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PhD THESIS

Design and agro-environmental evaluation of advanced fertilisers based on bio-residues, rhizobacteria and the circular economy principles

PROGRAMA DE DOCTORADO EN
CIENCIA Y TECNOLOGÍA DEL MEDIO AMBIENTE

Tesis doctoral presentada por	*Director y Tutor*
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“No hace falta ser un genio para ser una buena científica,
yo soy una persona normal”
Margarita Salas (1938 - 2019)

“If we knew what it was we were doing,
it would not be called research, would it?”
Albert Einstein (1879 - 1955)

“Dime y lo olvido, enséñame y lo recuerdo,
involúcrame y lo aprendo”
Benjamin Franklin (1706 - 1790)

“Queda prohibido llorar sin aprender, olvidar el ayer,
no pensar que mañana puede ser todo mejor,
algo mejor, mucho mejor…”
Once varas

A mi familia,

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Chapter 1. Introduction

ABSTRACT

To reduce the negative environmental consequences of agriculture intensification and to achieve sustainability, the European Commission has fostered strategies based on the circular economy to reduce dependence on non-renewable sources. The use of bio-residues is a leading strategy to provide nutrients to crops and improve nutrient use efficiency. The objective of this work was to design and evaluate advanced fertilisers based on different combinations of bio-residues and rhizobacteria, as part of a global strategy based on a circular economy aimed at reducing the use of conventional mineral fertilisers in agriculture. Two families of advanced fertilisers were designed: the first based on compost and the second based on biochar. The first one consisted of two products: i) compost + biochar; and ii) compost inoculated with *Bacillus siamensis* (SCFB3-1 strain) formulated with biochar as a carrier (‘doped compost’). The second family consisted of one product, namely biochar + anaerobic digestate (AD). The aqueous extracts of the designed products were analysed for phytotoxicity and toxicity in three species of soil rhizobacteria. All products were phytostimulants at the dilution ratio of 1:25 (w:v), whereas for the ratio 1:10, they had either no phytotoxic or moderately phytotoxic effects. Conversely, more concentrated ratios were phytotoxic. No toxicity to rhizosphere bacteria was observed. The products compost + biochar and biochar + AD were preliminary tested in microcosm conditions; in such a trial, maize plants treated with either of the two products produced higher biomass than the non-fertilised control, although the N content in the biomass decreased. In commercial fields, the ‘doped compost’ worked better in terms of crop yield than the combination of compost and biochar. Moreover, a reduced dose (minus 20%) of mineral fertiliser combined with ‘doped compost’ (2 t ha^{-1}) produced a higher yield in melon and pepper than the control that received a full mineral fertiliser dose (24% to 33% higher in melon and 2% to 4% in pepper). Furthermore, the same reduced dose of mineral fertiliser combined with biochar + AD (250 to 500 t ha^{-1}) produced a higher melon yield (2% to 16% higher) and a similar pepper yield compared with the control that received the full mineral dose. In addition to the positive agronomic effects on crop yield from the environmental side, the first advantage is the reduction in the dose of mineral

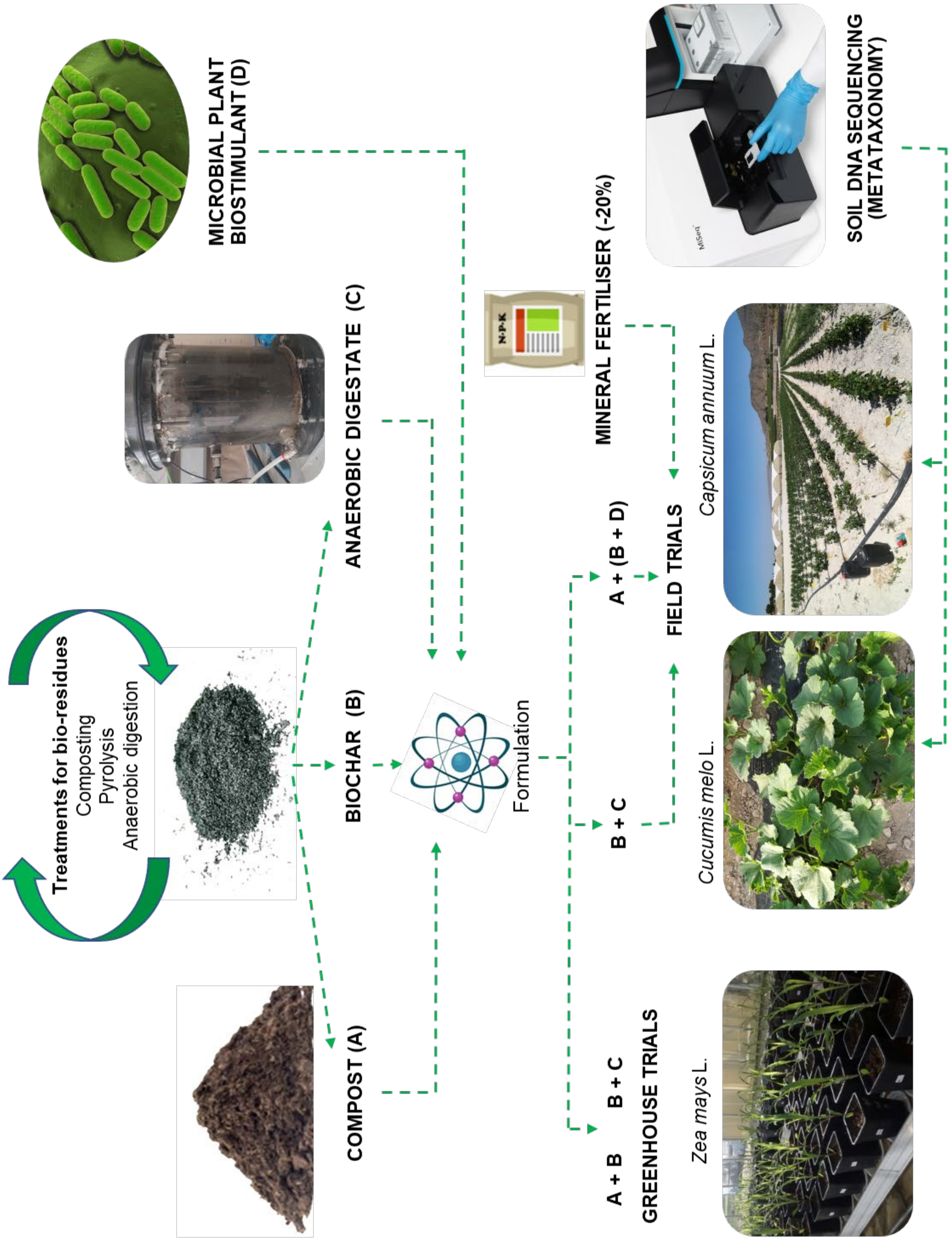
fertilisers. However, more relevant environmental benefits have been encountered in the soil microbiome, as revealed by the metataxonomic approach. Foremost, the composition of the bulk soil microbiome remained unaltered by the treatments. However, the treatments increased the soil microbiome activity, which consumed soil nitrogen (N); thus, the risk of N lixiviation was reduced, although it resulted in a lower N content in the crop biomass. We have demonstrated that the increased yield, even for less N available, is positively correlated with the enhanced microbiome activity but also with the presence of certain bacteria clusters considered plant growth promoting rhizobacteria (PGPR), whose relative abundance in the rhizosphere has been increased by the treatments. Moreover, microbiome diversity and species richness were also enhanced by the treatments, which theoretically has a positive effect on crop yield. We finally hypothesised that not only does the inoculated *B. siamensis* exert a direct effect on the crop, but it also causes changes in the rhizosphere. Further research is needed to understand the process involved in such a mechanism.

RESUMEN

Para reducir las consecuencias medioambientales negativas de la intensificación de la agricultura y lograr la sostenibilidad, la Comisión Europea ha fomentado estrategias basadas en la economía circular para reducir la dependencia de las fuentes no renovables. El uso de biorresiduos es una estrategia puntera para aportar nutrientes a los cultivos y mejorar la eficiencia en el uso de nutrientes. El objetivo de este trabajo fue diseñar y evaluar fertilizantes avanzados basados en diferentes combinaciones de biorresiduos y rizobacterias, como parte de una estrategia global basada en la economía circular dirigida a reducir el uso de fertilizantes minerales convencionales en la agricultura. Se diseñaron dos familias de fertilizantes avanzados: la primera basada en compost y la segunda en biochar. La primera constaba de dos productos: i) compost + biochar; y ii) compost inoculado con *Bacillus siamensis* (cepa SCFB3-1) formulado con biochar como soporte ('compost dopado'). La segunda familia constaba de un solo producto, concretamente, biochar + digerido anaerobio (DA). Los extractos acuosos de los productos diseñados fueron utilizados para analizar la fitotoxicidad y la toxicidad en tres especies de rizobacterias del suelo. Todos los productos fueron fitoestimulantes en la proporción de dilución 1:25 (p:v), mientras que en la proporción 1:10 no tenían efectos fitotóxicos o los tenían moderadamente. Por el contrario, las proporciones más concentradas resultaron fitotóxicas. No se observó toxicidad para las bacterias de la rizosfera. Los productos compost + biochar y biochar + AD se ensayaron preliminarmente en condiciones de microcosmos; en dicho ensayo, las plantas de maíz tratadas con cualquiera de los dos productos produjeron una biomasa superior a la del control no fertilizado, aunque el contenido de N en la biomasa disminuyó. En campo, el "compost dopado" funcionó mejor en términos de rendimiento de los cultivos que la combinación de compost y biochar. Además, una dosis reducida (menos 20%) de fertilizante mineral combinada con "compost dopado" (2 t ha^{-1}) produjo un mayor rendimiento en melón y pimiento que el control que recibió una dosis completa de fertilizante mineral (entre un 24% y un 33% mayor en melón y entre un 2% y un 4% en pimiento). Además, la misma dosis reducida de fertilizante mineral combinada con biochar + AD (250 a 500 t ha^{-1}) produjo un mayor rendimiento

en melón (2% a 16% mayor) y un rendimiento similar en pimiento en comparación con el control que recibió la dosis mineral completa. Además de los efectos agronómicos positivos sobre el rendimiento de los cultivos, desde el punto de vista ambiental, la primera ventaja es la reducción de la dosis de fertilizantes minerales. Sin embargo, se han encontrado beneficios ambientales más relevantes en el microbioma del suelo, como revela el enfoque metataxonómico. En primer lugar, la composición del microbioma del suelo permaneció inalterada por los tratamientos. Sin embargo, los tratamientos aumentaron la actividad del microbioma del suelo, que consumió nitrógeno (N) del suelo; por lo tanto, se redujo el riesgo de lixiviación de N, aunque resultó en un menor contenido de N en la biomasa del cultivo. Hemos demostrado que el aumento del rendimiento, incluso para menos N disponible, está positivamente correlacionado con la mayor actividad del microbioma, pero también con la presencia de ciertos grupos de bacterias consideradas rizobacterias promotoras del crecimiento vegetal (PGPR), cuya abundancia relativa en la rizosfera se ha visto incrementada por los tratamientos. Además, la diversidad del microbioma y la riqueza de especies también aumentaron con los tratamientos, lo que teóricamente tiene un efecto positivo en el rendimiento de los cultivos. Finalmente, nuestra hipótesis es que *B. siamensis* inoculado no sólo ejerce un efecto directo sobre el cultivo, sino que también provoca cambios en la rizosfera. Es necesario seguir investigando para comprender el proceso implicado en dicho mecanismo.

GRAPHICAL ABSTRACT



LIST OF ABBREVIATIONS

Abbreviations of bacterial strains

B. siamensis	*Bacillus siamensis*
P. brassicacearum	*Pseudomonas brassicacearum*
R. leguminosarum	*Rhizobium leguminosarum*

Other abbreviations through the document

AD	Anaerobic digestate
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
Bu	Bulk
Ca	Calcium
cfu	Colony-forming unit
C:N	Carbon : Nitrogen
CSTR	Continuosly stirred tank reactor
CO_2	Carbon dioxide
DNA	Deoxyribonucleic acid
EC	Electrycal conductivity
EU	European Union
FDA	Fluorescein diacetate hydrolytic activity
GHGs	Greenhouse gases
H'	Shannon index
GI	Germination index
HRT	Hydraulic retention time
IAA	Indole-3-acetic acid
IBM	International business machines
ITS	Internal transcribed spacer
K	Potassium
L	Litre
Mg	Magnesium
mg	milligram
mL	mililitre
MPB_s	Microbial plant biostimulants
MrDNA	Molecular research DNA

N	Nitrogen
NCBI	National center for biotechnology information
NGS	Next-generation sequencing
NH_3	Ammonia
nMDS	Non-metric multidimensional scaling
NOx	Nitrogen oxides
NPK	Nitrogen Phosphorus and Potassium
ns	Not significant
N_2O	Óxido nitroso
P	Phosphorus
PCR	Polymerase chain reaction
PGPB	Plant-growth promoting bacteria
PGPR	Plant-growth promoting rhizobacteria
pH	Potential of Hydrogen
Rh	Rhizospheric
RDP	Ribosomal database project
S	Species richness
SOC	Soil organic carbon
SPSS	Software platform of statistical analysis
TN	Total nitrogen
TSA	Tryptic soy agar
TSB	Tryptic soy broth
TOC	Total organic carbon
v/v	Volume/volume
w/v	Weight/volume
YMA	Yeast manitol agar

CHAPTER 1

Introduction and state of the art

1.1. Intensive agriculture: consequences and alternatives

The world's population is increasing year by year and as a consequence demands more food. The pressure of feeding an increasing number of people in combination with a change in diets towards more animal protein, puts a lot of additional pressure on the current available agricultural lands and nature areas (Erisman et al., 2016). This factor has led to an intensification of agriculture that started with the modern agriculture in the 20th century after the Second World War. To avoid a general shortage of food for the population, it is necessary to increase the use of chemical products, including mineral fertilisers, and other technological solutions as the use of heavy machinery, that make possible to increase crop production rapidly (Pellegrini and Fernández, 2018). The intensification of agriculture has allowed the production of more food in less time and space (Martin-Guay et al., 2018); but numerous environmental problems have arisen as a result of this intensification wherein amount produced takes precedence over quality and environmental preservation (Maxwell et al., 2016) as well as a loss of biodiversity (de Graaff et al., 2019) and many health problems for ensuring the world food security (Chandini et al., 2019). However, these effects were overlooked until recently, which has lead to issues like a decrease in agronomical biodiversity and an increase in the pollution of soil by chemical products as pesticides and fertilisers (Weldeslassie et al., 2018).

In brief, the negative effects of agricultural intensification are evident in terms of **soil fertility loss, water and air contamination** and **biodiversity decrease**.

Physical, chemical, and biological characteristics of soil encompass the broad notion of soil fertility which is linked to agricultural yield and food security (Kim and Bevis, 2019). Natural ecosystems may be able to sustain themselves with high productivity while conserving soil organic matter, nutrients, and water contents. By contrast, agrosystems reduce soil fertility (Nair, 2019), a condition that jeopardises crop quality and future crop yields (Bonanomi et al., 2020).

Although there are several causes of soil nutrient loss [mostly nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)], **soil erosion, crop consumption, and nutrient leaching are the main culprits**. First, it has been determined that soil erosion is the primary contributor to worldwide soil degradation, which endangers land, freshwater, and seas (Borrelli et al., 2020). The loss of crop-suitable soil from agricultural systems worldwide is projected to be 75 billion tonnes per year due to wind and water erosion, which harms the ecosystem and arable areas (Montanarella, 2015). Importantly, the natural soil fertility also declines when crop production intensifies and continuously removes the vital plant nutrients from the soil (Amanullah et al., 2019). Another negative well-known natural process is leaching which involves the loss of soluble materials from the soil as a result of the percolation of aqueous solutions (Tukey, 1970). Agronomic practises must be used to minimise nutrient leakage. Leached nutrients must be supplied in the soil in the form of fertilisers to make up for the leakage as this natural process cannot be totally avoided. Furthermore, an estimated 50% of inorganic fertilisers used on crops are not utilised by plants and are either stored in the soil or transferred to other ecosystems (Foley et al., 2011).

Moreover, the soil acidification can be caused by an accumulation of excess chemicals as fertilisers, fungicides and herbicides (Graversgaard et al., 2018). Acidification causes loss of fertility (Singh et al., 2020). This fact, together with increased osmotic potential and decreased organic matter resulting from the use of chemical in agriculture, suppresses microbial populations impoverishing the soils (Bruulsema, 2018). On the other hand, it is recognised that organic fertilisation enhances soil physical qualities, including porosity, aeration (Cercioglu, 2017), water-holding capacity (Zong and Lu, 2020) and provides nutrients, all of which are related to soil fertility. Mineral fertilisers replaced organic fertilisers after the Second World War, resulting in soil organic matter decrease.

Concerning water which is a vital resource that is both scarce and readily contaminated, only 1% of the water on Earth is deemed acceptable for human consumption, despite the fact that it covers three quarters of the planet (Ghaly and Ramakrishnan, 2015). Water availability is crucial to increase crop yields (Lu et al., 2015) and support agriculture and it uses about 70% of the world's freshwater withdrawals (Huang et al., 2019). According to Schweitzer and Noblet (2018), water pollution is the presence of external chemical, physical, or biological elements that degrade water bodies or alter their natural qualities; thus, it may be harmful to people, animals, and plants (FAO, 2018). When nutrients from fertilisers and manure, insecticides, or other chemical products are applied inefficiently or in excess to the soil and end up in water bodies cause water and soil pollution (Graversgaard et al., 2018). In the case of N fertilisers, when the excess reaches the food chain, it supposes a serious risk to the safety of the food chain (FAO and Intergovernmental Technical Panel on Soils, 2015). N can be absorbed by plants in various molecular forms. However, NO_3^-, is a potential source of water contamination, either directly through the incorporation of fertilisers or indirectly through the nitrification process.

Emissions of greenhouse gasses (GHGs) is one of the most important environmental problems nowadays. Agriculture contributes around 20% of global GHGs (United Nations, 2015). Ammonia (NH_3), nitrous oxide (N_2O), and N oxides (NO_x) are N species that are among the principal agricultural air pollutants (Bray et al., 2020). NH_3 released from agriculture and animal husbandry is contributing significantly to acidification (Wyer et al., 2022). Agriculture contributes over 81% of its total global NH_3 emissions (Van Damme et al., 2021). Respect to N_2O, human activity contributes about 30% of this gas in the atmosphere, primarily from agricultural sources (Velthof and Rietra, 2020). Regarding NOx, agriculture is another significant source with the highest soil emissions coming from areas with a high N fertiliser use (Almaraz et al., 2018), and these emissions have an impact on air quality by causing the formation of acid rain and ground-level ozone (Wang et al., 2020).

Biodiversity concept can be described as the richness and diversity of all life on earth, not only relevant in/for (semi-) natural areas, but also for agricultural areas which often have specific biodiversity which contributes to ecosystem services (Erisman et al., 2016). In addition to individual species, biodiversity also refers to the variety of habitats, and genes as well as the connections between them (United Nations, 1992). Maintaining biodiversity offers vital ecological services and functions for agricultural productivity, in fact it is crucial for ensuring global food and nutrition security (Chaudhary et al., 2019). Next to biodiversity loss due to habitat destruction by conversion of natural lands into agriculture, intensification of agriculture has led to a strong decline of specific farmland biodiversity (Erisman et al., 2016). Moreover, there is a lot of additional pressure on the currently accessible agricultural lands and natural areas due to the pressure of feeding a growing population and a change in diets toward more animal protein. This intensification has focussed on the cultivation and production of a few major staple crops, which are grown as large monocultures of genetically uniform individuals (Jacobsen et al., 2015). As a result, the genetic diversity of plants has gradually reduced (Besset-Manzoni et al., 2018).

Because of all the negative consequences of the intensification of agriculture mentioned above, humankind is increasingly aware that overexploitation has a negative impact on the environment in the current century. On other hand, modern agriculture is still strongly dependent on non-renewable energy sources, especially petroleum and its derivatives; thus, their continued use cannot be sustained for a longer time.

As a result, new initiatives for agriculture have emerged that focus on sustainable development while respecting the environment. Such “greener technologies” are supported by various types of European programs and legislation. The new technologies focus on the use of renewable biological raw materials, bio-residues, but also on the use of plant biostimulants, from microbial and non-microbial origin.

Both alternatives (bio-residues and microbial biostimulants) would constitute a **promising solution to the problem of agricultural intensification**. Moreover, these two alternatives would be framed within the second green revolution, whose objective is to maintain and improve crop yields, keeping the use of non-renewable resources, chemical inputs and water at as low levels as possible (Besset-Manzoni et al., 2018).

1.2. Transition to sustainable development in agriculture

The European Commission has emphasised in a large number of policy initiatives that were recently established in relation with sustainable agriculture: the EU Green Deal, Farm to Fork Strategy, Chemicals Strategy for Sustainability, New EU Strategy on Adaptation to Climate Change, Organic Farming Action Plan, Zero Pollution Action Plan for Air, Water and Soil, New Soil Strategy, Fertilising Product Regulation revision, and the Fit for 55 Climate Package (Hendriks et al., 2021). However, the Horizon Europe Program, the European Green Deal and the Circular Economy are more focused in sustainable fertilisers.

1.2.1. Horizon Europe Program and European Green Deal

The Horizon Europe (HE) Program is a scientific research initiative that replaces the Horizon 2020 program, with a period of action from 2021 to 2027 and a budget of 100 billion euros (European commission, 2019). Climate change and the transition to sustainable development will affect many aspects of today's European society and economy, especially those sectors and regions with high carbon emissions. Europe is focused on fostering competitiveness in a sustainable way with European companies, already representing five of the ten largest clean technology companies in the world (European commission, 2019). Therefore, the main objectives of the HE program through its second pillar are: (i) to generate knowledge; (ii) to strengthen the impact of research and innovation in the development, support and implementation of EU policies and (iii) to support access to innovative solutions with their adoption in European industry. In the latter sense linked to fertilisers, it has proposed by the HE program the recovery of nutrients from waste streams to produce bio-based fertilisers for reducing impacts associated to the production and use of synthetic fertilisers facilitating circularity (European commission, 2019). In summary, this pillar seeks to address global challenges such as climate change and sustainable development.

It must be achieved unheard-of technological, economic, and social reforms in order to protect our environment, biodiversity, and climate while also altering how we produce, trade, and consume by 2050. Through the European Green Deal (EGD), the EU will spearhead global efforts towards circular economies and clean green technologies, and work to decarbonize energy-intensive industries. In the case of the fertilisers, the EU policy priorities in the EGD include actions on reducing fertilisation input by 20% and nutrient losses by 50% without reducing the agricultural productivity (Panagos et al., 2022). Furthermore, the EU investments linked to the EGD will promote knowledge, build capacity and develop and demonstrate innovative solutions to accelerate the transition to sustainability in the areas of research and innovation in food, bioeconomy, natural resources, agriculture and environment. Thus, the following effects will be pursued with these investments:

- Improved knowledge and innovation will lay the foundation for climate neutrality by reducing greenhouse gas emissions and improving carbon sink and storage, as well as water resource management and production systems.
- A better knowledge of the frontiers for innovative solutions for the use of natural resources, as well as for the prevention and elimination of pollution, ensuring healthy soils, clean water and clean air for all.

- A better understanding of behavioural, socioeconomic and demographic changes leading to innovative approaches that drive sustainability and balanced development of rural and urban areas.

1.2.2. Circular economy concept

The concept of circular economy encompasses both economic growth and sustainable development. A circular economy is restorative and regenerative by design, and aims to always keep products, components and materials at their highest levels of use. Moreover, the circular economy is a positive continuous development cycle that preserves and increases natural capital, optimizes resource efficiency and minimizes risks to humanity's system by managing finite stocks and renewable flows being effectively at any scale (Cerdá and Khalilova, 2016). According to this concept, the following can be pointed out as key characteristics of a circular economy (De Wit et al., 2016):

- Reducing natural resources. Minimized and optimized exploitation of raw materials, providing more value with fewer materials. Reduced dependence on imports of natural resources. Efficient use of natural resources. Minimization of total water and energy consumption.
- Use more renewable and recyclable resources. Replace non-renewable resources with renewable resources at sustainable supply levels. Increased proportion of recyclable and recycled materials that can replace virgin materials. Extract raw materials sustainably, only when no other options are available.
- Reduce emissions. Reduced emissions throughout the entire material cycle. Less pollution thanks to clean material cycles.

- Reduce material losses and waste. Minimize waste accumulation. Limit and try to minimize the amount of waste incinerated and landfilled. Minimize losses due to resource dissipation.
- Maintain the value of products, components and materials in the economy. Extend the life of products, maintaining the value of products in use. Reuse components. Preserve the value of materials in the economy through high quality recycling.

One way to stimulate the transition towards sustainable and resilient farming systems is the circular economy and thus, circular agriculture, as it aims to minimise external inputs and negative discharges to the environment, and to close nutrient cycles. Therefore, it allows crop and livestock production without the depletion of non-renewable sources and harming the environment.

1.3. Specific actions to contribute sustainable agriculture from circular economy perspective

1.3.1. Application of treated bio-residues as organic fertilisers

1.3.1.1. Valorisation of bio-residues

As part of the package of measures focused on the transition to a circular economy, the EU has adopted the Directive (EU) 2018/851 on waste. This legislation reinforces the knowledge about the residue's hierarchy, and it requires each member state to adopt specific measures to prioritize waste prevention, reuse and recycling ahead of landfilling and incineration, thus making the circular economy a reality.

The management of residues in Spain is governed by the same principles as in the EU. Inadequate management or abandonment of residues is considered to affect ecosystems and human health, causing possible contamination of water, air and/or soil. However, when proper residues management is made, residues can be converted into resources (Figure 1.1) that contribute to sustainable development and conservation of raw materials (MITECO, 2013).

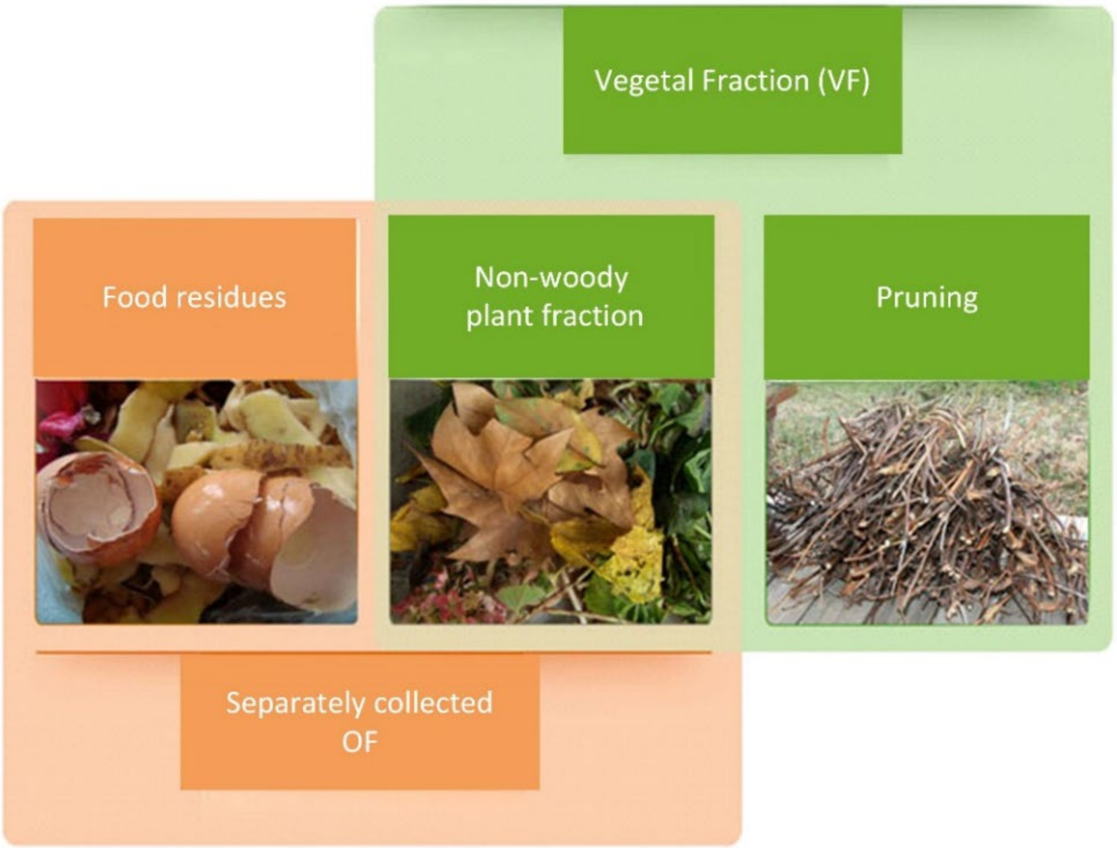


Figure 1.1. Types of bio-residues. Adapted from MITECO (2022).

1.3.1.2. Bio-residues classification and treatments required before application

According to the Directive (EU) 2018/851 on wastes, bio-residues are defined in the article 3 (4) as “biodegradable residues which come from gardens, and parks, food and kitchen wastes from households, offices, restaurants, wholesalers, canteens, restoration services and retail outlets, and comparable waste from food processing plants”. The Spanish Ministry for Ecological Transition and the Demographic Challenge (MITECO) has transposed that

European legislation and defines bio-residues as “biodegradable organic residues of plant and/or animal origin which are biodegradable generated at household and commercial level”.

These residues can be classified (Figure 1.1) according to their nature and from a waste management point of view (MITECO, 2022).

According to their nature, they are classified into:

- **Organic food and kitchen residues** (including food processing residues).
- **Vegetal residues or Vegetal Fraction (VF)** from private and public green areas and vegetation.

From a household organic residue management perspective, they are classified according to the following fractions:

- **Organic Fraction (OF).**

The OF components are: i) remains from food preparation or handling and processing of food products, leftover food remains, food in poor condition and food surpluses that have not been marketed or consumed. ii) Vegetable fraction in the form of small-sized, non-woody plant waste from gardening and pruning.

- **Pruning**

It consists of the VF, in the form of larger, woody plant waste from gardening, and pruning. Due to its characteristics, it requires specific management on account of issues related to collection logistics, treatment and the timing of generation.

The origin of these type of bio-residues is households, commercial activities, municipal facilities and restoration services, festivals and events (MITECO, 2022).

On the other hand, agricultural and forestry residues, as defined in the Spanish law 7/2022 (residues and contaminated soils for a circular economy) in the article 1 (ao), from agricultural, livestock and forestry activities are also considered bio-residues because of its biodegradable organic nature.

The European Directive (EU) 2018/851 on wastes, stipulates in the article 22 (2a) that bio-residues should be treated before further application using aerobic and anaerobic technologies as **composting** or **anaerobic digestion.** Failure to use these technologies for the treatment of these type of residues would pose threat to the quality of the receiving environment (Peng et al., 2020a; Peng et al., 2020b) because the biological processes, both anaerobic (anaerobic digestion) and aerobic (composting), are necessary to stabilise organic matter and maximise its potential before applying to the soil (Figure 1.2).

Pyrolysis and hydrothermal carbonization technologies to obtain biochar and char as amendments products for the land, are not contemplated by European and Spanish legislation as mandatory technologies for the treatment of bio-residues. However, these technologies are especially applied for the treatment of large-scale bio-residues with VF and pruning components coming from industrial activities mostly related to forestry and agriculture sectors. And indeed, it has been demonstrated their beneficial effect in the soil for agriculture (Ajeng et al., 2020; Hill et al., 2019) and bioremediation (Haider et al., 2022; Wang et al., 2022).

Figure 1.2. The organic matter cycle during bio-residues management. Adapted from MITECO (2022).

1.3.1.3. Bio-residues used in the present research

According to De Corato (2020), organic amendments as compost, biochar and digestate coming from the different kinds of agro-residues, are profitable sources of nutrients at lower costs for growers than chemical fertilisers and pesticides.

Therefore, the bio-residues used in this research were **anaerobic digestate, compost,** and **biochar.**

Anaerobic digestate is a bio-residue obtained through the process of anaerobic digestion (AD) which is an effective and environment-friendly process (Rada, 2015).

Compost is the final product obtained by composting. This is a low-cost technology, easy to handle where different biological and physico-chemical processes occurs improving the compost properties in terms of nutrients and organic matter. Compost can be used to recover degraded soils, to restore soil fertility by C-sequestration and as crop fertilisers, reducing the use of chemical inputs and the negative environmental impacts (De Corato, 2020). For that reason, fertilisation with compost has become an environmentally friendly technology for sustainable development (Vaverkova et al., 2020).

Biochar is a product of carbonization, characterized by a high content of organic carbon and low susceptibility to degradation, which is obtained through the pyrolysis of biomass and biodegradable waste (Saletnik et al., 2019). Recently, biochar has been recognized to comply with the objectives, criteria and principles of organic production in the EU's agriculture and has been admitted to the list of fertilisers/soil conditioners that can be used in organic farming. It has been included in the Annex I of the Regulation (EC) No. 889/2008 and the implementation of EU Regulation (EU) 2019/2164 (Official Journal of the European Union, L328/61, 18 December 2019), which has been in force since 2020.

The first scientific studies on the application of **compost** as an organic fertiliser were published around 1930s (Howard, 1933). The studies of **AD** applications in agricultural soils became more notable from the 1991s onwards with the European Council (EC) directive concerning the protection of waters against pollution caused by nitrates from agricultural sources, otherwise named "Nitrate's Directive" (Rizzioli et al., 2023). According to Lehman and Joseph

(2009), the earlier researchs on the effects of **biochar** on seedling growth was made by Retan (1915) and on soil chemistry by Tryon (1947), and next during the early 1980s by Kishimoto and Sugiura.

Although there is a history of **combining AD and biochar**, the main purpose so far has been to use the adsorption/desorption capacity of biochar to immobilise polluting cations or other polluting compounds such as herbicides (Peng and Pivato, 2017). Moreover, biochar also retains N-NH_4^+ (Kizito et al., 2015) and therefore the mixture with AD can help to retain the N contained in the AD, thus improving its properties and its positive effects on soil and plant.

Even if there are precedents in the **combination of compost and biochar** (Schmidt et al., 2014), the use of biochar as a carrier to add a beneficial PGPR to the compost in a protective environment provided by the biochar is a novelty in this work. It has been considered a promising strategy because some previous studies at microcosm scale have provided good results, but our work is the first that scale-up the technology to field trials (Nadeem et al., 2017; Rasool et al., 2021).

1.3.2. Organic fertilisers vs. mineral fertilisers from agronomic and environmental points of view

1.3.2.1. Agronomic effects

Organic amendments have been widely recognized by their positive effect in agroecosystems, e.g. improving the soil health, the grain yield (Yang et al., 2019), and other soil properties (Lin et al., 2019). Some studies have also suggested that the use of organic fertilisers, compared with mineral ones, resulted in higher seedling biomass (Sun et al., 2017) and crops yields (Li et al., 2018). Furthermore, from the biological point of view, organic matter increases soil microbial activities, which in turn improves crop growth and restraine pests and diseases incidence (Chang et al., 2010).

As an example, the use of digestate as a fertiliser is considered beneficial, as it provides nutrients (N, P, and K) and improves the soil structure with the addition of organic matter. Furthermore, the use of compost is recognized as beneficial because it increases soil organic matter content and decreases soil bulk density to mitigate the soil compaction issue (Akari and Uchida, 2020) and increase soil pH, electrical conductivity, and plant available P and NO_3^--N concentrations (Ouédraogo et al., 2001).

However, organic amendments alone may not offer sufficient nutrient supply to meet the demand for agriculture production. In Europe, 46% of the total N applied to agricultural soil comes from mineral fertiliser (Duan et al., 2020). Mineral fertilisers increase the plant growth and vigour and ensure the world food security (Chandini et al., 2019). Crop plants require N, P, and K to maintain the normal physiological function of the cell, and mineral fertilisers ensure all the nutrients crops need to complete their production cycle. However, whilst lack of N results in poor growth and slow growth, its excess results in delayed maturity and low quality of leaf.

1.3.2.2. Environmental effects

Application of organic fertiliser is thought to be a cost-effective way to increase soil organic carbon (SOC) (Maillard and Angers, 2014), which not only improves crop production (Oldfield et al., 2019) but also functions as a conditioner to enhance soil health and resilience, and improve water retention (Domingo et al., 2016; Oldfield et al., 2019). On other hand, there could be contaminants in the organic fertilisers made with bio-residues, such as heavy metals or antibiotics in the case of animal manure, that could limit the widespread use of animal wastes (Cobo et al., 2018). Previous treatments could be necessary to avoid environmental problems (Bai et al., 2016; Chandwich et al., 2015).

The overuse and/or used for the long-term of mineral fertilisers leds to soil mineralization and other derived issues as e.g. a low nutrients utilization rate and higher losses, which in turn initiates a series of eco-environmental problems, such as groundwater nitrate pollution, surface water contamination with loss of biodiversity, greenhouse gas emissions, soil acidification, and degradation (Dai et al., 2021). However, the nitrate discharged to water is not an exclusive issue of mineral fertilisers, as the excessive use of organic fertilisers with high N content can produce the same effect than mineral ones (Sutton et al., 2011; Wei et al., 2020). Moreover, the excessive use of mineral fertilisers leads to a deduction of soil fertility, affecting the structure of microbial communities (Lin et al., 2019). In addition, mineral fertilisers may represent a significant economic burden for farmers. Therefore, due to the high costs of mineral fertilisers, it is necessary to develop efficient and high-quality alternative methods at low cost.

Therefore, both organic and conventional mineral fertilisers have their respective advantages and drawbacks. The most updated agronomical practices use organic amendments to replace partially the mineral fertilisers (Wei et al., 2020). Such combination could possibly be the best solution to maximize the agronomic efficiency and crop productivity preserving life in the soil.

1.3.2.2.1 Effects of the fertilisation in the soil microbiome

The effect of fertilisation in soil microbiome is one of the most relevant environmental impacts, but it has been neglected up to very recent times because the use of traditional microbiological methods supposes a limiting condition to appraise the microbiome composition due to the fact that most microorganisms remain unculturable (Chen et al., 2012; Xi et al., 2019).

In 1998, the term metagenome came into general use when Handelsman et al. (1998) described soil microorganisms as essential sources of novel natural compounds. Recently, the emergence of a variety of molecular tools and the rapid development of next-generation DNA sequencing (NGS) technology have promoted the metagenomics analysis to resolve the challenge of assessing the entire microbial community, based on the 16S rRNA analysis extracted from the soil, but without requiring culturing methods. Besides, studies on functional analyses of soil microbiome are still relatively scarce and metagenomics as a non-culture approach encompasses a greater amount of microbial information than traditional approaches (Nwachukwu and Babalola, 2022).

Therefore, metagenomic analyses present an unprecedented opportunity to advance the understanding of the composition and function of soil microbial communities (Navarrete et al., 2015). Thus, metagenomics can contribute significantly to the study of agroecosystems and facilitate an understanding of the huge microbial diversities available in the soil environment, for biotechnological advancements and for sustainable agriculture (Garrido-Oter et al., 2018). The interactions of the microbial community members in the soil environment support not only the soil ecosystem integrity, furthermore nutrient recycling, habitat conservation and important physiological activities in the plants.

A highly diverse microbial taxa are also linked with plants in a singular entity that is known as holobiont (O'brien et al., 2019; Suárez and Stencel, 2020); these plants associated microbial taxa interactions could be neutral, pathogenic or beneficial (Igiehon and Babalola, 2018). The study of microbial communities associated with agricultural practices is not yet well-studied (Bevivino and Dalmastri, 2017). Thus, the emergence of metagenomics has offered exceptional opportunities to study the structure of the microbial community which provides information on the type of microorganisms present in soil (e.g, bacteria, archaea and eukaryotes) and their microbial composition (i.e. the identity and relative abundance of all taxa) in the community (Philippot et al.,2013) and their functional traits (Nwachukwu and Babalola, 2022). This approach is important to understand not only the microbial community structure in soils, furthermore the microbiome characteristics that are involved in plant-microbial interactions (Bulgarelli et al., 2015; Xu et al., 2018); as it has been proved to be an appropriate technique in understanding the different interactions occurring in the soil environment, rhizosphere, and plant tissues (Gupta et al., 2018) and the interactions when soil environments are negatively impacted by land preparation practices.

The NGS technique and metagenomics are also a valid technique to evaluate the microbial functional traits such as PGP actions on crops (Igiehon et al., 2019). Although, metagenomic analyses provide in-depth understanding of the functions of plant microbial community in the soil, evaluation of the associations between plant and microorganisms will only be comprehended when these functional characteristics or traits are studied in-situ (Nwachukwu and Babalola, 2022). On other hand, diverse microbial communities play important functional roles in biogeochemical cycles in soils being possible the identification of novel genomes involve in these cycles by metagenomics analysis application (Lo and Chong, 2020).

Therefore, the microbial communities from the soil are key players in agriculture, determining soil features, biogeochemical cycles, and plant health influencing yields and ultimately plant and fruit quality traits. Thus, metagenomic analysis has become an important tool for exploring microbiome and their potentials for improving sustainable agriculture by bringing to light many untapped soil microorganisms and their functionalities improving crop productivity, phytopathogen resistance, and nutrient cycling in plants.

Furthermore, applying metagenomics in soils treated with organic fertilisers would be useful in formulating strategies for fertilisation, reducing farmers’ dependence on chemical-based fertilisers and besides, being helpful in designing crop systems.

1.3.3. Bacterial inoculants for agriculture: Plant Growth Promoting Rhizobacteria

The term Plant Growth Promoting Rhizobacteria (PGPR) was defined by J.W. Kloepper and M.N. Schroth in 1978 to describe the rhizospheric bacteria which affect in positive terms the plant growth (Labra-Cardón, 2012). These bacteria have the ability to actively colonize the root system to improve its growth and performance (Moreno et al., 2018). PGPR represent around 2% to 5% of rhizospheric bacteria (Jha and Saraf, 2015). Bacterial genera that have been reported as PGPR are *Agrobacterium, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Micrococcous, Pantoea, Pseudomonas, Rhizobium* and *Serratia* among others (Ahemad y Kibret, 2013). However, the genera *Azospirillum, Azotobacter, Bacillus, Pseudomonas* and *Rhizobium* have been the most studied in the last few decades (Podile and Kishore, 2006; De Corato, 2020).

There are a variety of different rhizospheric and endophytic bacterial species that colonise plant roots to directly or indirectly support plant growth (Lugtenberg and Kamilova, 2009). The phytomicrobiome or plant-associated microbiota is the main source of bacterial strains to be used as inoculants in agriculture (Bettenfeld et al., 2020; Orozco-Mosqueda et al., 2018).

Bacillus is one of the genera that contributs a higher number of strains as PGPR. Several species show different PGP actions, even some of them have been described to be able to fix N, such as *Bacillus subtilis* and *Bacillus amyloliquefaciens* (Sun et al., 2020a; Sun et al., 2020b; Yang et al., 2020). *Bacillus siamensis* strain RGM 2529 and T20E-257 have been shown the ability to fix N (Altimira et al., 2022; Yoo et al., 2020). However, the ability of N fixation of the strain SCFB 3-1 from *B. siamensis* has not been evaluated, although Barquero (2014) found a high IAA production and ACC deaminase activity and Pastor-Bueis (2017) proved that the inoculant of the crops with this strain improves the nutrients use efficiency by the crop, particularly non-P, that produced a yield increase compared with the non-inoculated controls. Thus, the strain SCFB 3-1 from *B. siamensis* has been used as bacterial inoculant in the present work.

Despite the recognised advantages, microbial inoculants have been entering agriculture worldwide slowly. In the 1980s-1990s, the products based on microorganisms for agriculture entered the market and there was a massive sale, that vanished not long thereafter (Stamenkovic et al., 2018), due in part to the lack of proper regulation that ensures product quality, thus early items generally had poor quality and failed in field effectiveness. This fact caused farmers to lose faith in their abilities, and vanished from the market (Bashan et al., 2014). However, microbial inoculants have returned to the market recently, especially as a consequence of the second green revolution, also known as “Bio-Revolution” or “Fresh Green Revolution” (Backer et al., 2018). Many microbial inoculants are considered as eco-friendly and attractive alternatives for a partial replacement of mineral fertilisers and chemical pesticides (De Corato, 2020). Farmers and other agriculture stakeholders are now more receptive to the use of microbial inoculants, because legislation is constantly tightening, regulating, and even outlawing the use of synthetic fertilisers and pesticides (Keswani et al., 2019; Santos et al., 2019).

Several terms have been used during decades to denominate microbial inoculants aimed to improve plant growth by mechanisms different from biocontrol. They were frequently referred to as *biofertilisers* (Bhardwaj et al., 2014), *bioproducts* (Berg et al., 2013), *plant probiotics* (Flores-Félix et al., 2015), or *biostimulants* (Barquero et al., 2019). The most common usage has been to use "biofertiliser" but, there are disagreements among scientists as to what really qualifies as a biofertiliser. Nevertheless, the term biofertiliser has now been replaced by Microbial Plant Biostimulants.

1.3.4. Microbial Plant Biostimulants

The use of microorganisms that may be present in fertiliser products is regulated in Spain by the R.D. 999/2017 (Spain, 2017) and in Europe by the Regulation (EU) 2019/1009 (Europe, 2019) providing guidelines for the registration and use of microorganisms as fertilisers in agriculture. Both regulations acknowledge that microorganisms are, by nature, more similar to fertilising products than to other categories of plant protection products. Microorganisms that stimulate plant growth in different ways, enhance one or more of the following actions: increasing nutrients use efficiency, tolerance to abiotic stress, quality properties, or increasing the availability of confined nutrients in the soil or rhizosphere; thus, they must be covered by the fertiliser. Moreover, this European Regulation solves the controversies with the term biofertiliser for microbial products based on selected microorganisms, instead, the term used for such kind of products is Microbial Plant Biostimulants (MPBs) (Ricci et al., 2019).

The formulation of the inoculant plays a very important role in the success of the inoculants in the field (Bashan et al., 2014). There are two stages in the life cycle of an inoculant in which the formulation of the product plays a key role: i) the storage and distribution processes and ii) the field application process until the biostimulant exerts their action on the crop (Barquero et al., 2019). Formulations can be solid or liquid, although solid formulations provide physical protection to microorganisms. Although a variety of materials can be used as carriers in solid formulations, biochar is a good solution. The reason is due to its highly porous structure with nutrients naturally derived from the biomass, and high water and nutrient retention capacity which favour microbial immobilizing and later conservation to exert its functional action in the crop (Ajeng et al., 2020; Araujo et al., 2020). Moreover, using this material clean technologies as pyrolysis are being promoting assisting in the goal of sustainable agriculture.

1.4. Crop selection for the present research

Maize (*Zea mays*) is one of the most important and worldwide-distributed crops, due to its fast growth and higher biomass production (Ruiz et al. 2009; Wuana and Okieime, 2010). As a cereal crop ranks third globally in production after wheat and rice (Bawa, 2021) and it is the second most cultivated crop in the world (Guzzon et al., 2021). It is a versatile crop grown over a range of agroclimatic zones (Doebley, 1990) and economically important (Bawa, 2021). Moreover, it is one of the most important crops of Castille and Leon, being the first Spanish region in maize agricultural surface with 121,742 hectares cultivated and a production of 1,629,861 tons (t) in 2021. For these reasons, this crop was chosen for the greenhouse's trials.

Melon (*Cucumis melo* L.) and pepper (*Capsicum annuum* L.) are two horticultural crops that stand out for their nutritional properties, and these properties make them an interesting focus for their commercialization. Melon crop is considered as one of the ten most popular cultivated fruits in the world (Weng et al., 2021) and it is widely cultivated worldwide and welcomed by people because of its unique flavor and nutritional value (Shao et al., 2020). Considering economic incentives and the market demands, melon cultivation has rapidly expanded (Li et al., 2019; Zhang et al., 2016). In particular, Spain is the highest producer of melons in Europe, as well as the top exporter and the eighth-largest producer worldwide (FAOSTAT, 2022). Thus, melon crop require research to improve their quality and their agronomic performance because it has a great impact on the agriculture and the economy of Spain. Additionally, Spain is the fifth-largest pepper grower in the world and a major player in Europe (FAOSTAT, 2022). Thus, melon and pepper crop were chosen for the field assays due to these economical and commercialization reasons.

The present thesis has addressed the plant and soil effects for maize crop and the agronomic improvement for melon and pepper crop with the development of organic fertilisers based on treated bio-residues and MPBs, for a partial substitution of the mineral fertiliser.

1.5. References

Ahemad, M., Kibret, M., 2013. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University - Science 26 (1), 1-20. https://doi.org/10.1016/j.jksus.2013.05.001

Ajeng, A.A., Abdullah, R., Ling, T.C., Ismail, S., Lau, B.F., Ong, H.C., Chew, K.W., Show, P.L., Chang, J.S., 2020. Bioformulation of biochar as a potential inoculant carrier for sustainable agriculture. Environmental Technology & Innovation 20, 101168. https://doi.org/10.1016/j.eti.2020.101168

Altimira, F., Godoy, S., Arias-Aravena, M., Araya, B., Montes, C., Castro, J.F., Dardón, E., Montenegro, E., Pineda, W., Viteri, I., Tapia, E., 2022. Genomic and Experimental Analysis of the Biostimulant and Antagonistic Properties of Phytopathogens of Bacillus safensis and Bacillus siamensis. Microorganisms 10, 670. https://doi.org/10.3390/microorganisms10040670

Amanullah, Khalid, S., Imran, Khan, H. A., Arif, M., Altawaha, A. R., Adnan, M., Fahad, S., and Parmar, B., 2019. Organic Matter Management in Cereals Based System: Symbiosis for Improving Crop Productivity and Soil Health. in: Lal, R., Francaviglia, R. (Eds.), Sustainable Agriculture Reviews. Springer, Cham, pp. 67–92. https://doi.org/10.1007/978-3-030-26265-5_3

Araujo, J., Díaz-Alcántara, C. A., Urbano, B., González-Andrés, F. 2020. Inoculation with native Bradyrhizobium strains formulated with biochar as carrier improves the performance of pigeonpea (Cajanus cajan L.). *European Journal of Agronomy*, *113*, 125985. https://doi.org/10.1016/j.eja.2019.125985

Bai, Z.H., Ma, L., Jin, S.Q., Ma, W.Q., Velthof, G.L., Oenema, O., Liu, L., Chadwick, D., Zhang, F.S., 2016. Nitrogen, Phosphorus, and Potassium Flows through the Manure Management Chain in China. Environ. Sci. Technol. 50, 13409–13418. https://doi.org/10.1021/acs.est.6b03348

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D. L., 2018. Plant growth-promoting rhizobacteria:

Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science 9, 1473. https://doi.org/10.3389/fpls.2018.01473

Barquero, M. 2014. Caracterización y selección de bacterias y hongos micorrícicos aislados en raíces de alubia y pimiento, en la provincia de León, para el desarrollo de biofertilisantes. PhD thesis, University of Salamanca, Spain.

Barquero, M., Pastor-Buies, R., Urbano, B. and González-Andrés, F., 2019. Challenges, Regulations and Future Actions in Biofertilisers in the European Agriculture: From the Lab to the Field. in: Zúñiga- Dávila, D., González-Andrés, F., Ormeño-Orrillo, E. (Eds.), Microbial Probiotics for Agricultural Systems. Springer, New York, pp. 83-107. https://doi.org/10.1007/978-3-030-17597-9_6

Bashan, Y., De-Bashan, L. E., Prabhu, S. R., Hernandez, J. P., 2014. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). Plant and Soil 378, 1–33. https://doi.org/10.1007/s11104-013-1956-x

Bawa, A., 2021. Yield and Growth Response of Maize (Zea mays L.) to Varietal and Nitrogen Application in the Guinea Savanna Agro-Ecology of Ghana. Advances in Agriculture 2021, 1765251. https://doi.org/10.1155/2021/1765251

Bevivino, A., Dalmastri, C., 2017. Impact of agricultural land management on soil bacterial community: A case study in the mediterranean area. in: Lukax, P.G., Gamboni, M. (Eds.), Soil Biological Communities and Ecosystem Resilience. Springer, New York, pp 77–95. https://doi.org/10.1007/978-3-319-63336-7_5

Berg, G., Zachow, C., Müller, H., Philipps, J., Tilcher, R., 2013. Next-Generation Bio-Products Sowing the Seeds of Success for Sustainable Agriculture. *Agronomy 3* (4), 648–656. https://doi.org/10.3390/agronomy3040648

Besset-Manzoni, Y., Rieusset, L., Joly, P., Comte, G., Prigent-Combaret, C.

2018. Exploiting rhizosphere m*icrobial cooperation for developing sustainable agriculture strategies. Environ Sci Pollut Res* 25, 29953-29970. https://doi.org/10.1007/s11356-017-1152-2

Bettenfeld, P., Fontaine, F., Trouvelot, S., Fernandez, O. and Courty, P. E., 2020. Woody Plant Declines. What's Wrong with the Microbiome? *Trends Plant Sci* 25 (4), 381–394. https://doi.org/10.1016/j.tplants.2019.12.024

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., Tuteja, N., 2014. Biofertilisers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13, 66. https://doi.org/10.1186/1475-2859-13-66

Bonanomi, G., De Filippis, F., Zotti, M., Idbella, M., Cesarano, G., Al-Rowaily, S., Abd-ElGawad, A. 2020. Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield. Applied Soil Ecology 156(8), 103714. https://doi.org/10.1016/j.apsoil.2020.103714

Borrelli, P., Robinson, D. A., Panagos, P., Lugato, E., Yang, J. E., Alewell, C., Wuepper, D., Montanarella, L., Ballabio, C., 2020. Land use and climate change impacts on global soil erosion by water. Proceedings of the National Academy of Sciences of the United States of America 117(36), 21994–22001. https://doi.org/10.1073/pnas.2001403117

Bouhia, Y., Hafidi, M., Ouhdouch, Y., 2022. Conversion of waste into organo-mineral fertilisers: current technological trends and prospects. Rev Environ Sci Biotechnol 21, 425–446. https://doi.org/10.1007/s11157-022-09619-y

Bray, C. D., Battye, W. H., Aneja, V. P., Schlesinger, W. H. 2020. Global Emissions of NH 3 , NOx and N2O from Biomass Burning and the Impact of Climate Change. J Air Waste Manag Assoc 71(1), 102-114. https://doi.org/10.1080/10962247.2020.1842822

Bruulsema, T., 2018. Managing nutrients to mitigate soil pollution. Environmental Pollution 243, 1602–1605. https://doi.org/10.1016/j.envpol.2018.09.132

Bulgarelli, D., Garrido-Oter, R., Münch, P. C., Weiman, A., Dröge, J., Pan, Y., et al. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17, 392–403. https://doi.org/10.1016/j.chom.2015.01.011

Bulgari, R., Franzoni, G. and Ferrante, A. 2019. Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions. *Agronomy*, *9*, 1–30. https://doi.org/10.3390/agronomy9060306

Cercioglu, M. (2017). The Role of Organic Soil Amendments on Soil Physical Properties and Yield of Maize (Zea mays L.). *Communications in Soil Science and Plant Analysis*, *48*(6), 683–691. https://doi.org/10.1080/00103624.2017.1298787

Cerdá E, Khalilova A. 2016. Economía Circular, Estrategia Y Competitividad Empresarial. Econ Ind.

Chandini, R.K., Kumar, R. and Om, P. (2019) The Impact of Chemical Fertilisers on our Environment and Ecosystem. In: Research Trends in Environmental Sciences, 2nd Edition, 71-86.

Chang, K.H., Wu RY, Chuang KC, Hsieh TF, Chung RS. 2010. Effects of chemical and organic fertilisers on the growth, flower quality and nutrient uptake of *Anthurium andreanum*, cultivated for cut flower production. *Sci Hortic-Amsterdam*., 125:434–441.

Chaudhary, M., Pandey, A., Yadav, A., Naresh, R., Gangwar, L., Gupta, S., Kumar, A. (2019). *Improving sustainable food and nutrition systems with agro-biodiversity in recent paradigm of conservation agriculture: A review*.

Chen, M., Li, X., Yang, Q., Chi, X., Pan, L., Chen, N., et al. 2012. Soil eukaryotic microorganism succession as affected by continuous cropping of peanut – pathogenic and beneficial fungi were selected. PLoS One 7:e40659. https://doi.org/10.1371/journal.pone.0040659

Cobo, S.; Dominguez-Ramos, A.; Irabien, A. 2018. Minimization of Resource Consumption and Carbon Footprint of a Circular Organic Waste Valorization System. ACS Sustain. Chem. Eng. 6, 3493–3501.

Convention on Biological Diversity, United Nations. 1992. Available from: https://www.cbd.int/doc/legal/cbd-en.pdf.

Dai, X., Qiankun, G., Dali, S., Wei, Z., Guangrong, L., Guoqing, L., Ping, H., Gang, S., Fusheng, Y., Zengbing, L. 2021. Long-term mineral fertiliser substitution by organic fertiliser and the effect on the abundance and community structure of ammonia-oxidizing archaea and bacteria in paddy soil of south China. European Journal of Soil Biology, 103, 103288. https://doi.org/10.1016/j.ejsobi.2021.103288

De Corato, Ugo. 2020. Towards New Soil Management Strategies for Improving Soil Quality and Ecosystem Services in Sustainable Agriculture: Editorial Overview. *Sustainability* 12 (22): 9398. https://doi.org/10.3390/su12229398

de Graaff, M. A., Hornslein, N., Throop, H. L., Kardol, P., & van Diepen, L. T. A. (2019). Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis. In *Advances in Agronomy* (1st ed., Vol. 155). Elsevier Inc. https://doi.org/10.1016/bs.agron.2019.01.001

De Wit M, Bardout M, Ramkumar S, Kubbinga B. 2016. The Circular Dairy Economy: Exploring the business case for a farmer led, “net-postive” circular dairy sector.

Doebley, J. 1990. Molecular evidence for gene flow among Zea species. BioScience, vol. 40, no. 6, pp. 443–448.

Domingo-Olivé, F.; Bosch-Serra, À.D.; Yagüe,M.R.; Poch, R.M.; Boixadera, J. 2016. Long Term Application of Dairy CattleManure and Pig Slurry toWinter Cereals Improves Soil Quality. Nutr Cycl Agroecosyst 104, 39–51.

Duan, Y.F., Bruun, S., Stoumann Jensen, L., Van Gerven, L., Hendriks, C.M.J., Stokkermans, L., Groenedijk, P., Lesschen, J.P., Prado, J., Fangueiro, D., 2020. Nutri2Cycle: D1.5 Mapping and Characterization of CNP Flows and Their Stoichiometry in Main Farming Systems in Europe. Available from: https://www.nutri2cycle.eu/wp-content/uploads/2021/05/D1.5-Nutri2Cycle_Report-on-the-mapping-and-charcaterization-of-CNP-flows.pdf

Erisman, J.W., Nick van Eekeren, Jan de Wit, Chris Koopmans, Willemijn Cuijpers, Natasja Oerlemans, Ben J. Koks. 2016. Agriculture and biodiversity: a better balance benefits both[J]. AIMS Agriculture and Food, 1(2): 157-174. https://doi.org/10.3934/agrfood.2016.2.157

Europe, 2018. Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May 2018 amending Directive 2008/98/EC on waste.

Europe, 2019. Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003.

European Commission, 2019. Orientations towards the first Strategic Plan implementing the research and innovation framework programme Horizon Europe 2021-2027, 2019:142.

FAO. 2018. *Global Symposium on Soil Pollution (GSOP18).* Concept Note.

FAO and Intergovernmental Technical Panel on Soils. 2015. Status of the World's Soil Resources (SWSR) - Main Report. Available from: www.fao.org/3/a-i5199e.pdf.

FAOSTAT. 2022. Available from: http://www.fao.org/faostat/en/#home

Flores Félix, J. D., Silva, L. R. ., Rivera, L. P., Marcos-García, M., García-Fraile, P., Martínez-Molina, E., Mateos, P. F., Velázquez, E., Andrade, P. and Rivas, R. 2015. Plants probiotics as a tool to produce highly functional fruits: The case of Phyllobacterium and vitamin C in strawberries. PLoS ONE, 10 (4). https://doi.org/10.1371/journal.pone.0122281

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., Mueller, N. D., O'Connell, C., Ray, D. K., West, P. C., Balzer, C., Bennett, E. M., Carpenter, S. R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., … Zaks, D. P. M. (2011). Solutions for a cultivated planet. *Nature*, *478*(7369), 337–342. https://doi.org/10.1038/nature10452

Garrido-Oter, R., Nakano, R. T., Dombrowski, N., Ma, K. W., Team, T. A., Mchardy, A. C., et al. (2018). Modular traits of the rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. Cell Host Microbe 24, 155–167.e5. https://doi.org/10.1016/j.chom.2018.06.006

Ghaly, A. and Ramakrishnan, V. 2015. Nitrogen Sources and Cycling In the Ecosystem and its Role in Air, Water and Soil Pollution: A Critical Review. *Journal of Pollution Effects and* Chapter 116 *Control*. https://doi.org/10.4172/2375-4397.1000136.

Graversgaard, M., Hedelin, B., Smith, L., Gertz, F., Højberg, A. L., Langford, J., Martinez, G., Mostert, E., Ptak, E., Peterson, H., Stelljes, N., van den Brink, C., & Refsgaard, J. C. (2018). Opportunities and barriers for water co-governance-A critical analysis of seven cases of diffuse water pollution from agriculture in Europe, Australia and North America. Sustainability 10 (5). https://doi.org/10.3390/su10051634

Gupta, N., Vats, S., and Bhargava, P. (2018). “Sustainable agriculture: role of metagenomics and metabolomics in exploring the soil microbiota,” in Silico Approach for Sustainable Agriculture, eds D. K. Choudhary, M. Kumar, R. Prasad, and V. Kumar (New York, NY: Springer), 183–199.

Guzzon, F.; Arandia Rios, L.W.; Caviedes Cepeda, G.M.; Céspedes Polo, M.; Chavez Cabrera, A.; Muriel Figueroa, J.; Medina Hoyos, A.E.; Jara Calvo, T.W.; Molnar, T.L.; Narro León, L.A. 2021. Conservation and Use of Latin American Maize Diversity: Pillar of Nutrition Security and Cultural Heritage of Humanity. Agronomy, 11, 172. https://doi.org/10.3390/agronomy11010172

Haider, F. U., Wang, X., Zulfiqar, U., Farooq, M., Hussain, S., Mehmood, T., Naveed, M., Li, Y., Liqun, C., Saeed, Q., Ahmad, I. and Mustafa, A. 2022. Biochar application for remediation of organic toxic pollutants in contaminated soils; An update. Ecotoxicology and Environmental Safety 248, 114322. https://doi.org/10.1016/j.ecoenv.2022.114322

Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., and Goodman, R. M.

(1998). Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem. Biol. 5, R245–R249. https://doi.org/10.1016/S1074-5521(98)90108-9

Hendriks, C.M.J.; Shrivastava, V.; Sigurnjak, I.; Lesschen, J.P.; Meers, E.; Noort, R.v.; Yang, Z.; Rietra, R.P.J.J. 2022. Replacing Mineral Fertilisers for Bio-Based Fertilisers in Potato Growing on Sandy Soil: A Case Study. Appl. Sci., 12, 341. https://doi.org/10.3390/app12010341

Hill, R.A., Hunt, J., Sanders, E., Tran, M., Burk, G.A., Mlsna, T.E., Fitzkee, N.C. 2019. Effect of biochar on microbial growth: A metabolomics and bacteriological investigation in E. coli. Environ. Sci. Technol. http://doi.org/10.1021/acs.est.8b05024

Howard, A. 1933. The waste products of agriculture: their utilization as humus. Journal of the Royal Society of arts, 82 (4229), 84-121. Available from: https://www.jstor.org/stable/41360014

Huang, Z., Hejazi, M., Tang, Q., Vernon, C. R., Liu, Y., Chen, M., & Calvin, K. 2019. Global agricultural green and blue water consumption under future climate and land use changes. Journal of Hydrology *574*, 242–256. https://doi.org/10.1016/j.jhydrol.2019.04.046

Igiehon, N. O., and Babalola, O. O. 2018. Below-ground-above-ground plantmicrobial interactions: focusing on soybean, rhizobacteria and mycorrhizal fungi. Open Microbiol. J. 12, 261. https://doi.org/ 10.2174/1874285801812010261

Igiehon, N. O., Babalola, O. O., Aremu, B. R. 2019. Genomic insights into plant growth promoting rhizobia capable of enhancing soybean germination under drought stress. BMC Microbiol. 19, 159. https://doi.org/10.1186/s12866-019-1536-1.

Jacobsen, S. E., Sørensen, M., Pedersen, S. M., and Weiner, J. 2015. Using our agrobiodiversity: plant-based solutions to feed the world. *Agronomy for Sustainable Development 35* (4), 1217–1235. https://doi.org/10.1007/s13593-015-0325-y

Jha, C. K. and Saraf, M. 2015. Plant growth promoting Rhizobacteria (PGPR): a review. Journal of Agri-Biofertilisation and sustainable agricultura 82 cultural Research and Development, 5 (2). https://doi.org/10.13140/RG.2.1.5171.2164.

Jian Zhang, Jiajia Wang, Pengcheng Wang, and Tingting Guo. "Effect of no-tillage and tillage systems on melon (Cucumis melo L.) yield, nutrient uptake and microbial community structures in greenhouse soils" Folia horticulturae 32, no. 2 (2020): 265-278. https://doi.org/10.2478/fhort-2020-0024

Katie E. Wyer, David B. Kelleghan, Victoria Blanes-Vidal, Günther Schauberger, Thomas P. Curran (2022). Ammonia emissions from agriculture and their contribution to fine particulate matter: A review of implications for human health, Journal of Environmental Management, 323, 116285, https://doi.org/10.1016/j.jenvman.2022.116285

Keswani, C., Prakash, O., Bharti, N., Vílchez, J. I. ., Sansinenea, E., Lally, R. D. ., Borriss, R., Singh, S. P. ., Gupta, V. K. ., Fraceto, L. F. ., de Lima, R., and Singh, H. B. 2019. Re-addressing the biosafety issues of plant growth promoting rhizobacteria. *Science of the Total Environment*, *690*, 841–852. https://doi.org/10.1016/j.scitotenv.2019.07.046

Kim, K. and Bevis, L. 2019. Soil Fertility and Poverty in Developing Countries. *Source: Choices*, *34*(2), 1–8. https://doi.org/10.2307/26785776

Kizito, S., Wu, S., Kipkemoi, K.W., Lei, M., Lu, Q., Bah, H., Dong, R. 2015. Evaluation of slow pyrolyzed wood and rice husks biochar for adsorption of ammonium nitrogen from piggery manure anaerobic digestate slurry. Science of The Total Environment 505, 102-112.

Labra-Cardón, D., Guerrero-Zúñiga, L. A., Rodríguez-Tovae, A. V., Montes-Villafán, S., Pérez-Jiménez, S. y Rodríguez-Dorantes, A. 2012. Respuesta de crecimiento y tolerancia a metales pesados de *Cyperus elegans* y *Echinochloa polystachya* inoculadas con una rizobacteria aislada de un suelo contaminado con hidrocarburos deriva-dos del petróleo. Revista

Internacional de Conta-minación Ambiental 28 (1), 7-16.

Lehmann, J., Joseph, S., 2009. Biochar for environmental management: an introduction. En: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management: science and technology. Earthscan, London, pp. 1-9. https://doi.org/10.4324/9781849770552

Li, Y.C., Li Z.W., Lin WW, Jiang YH, Weng BQ, Lin WX. 2018. Effects of biochar and sheep manure on rhizospheric soil microbial community in continuous ratooning tea orchards. *Chin J Appl Ecol* 29, 1273–1282.

Li, S., Ni, X., XiA, Q., Li, Y., Dong, X., Hou, J., Li, Z., CHeng, S., CAo, D., Zhang, Z. 2019. Rapid characterization of the genetic loci controlling commodity traits of Chinese hami melon (Cucu-mis melo var. Saccharinensis Naud.) through mul-tiplexed shotgun genotyping. Agronomy 9 (8), 430. https://doi.org/10.3390/agronomy9080430

Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z., & Lin, W. (2019). The effects of chemical and organic fertiliser usage on rhizosphere soil in tea orchards. PLOS ONE, 14(5), e0217018.

Lo, R.L.S., Chong, K.P. 2020. Metagenomic data of soil microbial community in relation to basal stem rot disease. Data in Brief, 31, 106030. https://doi.org/10.1016/j.dib.2020.106030.

Lu, Y., Song, S., Wang, R., Liu, Z., Meng, J., Sweetman, A. J., Jenkins, A., Ferrier, R. C., Li, H., Luo, W., & Wang, T. (2015). *Impacts of soil and water pollution on food safety and health risks in China*. https://doi.org/10.1016/j.envint.2014.12.010.

Lugtenberg, B. and Kamilova, F. 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology*, *63* (1), 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918

Maillard, E.; Angers, D.A. 2014. Animal Manure Application and Soil Organic Carbon Stocks: A Meta-Analysis. Glob. Chang. Biol. 20, 666–679.

Martin-Guay, M. O., Paquette, A., Dupras, J., & Rivest, D. (2018). The new Green

Revolution: Sustainable intensification of agriculture by intercropping. *Science of the Total Environment*, *615*, 767–772. https://doi.org/10.1016/j.scitotenv.2017.10.024

Maxwell, S. L., Fuller, R. A., Brooks, T. M., & Watson, J. E. M. (2016). Biodiversity: The ravages of guns, nets and bulldozers. In *Nature* (Vol. 536, Issue 7615, pp. 143–145). Nature Publishing Group. https://doi.org/10.1038/536143a

MITECO. 2013. PROGRAMA ESTATAL MARCO DE GESTIÓN DE RESIDUOS 2016-2022. Available from: https://www.miteco.gob.es/es/calidad-y-evaluacion-ambiental/planes-y-estrategias/Planes-y-Programas.aspx.

MITECO. 2022. PROGRAMA ESTATAL MARCO DE PREVENCIÓN DE RESIDUOS. Available from: https://www.miteco.gob.es/es/calidad-y-evaluacionambiental/temas/prevencion-y-gestionresiduos/flujos/biorresiduos/Default.aspx.

Montanarella, L. 2015. Agricultural policy: Govern our soils. *Nature*, *528*(7580), 32–33. https://doi.org/10.1038/528032a

Moreno-Reséndez, A., Carda-Mendoza, V., Reyes-Carrillo, J.L., Vásquez-Arroyo, J., Cano-Ríos, P. 2018. Rizobacterias promotoras del crecimiento vegetal: una alternativa de biofertilisación para la agricultura sustentable. Revista Colombiana de Biotecnología, 20 (1), 68 83. https://doi.org/10.15446/rev.colomb.biote.v20n1.73707

Nadeem, S.M.; Imran, M.; Naveed, M.; Khan, M.Y.; Ahmad, M.; Zahir, Z.A.; Crowley, D.E. 2017. Synergistic Use of Biochar, Compost and Plant Growth-Promoting Rhizobacteria for Enhancing Cucumber Growth under Water Deficit Conditions. J. Sci. Food Agric. 97, 5139–5145.

Nair, K. P. 2019. Soil Fertility and Nutrient Management. In *Intelligent Soil Management for Sustainable Agriculture* (pp. 165–189). Springer International Publishing. https://doi.org/10.1007/978-3-030-15530-8_17

Navarrete, A. A., Diniz, T. R., Braga, L. P., Silva, G. G., Franchini, J. C., Rossetto, R., et al. 2015. Multi-analytical approach reveals potential microbial indicators in soil for sugarcan.

Nwachukwu, B.C., Babalola, O.O., 2022. Metagenomics: A Tool for Exploring Key Microbiome With the Potentials for Improving Sustainable Agriculture. Frontiers in Sustainable Food Systems, (6), 886987. https://doi.org/10.3389/fsufs.2022.886987

O'brien, P. A.,Webster, N. S.,Miller, D. J., and Bourne, D. G. 2019. Host-microbe coevolution: applying evidence from model systems to complex marine invertebrate holobionts.MBio 10, e02241–e02218. https://doi: 10.1128/mBio.02241-18

Oldfield, E.E.; Bradford, M.A.; Wood, S.A. Global Meta-Analysis of the Relationship Between Soil Organic Matter and Crop Yields. Soil 2019, 5, 15–32.

Ouédraogo, E., A. Mando, and N. P. Zombré, 2001. "Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa," *Agriculture, Ecosystems & Environment*, vol. 84, no. 3, pp. 259–266.

Orozco-Mosqueda, M. del C., Rocha-Granados, M. del C., Glick, B. R., and Santoyo, G. 2018. Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. In *Microbiological Research* (Vol. 208, pp. 25–31). Elsevier GmbH. https://doi.org/10.1016/j.micres.2018.01.005

Panagos, P., Köningner, J., Ballabio, C., Liakos, L., Muntwyler, A., Pasquale, B., Lugato, E. 2022. Improving the phosphorus budget of European agricultural soils, 853, 158706. https://doi.org/10.1016/j.scitotenv.2022.158706

Pastor-Bueis, R., Mulas, R., Gómez, X. and González-Andrés, F. 2017. Innovative liquid formulation of digestates for producing a biofertiliser based on Bacillus siamensis: Field testing on sweet pepper. Journal of Plant Nutrition and Soil Science, 180(6), 748–758. https://doi.org/10.1002/jpln.201700200

Peng, W., Pivato, A. 2017. Sustainable Management of Digestate from the

Organic Fraction of Municipal Solid Waste and Food Waste Under the Concepts of Back to Earth Alternatives and Circular Economy. Waste and Biomass Valorization, 1–17. https://doi:10.1007/s12649-017-0071-2

Peng, W., F. Lü, L. Hao, H. Zhang, L. Shao, P. He. 2020a. Digestate management for high-solid anaerobic digestion of organic wastes: a review. Bioresour. Technol., 297. Article 122485

Peng, W., A. Pivato, F. Garbo, T.F. Wang. 2020b. Effects of char from biomass gasification on carbon retention and nitrogen conversion in landfill simulation bioreactors. Environ. Sci. Pollut. Res., 27. pp. 6401-6410.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., and VanDer Putten,W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799. https://doi: 10.1038/nrmicro3109.

Podile AR and Kishore GK. 2006. Plant growth-promoting rhizobacteria. In: Gnanamanickam SS, editor. Plant-Associated Bacteria. Springer; Netherlands. pp. 195–230.

Rada, E.C. 2015. Biological Treatment of Solid Waste, Enhancing Sustainability, 1st ed.; Apple Academic Press, Inc.: Oakville, ON, Canada, pp. 3–103.

Rasool, M.; Akhter, A.; Soja, G.; Haider, M.S. 2021. Role of Biochar, Compost and Plant Growth Promoting Rhizobacteria in the Management of Tomato Early Blight Disease. Sci. Rep., 11, 6092.

Ricci, M., Tilbury, L., Daridon, B. and Sukalac, K. 2019. General principles to justify plant biostimulant claims. In *Frontiers in Plant Science* (Vol. 10). Frontiers Media S.A. https://doi.org/10.3389/fpls.2019.00494.

Rizzioli, F., Bertasini, D., Bolzonella, D., Frison, N., Battista. 2023. A critical review on the techno-economic feasibility of nutrients recovery from anaerobic digestate in the agricultural sector. Separation and Purification Technology, 306, 122690. https://doi.org/10.1016/j.seppur.2022.122690.

Ruiz E, Rodríguez L, Alonso-Azcaráte J et al. 2009. Phytoextraction of metal polluted soils around a Pb–Zn mine by crop plants. Inter J Phytorem 4:360–

384.

Saletnik, Bogdan, Grzegorz Zaguła, Marcin Bajcar, Maria Tarapatskyy, Gabriel Bobula, and Czesław Puchalski. 2019. Biochar as a Multifunctional Component of the Environment—A Review. *Applied Sciences* 9, no. 6: 1139. https://doi.org/10.3390/app9061139

Saletnik B, Zaguła G, Bajcar M, Tarapatskyy M, Bobula G, Puchalski C. 2019. Biochar as a Multifunctional Component of the Environment—A Review. *Applied Sciences*, 9(6):1139. https://doi.org/10.3390/app9061139

Santos, M. S., Nogueira, M. A. and Hungria, M. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. AMB Express, 9 (1). https://doi.org/10.1186/s13568-019-0932-0

Schmidt, H.P., Kammann, C., Niggli, C., Evangelou, M.W.H., Mackie, K.A., Abiven, S. 2014. Biochar and biochar-compost as soil amendments to a vineyard soil: Influences on plant growth, nutrient uptake, plant health and grape quality. Agriculture, Ecosystems & Environment, 191, 117-123.

Schweitzer, L. and Noblet, J. 2018. Water Contamination and Pollution. In *Green Chemistry: An Inclusive Approach* (pp. 261–290). https://doi.org/10.1016/B978-0-12-809270-5.00011-X

Shao, X., Li, M., Shen, Q., Fan, Y., Fang, X. 2020. Analysis of Nutritional Components and Comprehensive Evaluation of Quality of Different Varieties of. Science and Technology of Food Industry. 1-14. https://doi.org/10.13386/j.issn1002-0306.2020090009

Singh, T. B., Ali, A., Prasad, M., Yadav, A., Shrivastav, P., Goyal, D., & Dantu, P. K. (2020). Role of organic fertilisers in improving soil fertility. In *Contaminants in Agriculture: Sources, Impacts and Management* (pp. 61–77). Springer International Publishing. https://doi.org/10.1007/978-3-030-41552-5_3

Spain, 2017. R.D. 999/2017. *Ministerio de La Presidencia Y Para Las AdministracionesTerritoriales.* http://www.boe.es

Suárez, J., and Stencel, A. (2020). A part-dependent account of biological individuality: why holobionts are individuals and ecosystems simultaneously. Biol. Rev. 95, 1308–1324. https://doi.org/10.1111/brv.12610

Stamenković, S., Beškoski, V., Karabegović, I., Lazić, M. and Nikolić, N. 2018. Microbial fertilisers: A comprehensive review of current findings and future perspectives. *Spanish Journal of Agricultural Research*, *16* (1), 2171–9292. https://doi.org/10.5424/sjar/2018161-12117

Sun Q.R., Xu Y, Xiang L, Wang GS, Shen X, Chen XS, et al. 2017. Effects of a mixture of bacterial manureand biochar on soil environment and physiological characteristics of Mals huupehens seedlings. *Chin Agric Sci Bull.*, 33:52–59.

Sun, B., Bai, Z., Bao, L., Xue, L., Zhang, S., Wei, Y., Zhang, Z., Zhuang, G., & Zhuang, X. (2020a). Bacillus subtilis biofertiliser mitigating agricultural ammonia emission and shifting soil nitrogen cycling microbiomes. Environment International, 144, 105989. https://doi.org/10.1016/j.envint.2020.105989

Sun, B., Gu, L., Bao, L., Zhang, S., Wei, Y., Bai, Z., Zhuang, G., & Zhuang, X. (2020b). Application of biofertiliser containing Bacillus subtilis reduced the nitrogen loss in agricultural soil. Soil Biology and Biochemistry, 148, 107911. https://doi.org/10.1016/j.soilbio.2020.107911.

Sutton, M.A.; Howard, C.M.; Erisman, J.W.; Billen, G.; Bleeker, A.; Grennfelt, P.; Van Grinsven, H.; Grizzetti, B. 2011. The European Nitrogen Assessment: Sources, E_ects and Policy Perspectives; Cambridge University Press: Cambridge, UK.

Tukey, H. B. (1970). The Leaching of Substances from Plants. *Annual Review of Plant Physiology*, *21*(1), 305–324. https://doi.org/10.1146/annurev.pp.21.060170.001513.

United Nations. 2015. Transforming our world: The 2030 agenda for sustainable development. sustainabledevelopment.un.org

Van Damme, M., Clarisse, L., Franco, B., Sutton, M. A., Erisman, J. W., Kruit, R. W., and Coheur, P. F. 2021. Global, regional and national trends of atmospheric ammonia derived from a decadal (2008–2018) satellite record. Environmental Research Letters, 16(5), 055017.

Vaverková, M. D., Elbl, J., Voběrková, S., Koda, E., Adamcová, D., Mariusz Gusiatin, Z., Al Rahman, A., Radziemska, M., and Mazur, Z. 2020. Composting versus mechanical–biological treatment: Does it really make a difference in the final product parameters and maturity. *Waste Management*, *106*, 173-183. https://doi.org/10.1016/j.wasman.2020.03.030.

Velthof, G. L., and Rietra, R. P. J. J. 2020. Nitrous oxide emission from agricultural soils. *Wageningen Environmental Research*, 58. www.wur.eu/environmental-research.

Wang, W., Ganzeveld, L., Rossabi, S., Hueber, J., and Helmig, D. 2020. Measurement report: Leaf-scale gas exchange of atmospheric reactive trace species (NO2 , NO, O3) at a northern hardwood forest in Michigan. *Atmos. Chem. Phys*, *20*, 11287–11304. https://doi.org/10.5194/acp-20-11287-2020.

Wang, P., Cao, J., Mao, L., Zhu, L., Zhang, Y., Zhang, L., Jiang, H., Zheng, Y., and Liu, X. 2022. Effect of H3PO4-modified biochar on the fate of atrazine and remediation of bacterial community in atrazine-contaminated soil. *Science of The Total Environment*, *851*, 158278. https://doi.org/10.1016/j.scitotenv.2022.158278

Weldeslassie, T., Naz, H., Singh, B., & Oves, M. (2018). Chemical contaminants for soil, air and aquatic ecosystem. In *Modern Age Environmental Problems and their Remediation* (pp. 1–22). Springer International Publishing. https://doi.org/10.1007/978-3-319-64501-8_1

Weng, J., Li, P., Rehman, A., Wang, L., Gao, X. and Niu, Q. 2021. Physiological response and evaluation of melon (Cucumis melo L.) germplasm resources under high temperature and humidity stress at seedling stage. Scientia Horticulturae *288*, 110317. https://doi.org/10.1016/j.scienta.2021.110317

Wuana, R.A. and Okieimen, F.E. 2010. Phytoremediation potential of (Zea mays L.). A review. Afr J Gen Agric 6:275–287.

Xi, H., Shen, J., Qu, Z., Yang, D., Liu, S., Nie, X., et al. 2019. Effects of Long-term cotton continuous cropping on soil microbiome. Sci. Rep. 9:18297.

Yang, W.; Yang, Z.; Guan, Y.; Zhai, C.; Shi, D.; Chen, J.; Wang, T.; Gu, S. 2019. Dose-dependent effect of compost amendment on soil bacterial community composition and co-occurrence network patterns in soybean agroecosystem. *Arch. Agron. Soil Sci.*, *66*, 1027–1041. https://doi.org/10.1080/03650340.2019.1651450

Yang, Y. H., Xue, L. X., Sun, B., Zhang, B., Zhuang, X. L., Zhuang, G. Q., Bai, Z. H. 2020. Bacillus amyloliquefaciens Biofertiliser Mitigating Soil Ammonia Volatilization. Huanjing Kexue/Environmental Science, 41(10), 4711–4718. https://doi.org/10.13227/j.hjkx.201910157

Yoo, S.J., Weon, H.Y., Song, J., Sang, M.K. 2020. Effects of Chryseobacterium soldanellicola T16E-39 and Bacillus siamensis T20E-257 on Biocontrol against Phytophthora Blight and Bacterial Wilt and Growth Promotion in Tomato Plants. International Journal of Agriculture and Biology, 23(3), 534-540. https://doi.org/10.17957/IJAB/15.1320.

Zhang, C., lin, T., li, J., mA, G., WAng, Y., ZHu, P. and Xu, L. 2016. First report of the melon stem rot disease in protected cultivation caused by Pseudomonas fluorescens. Journal of Plant Diseases and Protection, 123, 247–255.

Zong, Y. and Lu, S. 2020. Does long-term inorganic and organic fertilisation affect soil structural and mechanical physical quality of paddy soil? Archives of Agronomy and Soil Science 66 (5), 625–637. https://doi.org/10.1080/03650340.2019.1630823

CHAPTER 2

Objectives

2.1. Objectives

The **general objective** was to design and evaluate advanced fertilisers based on different combinations of bio-residues and rhizobacteria, as part of a global strategy based on a circular economy aimed at reducing the use of conventional mineral fertilisers in agriculture. The **specific objectives** were as follows:

1. To select the bio-residual components that will be part of the advanced fertilisers, based on assessment of the potential phytotoxicity and toxicity over selected rhizobacteria.
2. To evaluate the effect of different combinations of bio-residues in maize under microcosm conditions, considering agronomical factors and the environmental impact on the soil.
3. To assess the agronomic effect of a reduced dose of mineral fertilisers combined with the bio-residue mixes selected in the previous objective and inoculated with a Microbial Plant Biostimulant (MPB) compared to a conventional fertilising programme in high added-value horticultural crops in large-scale field trials.
4. To explain, on a partial basis, the agronomic effect observed in large-scale field trials with rhizosphere microbiome modifications exerted by advanced fertilisers.
5. To evaluate the environmental effects of advanced fertilisers on soil microbiome composition.

CHAPTER 3

Materials and methods

In this section a general description of common materials and methods of each experimental chapter is described. Hence, the materials and methods are specified in detail in the 'Materials and methods' section of each experimental chapter (see in Chapter 4, Chapter 5 and Chapter 6).

3.1. Materials

3.1.1. Components to produce the organic fertilisers

The design and the different combinations of the components of organic fertilisers has been guided by the principles of the circular economy, whereby the components consist of bio-residues.

- **Compost** used derived from a mixture of de-alcoholized grape pomace combined with vinasses of lees and crushed plant biomass (Table S4.1 in Appendix 1.Chapter 4 and Table S6.1 in Appendix 3.Chapter 6).
- **Biochar** was obtained from the wood of vine shoots (Table S4.2 in Appendix 1.Chapter 4 and Table S6.2 in Appendix 3.Chapter 6) by slow pyrolysis in a pilot pyrolizer with an electrically heated reactor described by Rosas et al., (2015).
- **AD** used was obtained from a 25 L anaerobic continuously stirred tank reactor treating organic residues from local hotels, restaurants and cafes (Table S4.3 in Appendix 1 and Table S5.2 in Appendix 2).

3.1.2. Bacterial strains

The bacterial strains used in this thesis were the following:

- The strain SCFB3-1 from *B. siamensis*, which belongs to the IQUIMAB bacterial collection from the University of León (used in Chapter 4 and Chapter 6).

- The strain LCS0306 from *Rhizobium leguminosarum* which belongs to the IQUIMAB bacterial collection (used in Chapter 4).
- The strain RVPB2-2 from *Pseudomonas brassicacearum* which belongs to the IQUIMAB bacterial collection (used in Chapter 4).

3.1.3. Plant material

The maize crop used was cultivar 'Antalya' (Chapter 4), the melon crop used was cultivar ‘Piel de sapo’ (Chapter 5 and Chapter 6) and the pepper crop used was cultivar ‘Medrano’ (Chapter 5 and Chapter 6).

In addition, seeds of tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.) and cress (*Lepidium sativum* L.) were utilised to carry out the phytotoxicity test described in the Chapter 4.

3.2. Methods

3.2.1. Microbiological methods

3.2.1.1. Inoculant production

The inoculant SCFB3-1 for the field trials was selected due to their relevant growth-promoting activities in vitro (Barquero, 2014), unfrozen and grown at 28°C in a lab oven for 3 d. After, the inoculant broth was produced in a pilot fermenter (Sartorius BIOSTAT Bplus-MO, 5 L). Details can be obtained from the Chapter 5 and the Chapter 6. Finally, the resulting bacterial suspension was combined with two cell protectors, one polysaccharide and one disaccharide at 0.5% (w:v) each, to improve the bacterial shelf-life and the inoculant performance in the field when the fermentation was finished.

3.2.1.2. Toxicity Test on Soil Rhizobacteria

For small-scale bacterial production, each bacterium was produced in the optimal synthetic medium. More details can be obtained from the Chapter 4.

3.2.2. Molecular methods

3.2.2.1. DNA extraction

Total microbial DNA from the rhizospheric soil and the bulk soil of melon crop was extracted using the DNeasy Power Soil kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. These samples were collected to characterise soil microbial communities. More information can be obtained from the Chapter 5 and the Chapter 6. The DNA quality was quantified by NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific) and by Qubit^{TM} 4 fluorometer (Thermo Fisher Scientific).

3.2.2.2. Next Generation Sequencing

The entire DNA extracted was used for high-thoughput sequencing of 16S rRNA and ITS region. The primers for bacteria are specified in the Chapter 5 and 6 and the primers for fungi are specified in the Chapter 6. Samples from the Chapter 5 and Chapter 6 were analysed by Molecular Research DNA (MR DNA) Company (Shallowater, TX, USA) using the Illumina MiSeq high-throughput sequencing equipment (Illumina, San Diego, CT, USA). The details about the processed sequence data to obtain the taxonomy are shown in the Chapter 5 and the Chapter 6.

Microbial community analysis and plots were obtained using the Primer v7 and PERMANOVA+ software (Clarke and Gorley, 2015). Futhermore, Tukey's test was performed for the dependant variables: species richness, the number of reads and the Shannon index.

3.2.3. Test involving plants at different scales

3.2.3.1. Plant assays under controlled conditions

Two types of assays were carried out involving plants under controlled conditions: the phytotoxicity test and the greenhouse trials.

The potential phytotoxicity of the different bio-residues combinations was tested using the Zucconi test (Zucconi et al., 1981), modified by Varnero et al., (2006). More details can be seen in the Chapter 4. The effect of the different bio-residues combinations in maize plant and soil in greenhouse trials under microcosm conditions was also analysed. The details about the trials and the dependent variables analysed are shown in the Chapter 4.

The results were statistically analysed using analysis of variance (ANOVA) with the software IBM-SPSS v.26.0., IBM Corporation (Armonk-NY, USA) with the specifics explained in the Chapter 4.

3.2.3.2. Field assays

Two assays involving two different horticultural crops for two consecutive years were carried out, giving this thesis a clear agronomic character.

The experimental design for each assay was a randomized complete block with three blocks. The organic fertilisers were applied by hand before transplantation. The mineral N (27% N) fertiliser was applied in ten different applications along the crop’s cycles, each one corresponding to one tenth of the full dose. The mineral P and K fertilisers were applied by fertirrigation using the methodology indicated by Urbano-Terrón, (2008). The dependent variables analysed for both crops were: the yield, the yield components and variables related to the plant and fruit quality. More information on techniques and procedures in field assays can be found in the Chapter 5 and the Chapter 6.

The results were statistically analysed using analysis of variance (ANOVA) with the software IBM-SPSS v.26.0., IBM Corporation (Armonk-NY, USA) with the specifics explained in the Chapter 5 and the Chapter 6.

3.3. References

Barquero, M., 2014. Caracterización y selección de bacterias y hongos micorrícicos aislados en raíces de alubia y pimiento en la provincia de León, para el desarrollo de biofertilisantes. PhD thesis, University of Salamanca, Spain.

Clarke, K.R., Gorley, R.N., 2015. Getting started with PRIMER v7. Plymouth. Plymouth Marine Laboratory, PRIMER-E.

Rosas, J.G., Gómez, N., Cara, J., Ubalde, J., Sort, X., Sánchez, M.E., 2015. Assessment of sustainable biochar production for carbon abatement from vineyard residues. J Anal Appl Pyrolysis 113, 239–247. https://doi.org/10.1016/j.jaap.2015.01.011

Urbano-Terrón, P., 2008. Tratado De Fitotecnia General, Second edition. ed. Mundiprensa, Madrid.

Varnero, M.T., Orellana, R., Rojas, C., Santibáñez, C., Gallardo Lancho, J.F., 2006. Evaluación de especies sensibles a metabolitos fitotóxicos mediante bioensayos de germinación, in: Gallardo-Lancho, J.F. (Ed.), Medioambiente En Iberoamérica. Visión Desde La Física y La Química En Los Albores Del Siglo XXI. Cáceres, pp. 363–369.

Zucconi, F., Pera, A., Forte, M., DeBertolli, M., 1981. Evaluating Toxicity of Immature Compost. Biocycle 22, 54–57.

CHAPTER 4

Effects of compost, biochar and anaerobic digestate (AD) in a microcosm trial

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Abstract

Treated bio-residues can be used as biostimulants in crops within the circular economy approach to reduce the use of traditional fertilisers. In this work, we optimised the combination rates for three types of treated bio-residues (compost, biochar, and anaerobic digestate (AD)) in two microcosm trials, one with a combination of compost and biochar and other with biochar and AD. The crop used was maize, and the variables analysed were plant growth, and soil chemical and biological properties. The combination of bio-residues improved plant growth and soil biological activity to a greater extent than one product alone; that is, compost and biochar performed better than compost alone and biochar, and AD performed better than biochar alone. However, while the concentration in the plant biomass of several essential nutrients for crops increased in the treatments with compost and biochar, and with biochar and AD, compared to the untreated controls, the N concentration was reduced. This was due to the competition for N between the plant and the soil microbiome, whose activity was activated. Due to the importance of N in plant growth, the increase in biomass production could be explained not only by the higher availability of other nutrients but also by the plant-growth-promoting activity exerted by the more active soil microbiome. Further research should focus on validating this hypothesis and unravelling the mechanisms involved. From the environmental site, the presence of biochar in the mixtures of organic residues reduced the soil N at risk of lixiviation and sequestered carbon, which partially compensated for the increased CO_2 emissions because labile forms of carbon were present in the remaining organic residues.

CHAPTER 5

Field trial with biochar additivated with anaerobic digestate (AD)

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Ortiz-Liébana, N., Zotti, M., Barquero, M., González-Andrés, F. 2023. Biochar + AD exerts a biostimulant effect in the yield of horticultural crops and improves bacterial biodiversity and species richness in the rhizosphere. Scientia Horticulturae 321, 112277. https://doi.org/10.1016/j.scienta.2023.112277

Abstract

Organic fertilisers are gaining prominence in advanced agri-systems due to the need for alternatives to the most pollutant agricultural inputs, contributing to sustainable agriculture. The objective of this study was to analyse the agronomic effect of a biochar non-additivated and additivated with anaerobic digestate (AD) on the soil microbiome in melon and pepper crops at the field scale, hypothesising that the synergy between biochar and the additive confers additional benefits to the crop. Two doses of biochar (250 and 500 kg ha^{-1}) and two doses of additive with respect to biochar (5 and 10% v:w) were tested. The highest yield was observed for a reduced dose of mineral fertilisation (NPK −20%) with biochar + AD at the highest dose of additive: a biochar dose of 250 kg ha^{-1} with 10% AD for the melon crop and a biochar dose of 500 kg ha^{-1} with 10% AD for the pepper crop. Specifically, the yield increase compared with the control, which only received NPK, was a 33% increase in melon and 18% in pepper. The microbiome of the bulk soil was not modified by biochar + AD, but the composition of the rhizosphere microbiome changed, emerging plant growth-promoting rhizobacteria (PGPR) or increasing its relative abundance (e.g. *Arthrobacter, Mitsuaria* or *Bacillus* genus). We have demonstrated a positive correlation between yield and fruit quality parameters, and the presence of cluster of bacteria with predominance of known PGPR genera, that have been boosted by the treatments with biochar + AD. Thus, we hypothesise that the improved yield and fruit quality is in part due to the rhizosphere bacteria community enhancement.

CHAPTER 6

Field trial with compost inoculated with *Bacillus siamensis* formulated with biochar

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Ortiz-Liébana, N., Zotti, M., Barquero, M., González-Andrés, F. 2022. An organic fertiliser ‘doped’ with a Bacillus strain improves melon and pepper yield, modifying the rhizosphere microbiome with negligible changes in the bulk soil. Agronomy 12, 2620. https://doi.org/10.3390/agronomy12112620

Abstract

Doped compost consists of compost inoculated with *B. siamensis* SCFB3-1 that is formulated in biochar and then mixed with the compost. The study objective was to analyse at field scale, the effect of doped compost in the crop yield and on the soil microbiome. Two doses of compost (2 and 5 t ha^{-1}) and two doses of the inoculant (biochar+PGPR) with respect to the compost (3% and 6% w:w) were tested. The highest yield was observed for a reduced dose of mineral fertilisation (NPK -20%) with a compost dose of 2 t ha^{-1} with 6% of the inoculant. Specifically, the yield increase compared with the control, which only received NPK, resulted in 47% increase in melon and 28% in pepper. The microbiome of the bulk soil was not modified by the doped compost, but the composition of the rhizosphere microbiome changed, increasing in abundance *Bacillus* (the inoculated strain), but also changing the relative abundance of other genera in the bacterial community. We hypothesised that the improvement in crop performance could be due not only to the inoculated strain but also to other emerging microbial strains. Future works will be focused in unravelling the possible effects of phyto-hormones in the observed results.

CHAPTER 7

Conclusions

7.1. Conclusions

1. The aqueous extracts of compost + biochar and biochar + anaerobic digestate (AD) were phytostimulants when appropriately diluted and phytotoxic when highly concentrated. Thus, they are suitable as fertilisers as is or as feedstocks for others, if the doses are adequate.
2. Of the three bio-residues used as components of advanced fertilisers, namely compost, biochar and AD, compost and AD showed a higher phytostimulant effect than biochar at an appropriate concentration.

Conclusions derived from the microcosm trial with maize

3. The combination of bio-residues, compost with biochar and biochar with AD, worked better than compost or biochar alone in terms of plant biomass production, but they reduced the nitrogen (N) content in the biomass compared with the non-fertilised control.
4. The decrease in the N content in the biomass was most likely due to the increased microbial soil activity, which consumes N and competes with the crop but prevents N lixiviation from the soil.
5. The microcosm trial pointed out that the increased plant biomass despite reduced N availability could be partially due to the plant growth-promoting activity exerted by the more active soil microbiome, and this hypothesis was strengthened by metataxonomic analysis in the field trials

Conclusions derived from field trials with horticultural crops at a real field scale

6. A reduced dose of mineral fertiliser combined with 2 t per ha of compost inoculated with *Bacillus siamensis* formulated with biochar as a carrier, called

‘doped compost’, improved the yield of melon and pepper compared with the control, which was a full dose of mineral fertiliser.

7. A reduced dose of mineral fertiliser combined with the addition of biochar + AD at doses ranging between 250 and 500 kg per ha for melon and 500 kg per ha for pepper produced a higher melon yield and similar pepper yield than the control with a full dose of mineral fertiliser.
8. The advanced fertiliser based on compost produced, in general terms, higher yields than the one based on biochar.
9. The application of ‘doped compost’ or biochar + AD to melon crops did not affect the bacterial composition of the bulk soil but modified the rhizosphere soil.
10. The application of ‘doped compost’ or biochar + AD to melon crops enhanced the species richness and diversity of rhizosphere bacteria, increasing the presence of bacterial genera considered to promote plant growth.
11. The use of advanced organic fertilisers is a step forward from the environmental side because the use of mineral fertilisers up to 20% does not affect the microbial community of the bulk soil.
12. Increased melon yield with advanced fertilisers is partially due to changes in the rhizosphere bacterial community, and we also hypothesised that the addition of a specific plant growth promoting rhizobacteria (PGPR) strain not only exerts its own direct effect on the crop but also ameliorates the composition of the rhizosphere community.

7.2. Conclusiones (Spanish)

1. Los extractos acuosos de compost + biochar y biochar + digerido anaerobio (DA) fueron fitoestimulantes cuando se diluyeron adecuadamente y fitotóxicos cuando estuvieron muy concentrados. Así, son adecuados como fertilizantes como tal, o como materias primas para otros, siempre que las dosis sean las adecuadas.
2. De los tres biorresiduos utilizados como componentes de los fertilizantes avanzados, denominados, compost, biochar y DA; el compost y el DA mostraron un mayor efecto fitoestimulante que el biochar a una concentración apropiada.

Conclusiones derivadas del ensayo en microcosmos con maíz

3. La combinación de biorresiduos, compost con biochar y biochar con DA, funcionó mejor que el compost o el biochar solos, en términos de producción de biomasa vegetal, pero redujo el contenido de N en la biomasa comparado con el control no fertilizado.
4. La disminución del contenido de nitrógeno (N) en la biomasa se debió muy probablemente al aumento observado de la actividad microbiana del suelo, que consume N y compite con el cultivo, pero previene la lixiviación de N del suelo.
5. El ensayo en microcosmos señaló que el aumento de la biomasa vegetal, a pesar de una menor disponibilidad de N, podría deberse en parte a la actividad promotora del crecimiento vegetal ejercida por un microbioma del suelo más activo, y esta hipótesis se vio reforzada por los análisis metataxonómicos en los ensayos de campo.

Conclusiones derivadas de los ensayos de campo con cultivos hortícolas a escala real de campo

6. Una dosis reducida de fertilizante mineral combinada con 2 toneladas por hectárea de compost inoculado con *Bacillus siamensis* formulado con biochar como soporte, el cual se ha denominado ‘compost dopado’, mejoró el rendimiento del melón y el pimiento en comparación con el control con dosis completa de fertilizante mineral.
7. Una dosis reducida de fertilizante mineral combinada con la adicción de biochar + DA en la dosis que oscilan entre 250 y 500 kilogramos por hectárea para el melón y 500 kg por hectárea para el pimiento, produjo un rendimiento mayor para el melón y un rendimiento similar para el pimiento, comparado con el fertilizante mineral a dosis completa.
8. El abono avanzado a base de compost produjo, en términos generales, mayores rendimientos que el basado en biochar.
9. La aplicación de ‘compost dopado’ o biochar + DA al cultivo de melón, no afectó a la composición bacteriana del suelo de tipo bulk, pero modificó el suelo rizosférico.
10. La aplicación de ‘compost dopado’ o biochar + DA al cultivo de melón aumentó la riqueza y diversidad de especies de bacterias de la rizosfera, incrementando la presencia de géneros bacterianos considerados promotores del crecimiento vegetal.
11. El uso de fertilizantes orgánicos avanzados es dar un paso hacia adelante desde el punto de vista medioambiental, porque reduce hasta un 20% el uso de fertilizantes minerales y no afecta a la comunidad microbiana del suelo de tipo bulk.

12. El aumento del rendimiento del melón con los fertilizantes avanzados se debe en parte al impulso de un cambio en la comunidad bacteriana de la rizosfera, y también planteamos la hipótesis de que la adición de una cepa específica de PGPR no sólo ejerce en el cultivo su propio efecto directo, sino que también mejora la composición de la comunidad de la rizosfera.

APPENDIXES

Appendix I. Chapter 4: supporting information

Table S4.1. Composition of the compost used for the experiment.

Parameter	Compost	
N Kjeldahl	%	2.08
P	mg kg^{-1}	4,683.30
K	mg kg^{-1}	20,600.70
Ca	mg kg^{-1}	76,196.00
Mg	mg kg^{-1}	6,097.30
Na	mg kg^{-1}	2,387.00
Fe	mg kg^{-1}	1,868.30
Mn	mg kg^{-1}	101.20
Zn	mg kg^{-1}	111.10
Cu	mg kg^{-1}	7.49
Cr	mg kg^{-1}	8.40
Ni	mg kg^{-1}	4.90
Hg	mg kg^{-1}	0.09
Cd	mg kg^{-1}	0.19
Pb	mg kg^{-1}	2.62
Oxidizable organic carbon	%	15.08
Organic Matter	%	25.93
C/N Ratio	-	7.24
pH (soil:water)	-	8.55
Electrical Conductivity	ds m^{-1}	6.75
Particle size	mm	1.55

Table S4.2. Composition of the biochar used for the experiment**.**

Parameter		**Biochar**
Total nitrogen [a]	%	1.02
N-NH_4^+ [b]	%	< 0.03
N-NO_3^- [c]	mg kg^{-1}	3.21
Total phosphorous [d]	mg kg^{-1}	2,408.24
Total potassium [d]	mg kg^{-1}	18,618.22
Total calcium [d]	mg kg^{-1}	20,924.51
Total magnesium [d]	mg kg^{-1}	4,925.29
Assimilable phosphorous [e]	mg kg^{-1}	123.64
Assimilable potassium [f]	mg kg^{-1}	9,570.60
Assimilable calcium [f]	mg kg^{-1}	1,774.00
Assimilable magnesium [f]	mg kg^{-1}	674.40
Humidity [g]	%	8.00
Volatiles [g]	%	7.30
Ash [g]	%	8.99
Organic carbon	%	92.07
Fixed Carbon [g]	%	83.71
Hydrogen [g]	%	0.89
Sulphur [g]	%	0.01
Oxygen [g]	%	4.74
pH 1:5 (biochar:water)	-	10.82
Higher calorific value [g]		29.04
Lower calorific value [g]		28.94

[a] Kjeldahl method

[b] Destillation and titration

[c] Ion chromatography

[d] Acid digestion and ICP spectroscopy

[e] Olsen method

[f] Acid extraction and ICP spectroscopy

[g] results expressed on a dry matter basis

Table S4.3. Composition of the anaerobic digestate used for the experiment.

Parameter	Anaerobic Digestate	
Organic Matter	% of dry matter	62.41
Total Nitrogen	% of dry matter	12.38
N-NH_4^+	% of total N	34.10
C : N Ratio		5.14
Total phosphorus	% of dry matter	6.97
Potassium	% of dry matter	10.20
Calcium	% of dry matter	3.91
Magnesium	% of dry matter	2.35
Sodium	% of dry matter	0.05
Manganese	mg kg^{-1} of dry matter	235.02
Iron	mg kg^{-1} of dry matter	1,578.92
Copper	mg kg^{-1} of dry matter	48.12
Zinc	mg kg^{-1} of dry matter	147.04
Total solids	g L^{-1}	5.75
pH		7.71
Conductivity	dS m^{-1}	4.39

Table S4.4. Soil analysis prior to the experiment in the microcosm trial.

Parameter		Soil	
	Sand	%	60
Texture (%)	Silt	%	32
	Clay	%	8
pH 1:5 (soil:water)		-	6.23
Electric conductivity		dS m^{-1}	0.13
Organic matter		%	6.29
Cation exchange capacity		cmol (+) kg^{-1}	23.84
Mn		mg kg^{-1}	38.73
Fe		mg kg^{-1}	64.71
Cu		mg kg^{-1}	2.20
Zn		mg kg^{-1}	3.35
B		mg kg^{-1}	1.62

Table S4.5. ANOVA and contrasts for the dependent variables biomass production, height and in-plant ionomic analysis. The plot was considered a random factor. Values correspond to the F-statistic (ANOVA) and t-statistic (orthogonal contrasts) followed by the level of significance (*** *p* ≤ 0.001; ** 0.001 < *p* ≤ 0.01; * 0.01 < *p* ≤ 0.05; ns, not significant). df: degrees of freedom.

	Fresh Aerial Biomass (mg)	**Dry Aerial Biomass (mg)**	**Height (cm)**	**N (%)**	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Ca (mg kg^{-1})**	**Mg (mg kg^{-1})**
Treatment	37.9 ***	25.8 ***	0.681 ns	45.8 ***	15.8 ***	1.88 ns	343.9 ***	1.22 ns
df	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Control / Compost	-12.0 ***	-11.7 ***	-1.31 ns	-2.29 *	-2.84 *	-0.887 ns	-20.9 ***	-2.16 *
df	80.4	78.6	43.5	14.0	14.0	2.00	3.72	14.0
Control / Compost + B	-23.7 ***	-15.9 ***	-1.17 ns	9.09 ***	-6.64 ***	-1.21 ns	-95.4 ***	-1.05 ns
df	78.9	93.9	38.3	14.0	14.0	2.06	3.36	14.0
Compost / Compost + B	-3.23 **	-4.10 ***	0.314 ns	14.9 ***	-4.56 ***	-2.73 ns	-9.12 ***	1.69 ns
df	83.4	130.6	120.7	14.0	14.0	2.61	4.45	14.0

Table S4.6. ANOVA carried out for the independent variables compost dose, biochar dose and the interaction compost dose x biochar dose on different dependent variables measured in maize plants. Values correspond to the F-statistic, followed by the level of significance (*** p ⩽ 0.001; ** 0.001 < p ⩽ 0.01; ns not significant). df: degrees of freedom.

Doses	**df**	**Fresh Aerial Biomass (mg)**		**Dry Aerial Biomass (mg)**		**Height (cm)**		**N (%)**		**P (mg kg^{-1})**		**K (mg kg^{-1})**		**Ca (mg kg^{-1})**		**Mg (mg kg^{-1})**	
		Mean Square	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**
Compost dose (t ha^{-1})	1	57,638	45.6 ***	57,638	18.5 ***	17.9	1.19 ns	0.014	1.32 ns	122,766	45.6 ***	27,187,188	19.6 ***	33,133,259	919.6 ***	1.19	0.000 ns
Biochar dose (%)	2	28,323	18.4 ***	28,323	9.08 ***	1.72	0.114 ns	1.17	110.8 ***	49,510	18.4 ***	3,024,286	2.18 ns	2,789,308	77.4 ***	159,068	1.82 ns
Compost dose × biochar dose	2	3,800	2.59 ns	3,800	1.22 ns	3.56	0.236 ns	0.000	0.016 ns	6,989	2.59 ns	88,302	0.064 ns	265,155	7.36 **	28,351	0.324 ns

Table S4.7. ANOVA and contrasts for the dependant variables biomass production, height and in-plant ionomic analysis. The plot was considered a random factor. Values correspond to the F-statistic (ANOVA) and t-statistic (orthogonal contrasts), followed by the level of significance (*** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$; ns not significant). df: degrees of freedom.

	Fresh Aerial Biomass (mg)	**Dry Aerial Biomass (mg)**	**Height (cm)**	**N (%)**	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Ca (mg kg^{-1})**	**Mg (mg kg^{-1})**
Treatment	5.28 ***	4.19 ***	1.08 ns	40.7 ***	2.94 *	8.54 ***	2.93 *	1.16 ns
df	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Control / Biochar	-0.524 ns	-0.490 ns	2.05 *	7.19 **	-0.310 ns	-0.668 ns	2.86 **	1.53 ns
df	33.26	50.5	261.0	2.28	3.72	18.0	18.0	2.69
Control / Biochar + AD	-3.4 **	-3.28 **	1.38 ns	6.44 *	-3.34 *	-3.87 ***	3.92 ***	1.53 ns
df	40.9	39.6	261.0	2.10	3.29	18.0	18.0	2.06
Biochar / Biochar + AD	-6.12 ***	-3.88 ***	-1.25 ns	-2.99 *	-3.73 **	-4.12 ***	0.888 ns	-0.259 ns
df	183.2	64.9	261.0	5.80	4.26	18.0	18.0	3.33

Table S4.8. ANOVA performed for the independent variables biochar dose, AD dose and the interaction biochar x AD dose, on different dependent variables measured in maize plants. Values correspond to the F-statistic followed by the level of significance (*** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$; ns not significant). df: degrees of freedom.

Doses	**df**	**Fresh Aerial Biomass (mg)**		**Dry Aerial Biomass (mg)**		**Height (cm)**		**N (%)**		**P (mg kg^{-1})**		**K (mg kg^{-1})**		**Ca (mg kg^{-1})**		**Mg (mg kg^{-1})**	
		Mean Square	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**
Biochar dose (kg ha^{-1})	1	629,679.76	4.37 *	24,077.58	5.63 *	37.6	3.75 ns	1.51	241.9 ***	9,435.35	0.958 ns	4,272,387.24	10.38 **	6,664,568.51	14.4 **	24,786.69	0.399 ns
AD dose (%)	2	1,044,893.78	7.24 ***	25,371.06	5.93 ***	3.96	0.394 ns	0.027	4.40 *	54,386.48	5.52 **	14,436,259.92	35.1 ***	721,971.63	1.56 ns	2,752.08	0.044 ns
Biochar dose × AD dose	2	360,296.29	2.50 ns	2,448.58	0.573 ns	1.21	0.121 ns	0.009	1.43 ns	1,270.52	0.129 ns	1,510,580.52	3.67 *	542,704.32	1.18 ns	5,610.98	0.090 ns

Table S4.9. ANOVA and contrasts for the dependent variables pH, Electrical conductivity (EC), FDA, Carbon as CO_2 (C-CO_2) and Total organic carbon (TOC). The plot was considered a random factor. Values correspond to the F-statistic (ANOVA) and t-statistic (orthogonal contrasts) followed by the level of significance (*** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; ns not significant). df: degrees of freedom.

	pH	**EC (dS · m^{-1})**	**FDA (mg · kg^{-1}· 3 h^{-1})**	**C-CO_2 (mg · m^{-2}· soil · day^{-1})**	**TOC (%)**
Treatment	6.75 **	15.2 ***	67.8 ***	65.3 ***	16.4 ***
df	6.00	6.00	6.00	6.00	6.00
Control / Compost	-3.29 **	-5.85 ***	- 9.53 **	-12.5 ***	-31.7 ***
df	14.0	14.0	2.54	14.0	3.57
Control / Compost + B	-5.87 ***	-7.20 ***	- 33.8 ***	-18.6 ***	-9.08 ***
df	14.0	14.0	5.38	14.0	5.99
Compost / Compost + B	-3.00 ns	-1.02 ns	-8.09 **	-6.39 ***	6.27 ***
df	14.0	14.0	3.19	14.0	7.97

Table S4.10. ANOVA performed for the independent variables compost dose, biochar dose and the interaction compost x biochar dose, on different dependent variables measured in the soil. Values correspond to the F-statistic followed by the level of significance (*** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$; ns not significant). df: degrees of freedom.

Doses	**df**	**pH**		**EC (dS · m^{-1})**		**FDA (mg · kg^{-1}· 3 h^{-1})**		**C-CO_2 (mg · m^{-2}· soil · day^{-1})**		**TOC (%)**	
		Mean Square	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**
Compost dose (t ha^{-1})	1	0.012	0.514 ns	0.009	30.7 ***	7,010.554	43.3 ***	15,092.787	40.1 ***	0.747	11.1 **
Biochar dose (%)	2	0.130	5.66 *	0.001	1.86 ns	11,194.181	69.1 ***	9,574.094	25.4 ***	0.969	14.4 ***
Compost dose × biochar dose	2	0.001	0.032 ns	$8.4 \cdot 10^{-5}$	0.279 ns	72.2	0.446 ns	794.357	2.11 ns	0.148	2.21 ns

Table S4.11. ANOVA and contrasts for the dependant variables pH, Electrical conductivity (EC), FDA, Carbon as CO_2 (C-CO_2) and Total organic carbon (TOC). The plot was considered a random factor. Values correspond to the F-statistic (ANOVA) and t-statistic (orthogonal contrasts) followed by the level of significance (*** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; ns not significant). df: degrees of freedom.

	pH	**EC**	**FDA**	**C-CO_2**	**TOC**
Treatment	184.6 ***	63.3 ***	289.0 ***	40.9 ***	10.2 **
df	8.00	8.00	8.00	8.00	8.00
Control / Biochar	-13.3 ***	3.40 **	- 3.50 **	-1.41 ns	-2.60 ns
df	18.0	18.0	18.0	18.0	3.36
Control / Biochar + AD	-19.8 ***	-10.2 ***	- 23.8 ***	-10.5 ***	-6.74 **
df	18.0	18.0	18.0	18.0	2.63
Biochar / Biochar + AD	-6.18 ***	-18.6 ***	-26.2 ***	-11.8 ***	-1.31 ns
df	18.0	18.0	18.0	18.0	2.28

Table S4.12. ANOVA performed for the independent variables biochar dose, AD dose and the interaction biochar dose x AD dose, on different dependent variables measured in the soil. Values correspond to the F-statistic, followed by the level of significance (*** p ≤ 0.001; ** 0.001 < p ≤ 0.01; * 0.01 < p ≤ 0.05; ns not significant).df: degrees of freedom.

Doses	**df**	**pH**		**EC (dS · m^{-1})**		**FDA (mg · kg^{-1}· 3 h^{-1})**		**C-CO_2 (mg · m^{-2} · soil · day^{-1})**		**TOC (%)**	
		Mean Square	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**	**Mean Square**	**F Statistic**
Biochar dose (kg ha^{-1})	1	6.64	962.9 ***	0.000	4.82 *	3,224	190.2***	6,146	10.9 **	0.020	33.6 ***
AD dose (%)	3	0.159	23.0 ***	0.004	135.4 ***	9,080	535.6 ***	42,728	75.7 ***	0.004	7.70 **
Biochar dose × AD dose	3	0.001	0.162 ns	$8.19 \cdot 10^{-5}$	2.96 ns	352	20.7 ***	376.3	0.667 ns	0.000	0.323 ns

Appendix II. Chapter 5: supporting information

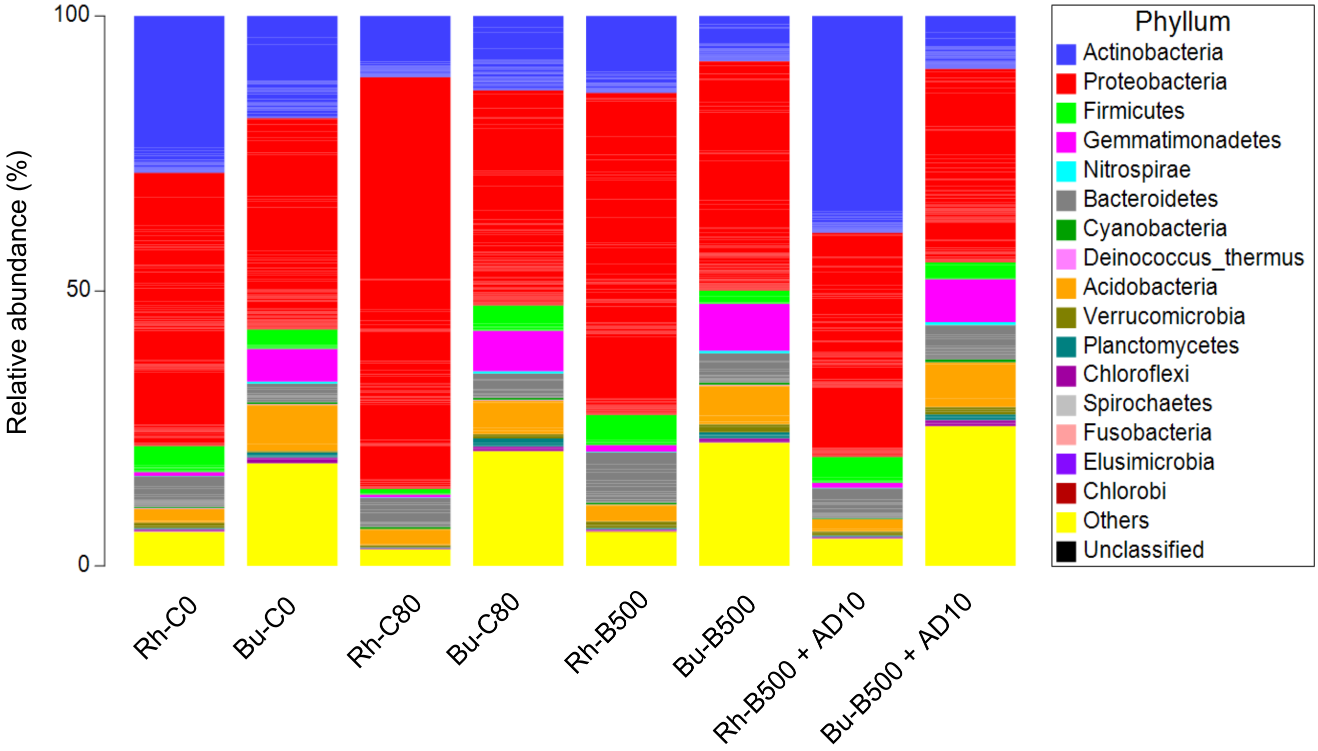


Figure S5.1. Stacked bar plot indicating the relative abundance of bacterial phyla for the interaction: soil location x treatment.

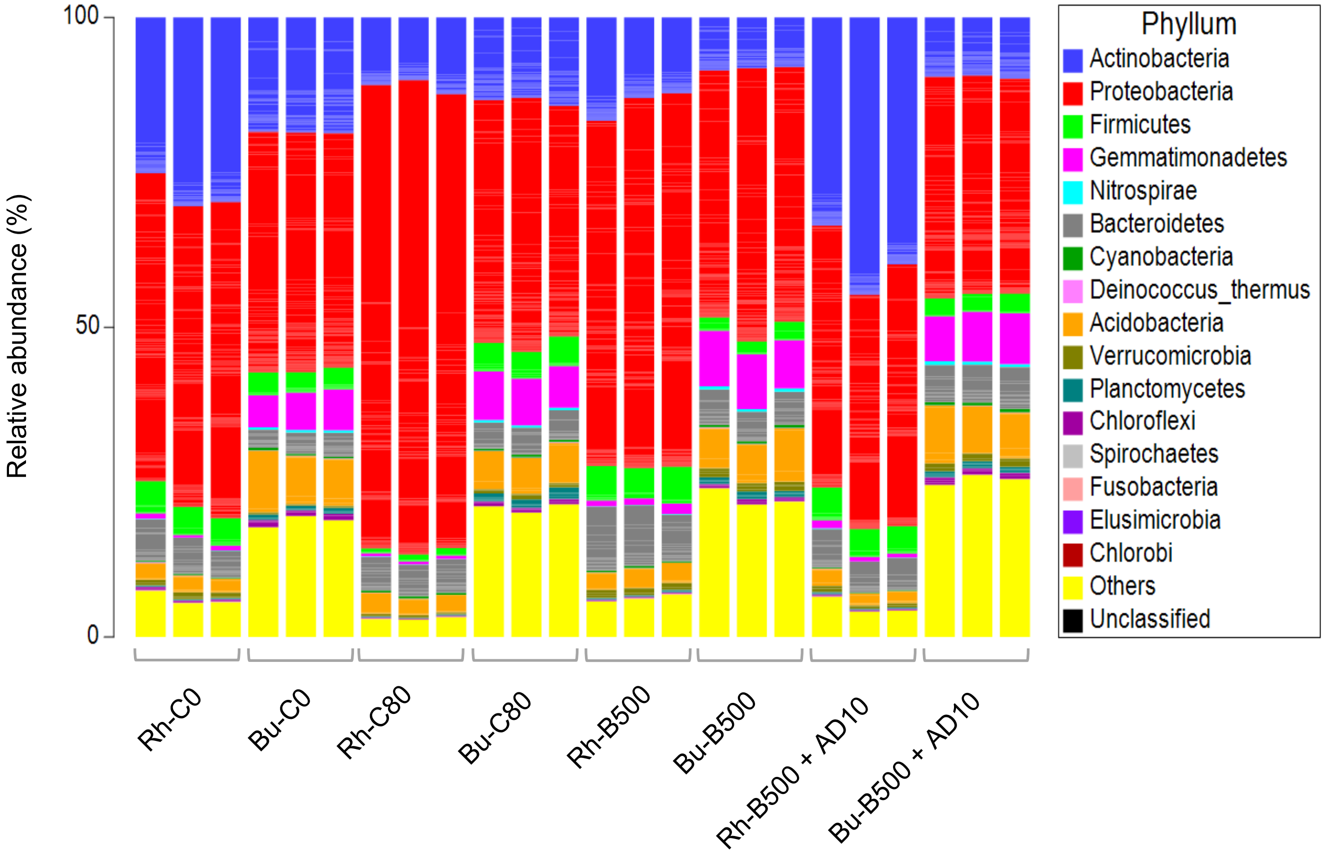
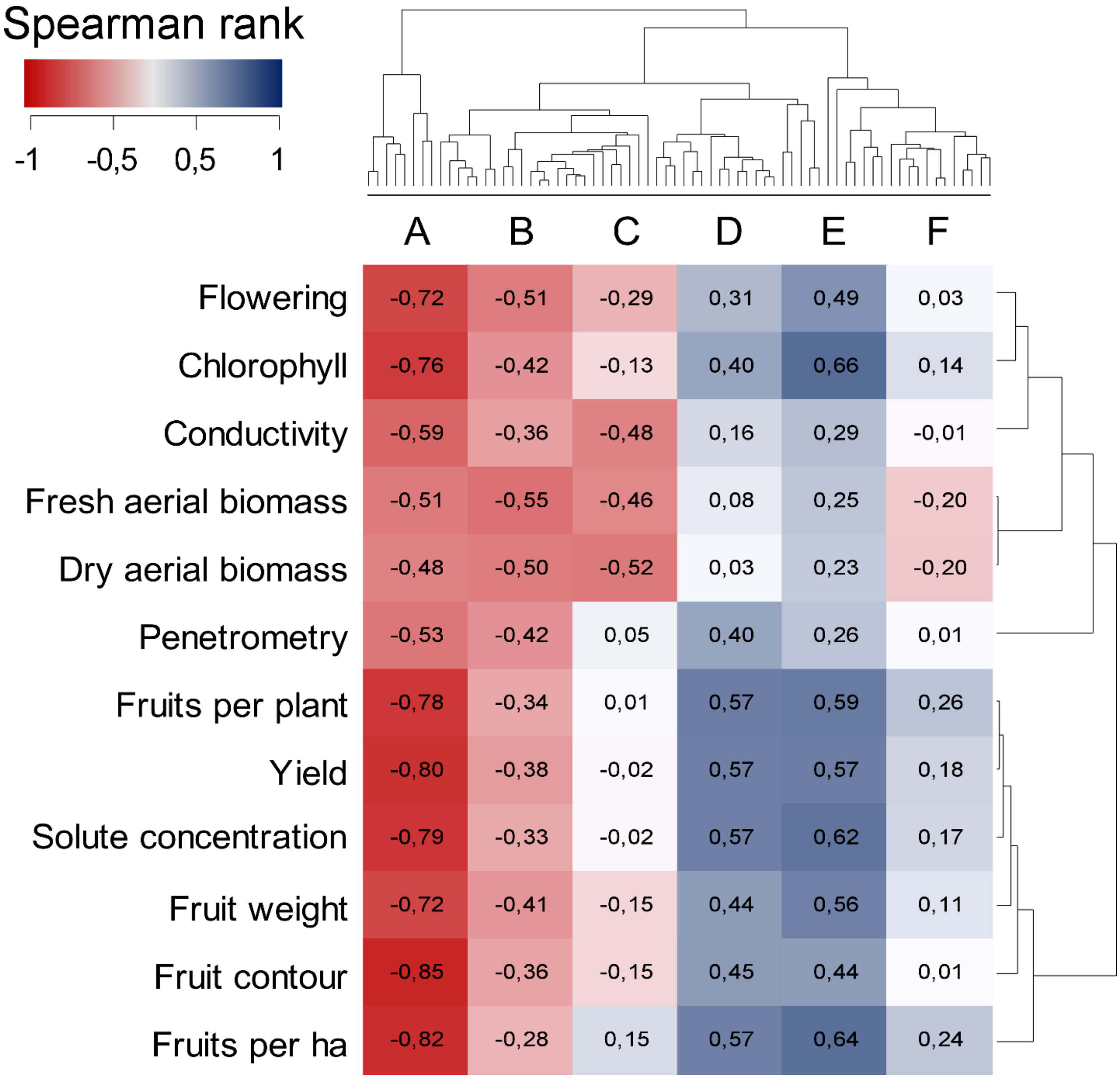


Figure S5.2. Stacked bar plot indicating the relative abundance of bacterial phyla for each replicate of each treatment applied for the interaction: soil location x treatment.



	A	B	C	D	E	F
Flowering	-0,72	-0,51	-0,29	0,31	0,49	0,03
Chlorophyll	-0,76	-0,42	-0,13	0,40	0,66	0,14
Conductivity	-0,59	-0,36	-0,48	0,16	0,29	-0,01
Fresh aerial biomass	-0,51	-0,55	-0,46	0,08	0,25	-0,20
Dry aerial biomass	-0,48	-0,50	-0,52	0,03	0,23	-0,20
Penetrometry	-0,53	-0,42	0,05	0,40	0,26	0,01
Fruits per plant	-0,78	-0,34	0,01	0,57	0,59	0,26
Yield	-0,80	-0,38	-0,02	0,57	0,57	0,18
Solute concentration	-0,79	-0,33	-0,02	0,57	0,62	0,17
Fruit weight	-0,72	-0,41	-0,15	0,44	0,56	0,11
Fruit contour	-0,85	-0,36	-0,15	0,45	0,44	0,01
Fruits per ha	-0,82	-0,28	0,15	0,57	0,64	0,24

Figure S5.3. Correlation heatmap between cluster identified as bacterial consortia and 12 agronomic variables based on Spearman rank r values. Yields and productivity data were ordered according to hierarchical clustering based on Euclidean distance metrics and complete distance methodology.

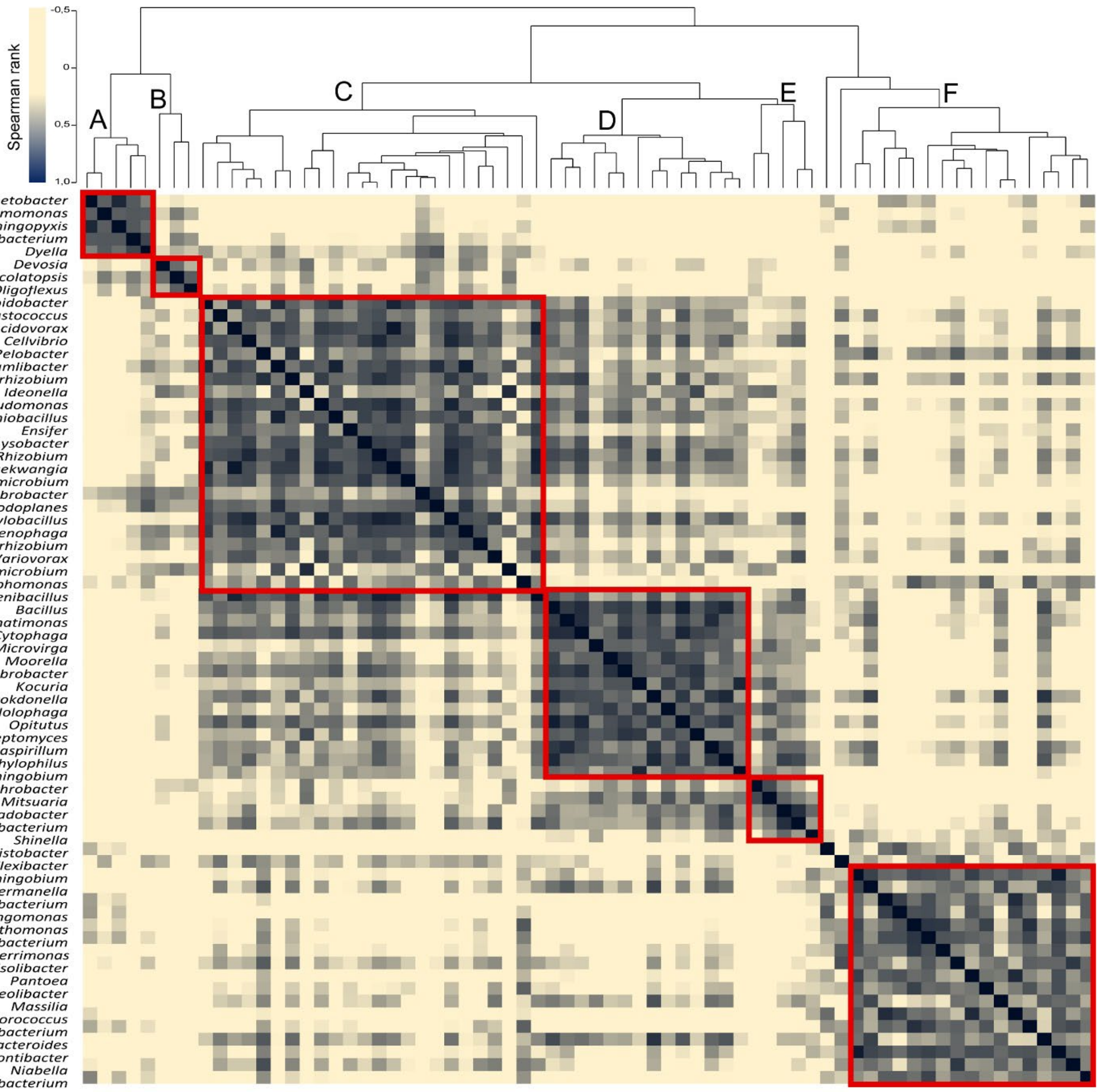


Figure S.5.4. Heatmap on contingency matrix showing Spearman rank-based correlation between bacterial frequencies in rhizospheric soil, variables were ordered according to hierarchical clustering of Spearman rank r values using complete linkage methodology. Red squared underline identified clusters of bacterial consortia. Letters in dendrograms identify the different clusters.

Table S5.1. Composition of the biochar used for the experiment.

Parameter	Biochar	
Total nitrogen ^{a}	%	1.02
N-NH_4^{+} ^{b}	%	< 0.03
N-NO_3^{-} ^{c}	mg kg^{-1}	3.21
Total phosphorous ^{d}	mg kg^{-1}	2,408.24
Total potassium ^{d}	mg kg^{-1}	18,618.22
Total calcium ^{d}	mg kg^{-1}	20,924.51
Total magnesium ^{d}	mg kg^{-1}	4,925.29
Assimilable phosphorous ^{e}	mg kg^{-1}	123.64
Assimilable potassium ^{f}	mg kg^{-1}	9,570.60
Assimilable calcium ^{f}	mg kg^{-1}	1,774.00
Assimilable magnesium ^{f}	mg kg^{-1}	674.40
Humidity ^{g}	%	8.00
Volatiles ^{g}	%	7.30
Ash ^{g}	%	8.99
Organic carbon	%	92.07
Fixed Carbon ^{g}	%	83.71
Hydrogen ^{g}	%	0.89
Sulphur ^{g}	%	0.01
Oxygen ^{g}	%	4.74
pH 1:5 (biochar:water)	-	10.82
Higher calorific value ^{g}		29.04
Lower calorific value ^{g}		28.94

^{a} Kjeldahl method

^{b} Destillation and titration

^{c} Ion chromatography

^{d} Acid digestion and ICP spectroscopy

^{e} Olsen method

^{f} Acid extraction and ICP spectroscopy

^{g} results expressed on a dry matter basis

Table S5.2. Composition of the anaerobic digestate used for the experiment.

Parameter	Anaerobic Digestate	
Organic Matter	% of dry matter	62.41
Total Nitrogen	% of dry matter	10.38
N-NH_4^+	% of total N	34.10
C : N Ratio		5.14
Total phosphorus	% of dry matter	6.97
Potassium	% of dry matter	8.20
Calcium	% of dry matter	0.91
Magnesium	% of dry matter	0.35
Sodium	% of dry matter	0.05
Manganese	mg kg^{-1} of dry matter	235.02
Iron	mg kg^{-1} of dry matter	1,578.92
Copper	mg kg^{-1} of dry matter	48.12
Zinc	mg kg^{-1} of dry matter	147.04
Total solids	g L^{-1}	5.75
pH		7.71
Conductivity	dS m^{-1}	4.39

Table S5.3. Soil analysis prior to the field trials. * Total N: organic + nitric + ammonia nitrogen.

Parameter		Melon crop		Pepper crop	
		2018	2019	2018	2019
Texture (%)	Sand	41.60	43.80	9.20	51.90
	Silt	28.10	28.90	25.40	31.10
	Clay	30.30	27.30	65.40	17.00
pH 1:2 (soil:water)		8.78	8.86	7.93	8.90
Electric conductivity (dS/m)		0.66	0.29	3.27	0.42
Organic matter (%)		1.04	1.24	0.98	1.34
Total nitrogen* (%)		0.069	0.068	0.071	0.076
Ratio C/N		8.72	10.62	8.02	10.26
P_2O_5 (mg kg^{-1})		69.70	20.60	12.50	58.60
K (meq 100g^{-1})		1.86	0.51	1.32	1.86
Ca (meq 100g^{-1})		6.90	11.12	21.52	6.74
Mg (meq 100g^{-1})		2.72	3.41	4.27	2.45
Na (meq 100g^{-1})		1.49	0.90	4.04	0.98

Table S5.4. Climatic conditions of the locations selected for field trials in 2018 and 2019. Hmax: maximum high temperature (°C); Havg: average high temperature (°C); Lmin: minimum low temperature (°C); Lavg: average low temperature (°C).

Crop	Location	Date		Temperatures (°C)				Monthly rainfall (mm)
				Hmax (°C)	Havg (°C)	Lmin (°C)	Lavg (°C)	
Melon	Rambla Salada	2018	April	25.8	22.6	8.3	12.8	7.4
			May	29.2	25.1	9.6	15.0	2.2
			June	33.6	29.2	14.7	19.1	10.2
			July	33.6	31.4	19.7	21.6	0.0
	Los Lorentes	2019	May	30.0	25.3	11.2	15.2	0.8
			June	36.9	29.0	15.5	18.7	3.0
			July	37.1	32.9	20.4	23.2	0.0
			August	35.2	32.7	19.5	22.7	4.4
Pepper	El Moaire	2018	June	33.6	29.2	14.7	19.1	10.2
			July	33.6	31.4	19.7	21.6	0.0
			August	36.8	33.6	22.2	23.7	0.0
			September	33.8	30.1	17.7	21.6	57.6
	Rambla Salada	2019	June	36.9	29.0	15.5	18.7	3.0
			July	37.1	32.9	20.4	23.2	0.0
			August	35.2	32.7	19.5	22.7	4.4
			September	32.9	29.4	16.3	20.5	50.4

Table S5.5. Mean values for the biomass production, the yield and the yield components, obtained in the field trial for the melon crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test.

Biochar dose (kg ha^{-1})	Additive dose (%)	Fresh aerial biomass (g per plant)	Dry aerial biomass (g per plant)	Yield (kg ha^{-1})	Number fruits per ha	Fruit weight (g)	Number fruits per plant
250	0	1,493 b	1,184 b	35,580 a	16,429 a	2,151 a	2.96 a
	5	1,190 a	945.2 a	38,202 a	17,327 ab	2,223 ab	3.19 a
	10	1,643 b	1,303 b	42,703 b	18,539 b	2,304 b	3.39 a
	average	1,442	1,144	38,828	17,732	2,226	3.18
500	0	1,089 a	863.5 a	38,381 a	17,446 a	2,205 a	3.12 a
	5	1,991 b	1,581 b	39,371 a	17,873 a	2,271 ab	3.27 a
	10	1,807 b	1,433 b	42,074 b	18,549 a	2,365 b	3.32 a
	average	1,629	1,293	39,942	17,956	2,280	3.23

Table S5.6. Mean values for the biomass production, the yield and the yield components, obtained in the field trial for the pepper crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test.

Biochar dose (kg ha^{-1})	Additive dose (%)	Fresh aerial biomass (g per plant)	Dry aerial biomass (g per plant)	Yield (kg ha^{-1})	Number fruits per ha	Fruit weight (g)	Number fruits per plant
250	0	255.1 a	202.2 a	40,480 a	531,752 a	70.2 a	21.3 a
	5	300.1 a	237.9 a	44,284 a	588,249 a	75.6 a	23.5 a
	10	271.4 a	215.2 a	44,297 a	600,215 a	77.2 a	24.0 a
	average	275.5	218.4	43,020	573,405	74.3	22.9
500	0	234.7 a	186.6 a	42,349 a	563,963 a	72.8 a	22.6 a
	5	308.5 b	244.3 b	46,823 b	594,102 a	82.4 a	23.8 a
	10	281.6 b	227.5 b	47,250 b	627,589 a	76.9 a	25.1 a
	average	274.9	219.5	45,474	595,218	77.4	23.8

Table S5.7. Mean values for leaf chlorophyll content, flowering and several fruit parameters obtained in the field trial for the melon crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test.

Biochar dose (kg ha^{-1})	Additive dose (%)	Chlorophyll (CCI)	Flowering (%)	Penetrometry (kg)	Fruit contour (cm)	Conductivity (µS cm^{-1})	Solute concentration (mg l^{-1})
250	0	27.3 a	9.17 a	2.20 a	42.7 a	4.68 a	1,511 a
	5	28.6 a	25.0 b	2.10 ab	46.2 b	5.03 ab	1,741 b
	10	33.8 b	35.4 b	2.38 b	46.9 b	5.20 b	1,787 b
	average	29.9	23.2	2.23	45.3	4.97	1,680
500	0	27.7 a	10.0 a	2.32 a	43.1 a	4.75 a	1,728 a
	5	28.3 a	23.3 b	2.35 a	47.9 b	5.38 b	1,764 a
	10	33.1 b	39.6 c	2.35 a	48.7 b	5.63 b	1,888 b
	average	29.7	24.3	2.34	46.6	5.25	1,793

Table S5.8. Mean values for leaf chlorophyll content and several fruit parameters obtained in the field trial for the pepper crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test.

Biochar dose (kg ha^{-1})	Additive dose (%)	Chlorophyll (CCI)	Fruit contour (mm)	Conductivity (µS cm^{-1})	Solute concentration (mg l^{-1})
250	0	79.9 a	11.3 a	4.02 a	1,849 a
	5	78.2 a	13.0 a	4.06 a	1,951 a
	10	78.9 a	13.4 a	4.12 a	1,992 a
	average	79.0	12.6	4.07	1,931
500	0	76.6 a	11.8 a	4.02 a	1,854 a
	5	82.2 b	13.7 a	4.35 a	2,068 ab
	10	77.4 ab	13.9 a	4.38 a	2,118 b
	average	78.7	13.1	4.25	2,013

Appendix III. Chapter 6: supporting information

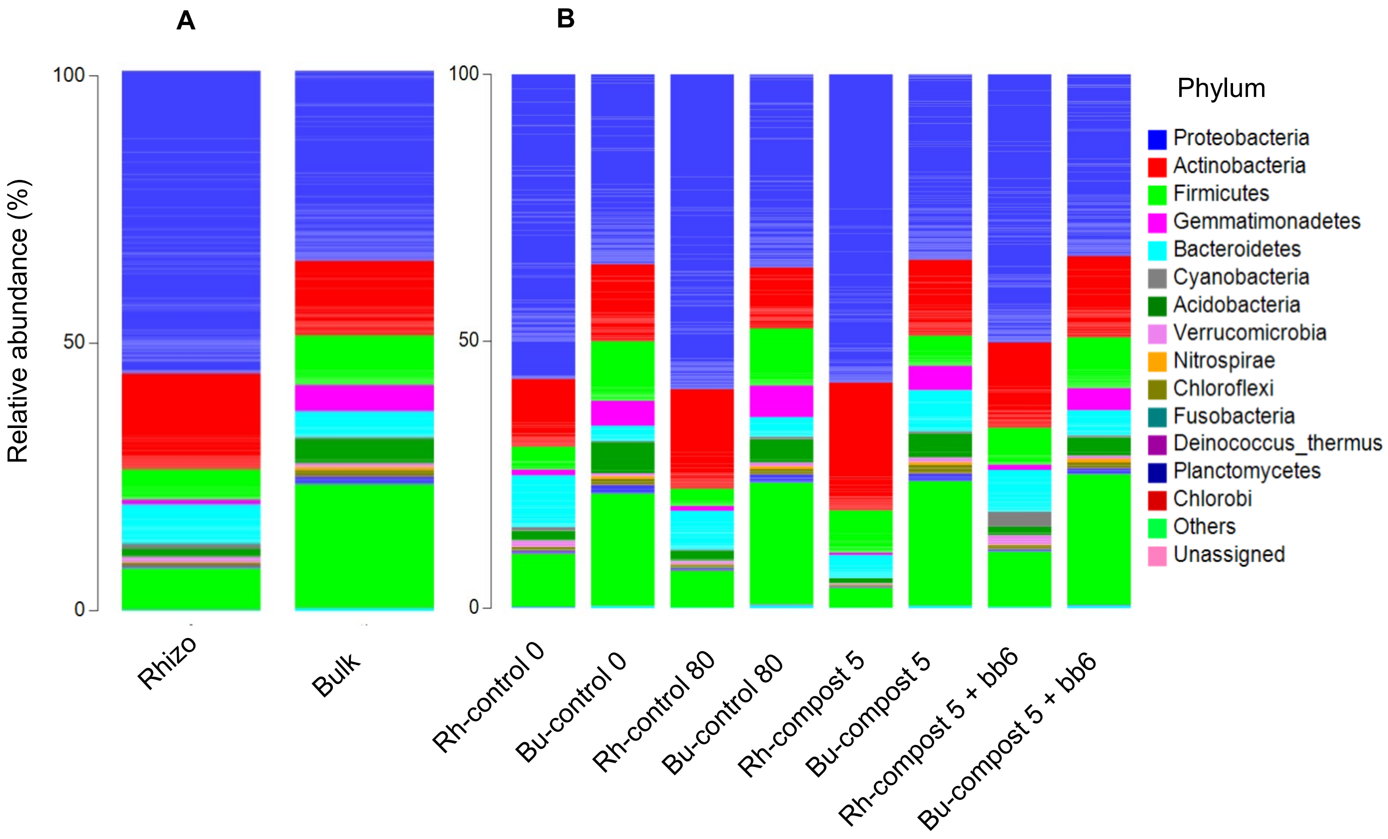


Figure S6.1. Stacked bar plot indicating the relative abundance of bacterial phyla for the factors: A) type of soil B) type of soil and treatment.

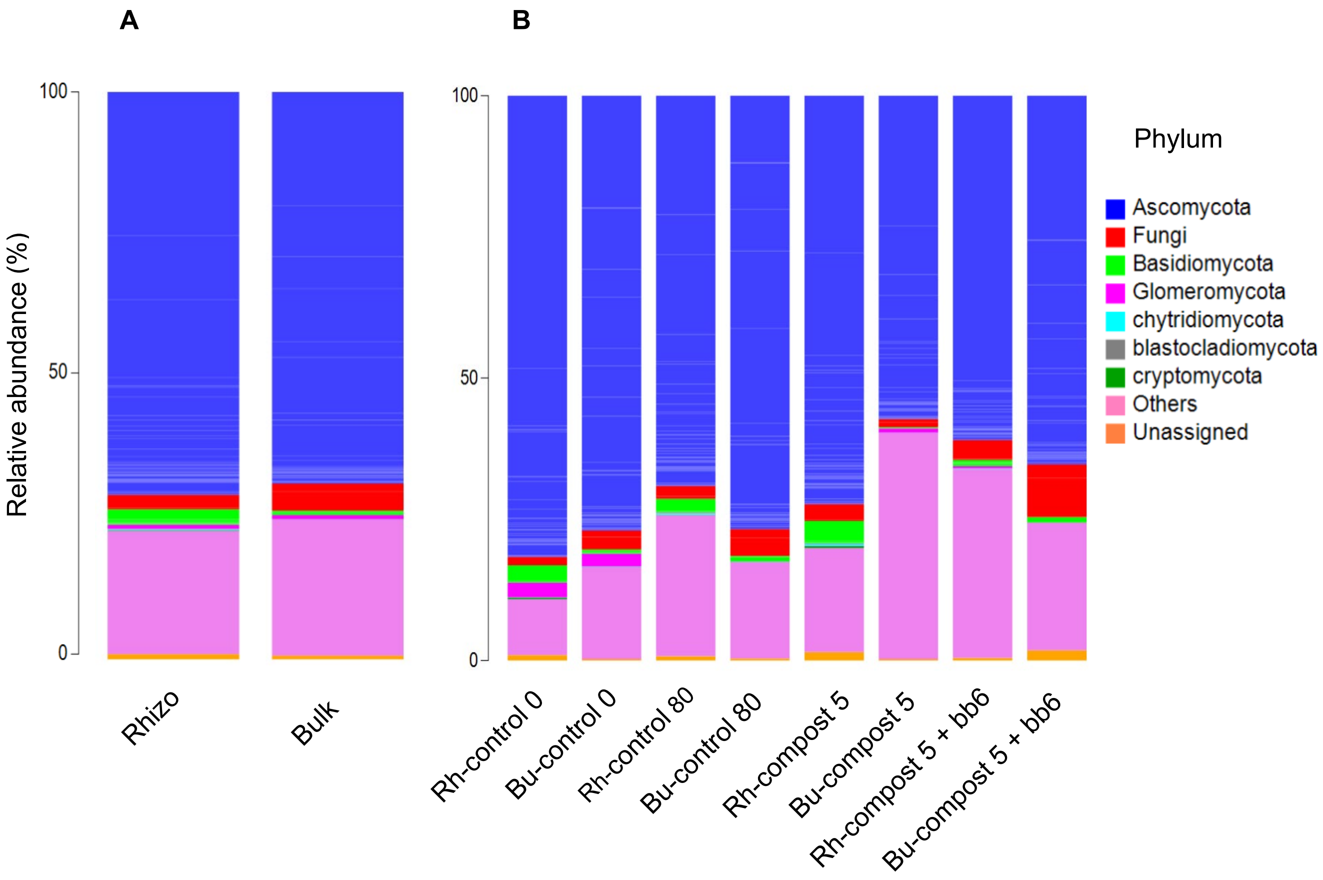


Figure S6.2. Stacked bar plot indicating the relative abundance of fungal phyla for the factors: A) type of soil B) type of soil and treatment.

Table S6.1. Composition of the compost used.

Parameter	**Compost**	
N Kjeldahl	%	2.08
P	mg kg^{-1}	4,683.30
K	mg kg^{-1}	20,600.70
Ca	mg kg^{-1}	76,196.00
Mg	mg kg^{-1}	6,097.30
Na	mg kg^{-1}	2,387.00
Fe	mg kg^{-1}	1,868.30
Mn	mg kg^{-1}	101.20
Zn	mg kg^{-1}	111.10
Cu	mg kg^{-1}	7.49
Cr	mg kg^{-1}	8.40
Ni	mg kg^{-1}	4.90
Hg	mg kg^{-1}	0.09
Cd	mg kg^{-1}	0.19
Pb	mg kg^{-1}	2.62
Oxidizable organic carbon	%	15.08
Organic Matter	%	25.93
C/N Ratio	-	7.24
pH (soil:water)	-	8.55
Electrical Conductivity	ds m^{-1}	6.75
Particle size	mm	1.55

Table S6.2. Composition of the biochar used for the experiment.

Parameter		**Biochar**
Total nitrogen [a]	%	1.02
$N\text{-}NH_4^+$ [b]	%	< 0.03
$N\text{-}NO_3^-$ [c]	$mg\ kg^{-1}$	3.21
Total phosphorous [d]	$mg\ kg^{-1}$	2,408.24
Total potassium [d]	$mg\ kg^{-1}$	18,618.22
Total calcium [d]	$mg\ kg^{-1}$	20,924.51
Total magnesium [d]	$mg\ kg^{-1}$	4,925.29
Assimilable phosphorous [e]	$mg\ kg^{-1}$	123.64
Assimilable potassium [f]	$mg\ kg^{-1}$	9,570.60
Assimilable calcium [f]	$mg\ kg^{-1}$	1,774.00
Assimilable magnesium [f]	$mg\ kg^{-1}$	674.40
Humidity [g]	%	8.00
Volatiles [g]	%	7.30
Ash [g]	%	8.99
Organic carbon	%	92.07
Fixed Carbon [g]	%	83.71
Hydrogen [g]	%	0.89
Sulphur [g]	%	0.01
Oxygen [g]	%	4.74
pH 1:5 (biochar:water)	-	10.82
Higher calorific value [g]		29.04
Lower calorific value [g]		28.94

[a] Kjeldahl method

[b] Destillation and titration

[c] Ion chromatography

[d] Acid digestion and ICP spectroscopy

[e] Olsen method

[f] Acid extraction and ICP spectroscopy

[g] results expressed on a dry matter basis

Table S6.3. Soil analysis prior to the field trials. * Total N: organic + nitric + ammonia nitrogen.

Parameter		Melon crop		Pepper crop	
		2018	**2019**	**2018**	**2019**
	Sand	41.60	43.80	9.20	51.90
Texture (%)	Silt	28.10	28.90	25.40	31.10
	Clay	30.30	27.30	65.40	17.00
pH 1:2 (soil:water)		8.78	8.86	7.93	8.90
Electric conductivity (dS/m)		0.661	0.295	3.27	0.427
Organic matter (%)		1.04	1.24	0.986	1.34
Total nitrogen* (%)		0.069	0.068	0.071	0.076
Ratio C/N		8.72	10.62	8.02	10.26
P_2O_5 (mg kg^{-1})		69.70	20.60	12.50	58.60
K (meq 100g^{-1})		1.86	0.51	1.32	1.86
Ca (meq 100g^{-1})		6.90	11.12	21.52	6.74
Mg (meq 100g^{-1})		2.72	3.41	4.27	2.45
Na (meq 100g^{-1})		1.49	0.907	4.04	0.981

Table S6.4. Climatic conditions of the locations selected for field trials in 2018 and 2019. Hmax: maximum high temperature (°C); Havg: average high temperature (°C); Lmin: minimum low temperature (°C); Lavg: average low temperature (°C).

Crop	Location		Date	Temperatures (°C)				Monthly rainfall (mm)
				Hmax (°C)	**Havg (°C)**	**Lmin (°C)**	**Lavg (°C)**	
Melon	Rambla Salada	2018	April	25.8	22.6	8.3	12.8	7.4
			May	29.2	25.1	9.6	15.0	2.2
			June	33.6	29.2	14.7	19.1	10.2
			July	33.6	31.4	19.7	21.6	0.0
	Los Lorentes	2019	May	30.0	25.3	11.2	15.2	0.8
			June	36.9	29.0	15.5	18.7	3.0
			July	37.1	32.9	20.4	23.2	0.0
			August	35.2	32.7	19.5	22.7	4.4
Pepper	El Moaire	2018	June	33.6	29.2	14.7	19.1	10.2
			July	33.6	31.4	19.7	21.6	0.0
			August	36.8	33.6	22.2	23.7	0.0
			September	33.8	30.1	17.7	21.6	57.6
	Rambla Salada	2019	June	36.9	29.0	15.5	18.7	3.0
			July	37.1	32.9	20.4	23.2	0.0
			August	35.2	32.7	19.5	22.7	4.4
			September	32.9	29.4	16.3	20.5	50.4

Table S6.5. Mean values for the biomass production, the yield and the yield components, obtained in the field trial for the melon crop. Means followed by the same letter did not differ significantly at *p* ≤ 0.05 in Tukey's test.

Compost dose (t ha^{-1})	Additive dose (%)	Fresh aerial biomass (g per plant)	Dry aerial biomass (g per plant)	Yield (kg ha^{-1})	Number fruits per ha	Fruit weight (g)	Number fruits per plant
2	0	1,759 a	1,315 a	37,079 a	3.08 a	2,193 a	16,898 a
	3	1,888 ab	1,395 ab	38,118 a	2.92 a	2,387 b	15,973 a
	6	2,083 b	1,553 b	48,235 b	3.73 b	2,371 b	20,327 b
	average	1,910	1,421	41,144	3.24	2,317	17,732
5	0	1,638 a	1,210 a	37,488 a	3.11 a	2,200 a	17,034 a
	3	2,264 b	1,679 b	42,933 b	3.37 a	2,346 b	18,319 ab
	6	2,450 b	1,806 b	45,245 b	3.44 a	2,400 b	18,842 b
	average	2,117	1,565	41,889	3.31	2,315	18,065

Table S6.6. Mean values for the biomass production, the yield and the yield components, obtained in the field trial for the pepper crop. Means followed by the same letter did not differ significantly at *p* ≤ 0.05 in Tukey's test.

Compost dose (t ha^{-1})	Additive dose (%)	Fresh aerial biomass (g per plant)	Dry aerial biomass (g per plant)	Yield (kg ha^{-1})	Number fruits per ha	Fruit weight (g)	Number fruits per plant
2	0	281.2 a	-	43,131 a	21.1 a	82.3 a	526,806 a
	3	332.3 b	-	47,586 ab	25.2 b	76.1 a	629,869 b
	6	362.5 b	-	50,593 b	24.7 b	82.5 a	617,224 b
	average	325.3	-	47,103	23.7	80.3	591,300
5	0	294.3 a	-	44,786 a	24.9 a	72.0 a	623,534 a
	3	320.8 b	-	47,932 ab	22.1 a	87.3 b	553,266 a
	6	330.1 b	-	51,298 b	23.5 a	88.4 b	587,018 a
	average	315.1	-	48,005	23.5	82.6	587,939

Table S6.7. Mean values for the leaf chlorophyll content, the flowering and several fruit parameters obtained in the field trial for the melon crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test.

Compost dose (t ha^{-1})	Additive dose (%)	Chlorophyll (CCI)	Flowering (%)	Penetrometry (kg)	Fruit contour (cm)	Conductivity (µS cm^{-1})	Solute concentration (mg l^{-1})
2	0	25.8 a	10.0 a	2.18 a	41.8 a	4.87 a	1,567 a
	3	26.2 a	12.1 a	2.14 a	43.7 b	5.04 ab	1,652 b
	6	30.3 b	18.6 b	2.17 a	43.2 b	5.23 b	1,778 c
	average	27.4	13.6	2.2	42.9	5.05	1,666
5	0	34.1 a	37.9 a	2.45 b	49.3 a	5.10 a	1,630 a
	3	37.1 b	37.9 a	2.06 a	47.9 a	5.31 a	1,685 a
	6	38.5 b	41.4 a	1.97 a	49.1 a	5.50 b	1,909 b
	average	36.6	39.1	2.16	48.9	5.30	1,742

Table S6.8. Mean values for the leaf chlorophyll content and several fruit parameters obtained in the field trial for the pepper crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test.

Compost dose (t ha^{-1})	Additive dose (%)	Chlorophyll (CCI)	Fruit contour (mm)	Conductivity (µS cm^{-1})	Solute concentration (mg l^{-1})
2	0	79.6 a	46.1 a	4.51 a	2,753 b
	3	74.8 a	46.2 a	4.40 a	2,330 a
	6	79.9 a	44.8 a	3.92 a	2,408 a
	average	78.1	45.7	4.28	2,497
5	0	83.5 a	46.2 a	4.20 a	2,610 b
	3	86.3 a	45.9 a	3.90 a	2,408 a
	6	85.9 a	47.1 a	3.87 a	2,382 a
	average	85.2	46.4	3.99	2,467