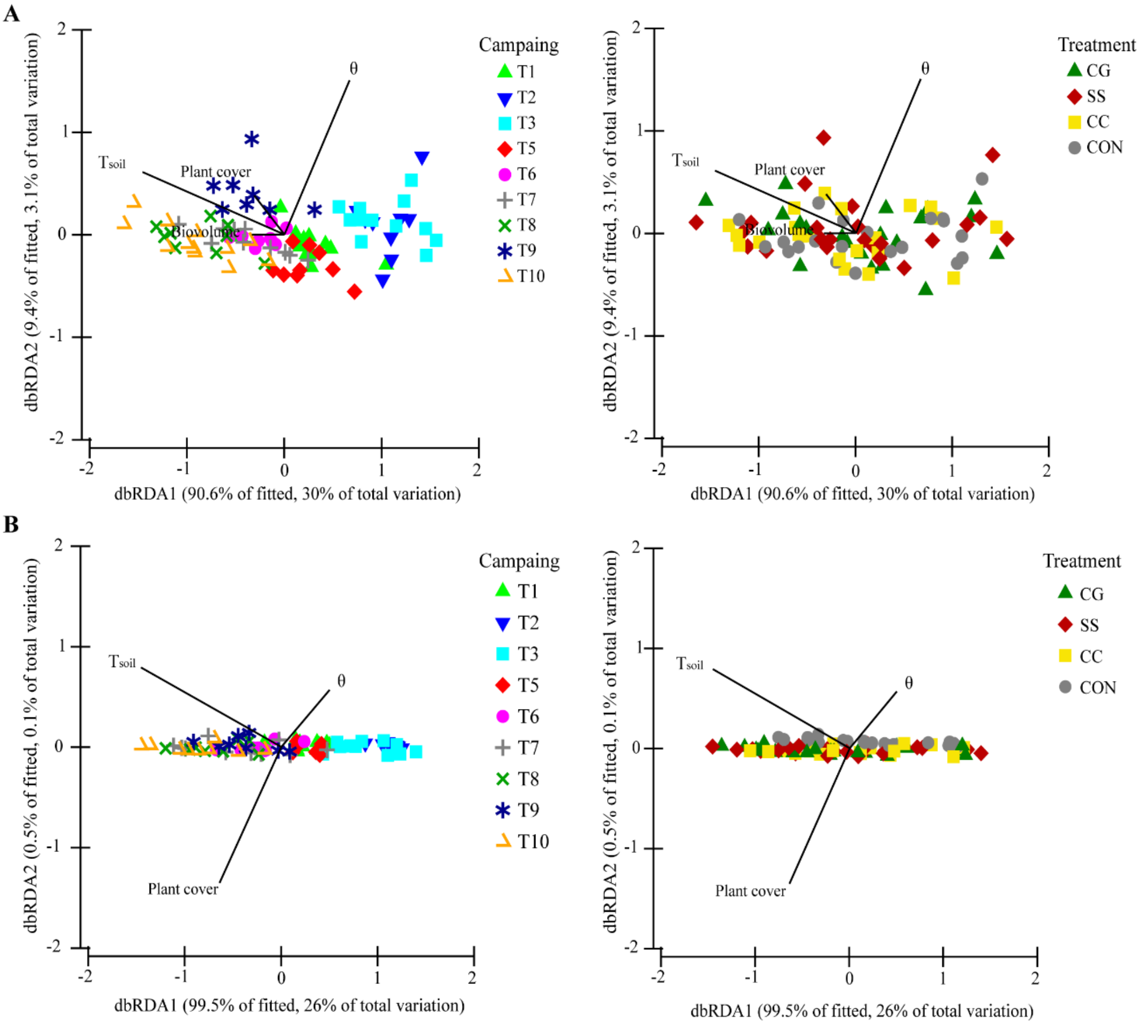


Figure 4.8 A and B. Redundancy analysis (RDA) ordination biplot of net ecosystem CO_2 exchanges (NEE) and water vapor pressure deficit (VDP) and environmental (soil moisture and temperature) and ecological (biovolume and plant cover) variables for the different field campaigns in single treatments (-T-) in both types of microcosms (St and Sc). A) St-microcosms in each T-campaing (right) and per T-restored soils (left); B) Sc-microcosms in each T-campaigns (right) and per M-restored soil (left). SM: soil moisture; ST: soil temperature. CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; T-CON: no-amendment soils measured in the same campaign types to be taken as control versus restored soils with single treatments.

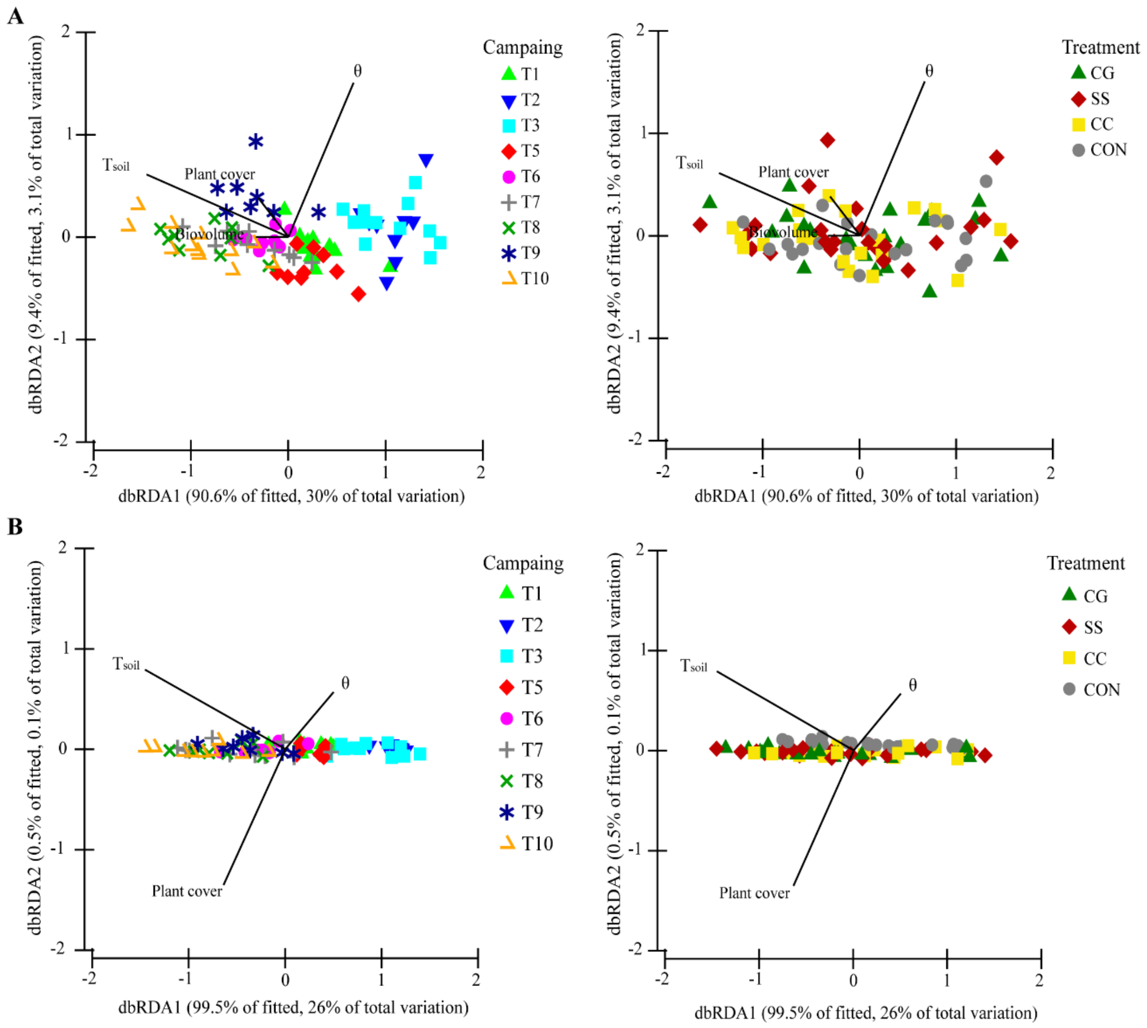


Figure 4.8C and 4.8D. Redundancy analysis (RDA) ordination biplot of net ecosystem CO_2 exchanges (NEE) and water vapor pressure deficit (VDP) and environmental (soil moisture and temperature) and ecological (biovolume and plant cover) variables for the different field campaigns in mixtures treatments (-M-) in both types of microcosms (St and Sc). C) St-microcosms in each M-campaing (right) and per M-restored soil (left); D) Sc-microcosms in each M-campaing (right) and per M-restored soil (left). SM: soil moisture; ST: soil temperature. Mix1: SS + CG; Mix2: SS + CG; M-CON: no-amendment soils measured in the same campaign types to be taken as control versus restored soils mixtures.

The marginal analysis showed that θ, T_{soil} and plant cover significantly influenced NEE and VPD in the microcosm types of the different campaigns, except for the St-microcosm of the soil restored with mixtures (M-campaigns) campaign where plant cover did not significantly influence (Table 4.4).

Table 4.4. Results of the DistLM marginal test to study the environmental and ecological variables that influence the behavior of net ecosystem CO_2 exchange (NEE) and vapor pressure deficit (VPD) in the different field campaigns, treatments and microcosms at 95% of significance ($p < 0.05$).

T- field measurement campaigns				***M- field measurement campaigns***			
St-microcosm				**St-microcosm**			
AICc		R^2		AICc		R^2	
34.190		0.327		18.189		0.398	
Variable	SS (trace)	Pseudo-F	P	Variable	SS (trace)	Pseudo-F	P
θ	27.631	16.171	0.001	θ	13.553	7.416	0.002
T_{soil}	52.663	36.975	0.001	T_{soil}	51.417	40.728	0.001
Biovolume	5.288	2.6944	0.07	Biovolume	3.087	1.556	0.210
Plant cover	8.867	4.614	0.009	Plant cover	2.603	1.307	0.285
Sc-microcosm				**Sc-microcosm**			
AICc		R^2		AICc		R^2	
39.562		0.261		26.307		0.334	
Variable	SS (trace)	Pseudo-F	P	Variable	SS (trace)	Pseudo-F	P
θ	16.346	8.978	0.001	θ	13.491	7.422	0.001
T_{soil}	40.152	26.362	0.001	T_{soil}	39.658	28.28	0.001
Plant cover	13.641	7.356	0.002	Plant cover	11.069	5.964	0.005

θ: soil moisture; T_{soil}: soil temperature.

4.4. Discussion

This study shows the importance of organic amendments management to favor atmospheric CO_2 sequestration and the increase vegetation cover. Overall, our results show that restored soil with organic amendments can enhance CO_2 sequestration compared to soils without organic inputs, but NEE rates showed clearly differences depend to organic amendments applicated (Figure 4.3).

It is known that NEE is mainly the difference between CO_2 sequestration by plants through the photosynthesis process and CO_2 emissions due to heterotrophic respiration by soil microorganisms and roots of plants, among others (Azeem et al., 2019; Norman et al., 1992). Moreover, it is important to note that the chemical composition of the organic matter provided by the amendment used modulates the size of the CO_2 emission from amended soils (Brenzinger et al., 2018; Ray et al., 2020) and it has been tested in previous studies for our restored soils (Soria et al., 2022, 2021b). Similarly, the NEE of the restored experimental plots was due to complex interactions between soil properties, biological variables and soil temperature and moisture conditions, which in turn were conditioned by microclimatic conditions. This complex interrelationship has been backed by our statistical analyses, showing the redundancy analyses a clear separation between campaigns with abundant rainfall and lower temperatures from the drier and more water-stressed measurement campaigns leading NEE behavior (Figure 4.8). This is consistent with observations in other semiarid zones, being the rainfall one of the main drivers that influence NEE (Liu et al., 2016; López-Ballesteros et al., 2016). Tendency observed in NEE were clear for all treatments during the measurements, independently of microcosm type (Figure 4.4), showing higher cumulative CO_2 fixation in presence of *Stipa tenacissima* (Figure 4.4A, 4.4B; Supplementary Table 4.3). Although, some authors have observed in arid ecosystems that autotrophic respiration is limited by drought conditions to a greater extent than heterotrophic respiration (Niu et al., 2020), the days of measurement difference between both groups of campaigns (T and M) had no influence as they showed similar trend. However, the slight change observed in the microclimatic conditions of the ecosystem could have had some influence on soil respiration, especially in soils treated with organic amendments. Therefore, humidity and vegetation, both planted and spontaneous, could have had an important influence on environmental conditions within the microcosms modifying NEE (Table 4). After rain events T- and later M-campaigns were measured, suggesting that *S. tenacissima* plants might be assimilating CO_2 through photosynthesis and compensating gas emissions due to the respiration of heterotrophic microorganisms. Whereas sporadic rainfall can produce pulses of water in the soil and nutrient availability (Huxman et al., 2004b; Zhou et al., 2020), which will be different in our case in terms of the chemical composition of the amendment used (Ray et al., 2020; Soria et al., 2022), favoring a greater CO_2 emissions by soil respiration in T campaigns than in M-campaigns. Furthermore, the type of precipitation event modulates θ and soil infiltration capacity, while buffering temperatures in semi-arid ecosystems (Li and Zhou, 2012; Niu et al., 2020), matching with rainfall. Although the most influential variables on NEE and VPD were θ and T_{soil}

(Table 4), a strong variability in NEE patterns in all restored experimental plots with the organic amendments in the different field measurement campaigns, suggesting that NEE patterns could be influenced by complex interactions between different variable studied, which condition the microclimate in both types of microcosms (St and Sc). Also, because soil respiration in arid zones responds to rainfall pulses that increase soil moisture (Fan et al., 2012; Soria et al., 2021a; Vargas et al., 2018) and this is increased in the presence of a higher amount and type of organic matter (Gelfand et al., 2015; Ray et al., 2020).

On the other hand, soil properties had an indirect influence on ecological variables studied (biovolume and plant cover). In addition, organic amendment treatments favored *S. tenacissima* growth and had better plant cover respect to CON (Figure 4.6). Moreover, the properties of the amended soils clearly differed from the control soils (Figure 4.3), indicating an improvement in soil quality and fertility of the amended soils respect to unamended soils (Soria et al., 2021a), and mainly due to the increase in SOC and TN content (Figure 4.3 and Table 4.2) that showed a clear influence on the growth of *S. tenacissima* and plant cover development, as indicated by the correlations results o found between them (Table 4.3). Soils amended with compost from plant residues (CG and CC) showed a C/N ratio that could indicate an equilibrium between humification and mineralization, while in SS and its mixtures (Mix1 and Mix2) the mineralization processes were dominant in the first campaign and reached equilibrium later (Table 4.2). Higher SOC and TN values in restored soils, changes in pH and higher EC values compared to control soils (Table 4.2) influence the composition and structure of soil microbial communities (Rodríguez-Berbel et al., 2021a; Sánchez-Marañón et al., 2017). Likewise, soil properties modulate the activity of microorganisms in the carbon cycle and mineralization processes of organic matter (Rodríguez-Berbel et al., 2021a) that could influence the NEE monitored in this study. Interestingly, initial SOC levels were maintained even two and a half years after application but increased during the winter and decreased again in the spring (Table 4.2). This increase in SOC levels could be explained by the incorporation of plant biomass remains from *S. tenacissima* and spontaneous annual plants that dried out during the summer, which could also favor the organic matter decomposition by soil microorganisms (Coleman and Whitman, 2005; Ortega et al., 2020; Soria et al., 2021b). In addition, vegetation cover has been shown to be an influential variable of NEE in the marginal test (Table 4.4) and soil respiration depends on photosynthesis because leaf litter and root exudates released by plants are essential for microbial metabolism (Niu et al., 2020). On the contrary, in soil without organic amendments (CON) the growth of *S. tenacissima* plants was minimal and there was little spontaneous vegetation cover development (Figure 4.6), which could be due to the fact that mining activity generates changes in physical and chemical soil properties that do not favor the development of vegetation (Sort and Alcañiz, 1996). In turn, the loss of the fertile soil layer due to mining activity causes the loss of organic carbon and other essential plant nutrients. In fact, these soils presented very low SOC and TN levels (Table 4.2) despite the time elapsed since the degradation by mining activity, which in turn negatively affects the nutrient cycling and biological activity of the soil (Luna et al. 2016b).

Specifically, soils amended with CC and CG plant compost were more effective in CO_2 sequestration than the other amendments, especially in the presence of *S. tenacissima* (Figure 4.7), and at the time of more intense rainfall (Figure 4.2). However, CG showed CO_2 emissions in dry measurements, while CC continued fixing C, being the most effective treatment for sequestration among the used (Figure 4.7). These values coincide with those determined by López-Ballesteros et al. (2016) who showed that in semi-arid *S. tenacissima* grasslands on calcareous soils of SE Spain a rainfall event of 13 mm was not sufficient to stimulate the photosynthetic process of plants. NEE fluctuated more during the rainy period in Sc microcosms (Figure 4.7), although mainly emission rates were higher than sequestration rates. Therefore, the planted vegetation may have played a key role in favoring its activation in conditions of lower water stress, while it may be dormant when environmental conditions and water stress are not favorable (Huxman et al., 2004b). These results are consistent with those reported by Pugnaire et al. (2006), which indicated the activation of the photosynthetic process of *S. tenacissima* during high rainfall pulses and were later confirmed by López-Ballesteros et al. (2016) in a semi-arid grassland ecosystem with calcareous soils close to our study area. However, under conditions of water stress or prolonged drought, typical in our study area, *S. tenacissima* has shown complex morphological and physiological strategies that could minimize its photosynthetic processes by reducing its CO_2 assimilation capacity (Maestre et al., 2007; López-Ballesteros et al., 2016). Such variations could be related to the behavior of *S. tenacissima* plants randomly selected in the plots, which could present a high variability in the senescence of their leaves, decreasing their photosynthetic capacity (Ramírez et al., 2006), especially in dry seasons (Haase et al., 1999), which could affect the photosynthesis performance. It could justify the weak negative NEE or even NEE variations observed in St-microcosms in CG and CC soils during campaigns with lighter rainfall and high-water stress. In addition, the correlation between soil moisture (θ) and plant water availability (AW) in dry seasons (Table S1), could indicate an important influence on stomatal conductance within the plant-soil-atmosphere model, with the plant being able to perform stomatal closure when soil water conditions are critical (Tuzet et al., 2003).

On the contrary, SS showed higher CO_2 emission rates than sequestration in campaigns with heavy rainfall and sequestration in light rainfall events in the presence of *S. tenacissima*. In Sc-microcosm NEE was more variable but predominate CO_2 emissions (Figure 4.7). It has been described in previous studies that SS amendment provided an organic matter with a high content of labile fractions (Soria et al., 2022). On the other hand, GC and CC provided more recalcitrant organic matter with lignin-rich compounds that are more difficult to biodegrade (Soria et al., 2022). The labile organic matter in SS soils could have been responsible for the metabolic activation of soil microbial communities, resulting in a higher rate of heterotrophic respiration (Abdelhafez et al., 2018; Abdelrahman et al., 2020; Selivanovskaya and Latypova, 2006). In fact, Rodríguez-Berbel et al. (2021a) found the presence of bacterial communities involved in the mineralization of organic matter in these same SS restored soils. The mineralization of labile organic matter fractions by soil microorganisms in SS soils (Soria et al., 2021a), activated by soil moisture conditions after rainfall and the concomitant CO_2 emission (Soria et al., 2021b) could exceed CO_2 assimilation by plant photosynthetic processes, explaining the positive NEE found in both types of St-microcosms (Figure 4.7).

The amendment mixture soils (Mix1 and Mix2) showed an intermediate NEE trend based on the amendments that comprise it. During heavy rainfall campaigns, CO_2 emissions predominated in both St-microcosm and Sc-microcosm (Figure 4.7). Nevertheless, Mix1 presented negative NEE values in period with pulses of rain and positive in dry period in Sc as well as in St. Mix2 showed a net CO_2 assimilation in St-microcosm in the rest of study period, but there were more fluctuations for the Sc-microcosm depend to rainfall pulses. Therefore, NEE is a result of labile and recalcitrant forms of organic matter that make up a portion of the compost-soil matrix in terms of soil respiration behavior, while the plants behave in a similar way as described above.

Soil microcosms without organic amendments (CON), interestingly also showed negative NEE during wet campaigns, especially in microcosms with *S. tenacissima* plants, although it was comparatively lower than in soils plant compost (CG and especially CC) (Figure 4.7). This apparent sequestration of CO_2 in St-microcosms could be explained by a very low mineralization of organic matter by soil microorganisms and in parallel lower CO_2 emission from heterotrophic respiration, since the SOC content was significantly lower by CON soils than the amended soils (Table 4.2). Therefore, the chamber could be capturing mainly photosynthetic fixation of the *S. tenacissima* plant, given the reduced microbial activity previously found in these soils (Soria et al., 2021a). However, in drier conditions, greater NEE variability was observed, with occasional CO_2 emission mostly in T-CON and less in M-CON, and only in some St-microcosm campaigns. Soria et al., (2021b) also recorded small CO_2 emission from CON soils in our study area. Interestingly, although only *S. tenacissima* plants existed in St-microcosms in CON soils and its size were significantly smaller than in amended soils (Figure 4.6). It suggests that the plants could have performed a photosynthetic activity sufficient to compensate CO_2 emissions under water deficit conditions (Figure 4.7). Alternatively, CO_2 fixation by *Cyanobacteria* and autotrophic bacteria were identified in CON plots (Miralles et al., 2021b) and it could also explain the small CO_2 sequestration in Sc microcosms in CON soils during rainy seasons. On the other hand, it should also be taken into account that CO_2 fixation by *Cyanobacteria* also depends on the conjugation of several environmental factors (air humidity, photosynthetically active radiation, etc.) being limited to specific times of day, as thus observed by Miralles et al. (2018) in similar degraded marl soils in the Tabernas desert.

Other possible reasons for negative NEE in CON soils, especially on bare soil (Sc), could be due to geochemical processes other than biological ones. Sánchez-Cañete et al. (2018) found important CO_2 losses attributable to dissolution in water, biological activities and chemical reactions, and conclude that we must change our point of view from an inappropriately conceived system in which all CO_2 is produced by biology, to a dynamic system where the soil CO_2 is produced and removed by the interaction of combinatorial biological processes, hydrologic transport, and associated geochemical reactions. For this ecosystem, the most plausible reason to register negative NEE is due to the CO_2 assimilation attributed to a dissolution of soil calcite ($CaCO_3$) causing a geochemical reaction that consumes CO_2 in the soil pore space (Hamerlynck et al., 2013), induced by water vapor absorption by soil (Lopez-Canfin et al., 2022). This would be in agreement with the high amount of calcium carbonates found in the study area (García-Ávalos et al., 2018; Luna et al., 2016a) as well as active limestone resulting from mining activity (Soria et al., 2021a). Or, it could respond to $CaCO_3$ biomineralization processes from atmospheric CO_2 carried out by *Cyanobacteria* (Benzerara et al., 2014). In any case, the existence of these geochemical processes should also occur in the rest of the experimental plots with organic amendments, these CO_2 consumption processes should be lower than the CO_2 emission processes and therefore they could change the sign of the NEE.

4.5. Conclusions

The soil-forming factors in the study area caused all organic amendment to increase the quality of soils and favored *S. tenacissima* growth and the development of spontaneous plant cover with respect to control soils showing an improvement of carbon sequestration in plant structure. So, initially a single dose of the amendments of approximately 3% organic matter content may be adequate to restore highly degraded soils of open-pit lime mines. The greenhouse compost could be more suitable for application in the restoration of soils degraded by mining, at least in terms of its better CO_2 sequestration capacity and the development of vegetation with greater efficiency than the rest of the amendments tested. The same happened with the amendment mixtures whose contribution together with the vegetable compost from greenhouse crop residues improved the CO_2 sequestration capacity, exceeding the rates observed for the mixture of sludge with garden waste compost. The soil moisture, closely linked to precipitation, influenced net CO_2 exchange (NEE) throughout the sampling campaigns. More CO_2 was sequestered at higher precipitation levels in treatments with more resilient organic matter forms such as both greenhouse and garden plant compost, over in the presence of *S. tenacissima*. However, in periods with milder temperatures and rain pulses, NEE was mainly affected by the composition of amendments, while plants showed dormant periods due to elevated temperatures. This was more prominent in the sewage sludge amendments, whose chemical composition could favor heterotrophic respiration, which were the least efficient respect CO_2 sequestration. Our results demonstrated the importance of short-term, high-frequency field monitoring on land restored using composted residues in Mediterranean semiarid grassland recovery degraded by mining in order to improve our understanding of CO_2 cycling and its likely responses to a changing climate. But it will be necessary to study ecosystem NEE over a longer period to reveal its long-term response to environmental and biological factors and be able to obtain annual CO_2 balances.

General conclusions

1. The application of organic amendments has a significant influence on the physical, chemical and microbiological properties of technosols developed in a quarry in a semi-arid Mediterranean area, as well as on the vegetation cover, when compared to unrestored control soils. Thus, the application of organic amendments shows a positive feedback impact on the quality and short-term functional recovery of the restored soils.

2. Organic amendments improve nutrient availability, increase enzymatic activities linked to C, N, and P cycles, and favour growth and proliferation of microorganisms, thus improving the functionality of restored soils in the first six months after their application. In the sludge treatments and their mixtures, this improvement is more evident, while the soil restoration treatments with plant compost show intermediate functional responses between soils that were not amended and soils restored with sludge.

3. The composition of organic matter in the restored soils is characterized on a molecular point of view using thermogavimetric and analytical pyrolysis techniques, showing that soils restored with amendments produced from sewage sludge are richer in labile organic compounds, while plant composts show a combination of labile and recalcitrant organic matter that are more similar as those in the surrounding natural soils. Thus, the latter amendments develop soils that are more balanced in terms of organic matter composition and quality, and could therefore result in a reservoir of nutrients in both the short and long term.

4. After the application of the amendments, CO_2 emission peaks are observed due to the stimulation of microbial communities, due to nutrient enrichment of the technosols, especially due to the rapid consumption of easily-mineralized labile compounds. This effect, known as "priming effect", is much lower in soils treated with plant composts than in soils treated with sludge and its mixtures (richer in labile compounds). Therefore, the chemical composition of organic matter also plays an important role in the regulation of CO_2 emissions from restored soils.

5. The magnitude and temporal evolution of CO_2 emissions during the first 18 months are still different depending on the type of amendment used, although they show a significantly decreasing trend during the experimental period, and emission peaks are observed in times of rainy events. Technosols with sludge and its mixtures result in emissions by 80-90% higher than the other restored soils, soils without amendments and natural soils. Soils amended with vegetable compost from greenhouse wastes show emission dynamics that are similar as the surrounding natural soils, while compost from pruning and gardens have hardly any emissions, as the control soils, in which biological activity is practically absent.

6. On the other hand, once the priming effect depletes, the restored soils could become CO_2 sinks, due to the establishment of a vegetation cover that counteracted the release of CO_2 due to plant respiration and microorganisms activity. Thus, the treatments with compost from greenhouse residue are the most promising under this aspect, followed by garden and pruning compost. However, the latter show a dual behaviour, showing CO_2 emissions during dry periods, while the greenhouse compost continues to fix carbon. In contrast, in soils treated with sludge the net CO_2 exchange shows fixation values in dry periods, while especially after inputs of rainfall and high temperatures, this is affected mainly by the composition of the amendments, showing emission values, which counteract the sequestration by vegetation, making them less efficient with respect to CO_2 sequestration.

7. Apart from the chemical composition of organic matter, environmental factors and the physical and chemical properties of the soil that influence its biological processes also play a key role in CO_2 emission/sequestration, with moisture being the factor with the greatest influence.

8. Finally, as a general recommendation, vegetable compost from greenhouse plant waste, followed by compost from garden and pruning waste, prove to be the most suitable for application in the restoration of soils degraded by open-pit mining in limestone quarries in semi-arid areas. These composts are more beneficial compared to stabilized sewage sludge in terms of soil properties, priming effect, and temporal evolution of CO_2 emission/fixation, organic matter quality and effect on vegetation cover development.

9. The studies shown in the present thesis demonstrate the good short-term results obtained using composted organic wastes in the restoration of semiarid Mediterranean grasslands degraded by mining. Furthermore, they emphasize the importance of selecting an adequate amendment type for the application of organic wastes in soil restoration, which should take into account the different behaviour of the soils. However, it would be of interest to extend our knowledge more in the long term about the response of ecosystems to restorations based on organic amendments.

Conclusiones generales

1. La aplicación de las enmiendas orgánicas tuvo una influencia significativa en las propiedades físicas, químicas y microbiológicas de tecnosoles desarrollados en una cantera en área semiárida mediterránea, así como en la cubierta vegetal, en comparación con suelos control sin restaurar. Así, la aplicación de enmiendas orgánicas mostró un proceso de retroalimentación positivo en la calidad y recuperación la funcionalidad a corto plazo de los suelos restaurados.

2. Las enmiendas orgánicas mejoraron la disponibilidad de nutrientes, incrementaron las actividades enzimáticas implicadas en los ciclos de C, N y P, y favorecieron el crecimiento y proliferación de microorganismos, mejorando así la funcionalidad de los suelos restaurados en los primeros seis meses tras su aplicación. En los tratamientos de lodos, y sus mezclas, es donde esta mejoría fue más evidente, mientras que los tratamientos de restauración de suelos con compost vegetales mostraron respuestas de funcionalidad intermedias entre suelos los suelos no emendados y los suelos restaurados con lodos.

3. Se caracterizó molecularmente la composición de la materia orgánica en los suelos restaurados mediante técnicas termogavimétricas y de pirólisis analítica, observándose que los suelos restaurados con enmiendas producidas a partir de lodos de depuradora eran más ricos en compuestos orgánicos lábiles, mientras que los compost vegetales presentaron una combinación de materia orgánica lábil y recalcitrante más similar a la de los suelos naturales de los alrededores. Así, estas últimas enmiendas desarrollaron unos suelos más equilibrados en cuanto a la composición y calidad dela materia orgánica, y por tanto, podrían garantizar un reservorio de nutrientes tanto a corto como a largo plazo.

4. Tras la aplicación de las enmiendas se observaron picos de emisión de CO_2 en base a la estimulación de las comunidades microbianas como consecuencia del enriquecimiento en nutrientes en los tecnosuelos, debido especialmente al rápido consumo de los compuestos lábiles fácilmente mineralizables. Este efecto, conocido como "efecto de cebado" (priming effect) fue bastante inferior en los suelos tratados con composts vegetales respecto a los suelos tratados con lodos y sus mezclas (más ricos en compuestos lábiles). Por tanto, la composición química de la materia orgánica también juega un importante papel en la regulación de las emisiones de CO_2 de los suelos restaurados.

5. La magnitud y evolución temporal de las emisiones de CO_2 a lo largo de los primeros 18 meses siguió siendo diferente en función del tipo de enmienda utilizada, aunque presentaron una tendencia significativamente decreciente durante el periodo experimental, se observaron picos de emisión en épocas de eventos lluviosos. Los tecnosuelos con lodo y sus mezclas presentaron emisiones entre un 80-90% superiores

que el resto de suelos restaurados, suelos sin enmiendas y naturales. Los suelos enmendados con compost vegetales a partir de residuos de invernadero tuvieron una dinámica de emisiones similar a los suelos naturales del entorno, mientras que los compost procedentes de podas y jardines apenas tuvieron emisión asemejándose a los suelos de control, en los que la actividad biológica es prácticamente nula.

6. Por otro lado, una vez superado el efecto cebado, los suelos restaurados podían convertirse en sumideros de CO_2, debido al establecimiento de una cobertura vegetal que contrarrestara la liberación de CO_2 debido a la respiración de las plantas y los microorganismos. Así, los tratamientos de compost de residuos de cultivo de invernadero fueron el tratamiento más destacado en este aspecto, seguido del compost de jardines y podas. Sin embargo, estos últimos tuvieron un comportamiento dual, mostrando emisiones de CO_2 durante periodos secos, mientras que el compost de invernadero continuó fijando carbono. Por el contrario, en los suelos tratados con lodos el intercambio neto de CO_2 mostró valores de fijación en periodos secos, mientras que especialmente tras pulsos de lluvias y temperaturas elevadas, este se vio afectado principalmente por la composición de las enmiendas, mostrando valores de emisión, que contrarrestaron el secuestro por parte de la vegetación, haciéndolos menos eficientes respecto a la captura de CO_2.

7. Aparte de la composición química de la materia orgánica, los factores ambientales y las propiedades físicas y químicas del suelo que influyen en sus procesos biológicos, también tuvieron papel clave en la emisión/secuestro de CO_2, siendo la humedad el factor con mayor peso.

8. Finalmente, como recomendación general, el compost vegetal procedente de residuos vegetales de invernadero, seguido del de los restos de jardinería y podas resultaron los más adecuados para su aplicación en la restauración de suelos degradados por la minería a cielo abierto en canteras de caliza de zonas semiáridas. Estos mostraron ventajas en comparación a los lodos de depuradora estabilizados en cuanto a las propiedades de los suelos, el efecto cebado, y la evolución temporal de la emisión / fijación de CO_2, la calidad de la materia orgánica y el efecto sobre el desarrollo de la cubierta vegetal.

9. Los estudios mostrados en el presente trabajo ponen de manifiesto los buenos resultados obtenidos a corto plazo usando residuos orgánicos compostados en la restauración de pastizales semiáridos mediterráneos degradados por la minería. Además, enfatizan la importancia de considerar un tipo de enmienda adecuada en la toma de decisiones para la aplicación de residuos orgánicos en las restauraciones de suelos, que debería tener en cuenta el diferente comportamiento de los mismos. Sin embargo, sería de interés ampliar nuestro conocimiento más a largo plazo acerca de la respuesta de los ecosistemas a las restauraciones basadas en enmiendas orgánicas.

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Annexes

Supplementary material Chapter 1

Supplementary Table 1.1. Chemical characteristics of organic amendments measured before their application.

	CG	CC	SS
pH	8.6	8.02	7.19
EC (mScm^{-1})	4.28	6.37	3.41
TOC (%)	31.4	59.15	66.4
TN (%)	1.64	1.87	5.49
TP (%)	1.8	0.85	6.29
C:N	14.3	18.34	7.01

[†] CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C.

[‡] EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon to nitrogen ratio.

Supplementary Table 1.2. Main chemical and physical characteristics of soils with restored organic amendments and control soils 2 years after organic amendment application and coinding with plant sampling. Average of three replicates (average ± standard deviation).

	CON	**CG**	**SS**	**CC**	**Mix1**	**Mix2**
pH	9.02 ± 0.15a	8.23 ± 0.11b	7.91 ± 0.17b	8.80 ± 0.03a	8.08 ± 0.11b	8.08 ± 0.06b
EC (mScm^{-1})	2.13 ± 0.51a	2.41 ± 0.29a	2.34 ± 0.40a	2.71 ± 0.57a	2.08 ± 0.05a	2.28 ± 0.57a
TOC (%)	0.44 ± 0.09a	2.16 ± 0.43b	2.66 ± 0.50b	3.20 ± 0.76b	3.17 ± 0.48b	2.73 ± 0.29b
TN (%)	0.06 ± 0.01a	0.33 ± 0.04b	0.60 ± 0.12b	0.40 ± 0.09b	0.55 ± 0.10b	0.47 ± 0.08b
C:N	9.01 ± 2.85ab	6.42 ± 0.68ab	4.94 ± 0.50a	7.94 ± 0.37b	5.78 ± 0.25a	5.90 ± 0.47a
pF -1500 kPa	13.99 ± 2.51a	17.62 ± 0.36a	19.51 ± 1.49a	16.85 ± 1.20a	18.48 ± 0.50a	13.79 ± 2.52a
pF -33 kPa	34.21 ± 0.53a	35.27 ± 1.77a	36.84 ± 2.22a	33.78 ± 2.96a	35.32 ± 1.21a	35.17 ± 0.50a

CON: unamended control soils; CG: 100 % vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70º C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost.

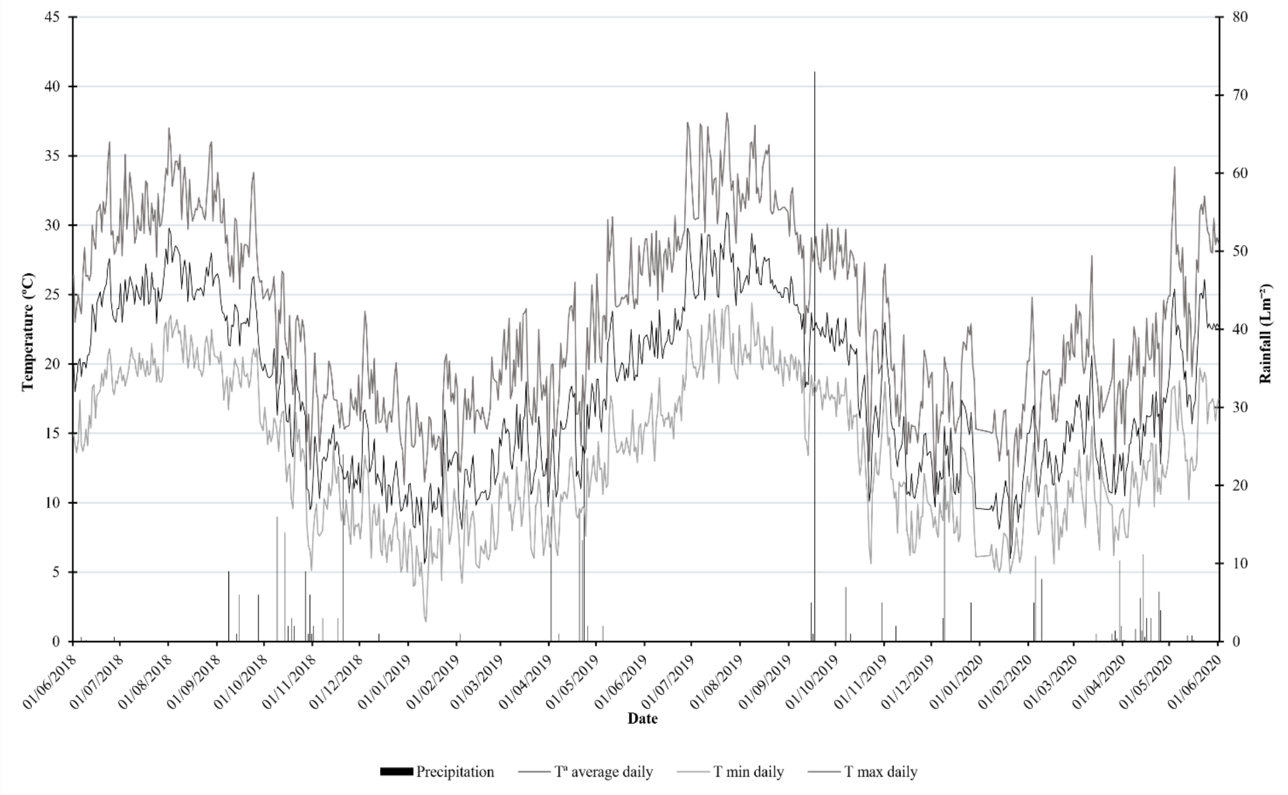
EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen;; C:N: carbon to nitrogen ratio; pF: water retention in soil at different pressures. Across treatments data with different lowercase letters are significantly different, *$P < 0.05$* [PERMANOVA].

Supplementary Table 1.3. Component loadings on a three-principal-component-analysis solution for chemical, biochemical and microbiological properties in restored and control soils.

Variables	PC1	PC2	PC3
EC	0.227	0.212	-0.003
pH	-0.202	0.126	0.436
TOC	0.240	0.324	0.048
pF -1500	0.240	-0.066	0.027
TN	0.286	0.134	-0.036
C:N	-0.170	-0.240	0.239
AP	0.258	0.033	0.314
Lime	-0.011	-0.244	0.674
CO_2	0.262	-0.109	-0.057
BR	0.278	-0.147	0.052
Fungi	0.216	-0.206	-0.003
Bacteria	0.248	-0.105	-0.240
Dehydrogenase	0.256	0.081	0.237
β-glucosidase	0.284	-0.125	0.0897
Phospatase	0.264	-0.193	0.085
Urease	-0.016	0.481	0.125
CHs	0.262	-0.196	0.032
POLs	0.076	0.485	0.195

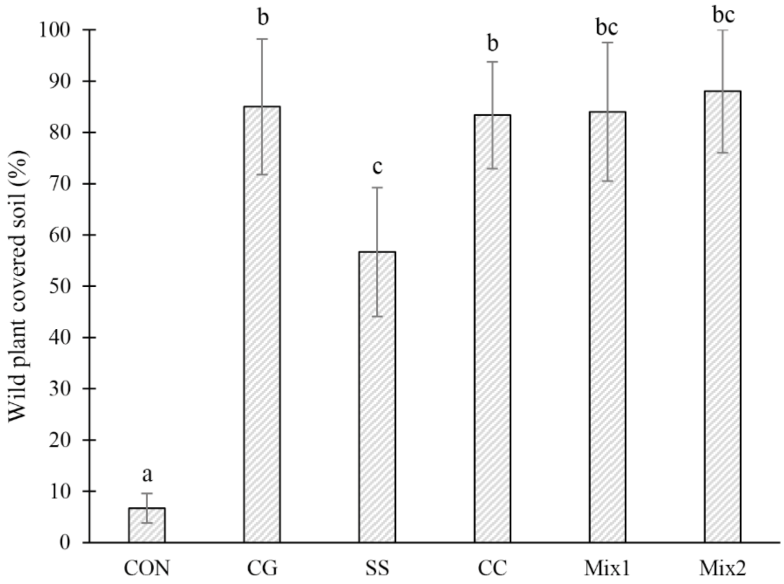
EC: Electrical conductivity; TOC: Total Organic Carbon; pF-1500: water retention in soil at -1500 kPa; TN: Total Nitrogen; C:N: carbon to nitrogen ratio; AP: Available phosphorus; Lime: active lime; CO_2: emission of CO_2; BR: Basal Respiration; Bacteria: Bacteria fatty acids; Fungi: fungal fatty acids; CHs: carbohydrates content; POLs: Polyphenols content; Mineralization: glucose mineralized.

Supplementary Figure 1.1. Local climate diagram of the experiment site.



Precipitation (rainfall) monitored by a rain sensor (Rain-O-Matic Small, Pronamic ApS, Denmark) located in the experimental area; Temperature mean daily, maximum and minimum from weather station RAIFALL003 of Junta de Andalucía.

Supplementary Figure 1.2. Percentage of spontaneous plant per treatment.



CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues: SS: wastewater treatment station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70 °C; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. Different letters in this panel indicate significant differences between treatments (P < 0.05) [PERMANOVA].

Supplementary material Chapter 2

Supplementary Table 2.1. Results of multivariante analysis of variance **(**PERMANOVA). Significant differences (P < 0.05) in soil properties by two factor: Treatment (different organic amendments applied, untreated soil and natural reference soil) and sampling date (different sampling dates, T6 (17-12-2018 and T18 (24-11-2019)).

		PERMANOVA		
		df	PS-F	P
Soil properties	Treatment	6	5.7465	0.001
	Date	1	5.2679	0.002
	Treatment x Date	6	1.0254	0.435

Supplementary material Chapter 3

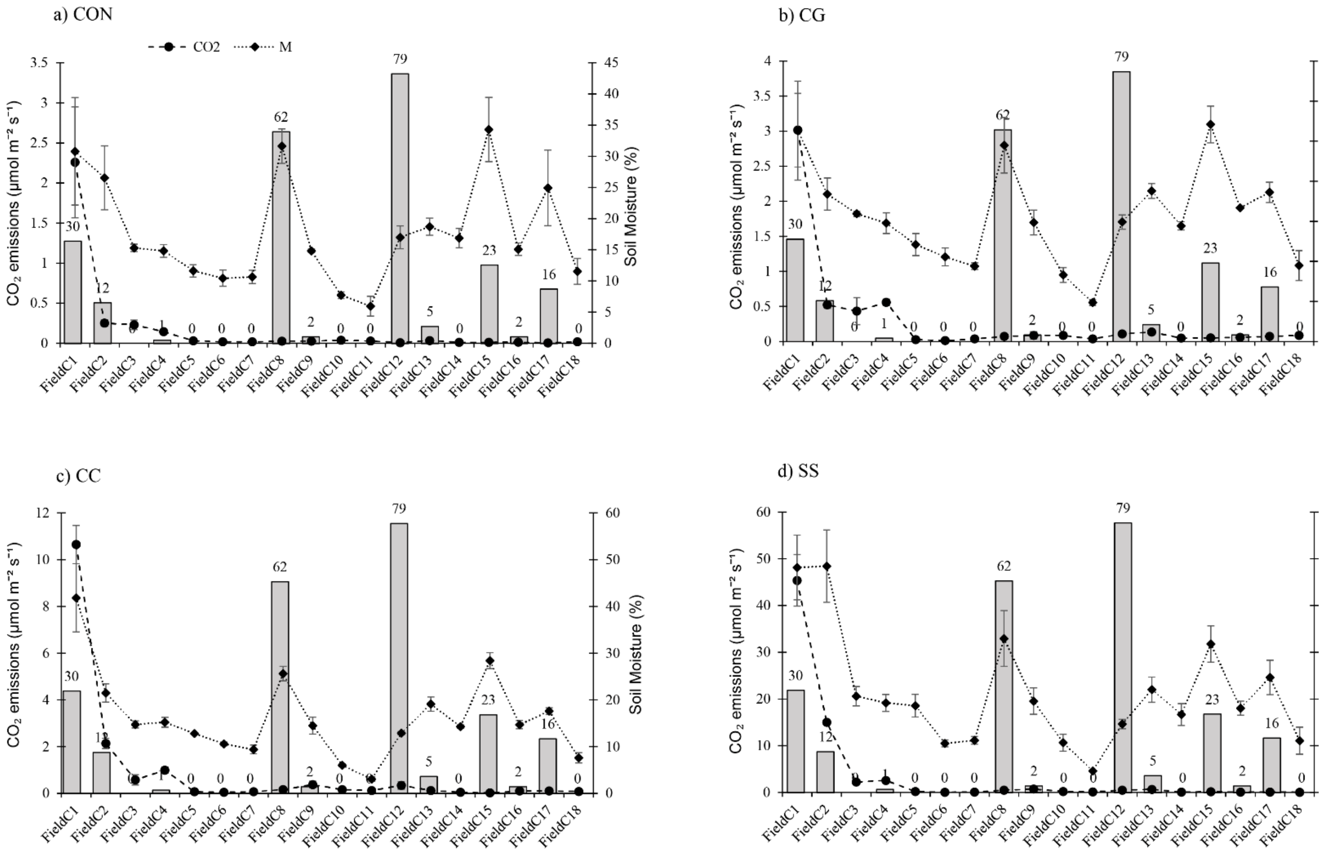
Supplementary Table 3.1. Characterization of field CO_2 measurement campaigns: date, season and environmental conditions in which the field campaigns for the measurement of CO_2 emissions were carried out.

Field Campaign	Date	Season	Precipitation (mm)	T min day* (ºC)	T max day* (ºC)	T mean day* (ºC)
FieldC1	15-10-18	autumn	30	16.9	24.9	15.9
FieldC2	29-10-18	autumn	12	6.9	16.4	10.9
FieldC3	05-12-18	autumn	0.2	12.5	22.8	16
FieldC4	17-12-18	autumn	0	7.00	16	11.10
FieldC5	16-01-19	winter	0	5.60	14	9.30
FieldC6	18-02-19	winter	0	6.30	16.2	10.70
FieldC7	14-03-19	winter	0	8.30	18.8	13.00
FieldC8	23-04-19	spring	62	9.60	16.8	13.50
FieldC9	02-05-19	spring	2	14.40	24.3	18.90
FieldC10	11-06-19	spring	0	16.30	27.2	21.80
FieldC11	23-07-19	summer	0	24.20	38.1	30.90
FieldC12	19-09-19	summer	79	18.50	27.8	22.60
FieldC13	25-10-19	autumn	5	11.90	22.3	16.00
FieldC14	24-11-19	autumn	0	9.00	17.3	12.20
FieldC15	04-12-19	autumn	23	7.90	17.1	12.20
FieldC16	23-12-19	winter	2	13.10	21	16.40
FieldC17	24-01-20	winter	16	8.20	15.4	10.60
FieldC18	24-02-20	winter	0	9.60	18.6	13.50

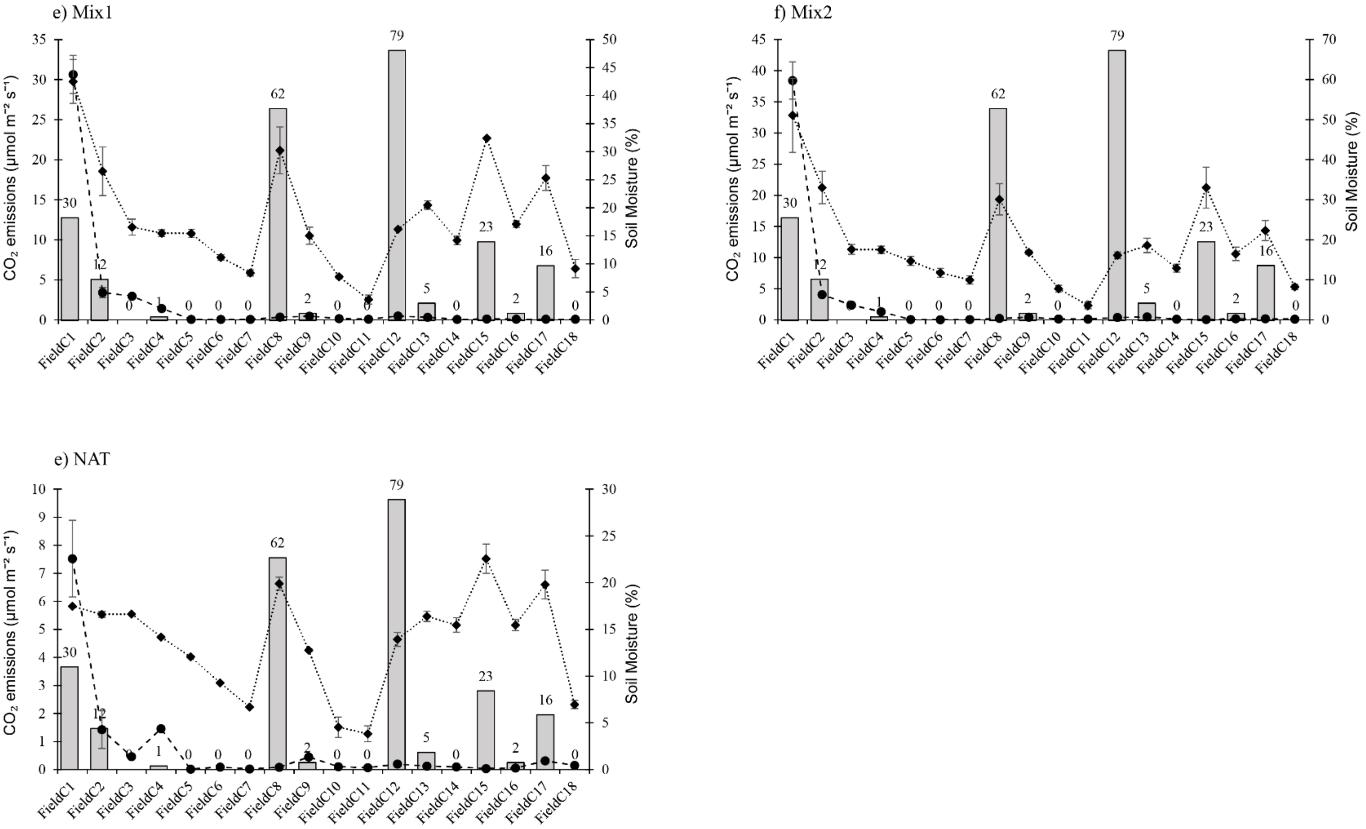
Date: day of measurement in the field campaign; precipitation: accumulated rainfall 10 days before field campaign measured on site with digital rain sensor[2]; T min day: minimum ambient temperature per day; T max day: maximum daily ambient temperature; T mean day: mean daily ambient temperature. * Data from RAIFALL003 (Junta de Andalucía).

Supplementary Table 3.2. PERMANOVA analysis. Significant differences ($P < 0.05$) in CO_2 fluxes and in soil properties based on two factors: treatment (different organic amendments applied, untreated soil, and natural reference soil) and date (different dates on which the measurement has been made throughout the chronological sequence).

	PERMANOVA			
		df	PS-F	P
CO_2 emissions	Treatment	6	44.34	0.0001
	Date	18	166.65	0.0001
	Treatment x Date	108	23.807	0.0001
		df	PS-F	P
Soil properties	Treatment	6	27.26	0.001
	Date	5	19.79	0.001
	Treatment x Date	30	1.51	0.002



Supplementary Figure 3.1. Temporal distribution of CO_2 emissions, rainfall and soil moisture during the experiment in the different field measurement campaigns. Footnotes: The round black dots (CO_2 in legend) represent the CO_2 emissions in each of the sampling campaigns. Grey bars represent rainfall and the number at the top represents the amount of accumulated precipitation 10 days before the measurement campaign (mm m^{-2}). The diamonds represent the soil moisture at 3 cm depth on day of measurement (M in legend). CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues, SS: wastewater station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70 °C compost; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. NAT: natural reference soils.



Supplementary Figure 3.1. (continuation). Temporal distribution of CO_2 emissions, rainfall and soil moisture during the experiment in the different field measurement campaigns. Footnotes: The round black dots (CO_2 in legend) represent the CO_2 emissions in each of the sampling campaigns. Grey bars represent rainfall and the number at the top represents the amount of accumulated precipitation 10 days before the measurement campaign (mm m^{-2}). The diamonds represent the soil moisture at 3 cm depth on day of measurement (M in legend). CON: unamended control soils; CG: 100% vegetable compost from garden waste; CC: vegetable compost from greenhouse crop residues, SS: wastewater station sludges from anaerobic mesophilic digestion, dehydrated by centrifuges and heat-dried at 70 °C compost; Mix1 (CG + SS) and Mix2 (CC + SS): mixture amendments from different vegetal compost and sludge compost. NAT: natural reference soils.

Supplementary material Chapter 4

Supplementary Table 4.1. Dates, precipitation (recorded 10 days before) and maximum, minimum and average daily temperature recorded in each of the net ecosystem CO_2 exchange measuring campaigns.

Campaing	**Date**	**Rainfall**	**Air temperature (ºC)**		
		(mm)	Max	Min	Average
T1	30/11/20	12	20.8	13.3	17.1
T2	12/01/21	142	14.2	6.3	9.8
T3	14/01/21	142	15.4	5.1	9.6
T4	18/01/21	100	18.1	5.2	11.6
T5	17/02/21	0	17.3	5.1	12.1
T6	25/03/21	27	18.9	11.2	14.8
T7	28/04/21	4	22.8	12.5	18.3
T8	20/05/21	0	27.3	17.5	22.1
T9	24/05/21	26	23.6	13.4	18.6
T10	25/06/21	3	31.6	20.3	26.5
M1	02/12/20	12	20	7.7	14.5
M2	13/01/21	142	15.5	5.5	9.7
M3	15/01/21	142	15.7	3.9	9.5
M4	19/01/21	29	15.8	7.1	11.5
M5	18/02/21	0	18	5.9	12.5
M6	05/04/21	5	18.6	12.1	16.2
M7	29/04/21	4	21.4	13.9	18.1
M9	02/06/21	36	25.7	17.3	21.5
M10	18/06/21	3	26.2	19.8	23

For two different types of campaigns: "T" includes measurements in experimental plots: CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost and T-CON: unamended soils. Campaigns "M" includes measurements in experimental plots: Mix1: SS + CG; Mix2: SS + CG; M-CON: no-amendment soils.

Supplementary Table 4.2. Component loadings on a two-principal-component-analysis solution for physical and chemical soil conditions in restored soils with organic amendments and control soils without any treatment. Variables included in the PCA analysis. EC: Electrical conductivity; AW: Available water; SOC: Soil Organic Carbon; TN: Total Nitrogen; C/N: carbon to nitrogen ratio. Variables included in the PCA analysis. EC: Electrical conductivity; AW: Available water; SOC: Soil Organic Carbon; TN: Total Nitrogen; C/N: carbon to nitrogen ratio.

Variables†	PC1	PC2
pH	0.399	0.305
EC	-0.316	-0.566
AW	0.258	0.371
SOC	-0.410	0.576
TN	-0.514	0.342
C/N ratio	0.491	0.032

Supplementary Table 4.3. Results (mean ± SEM; n = 3) and significant differences of ANOVA test ($p < 0.05$) for data presented in Figure 4.4. T-campaigns include: CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; T-CON: no-amendment soils. M-campaigns include: Mix1: SS + CG; Mix2: SS + CG; CON: no-amendment soils; θ: soil moisture; T_{soil}: soil temperature. The letters indicate the differences between treatments by field sampling campaign.

T-Campaings	**Microcosm**	**Treatment**	**θ** (%)	**T_{soil}** (ºC)
T1	St	CG	10.8 ± 1.94 a	21.1 ± 0.43 a
		SS	14.5 ± 3.37 a	22.5 ± 1.66 a
		CC	10.8 ± 0.34 a	24.3 ± 2.70 a
		T-CON	14.4 ± 0.34 a	19.3 ± 3.15 a
	Sc	CG	14.8 ± 0.86 a	20.9 ± 0.29 a
		SS	13.8 ± 2.31 a	22.1 ± 1.50 a
		CC	13.3 ± 1.36 a	24.2 ± 1.86 a
		T-CON	15.1 ± 1.38 a	23.1 ± 1.08 a
T2	St	CG	25.3 ± 1.46 ab	14.8 ± 1.64 a
		SS	34.6 ± 5.98 b	12.7 ± 1.23 a
		CC	23.4 ± 5.04 ab	14.6 ± 1.15 a
		T-CON	23.1 ± 2.68 a	13.9 ± 0.7 a
	Sc	CG	29.6 ± 3.38 ab	12.6 ± 1.64 a
		SS	34.6 ± 4.50 ab	12.7 ± 1.18 a
		CC	28.8 ± 1.32 ab	14.2 ± 1.27 a
		T-CON	27.8 ± 3.57 ab	13.5 ± 0.35 a
T3	St	CG	27.4 ± 5.5 ab	12.3 ± 2.88 a
		SS	23.2 ± 1.65 a	11.7 ± 2.48 a
		CC	26.7 ± 1.47 ab	14.6 ± 3.37 a

		T-CON	29.8 ± 4.68 ab	15.5 ± 1.27 a
	Sc	CG	28.2 ± 2.2 ab	12.5 ± 2.64 a
		SS	35.3 ± 4.74 b	12.3 ± 2.21 a
		CC	27.4 ± 3.40 ab	14.9 ± 2.96 a
		T-CON	31.7 ± 4.07 ab	15.8 ± 1.16 a
T5	St	CG	4.26 ± 0.63 a	21.2 ± 2.77 a
		SS	10.3 ± 1.86 bc	21.9 ± 1.58 a
		CC	4.93 ± 1.19 ab	24.6 ± 0.90 a
		T-CON	8.56 ± 2.98 abc	22.8 ± 1.02 a
	Sc	CG	11.7 ± 0.75 c	21.9 ± 2.06 a
		SS	11.4 ± 2.53 c	22.2 ± 1.18 a
		CC	10.8 ± 1.8 c	24.1 ± 0.86 a
		T-CON	13.4 ± 1.27 c	23.0 ± 0.92 a
T6	St	CG	6.86 ± 0.56 ab	26.0 ± 0.21 abc
		SS	9.43 ± 2.12 ab	26.3 ± 1.25 abc
		CC	4.46 ± 1.20 a	28.8 ± 1.50 c
		T-CON	10 ± 2.17 ab	24.7 ± 1.27 a
	Sc	CG	10.4 ± 1.21 abc	24.8 ± 0.87 a
		SS	12.5 ± 3.70 bc	26.4 ± 1.06 abc
		CC	5.4 ± 1.75 a	28.5 ± 1.65 bc
		T-CON	16.3 ± 2.27 c	25.1 ± 1.03 ab
T7	St	CG	7.16 ± 0.86 ab	27.5 ± 2.17 a
		SS	6.36 ± 0.08 a	28.7 ± 4.13 a
		CC	5.76 ± 0.66 a	24.9 ± 3.46 a
		T-CON	7.56 ± 0.71 ab	29.7 ± 1.79 a
	Sc	CG	10.3 ± 0.61 bc	31.6 ± 3.49 a
		SS	8.7 ± 1.37 abc	28.4 ± 3.71 a
		CC	6 ± 1.21 a	25.2 ± 2.62 a
		T-CON	11.4 ± 2.42 c	31.9 ± 2.60 a
T8	St	CG	6.06 ± 1.72 a	32.1 ± 1.03 a
		SS	3.33 ± 0.73 a	32.0 ± 3.49 a
		CC	4.33 ± 0.82 a	35.1 ± 2.43 a
		T-CON	3.86 ± 0.48 a	30.5 ± 1.72 a
	Sc	CG	5.56 ± 0.43 a	31.8 ± 1.21 a
		SS	5.7 ± 0.37 a	31.8 ± 3.44 a
		CC	4.03 ± 0.54 a	34.6 ± 2.19 a
		T-CON	5 ± 1.49 a	30.2 ± 1.83 a
T9	St	CG	20.6 ± 1.15 a	26.8 ± 3.26 a
		SS	28.1 ± 7.49 a	26.5 ± 2.17 a
		CC	19.1 ± 1.05 a	27.9 ± 1.49 a
		T-CON	19.8 ± 2.62 a	30 ± 1.81 a
	Sc	CG	26.4 ± 3.33 a	26.8 ± 3.32 a
		SS	28.7 ± 4.71 a	26.6 ± 2.01 a
		CC	23.9 ± 1.28 a	27.5 ± 1.48 a
		T-CON	29.5 ± 6.38 a	29.7 ± 1.70 a
T10	St	CG	5.53 ± 0.60 a	34.5 ± 3.85 a
		SS	4.83 ± 0.43 a	33.8 ± 3.78 a
		CC	4.3 ± 0.41 a	31.4 ± 2.93 a
		T-CON	4.76 ± 1.33 a	35.4 ± 2.28 a
	Sc	CG	6.03 ± 1.59 a	33.8 ± 3.00 a
		SS	7.36 ± 2.39 a	33.6 ± 3.16 a
		CC	3.7 ± 1.21 a	31.6 ± 2.65 a
		T-CON	7.46 ± 3.04 a	35.4 ± 2.29 a

M-Campaing	Microcosm	Treatment	θ (%)	T_{soil} (ºC)
M1	St	Mix1	11.6 ± 1.24 ab	21.8 ± 2.65 a
		Mix2	9.5 ± 1.12 a	21.0 ± 1.51 a
		M-CON	12.7 ± 0.40 ab	22.8 ± 1.38 a
	Sc	Mix1	11.6 ± 0.75 ab	23.6 ± 0.29 a
		Mix2	10.9 ± 1.42 ab	22.4 ± 3.26 a
		M-CON	15.6 ± 2.80 b	20.5 ± 2.21 a
M2	St	Mix1	25.2 ± 0.56 a	17 ± 1.58 a
		Mix2	29.4 ± 6.40 a	16.7 ± 1.31 a
		M-CON	29.5 ± 3.01 a	16.9 ± 1.76 a
	Sc	Mix1	29.0 ± 0.73 a	17.2 ± 0.88 a
		Mix2	27.6 ± 1.76 a	17.2 ± 1.08 a
		M-CON	29.5 ± 0.89 a	16.9 ± 0.40 a
M3	St	Mix1	26.7 ± 1.84 a	15.2 ± 1.82 a
		Mix2	23.3 ± 4.02 a	14.9 ± 2.28 a
		M-CON	21.4 ± 2.60 a	13.4 ± 1.56 a
	Sc	Mix1	27.6 ± 3.37 a	15.4 ± 1.17 a
		Mix2	32.0 ± 4.56 a	15.6 ± 1.75 a
		M-CON	28.9 ± 4.30 a	13.3 ± 2.05 a
M4	St	Mix1	22.7 ± 1.12 a	14.4 ± 2.68 a
		Mix2	24.6 ± 3.58 a	15.7 ± 1.86 a
		M-CON	20.4 ± 2.29 a	15.6 ± 1.35 a
	Sc	Mix1	23.5 ± 1.73 a	14.6 ± 2.45 a
		Mix2	26.8 ± 3.83 a	15.7 ± 1.88 a
		M-CON	27.2 ± 4.05 a	15.4 ± 1.37 a
M5	St	Mix1	9 ± 2.63 a	20.1 ± 2.34 a
		Mix2	10.9 ± 3.06 a	21.2 ± 1.66 a
		M-CON	9.33 ± 2.18 a	20.3 ± 2.07 a
	Sc	Mix1	9.43 ± 1.10 a	19.8 ± 2.28 a
		Mix2	12.5 ± 1.91 a	20.5 ± 2.02 a
		M-CON	12 ± 4 a	21.8 ± 1.06 a
M6	St	Mix1	8.3 ± 0.1 ab	17.8 ± 1.22 a
		Mix2	6.8 ± 1.24 a	17.9 ± 0.97 a
		M-CON	8.9 ± 1.73 ab	17.1 ± 0.89 a
	Sc	Mix1	13.8 ± 2.68 b	17.7 ± 1.26 a
		Mix2	13.3 ± 2.39 ab	17.8 ± 0.93 a
		M-CON	14.4 ± 3.29 b	17.1 ± 0.85 a
M7	St	Mix1	13.8 ± 2.29 b	22.4 ± 2.40 a
		Mix2	6.6 ± 2.65 a	27.0 ± 2.24 a
		M-CON	11 ± 1.04 ab	28.4 ± 1.14 a
	Sc	Mix1	14.0 ± 1.12 b	24.9 ± 3.35 a
		Mix2	10.5 ± 1.28 ab	29.1 ± 2.53 a
		M-CON	12.6 ± 2.6 ab	25.8 ± 4.40 a
M9	St	Mix1	17.6 ± 2.80 b	26.4 ± 2.87 a
		Mix2	13.2 ± 1.44 ab	28.6 ± 2.83 a
		M-CON	11.8 ± 1.66 a	25.7 ± 3.26 a
	Sc	Mix1	16.6 ± 1.11 ab	26.9 ± 2.50 a
		Mix2	16.3 ± 0.81 ab	28.4 ± 2.65 a
		M-CON	15.1 ± 0.55 ab	26.3 ± 3.43 a
M10	St	Mix1	9.06 ± 2.39 a	29.6 ± 1.25 a
		Mix2	7.03 ± 0.63 a	30.4 ± 2.73 a
		M-CON	9.06 ± 1.10 a	29.5 ± 2.02 a
	Sc	Mix1	13.2 ± 2.97 a	30.0 ± 1.55 a
		Mix2	14.2 ± 4.70 a	30.4 ± 2.55 a
		M-CON	14.2 ± 1.44 a	29.4 ± 1.79 a

Supplementary Table 4.4. Results of ANOVA test ($p < 0.05$) for data presented in Figure 4.5.

ANOVA analysis comparing treatments (CG, SS, CC and T-CON):

Biovolume					
Source	DF	SS	MS	F-Value	P-Value
Factor: Campaign	8	0.000027	0.000003	0.00	0.9999
Factor: Treatment	3	922.353	307.451	12.68	0.0000
Residual	96	2328.55	242.558		
Total	107	3250.91			
Plant cover					
Source	DF	SS	MS	F-Value	P-Value
Factor: Campaign	8	25929.3	3241.16	7.91	0.0000
Factor: Treatment	3	75165.7	25055.2	61.18	0.0000
Residual	96	39316.9	409.551		
Total	107	140412.			

ANOVA analysis comparing mixtures (Mix1, Mix2 and M-CON):

Biovolume					
Source	DF	SS	MS	F-Value	P-Value
Factor: Campaign	2	662.253	331.126	17.24	0.0001
Factor: Treatment	8	1,56E+05	194.748	1.01	0.4638
Residual	16	3,07E+05	192.083		
Total	26	1125.38			
Plant cover					
Source	DF	SS	MS	F-Value	P-Value
Factor: Campaign	2	30077.9	15038.9	224.43	0.0000
Factor: Treatment	8	2730.07	341.259	5.09	0.0028
Residual	16	1072.15	670.093		
Total	26	33880.1			

Supplementary Table 4.5. Results (mean ± SEM; n = 3) and significant differences of ANOVA test ($p < 0.05$) for data presented in Figure 4.6. T-campaigns include: CG: soils restored with garden compost; SS: soils restored with sewage sludge; CC: soils restored with greenhouse crop compost; T-CON: no-amendment soils. M-campaigns include: Mix1: SS + CG; Mix2: SS + CG; CON: no-amendment soils; NEE: net ecosystem CO_2 exchange; VPD: vapor pressure deficit. The letters indicate the differences between treatments by field sampling campaign.

T-Campaing	**Microcosm**	**Treatment**	**NEE** (μmol m^{-2} s^{-1})	**VPD** (kPa)
T1	St	CG	0.3 ± 0.14 b	3.87 ± 0.34 ab
		SS	0.48 ± 0.87 b	3.75 ± 0.69 ab
		CC	-0.3 ± 0.30 ab	3.51 ± 0.21 ab
		T-CON	-1.4 ± 1.02 a	3.25 ± 0.40 ab
	Sc	CG	0.89 ± 0.48 b	3.83 ± 0.10 ab
		SS	0.09 ± 0.28 ab	3.85 ± 0.22 ab
		CC	0.62 ± 0.57 b	4.14 ± 0.28 b
		T-CON	-0.2 ± 0.09 ab	3.02 ± 0.32 a
T2	St	CG	-0.1 ± 0.29 a	1.94 ± 0.45 a
		SS	0.35 ± 1.48 a	0.92 ± 0.56 a
		CC	0.14 ± 1.87 a	2.81 ± 0.05 a
		T-CON	-1.2 ± 1.70 a	2.09 ± 0.93 a
	Sc	CG	0.27 ± 0.13 a	2.14 ± 0.08 a
		SS	no data	no data
		CC	-1.8 ± 1.68 a	2.21 ± 1.87 a
		T-CON	0.08 ± 0.34 a	1.61 ± 0.72 a
T3	St	CG	-1.9 ± 2.19 a	2.56 ± 0.15 a
		SS	2.32 ± 1.69 b	2.28 ± 0.25 a
		CC	-2.4 ± 1.43 a	2.79 ± 0.50 a
		T-CON	-1.0 ± 0.62 ab	2.46 ± 0.23 a
	Sc	CG	-0.4 ± 0.36 ab	2.53 ± 0.28 a
		SS	0.49 ± 0.81 ab	2.65 ± 0.25 a
		CC	-0.4 ± 0.21 ab	2.79 ± 0.13 a
		T-CON	-0.2 ± 0.44 ab	2.42 ± 0.10 a
T5	St	CG	-2.7 ± 2.25 ab	3.77 ± 1.55 a
		SS	-3.6 ± 1.08 a	2.55 ± 1.31 a
		CC	-2.4 ± 1.29 ab	3.84 ± 0.52 a
		T-CON	-0.8 ± 0.66 ab	5.53 ± 0.75 a
	Sc	CG	no data	no data
		SS	0.36 ± 0.37 ab	4.41 ± 0.09 a
		CC	no data	no data
		T-CON	1.41 ± 1.21 b	3.88 ± 0.87 a
T6	St	CG	-2.2 ± 1.30 a	4.66 ± 0.59 a
		SS	-0.9 ± 2.24 ab	4.84 ± 0.67 a
		CC	-0.7 ± 1.52 ab	4.65 ± 1.53 a
		T-CON	no data	no data
	Sc	CG	2.81 ± 0.43 b	5.23 ± 0.89 a
		SS	1.07 ± 0.94 ab	4.17 ± 1.34 a
		CC	-0.4 ± 0.64 ab	5.25 ± 1.35 a
		T-CON	0.08 ± 0.05 ab	4.94 ± 0.52 a
T7	St	CG	1.32 ± 0.95 b	4.79 ± 0.34 a
		SS	-2.0 ± 0.44 a	3.99 ± 0.92 a
		CC	-0.2 ± 1.05 ab	5.06 ± 0.44 a
		T-CON	0.84 ± 0.50 b	4.42 ± 0.89 a
	Sc	CG	-0.3 ± 0.27 ab	5.51 ± 0.75 a
		SS	-0.0 ± 1.52 ab	3.76 ± 0.73 a
		CC	0.89 ± 0.44 b	4.55 ± 0.86 a
		T-CON	0.38 ± 1.57 ab	6.39 ± 0.88 a
T8	St	CG	-0.3 ± 0.26 a	5.96 ± 1.37 a

		SS	-1.0 ± 3.30 a	8.11 ± 0.00 a
		CC	-1.9 ± 0.31 a	6.92 ± 1.22 a
		T-CON	0.18 ± 0.05 a	8.03 ± 1.33 a
	Sc	CG	-1.1 ± 1.08 a	6.50 ± 1.25 a
		SS	-0.2 ± 0.39 a	7.39 ± 1.05 a
		CC	no data	no data
		T-CON	-0.9 ± 0.82 a	5.23 ± 3.17 a
T9	St	CG	3.62 ± 0.21 b	5.68 ± 1.68 a
		SS	2.92 ± 1.71 b	6.36 ± 0.66 a
		CC	-3.6 ± 0.90 a	5.85 ± 0.69 a
		T-CON	no data	no data
	Sc	CG	1.70 ± 0.26 b	5.95 ± 0.99 a
		SS	-1.3 ± 2.60 ab	6.19 ± 0.68 a
		CC	no data	no data
		T-CON	-0.0 ± 0.59 ab	6.48 ± 0.03 a
T10	St	CG	-0.5 ± 0.60 a	6.30 ± 1.08 a
		SS	-1.5 ± 1.03 a	5.93 ± 1.18 a
		CC	-0.9 ± 0.06 a	7.88 ± 1.15 a
		T-CON	-2.2 ± 1.12 a	6.07 ± 1.38 a
	Sc	CG	-1.5 ± 0.42 a	5.56 ± 1.78 a
		SS	-0.2 ± 0.13 a	6.08 ± 1.46 a
		CC	-1.4 ± 0.70 a	6.80 ± 0.71 a
		T-CON	no data	no data

M-Campaing	**Microcosm**	**Treatment**	**NEE** (µmol m^{-2} s^{-1})	**VPD** (kPa)
M1	St	Mix1	0.6 ± 0.12 abc	3.05 ± 0.11 a
		Mix2	0.66 ± 0.39 bc	2.18 ± 0.20 a
		M-CON	0.68 ± 0.31 bc	3.00 ± 0.71 a
	Sc	Mix1	1.07 ± 0.34 c	3.32 ± 0.44 a
		Mix2	0.33 ± 0.72 ab	2.95 ± 0.67 a
		M-CON	-0.3 ± 0.29 a	3.16 ± 0.88 a
M2	St	Mix1	0.43 ± 0.24 b	2.54 ± 0.07 a
		Mix2	-0.1 ± 0.48 ab	2.12 ± 0.25 a
		M-CON	-1.2 ± 0.79 a	2.52 ± 0.03 a
	Sc	Mix1	0.10 ± 0.39 ab	2.69 ± 0.52 a
		Mix2	0.12 ± 0.34 ab	2.63 ± 0.13 a
		M-CON	no data	no data
M3	St	Mix1	0.35 ± 0.14 a	1.82 ± 0.11 a
		Mix2	-0.7 ± 0.45 a	1.89 ± 0.35 a
		M-CON	-1.1 ± 0.47 a	1.44 ± 0.22 a
	Sc	Mix1	0.46 ± 0.40 a	2.07 ± 0.04 a
		Mix2	-0.2 ± 0.85 a	1.95 ± 0.30 a
		M-CON	-1.0 ± 0.43 a	1.99 ± 0.31 a
M4	St	Mix1	-0.9 ± 1.21 a	2.15 ± 0.07 a
		Mix2	1.15 ± 0.12 a	2.46 ± 0.22 a
		M-CON	no data	no data
	Sc	Mix1	-0.4 ± 0.04 a	2.51 ± 0.05 a
		Mix2	0.51 ± 0.12 a	2.18 ± 0.17 a
		M-CON	-0.7 ± 0.64 a	2.16 ± 0.25 a
M5	St	Mix1	-1.2 ± 0.31 a	3.61 ± 0.15 a
		Mix2	-2.0 ± 1.89 a	2.96 ± 0.21 a
		M-CON	-0.7 ± 0.34 a	3.49 ± 0.28 a
	Sc	Mix1	-0.8 ± 0.42 a	3.96 ± 0.60 a
		Mix2	-1.5 ± 0.26 a	3.88 ± 0.43 a
		M-CON	0.19 ± 0.17 a	3.23 ± 0.40 a
M6	St	Mix1	-6.3 ± 0.89 a	2.54 ± 0.29 a
		Mix2	no data	no data
		M-CON	-0.9 ± 0.56 bc	2.20 ± 0.35 a
	Sc	Mix1	-2.3 ± 1.00 b	2.67 ± 0.27 a
		Mix2	2.07 ± 1.86 c	2.18 ± 0.41 a
		M-CON	no data	no data
M7	St	Mix1	1.06 ± 0.40 a	4.67 ± 0.38 a
		Mix2	-0.1 ± 1.20 a	4.98 ± 0.89 a

		M-CON	-0.9 ± 0.53 a	5.42 ± 0.77 a
	Sc	Mix1	-0.4 ± 0.55 a	4.89 ± 0.93 a
		Mix2	0.32 ± 1.23 a	5.73 ± 0.84 a
		M-CON	-0.3 ± 0.96 a	3.21 ± 1.05 a
M9	St	Mix1	no data	no data
		Mix2	-3.8 ± 0.20 a	5.71 ± 0.76 a
		M-CON	no data	no data
	Sc	Mix1	no data	no data
		Mix2	no data	no data
		M-CON	no data	no data
M10	St	Mix1	2.05 ± 1.36 a	5.07 ± 0.35 a
		Mix2	-0.6 ± 1.46 a	4.98 ± 0.68 a
		M-CON	-0.7 ± 1.30 a	4.46 ± 0.46 a
	Sc	Mix1	0.05 ± 0.08 a	5.80 ± 0.74 a
		Mix2	-0.5 ± 1.95 a	4.65 ± 0.36 a
		M-CON	2.40 ± 1.48 a	5.40 ± 0.22 a

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