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IMPACTO DE CONCENTRACIONES AMBIENTALMENTE RELEVANTES DE NANOPARTÍCULAS DE PLATA EN LA COMUNIDAD MICROBIANA DEL SUELO

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Declaración de autenticidad

Yo Gabriela Montes de Oca Vásquez, estudiante del Doctorado en Ciencias Naturales para el Desarrollo, declaro que la Tesis Doctoral que presento para su exposición y defensa titulada "Impacto de concentraciones ambientalmente relevantes de nanopartículas de plata en la comunidad microbiana del suelo" y cuyos tutores son el Dr. José Roberto Vega Baudrit, Dr. Felipe Bastida López y el Dr. José Luis Moreno Ortego, es original y que todas las fuentes utilizadas para su realización han sido debidamente citadas en el mismo.

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1. Introducción

La nanotecnología es un área de la ciencia emergente que involucra la ingeniería de partículas nanométricas de diversos materiales (Khare et al., 2014). De acuerdo con la Agencia de Protección Ambiental de Estados Unidos (EPA, por sus siglas en inglés), la nanotecnología se define como "la creación y uso de estructuras, dispositivos y sistemas que tienen propiedades y funciones novedosas debido a su pequeño tamaño". Estas partículas y estructuras moleculares tienen dimensiones en el rango nanométrico (típicamente entre 1 y 100 nm en al menos una dimensión) (Hobson, 2011). Es debido a esta escala que los materiales pueden adquirir propiedades físicas y químicas diferentes a las que tienen en la escala macroscópica (Ivask et al., 2014; Tortella et al., 2020), como propiedades ópticas, electrónicas, antimicrobianas, magnéticas y catalíticas (Capek, 2006).

Debido a estas características la producción global de las nanopartículas (NPs) continúa aumentando, y se han utilizado en numerosos productos comerciales con diversas aplicaciones industriales (Sharma et al., 2019; Tortella et al., 2020). Se han utilizado en el área agrícola, como fertilizantes y pesticidas (Madbouly et al., 2017), en el área médica (Her et al., 2017), principalmente con el desarrollo de NPs para la liberación controlada de fármacos (Pang et al., 2019), en el desarrollo de dispositivos de diagnóstico médico (McNamara & Tofail, 2015), además, de su uso para mejorar las imágenes médicas (Ryan et al., 2019). Asimismo, las NPs se han utilizado en dispositivos electrónicos (Khanra et al., 2018), en cosméticos (de la Calle et al., 2017), en la nanoremediación (Moustafa, 2017), en el sector agroalimentario (Dasgupta et al., 2017), entre otros.

Dentro de los diferentes tipos de NPs, las nanopartículas de plata (AgNPs), son unas de las más utilizadas actualmente en diversos productos comerciales (Ottoni et al., 2020; Temizel-Sekeryan & Hicks, 2020). La síntesis y usos comerciales de las AgNPs ha aumentado principalmente debido a sus conocidas propiedades antimicrobianas en contra de un gran rango de microorganismos como hongos, bacterias y arqueas (Grün & Emmerling, 2018; Shen et al., 2020). Sin embargo, con el desarrollo de numerosos productos comerciales surge la necesidad del estudio de la toxicidad de los nanomateriales o NPs, en un área denominada como nanotoxicología, cuyo objetivo principal es determinar los efectos tóxicos o dañinos de los diferentes tipos de NPs en los seres humanos y el medio ambiente (incluyendo el aire, el agua y el suelo) (Erkekoglu & Kocer-Gumusel, 2018). Se ha reportado que las AgNPs o su ion Ag^+

liberado, pueden causar efecto directo sobre los organismos, incluyendo impacto sobre las paredes y membranas celulares. Además, pueden inducir la producción de especies de oxígeno reactivo (ROS) y producir cambios en la estructura de componentes celulares como ácidos nucleicos y proteínas, indispensables para el funcionamiento celular (Tang & Zheng, 2018).

De acuerdo a la base de datos de productos con nanotecnología se ha estimado que estas NPs han sido utilizadas en 310 diferentes tipos de productos, en 37 países y en 15 tipos de industrias como la biosanitaria, electrónica, textil, cosmética, construcción, entre otros, con diversos tipos de aplicaciones y propiedades (StatNano, 2020). Debido a este uso extensivo, las NPs pueden llegar al medio ambiente (aire, agua y suelo) en cualquiera de sus fases de producción (Wang et al., 2018), y puede actuar como un contaminante a lo largo de su ciclo de vida, afectando el equilibrio de las comunidades de microorganismos (Pachapur et al., 2016). Se ha reportado que el suelo es el mayor reservorio de NPs comparado con los otros ambientes (Wang et al., 2018; He et al., 2019). Se ha identificado que las NPs pueden llegar al suelo principalmente mediante la aplicación de biosólidos, aguas residuales, o aplicación de productos con AgNPs para la protección de las plantas contra plagas y enfermedades (Hänsch & Emmerling, 2010). Lo anterior plantea una preocupación acerca de la toxicidad potencial que pueden tener las AgNPs sobre el medio ambiente, y especialmente para las comunidades microbianas de los suelos agrícolas (McGee et al., 2020; Meier et al., 2020). Sin embargo, los efectos adversos producidos por las AgNPs sobre las comunidades de microorganismos y las propiedades físico-químicas del suelo todavía no están claros (Xu et al., 2016; Tortella et al., 2020).

Las comunidades microbianas del suelo son muy diversas y juegan un papel fundamental en el mantenimiento de la calidad del suelo (Delgado-Baquerizo et al., 2016). Algunos estudios han demostrado que las comunidades microbianas del suelo se ven afectadas por las AgNPs (Samarajeewa et al., 2017; Grün et al., 2018; Zhang et al., 2020). La biomasa microbiana, las actividades enzimáticas y la diversidad microbiana, han sido utilizados como indicadores de la calidad, debido a que perturbaciones en los suelos son rápidamente detectables por estos métodos (Bastida et al., 2006; Schloter et al., 2018). Por otra parte, diversos parámetros físico-químicos, como el pH, el contenido de nitrógeno, fósforo y carbono se han utilizado para determinar la calidad del suelo (Xu et al., 2016). Sin embargo, hasta la fecha son pocos los estudios que se encuentran relacionados con el efecto de las NPs sobre las características físico-químicas del suelo.

Los efectos de las AgNPs sobre las comunidades microbianas del suelo dependen de diversas características, tales como las propiedades fisicoquímicas del suelo, entre ellas el pH, el contenido de materia orgánica y de arcilla, y la fuerza iónica (McGee et al., 2020). Además, la toxicidad depende de las características inherentes a las NPs, como el agente estabilizante, la carga, el tamaño, el tipo de NPs, y la forma, así como las condiciones de exposición, como el tiempo de exposición (Grün et al., 2018), la concentración de las AgNPs (Huang et al., 2018; Shen et al., 2020), la temperatura (Huang et al., 2018), los cuales influyen sobre el destino, la biodisponibilidad y transformaciones de las NPs en el suelo, incidiendo sobre la potencial toxicidad en los microorganismos.

A pesar de estudios recientes, el conocimiento sobre los impactos de AgNPs en la actividad y función de la comunidad microbianas del suelo sigue siendo limitado (Ivask et al., 2014; Weber et al., 2014; Moreno-Garrido et al., 2015; Simonin & Richaume, 2015); principalmente, debido a que evaluar los posibles efectos ecotoxicológicos en una gran variedad de suelos, con los diferentes tipos de AgNPs que existen en el mercado, y bajo diferentes condiciones, es un desafío mundial. Además, aunque se han realizado estudios del impacto de las AgNPs sobre las comunidades microbianas, la mayoría se han realizado bajo condiciones de altas concentraciones de AgNPs (Rahmatpour et al., 2017; Asadishad et al., 2018; Grün et al., 2018). Por lo tanto, estudios basados en el efecto de las concentraciones probabilísticas de AgNPs, que se podrían encontrar en los suelos agrícolas, sobre las propiedades físico-químicas del suelo y sobre las comunidades microbianas, utilizando diferentes técnicas bioquímicas y moleculares, es fundamental (Grün et al., 2018).

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2. Marco teórico

2.1. El suelo

2.1.1. Definición y función dentro del ecosistema

Los suelos constituyen un recurso no renovable en la escala de tiempo humana, es por esto que es un recurso que debemos de mantener y conservar (Breure et al., 2018). El suelo ha sido definido como "La parte sólida de la tierra, que incluye componentes líquidos, gaseosos y los organismos" (Römbke et al., 2005). Aquí se incluye tanto la capa superior, así como las capas más profundas del suelo. Es, por lo tanto, un sistema dinámico, vivo, y constituye una interfase que permite intercambios entre la litosfera, la biosfera y la atmósfera (Porta Casanellas, 2015). El suelo, es el producto final a través del tiempo, de la acción combinada de diversos factores ecológicos, como el clima, la geomorfología, los organismos vivos, y los materiales parentales (Porta Casanellas, 2015). Como resultado, el suelo difiere de su material parental, en su textura, color, estructura y características biológicas, físicas y químicas.

Los suelos son importantes desde una perspectiva ecológica y agroforestal, asimismo son indispensables para el desarrollo de las actividades humanas, debido a que proveen muchos servicios biológicos que son esenciales para la vida en la tierra (Schloter et al., 2018). Dentro de sus múltiples funciones fundamentales cabe citar que son indispensables para la producción de alimentos y fibras; ayudan en el mantenimiento y mejora de la calidad de las aguas; regulan el ciclo hidrológico; degradan sustancias contaminantes; están involucrados en la fijación de gases de efecto invernadero y la regulación del clima; son esenciales para el control de la erosión; constituyen una reserva de biodiversidad, y son un soporte físico para las diversas actividades humanas (Maestre et al., 2015; Porta Casanellas, 2015; Schloter et al., 2018).

El suelo es una materia compleja y está constituido de minerales, materia orgánica, agua y aire (Voroney, 2018), los cuales influyen en la textura, estructura y porosidad del suelo (Naga Raju et al., 2017). Las partículas minerales se derivan de fragmentos de roca y minerales triturados y desgastados y constituyen más de 90% del peso seco del suelo (Voroney, 2018). Las proporciones de arena, limo y arcilla en la fracción de tierra fina determinan la textura del suelo (Naga Raju et al., 2017; Voroney, 2018). Por otra parte, la materia orgánica del suelo representa entre el 1% y el 10% de la masa total del suelo y esta formada por residuos vegetales, fauna y microorganismos en varios estados de descomposición, desde hojarasca hasta humus. El

componente vivo de la materia orgánica del suelo consiste en raíces de plantas, microorganismos, y fauna. La química del componente no vivo de la materia orgánica del suelo, está compuesta por carbohidratos, proteínas y lípidos, y biomoléculas simples como azúcares, aminoácidos y ácidos grasos (Voroney, 2018) (Fig. 1).
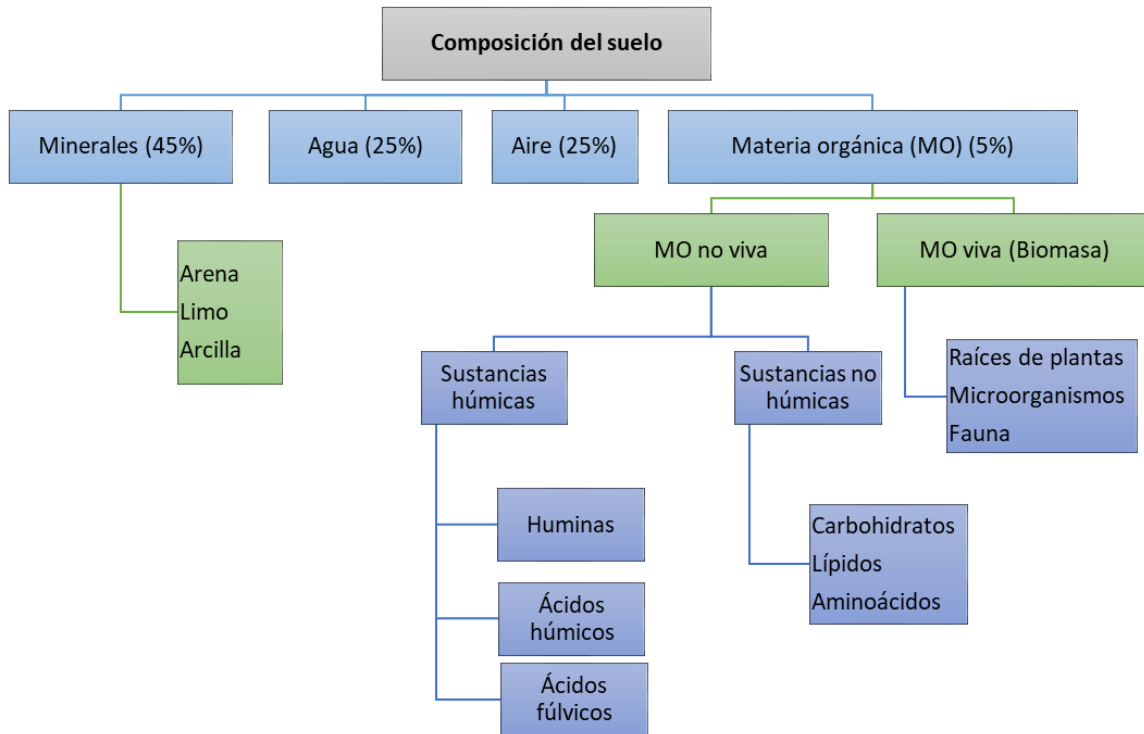
Fig. 1. Composición general del suelo.

2.1.2. Calidad del suelo

Debido a la constante degradación del suelo, por factores climáticos y antropogénicos, como el inadecuado uso del suelo, y el uso constante de agroquímicos y fertilizantes, se ha priorizado en el desarrollo de indicadores físicos, químicos y biológicos robustos y sensibles, que permitan monitorear la calidad del suelo (Zornoza et al., 2015; Muñoz-Rojas et al., 2016; Schloter et al. 2018). La calidad de un suelo, se define como "la capacidad de un suelo para funcionar dentro de los límites del ecosistema y mantener su productividad biológica y calidad del medioambiente, mientras que promueve la salud animal, de plantas y de los seres humanos." (Doran & Parkin, 1994). Según esta definición, por tanto, se requiere que se evalúen de manera simultánea varios

parámetros para describir la calidad del suelo (Bastida et al., 2008). Diversos autores han resaltado la importancia de usar parámetros físicos, químicos y biológicos (Herrick et al., 2002; Aparicio & Costa, 2007), (Tabla 1), con el fin de lograr una comprensión clara de la calidad del suelo (Zornoza et al., 2015; Muñoz-Rojas et al., 2016).

Tabla 1. Parámetros físicos, físico-químicos, químicos, microbiológicos y bioquímicos para evaluar la calidad del suelo.

Físicos
• Densidad • Textura • Porosidad • Retención hídrica
Físico - Químicos y Químicos
• Conductividad eléctrica • pH • Capacidad de intercambio catiónico • Macro y micronutrientes • Carbono y nitrógeno
Microbiológicos y Bioquímicos
• Actividad y funcionalidad: Actividades enzimáticas, metagenómica • Biomasa microbiana: ácidos grasos microbianos • Diversidad y composición: secuenciación de amplicones

Tradicionalmente, los parámetros físicos y químicos han sido principalmente utilizados para evaluar la calidad del suelo. Entre los parámetros químicos utilizados se encuentran, el carbono orgánico del suelo, el nitrógeno total, el pH y entre los parámetros físicos, la distribución del tamaño de partícula, la densidad aparente, el agua disponible, la estructura del suelo y la estabilidad del agregado (Rabot et al., 2018). Entre ellos, el carbono orgánico del suelo ha sido durante muchas décadas utilizado para determinar la calidad del suelo (Hueso-González et al., 2018). Muchos parámetros utilizados para evaluar la calidad del suelo se relacionan con el ciclo de la materia orgánica del suelo, ya que dicha materia orgánica está estrechamente relacionada con muchas funciones edáficas, como la estructura del suelo, la infiltración de aire y agua, la retención del agua, la disponibilidad de nutrientes, la erosión y el transporte o inmovilización de contaminantes (Knoepp et al., 2000).

2.2. Comunidades microbianas del suelo

Se han detectado una amplia variedad de organismos procariotas (bacterias y arqueas) y eucariotas (algas, protozoos, nemátodos y hongos) en el suelo (Buee et al., 2009; Pressler et al., 2019), siendo los más abundantes las bacterias, arqueas y hongos (Voroney, 2018). A pesar de la aparente y relativa simplicidad de las células procariotas, como grupo tienen la mayor diversidad taxonómica y funcional. A nivel mundial, el carbono orgánico contenido en los procariotas es equivalente al de las plantas y contienen 10 veces más nitrógeno. También poseen los mecanismos de dispersión y supervivencia más eficientes, lo que los hace organismos muy ampliamente distribuidos en todos los suelos del planeta. En consecuencia, los procariotas son de enorme importancia en el funcionamiento del suelo (Killham & Prosser, 2007; Zhou et al., 2020). Por su parte, eucariotas como los hongos, también cumplen funciones muy importantes, ya que son indispensables para la descomposición de compuestos de carbono recalcitrantes en los ecosistemas (Treseder et al., 2016), están relacionados con el ciclo de nutrientes y la regulación del clima (Li et al., 2019).

Las comunidades microbianas del suelo son sorprendentemente diversas y abundantes. Se ha estimado que 1 g de suelo contiene hasta 1 billón (10^9) de células bacterianas y hasta 1 millón (10^6) de hongos (Dindal, 1990; Zhou et al., 2020), lo que plantea grandes desafíos para investigar la diversidad microbiana presente en los suelos. La gran diversidad de comunidades microbianas que posee el suelo, son indispensables, ya que son responsables de muchos servicios ecosistémicos, como la mineralización de nutrientes, los ciclos biogeoquímicos como el ciclo del nitrógeno, carbono, fósforo, entre otros, la degradación de la materia orgánica, así como el control de patógenos (Kallenbach et al., 2016; Ma et al. 2016; Lee et al., 2019). Por lo tanto, muchos servicios del ecosistema, como el suministro de agua subterránea limpia, la degradación de contaminantes y la producción agrícola, dependen de la comunidad microbiana presente en el suelo, y por ende, los cambios en la diversidad y abundancia microbiana pueden afectar a diversos servicios ecosistémicos (Frenk et al., 2013).

Las comunidades microbianas del suelo pueden ser estudiadas a través de diferentes técnicas, entre ellas, mediante la evaluación de las actividades enzimáticas (Rahmatpour et al., 2017; McGee et al., 2018; Samarajeewa et al., 2019), que determinan la función de las comunidades microbianas, la biomasa microbiana (Bastida et al., 2019; Hinojosa et al., 2019; Moreno et al., 2019), que determina el tamaño y la estructura de las comunidades microbianas, y finalmente

mediante la evaluación de la diversidad microbiana (Forstner et al., 2019; Vera et al., 2019; Zhang et al., 2020), que se determina usualmente por técnicas moleculares como la secuenciación de amplicones.

2.2.1. Actividad microbiana

Los diversos parámetros relacionados con la actividad microbiana han sido muy utilizados para evaluar el estado del suelo y su calidad. Entre otras técnicas de actividad microbiana general destacan la respiración basal y la respiración inducida por sustrato (Rahmatpour et al., 2017). La respiración microbiana, indica la capacidad oxidativa de los microorganismos del suelo, y por lo tanto está influenciada tanto por las fuentes de energía (compuesos de C) que hay en el suelo, como por la cantidad de microorganismos (Bastida et al., 2008). Otros parámetros marcadores de actividad microbiana del suelo son las actividades enzimáticas: i) actividad deshidrogenasa (fundamentalmente intracelular) que es un marcador de la actividad metabólica de los microorganismos (Garcia et al., 1997); y, ii) las actividades de enzimas hidrolasas extracelulares que son liberadas por los microorganismos en el medio edáfico para descomponer el material orgánico de composición y complejidad variables (macromoléculas), en moléculas mas sencilles y biodisponibles las cuales pueden ser asimilables por los microorganismos y las plantas (Jian et al., 2016). Las actividades de enzimas extracelulares son muy utilizadas como indicadores de la calidad de los suelos, ya que sirven para detectar cambios en la actividad de la comunidad microbiana (Bastida et al., 2008) y se han usado durante mucho tiempo como indicadores de la diversidad funcional de los microorganismos, y de la contaminación ambiental del suelo en diversos ecosistemas (Sanchez-Hernandez et al., 2017). Constituyen el principal medio para controlar los procesos bioquímicos, como la descomposición del carbono orgánico y el ciclo de nutrientes (Qi et al., 2016), por lo que ofrecen información acerca de la capacidad de los microorganismos del suelo para conducir las reacciones de los ciclos del Nitrógeno, Carbono, Fosforo y Azufre (García et al., 2003). Estas actividades enzimáticas además, están influenciadas por las características físico-químicas (temperatura, pH y contenido de materia orgánica) del suelo (Xian et al., 2015; Schimel et al., 2017).

Además de las hidrolasas se pueden incluir una gran parte de las oxidoreductasas como enzimas del suelo que actúan extracelularmente (Sinsabaugh & Shah, 2012). Dentro de las oxidoreductasas se encuentran, las catalasas, las fenoloxidasas, las peroxidasas, y las anteriormente mencionadas deshidrogenasas que particularmente actúan intracelularmente.

Entre las hidrolasas mas estudiadas en el suelo se encuentran las fosfatasas, las proteasas, las glucosidasas y la ureasa (García et al., 2003). Las enzimas oxidoreductasas son producidas por los microorganismos para descomponer sustratos que deben oxidarse para despolimerizarse, como la lignina (Sinsabaugh, 2010). Dentro de este grupo se encuentra, la fenol oxidasas y las peroxidasas (Jian et al., 2016). Las celulasas son un grupo de enzimas hidrolíticas que son producidas por los microorganismos del suelo para descomponer polisacáridos; incluyen la endo-1,4-glucanasa, la exo-1,4-glucanasa (Celobiohidrolasa) y la β-D-glucosidasa (Deng & Tabatabai, 1994). La enzima β-glucosidasa, tiene un papel primordial en el ciclo del carbono, y es importante para la descomposición de los hidratos de carbono lábil que componen los restos de los tejidos vegetales depositados en el suelo (Mangalassery et al., 2015). Por otra parte, las enzimas asociadas con la adquisición del N incluyen la β-1,4-N-acetil-glucosaminidasa, la leucina amino peptidasa y la ureasa, que utilizan a la quitina, un polipéptido y la urea como sustratos, respectivamente (Tabatabai & Bremner, 1972). La actividad de la ureasa es considerada como un indicador de la mineralización del nitrógeno orgánico (principalmente urea) e interviene en la regulación de la dinámica del nitrógeno, produciendo amoniaco (NH_3), el cual es posteriormente transformado a amonio (NH_4^*) cuando pasa a formar parte de la disolución o del complejo de cambio del suelo. Esta especie química de N es considerada una fuente de nitrógeno disponible para las plantas (Li et al., 2017). Por su parte, la fosfatasa ácida y alcalina, son las enzimas que catalizan la reacción de hidrólisis que produce la liberación del grupo $PO4^{3-}$ de los compuestos orgánicos que contienen P (Eivazi & Tabatabai, 1977; Tabatabai & Bremner, 1972). La actividad fosfatasa está involucrada en el ciclo del fósforo, y depende del contenido de carbono orgánico del suelo y del fósforo inorgánico (Adamczyk et al., 2015). Por todo ello esta enzima extracelular cataliza en el suelo la mineralización del fósforo orgánico produciendo anión fosfato que es una especie química de P asimilable por la planta (Li et al., 2017). Es importante recalcar que en la mayoría de los ecosistemas la actividad de una sola enzima no refleja la tasa completa de los procesos metabólicos, en consecuencia, se deben usar distintas actividades enzimáticas que responden a diferentes procesos metabólicos para obtener una visión más holística de la actividad microbiana del suelo y de los procesos que tienen lugar en él (Adamczyk et al., 2015).

2.2.2. Biomasa y estructura de la comunidad microbiana

La biomasa microbiana del suelo se ha utilizado como un indicador de la calidad del suelo, y ha sido usada en una gran cantidad de estudios comparativos en diferentes ecosistemas, tropicales y subtropicales (Maharjan et al., 2017). Diferentes métodos se han utilizado para la determinación

de la biomasa bacteriana y fúngica. Dentro de los principales métodos se encuentran, el conteo directo mediante análisis de imágenes y microscopía de epifluorescencia (Bloem, 1995; Van Leeuwen et al., 2015), el método de extracción y fumigación con cloroformo (Vance et al., 1987), el método de respiración inducida por sustrato (SIR), el cual mide la biomasa microbiana activa (Anderson & Domsch, 1978), y la PCR cuantitativa (Fajardo et al., 2012). No obstante, uno de los métodos más robustos para la evaluación de la biomasa microbiana es el análisis de ácidos grasos fosfofolipídicos microbianos (PLFA, por sus siglas en inglés), que se han utilizado durante muchos años para estimar la biomasa y la estructura de la comunidad microbiana en suelos (Jin et al., 2014; Durenkamp et al., 2016). Este método, se ha empleado para medir el efecto de las enmiendas orgánicas en suelos agrícolas (Zhou et al., 2017), para determinar cambios en la biomasa microbiana en diferentes gradientes altitudinales (Ren et al., 2018), y con diferentes alteraciones de los regímenes de precipitación (Ren et al., 2017), así como un indicador para evaluar el efecto del uso del suelo de zonas semiáridas (Moreno et al., 2019) y las prácticas de manejo en suelos subtropicales (Moreno-Garrido et al., 2015; Maharjan et al., 2017).

Sin embargo, este es un método que consume mucho tiempo (Hinojosa et al., 2005). Debido a lo anterior, se han desarrollado otros métodos para extraer los ácidos grasos microbianos directamente de los suelos (Schutter & Dick, 2000; Miura et al., 2017), entre ellos, el método de MIDI (Microbial ID, Inc., Newark, DE) y el método de los éster metílicos de los ácidos grasos ligados a éster (EL-FAME, por siglas en inglés) (Schutter & Dick, 2000). Este último método utiliza un reactivo alcalino suave para lisar las células y liberar los ácidos grasos de los lípidos una vez que se rompen los enlaces éster. En teoría, solo los ácidos grasos ligados a éster y no los libres son extraídos con este método (Schutter & Dick, 2000). Este protocolo, se ha utilizado para la caracterización de comunidades microbianas en suelos agrícolas y pastizales (Ghimire et al., 2019), en suelos de bosques y arables (Miura et al., 2017). Además, se ha utilizado para determinar la respuesta de las comunidades microbianas a eventos como sequía posterior a un incendio en suelos cultivados con el arbusto *Cistus-Erica* (Hinojosa et al., 2019), perturbaciones provocadas por eventos naturales, como huracanes (Cantrell et al., 2014), contaminación por metales pesados (Hinojosa et al., 2005), etc.

2.2.3. Diversidad microbiana

El término "diversidad microbiana" es complejo y, de manera general, puede incluir todos aquelllos parámetros que informan sobre el estado de la comunidad microbiana. De forma más

concreta, en esta Tesis Doctoral nos referiremos al nivel de diversidad microbiana (número de filos/clases y géneros microbianos y abundancia relativa de cada una de ellas) y la composición de la comunidad microbiana. Los indicadores de calidad anteriormente mencionados se complementan con técnicas moleculares más novedosas, que aportan una información relevante sobre diversidad genética de las comunidades microbianas (Muñoz-Rojas, 2018). La detección de ADN genómico de especies microbianas en el suelo ha mejorado el conocimiento de microorganismos no cultivables y ha llevado a una mayor comprensión sobre la diversidad microbiana y su relación con las funciones del ecosistema (Nesme et al., 2016). El estudio de la diversidad microbiana es muy importante ya que el suelo alberga una inmensa diversidad de microorganismos (taxones individuales) comúnmente descritos como unidades taxonómicas operacionales (OTUs, por sus siglas en inglés) de bacterias, hongos y arqueas (Simonin & Richaume, 2015).

En las últimas dos décadas se han utilizado una serie de técnicas moleculares basadas en genómica para evaluar la diversidad genética. La genómica tiene la ventaja de poder identificar microorganismos que no pueden ser cultivados utilizando medios de cultivo. De hecho, la gran mayoría de microorganismos (aproximadamente el 95-99%) no son cultivables y, por tanto, para su estudio, las técnicas moleculares de tipo genómico están suponiendo un verdadero avance en la ecología microbiana de suelos. Entre estas técnicas cabe destacar algunas como la electroforesis en gel con gradiente de desnaturalización (PCR-DGGE), los polimorfismos de longitud de fragmentos de restricción terminal (T-RFLP), y la secuenciación de próxima generación (NGS, por sus siglas en inglés) (Doolette et al., 2016; Xu et al., 2016; Zhang et al., 2016; McGee et al., 2018). Dentro de estas técnicas, la NGS constituye una metodología muy ventajosa para realizar estudios a gran escala con el objetivo de caracterizar la estructura y función de las comunidades microbianas en muchos ecosistemas (Forstner et al., 2019; Zhang et al., 2020). Cabe recalcar que la NGS ha revolucionado el campo de la genómica taxonómica, y ha facilitado el estudio de la composición de comunidades microbianas. Con la ayuda de programas bioinformáticos de acceso libre (Huson et al., 2011; Caporaso et al., 2012) es posible analizar los datos generados mediante secuenciación para responder preguntas como: ¿cuáles microorganismos se encuentran en el suelo?, ¿cómo se correlacionan determinadas poblaciones con funciones del suelo?, ¿cuál es la estructura y composición de la comunidad microbiana del suelo?, ¿cómo de diverso microbiológicamente es un suelo?

Entre las tecnologías mejor utilizadas en los instrumentos de NGS, la secuenciación por síntesis (Ronaghi et al., 1998), la secuenciación por ligadura (Shendure et al., 2005), la secuenciación

basada en pirofosfato (Margulies et al., 2005), y la secuenciación de semiconductores de iones (Rothberg et al., 2011) son las más destacados, siendo la secuenciación por síntesis la más utilizada. Dentro de las principales plataformas que utilizan NGS para el análisis de las comunidades microbianas del suelo expuestas a AgNPs se encuentran, la plataforma de secuenciación 454 (Kumar et al., 2014; Shah, 2014), la cual se basa en la detección quimioluminiscente del pirofosfato liberado durante la elongación de la cadena complementaria de ADN, sin embargo, esta tecnología prácticamente se encuentra en desuso. Por otra parte esta disponible la plataforma Ion Torrent™ DNA sequencing (Samarajeewa et al., 2017; Samarajeewa et al., 2019), la cual es una tecnología que se basa en la detección de los protones liberados durante el proceso de polimerización del ADN. Finalmente hay que destacar como una de las tecnologías más potentes en este ámbito a la plataforma de Illumina MiSeq, que se basa en la secuenciación por síntesis, y que es una de las más utilizadas actualmente para el estudio de la secuenciación de amplicones de las comunidades microbianas de hongos y bacterias, en diferentes tipos de suelos (Huang et al., 2017; McGee et al., 2017; Asadishad et al., 2018; Bastida et al., 2019; Forstner et al., 2019; Moreno et al., 2019; Vera et al., 2019; Zhang et al., 2020).

2.3. Características e importancia de los suelos tropicales

La zona tropical de la Tierra se ubica entre los 23,5 grados norte y sur del Ecuador y abarcan el 38% de la superficie terrestre (Arias, 2001). Los suelos tropicales, corresponden a suelos formados entre los trópicos de Cáncer y Capricornio (Igwe, 2011). Son suelos muy diversos debido a la gran diversidad en climas, vegetación, material orgánico y materiales parentales, lo que resulta en suelos con diferentes propiedades físico-químicas. Además, son suelos que han sido influenciados por diversos fenómenos geológicos como la tectónica de placas, la meteorización, el levantamiento de cadenas montañosas, salidas volcánicas y sumersión o emergencia oceánica (Arias, 2001; Hartshorn, 2013). La única característica común presente en todos los suelos tropicales es que presentan un régimen uniforme de la temperatura del suelo (Arias, 2001).

En general, los suelos tropicales se carcterizan por presentar altos contenidos de óxidos y óxidos hidratados de hierro y aluminio que poseen carga variable que dependen del pH del suelo, por lo tanto, en condiciones de acidez, estos suelos exhiben carga positiva, debido a la asociación de los iones H^+ con los grupos hidróxido de la superficie del suelo (Schlesinger & Bernhardt, 2013). A nivel ecosistémico global, es importante destacar que, dado el alto contenido en materia

orgánica de los suelos tropicales, estos son fundamentales en el ciclo global del carbono (Pan et al., 2013). En estos suelos, la estabilización de la materia orgánica por minerales puede tener un papel mucho más importante comparado con los suelos templados. En los trópicos, las condiciones climáticas favorecen la descomposición de materia orgánica, por lo tanto, los suelos tropicales serían muy pobres en materia orgánica sin este proceso de estabilización mineral (Zech et al., 1997). Por otra parte, en los suelos ácidos de los trópicos, existe una disponibilidad limitada de P, lo cual limita el crecimiento de las plantas. La deficiencia de P es causada principalmente por la adsorción de H_2PO_4 a los óxidos e hidróxidos de aluminio (Al) y hierro (Fe), que convierte grandes proporciones de P total en formas que no están disponibles para las plantas (Cardoso & Kuyper, 2006).

De acuerdo con la minerología, los suelos tropicales, se pueden clasificar en 1) suelos caoliníticos/halloisíticos o arcillosos de baja actividad (Ultisoles y Oxisoles), los cuales representan el 60-70% de las áreas terrestres tropicales, (2) suelos esmectíticos o arcillosos de alta actividad (Vertisoles) y, (3) suelos más o menos alofánicos (Andisoles) con grandes cantidades de minerales amorfos o cripto-cristalizados (Feller & Beare, 1997). A nivel mundial, los Andisoles cubren aproximadamente 110 millones de hectáreas (Sanchez, 2019). Estos se desarrollaron debido a que en las partes altas del trópico existen montañas, que anteriormente fueron volcanes activos o que cíclicamente se activan, por lo tanto, son suelos formados a partir de material parental volcánico, como ceniza volcánica, piedra pómez, y lava (Arias, 2001). El material parental más común de los andisoles se denomina tefra. Tefra, es un término común que incluye todas las eyecciones volcánicas en el aire, independientemente de su morfología, tamaño y composición. La tefra se desgasta rápidamente, lo que produce altas concentraciones de Al, Fe y Si (Chesworth, 2007). Esto da como resultado la formación de alofano, una mezcla amorfa de aluminio y silicato, inestable, muy reactivo, y que tiene la capacidad de fijarse muy fácilmente a la materia orgánica formando complejos órgano-minerales (Buringh, 1979), razón por la cual son suelos con altos contenidos de materia orgánica, debido a que la alofana reacciona con los grupos carboxílicos y aromáticos de la materia orgánica, impidiendo la mineralización o transformación de la materia orgánica a nutrientes minerales (Arias, 2001). Además, estos suelos poseen otras fracciones coloidales como imogolita, ferrihidrita o complejos aluminio-humus (FAO, 2007). Además, son suelos porosos, con alta retención de fósforo, generalmente presentan un pH entre 5,0 y 6,0, son friables, poseen buenas características físicas, como baja densidad aparente, buen drenaje pero con alta capacidad de retención de

humedad, y por lo general son suelos de color negro en la superficie y de colores café con la profundidad (Alvarado et al., 2001; Arias, 2001; Sanchez, 2019).

2.4. Características e importancia de los suelos Mediterráneos-semiáridos

Según la FAO (1991), a nivel mundial se estima que la extensión de los suelos Mediterráneos es de aproximadamente 420 millones de hectáreas. La principal característica de las zonas Mediterráneas es que tienen dos estaciones bien definidas en el año, coincidiendo el período de lluvias y las bajas temperaturas con el invierno, y los periodos calurosos y secos con el verano. Las zonas Mediterráneas son ecosistemas frágiles, con una gran variedad de paisajes, suelos, vegetación, geología, clima, y biodiversidad (Zdruli et al., 2011). Esta región está sujeta a fuertes presiones ambientales como el estrés climático ocasionado por sequías intensas y prolongadas. En general, los suelos mediterránes son suelos delgados, arenosos, pobres y xéricos y están sujetos a fuertes perturbaciones antropogénicas, principalmente vinculadas al desarrollo de las poblaciones humanas y a la agricultura (Mohamed et al., 2019). Lo anterior, provoca severos procesos de degradación acompañados de una disminución del contenido de materia orgánica, lo que contribuye a una pérdida de fertilidad del suelo (García-Gil et al., 2004), que desemboca en una desertificación que resulta especialmente problemática en las zonas áridas y semiáridas de alto estrés por su mayor vulnerabilidad (López-Bermúdez et al., 1998).

Las regiones semiáridas, cubren aproximadamente el 15% de la superficie de la tierra, y son un subtipo de tierra seca con un índice de aridez entre 0,20 y 0,50 (Lal, 2004). La falta de precipitaciones adecuadas durante muchos meses del año es típico en zonas semiáridas (Bailey, 1979). Estos suelos se caracterizan por tener un bajo contenido de carbono orgánico, y una estructura deficiente (Garcia-Franco et al., 2018). De hecho, suelen presentar un contenido de materia orgánica considerablemente inferior a los suelos tropicales. Las principales amenazas de los suelos mediterráneos incluyen la erosión, la degradación ocasionada por las actividades humanas y la salinidad (FAO, 2016). Por otra parte, en estos suelos, existe mucha variedad de rocas madre o material parental, aunque las rocas carbonáceas, son las más extensas (Verheye & De la Rosa, 2005).

2.5. Nanotecnología

2.5.1. Definición y usos

La nanotecnología es una disciplina que implica la medición, el modelado y la manipulación de la materia en una escala nanométrica, en el intervalo de 1 a 100 nanómetros. Su importancia radica en que a esa escala los materiales pueden adquirir propiedades físicas y químicas diferentes a las que tienen en la escala macroscópica (Ivask et al., 2014). Por sus propiedades únicas, como las ópticas, electrónicas, antimicrobianas, magnéticas y catalíticas, el desarrollo de las nanopartículas (NPs) se ha extendido muy rápidamente (Nogueira et al., 2012). Los diversos tipos de NPs poseen muchas aplicaciones, y actualmente se usan como vectores en la terapia del cáncer (Her et al., 2017), en el sector agroalimentario (Dasgupta et al., 2017), como catalizadores (Wang et al., 2020), en productos cosméticos (de la Calle et al., 2017), en dispositivos electrónicos (Khanra et al., 2018), biomédicos (McNamara & Tofail, 2015), entre otros. Lo anterior hace que las NPs sean parte de un gran número de productos industriales (Lee et al., 2010; Simonin & Richaume, 2015).

Las NPs pueden sintetizarse a partir de métodos físicos, químicos o biológicos. Estos a su vez involucran dos tipos de síntesis: "top-down" y "bottom-up". En la síntesis "top-down", las NPs son producidas por reducción de tamaño a partir de un material inicial adecuado. Entre las técnicas más comunes se encuentra la fotolitografía (Zhang et al., 2014). Por otro lado, en la síntesis "bottom-up" las NPs son formadas a partir de entidades más pequeñas, por ejemplo, al unir átomos, moléculas y partículas más pequeñas en bloques. En este tipo de síntesis los bloques nanoestructurados son formados primero y luego ensamblados para producir la partícula final. Este tipo de síntesis involucra principalmente métodos de producción químicos y biológicos (Ahmed et al., 2016). Dentro de los métodos químicos, se encuentra la síntesis electroquímica, fotoquímica, solvotermal, interfacial, sonoquímicas, el uso de micelas y microemulsiones, termólisis, entre otros (Iravani et al., 2014). Entre los métodos biológicos se encuentran, la síntesis utilizando plantas, extractos de plantas (Alsammarraie et al., 2018), levaduras (Fernández et al., 2016), hongos (Aziz et al., 2019), algas (Dandamudi et al., 2016) y bacterias (Lateef et al., 2015).

Las NPs pueden ser de dos tipos: sintetizadas y naturales. Las naturales son las que se producen por las erupciones volcánicas, tormentas de arena, erosión e incendios forestales (Cupaioli et al.,

2014). Por su parte, las nanopartículas sintetizadas son producidas por el hombre, y se clasifican en NPs metálicas (Ag, Zn, Au, Ni, Fe, Cu, Pd, Si), NPs óxido metálicas (TiO_2, ZnO, CuO, Fe_3O_4, SiO_2, CeO_2, Al_2O_3, y SnO_2), NPs no metálicas (sílice y quantum dots), NPs de carbono (nanotubos y fullereno), NPs polímericas (alginato, chitosano, hidroximetilcelulosa, polihidroxialkanoatos, y Poli(e-caprolactona) y NPs de lípidos (lecitina de soja) (Simonin & Richaume, 2015).

2.5.2. Usos de las nanopartículas de plata

En la última década la síntesis y uso de las NPs metálicas, principalmente las de plata (AgNPs) se ha extendido rápidamente y podrían ser consideradas unas de las más importantes (Akter et al., 2018), debido, a que estas NPs poseen diversas características que las hacen atractivas para su aplicación en diversos productos comerciales (Mackevica et al., 2017). Una de las aplicaciones más reportadas es su uso como agente antimicrobiano (Durán et al., 2016). Asimismo, se han utilizado en otros campos como la electrónica, las tecnologías energéticas, y la remediación, además de su uso en productos cosméticos, pinturas, textiles, recipientes de almacenamiento de alimentos, aerosoles antisépticos, y como pesticidas y fertilizantes (Simonin & Richaume 2015, Lu et al., 2020) (Fig. 2).
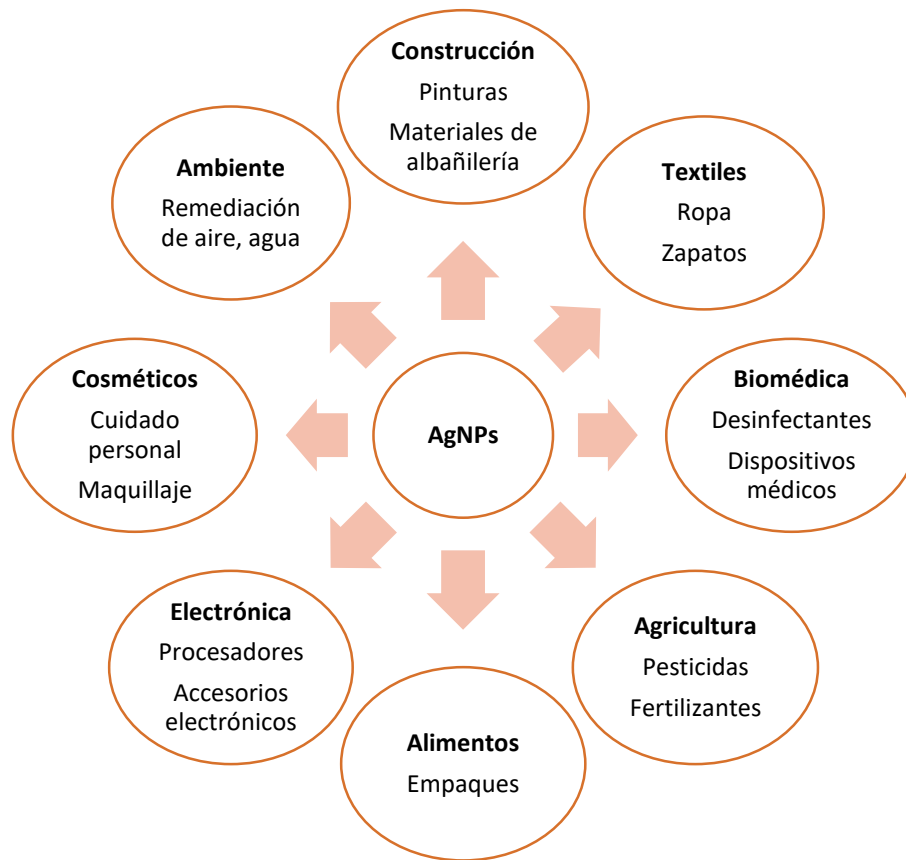
Fig. 2. Aplicaciones de las AgNPs en diferentes sectores industriales.

Debido a los efectos inhibitorios que presentan las AgNPs contra varios microorganismos, incluyendo algunas cepas bacterianas resistentes, es que ha habido un gran interés en el uso de estas NPs en la industria biomédica (Marassi et al., 2018). Las aplicaciones más conocidas en este campo incluyen pomadas tópicas y cremas para prevenir infecciones de quemaduras y heridas, dispositivos médicos e implantes con polímeros impregnados de plata, catéteres y vendajes, entre otros (Firdhouse & Lalitha, 2016).

2.5.3. Nanopartículas de plata: mecanismos antimicrobianos

Dentro de las anteriores aplicaciones, la actividad antibacteriana de las AgNPs ha hecho que su uso se haya extendido muy rápidamente. Diversos autores han determinado que esta actividad depende principalmente de su tamaño, forma, carga superficial, concentración y agentes estabilizantes (Grün et al., 2019). Se ha determinado que existe una relación directa entre el

tamaño y la concentración de las NPs, sobre su actividad antimicrobiana (Ivask et al. 2014; Zhang et al., 2019). Asímismo, según ha sido demostrado en diversas publicaciones, las AgNPs, poseen actividad antibacteriana, tanto en bacterias Gram + como en Gram – (Gurunathan et al., 2014). También se ha reportado actividad contra nemátodos entomopatógenos (Kleiven et al., 2018), virus (Wei et al., 2015) y recientemente, han ganado gran atención debido a sus prometedoras aplicaciones como agente anticancerígeno (Blanco et al., 2017). La toxicidad de las AgNPs también depende de los organismos o especies, lo que está relacionado con los mecanismos de defensa que utilizan los microorganismos para eliminar compuestos indeseables (Tortella et al., 2020).

A pesar de su uso generalizado y la creciente incidencia sobre el medio ambiente, el mecanismo de actividad antimicrobiana de las AgNPs sigue siendo tema de discusión (Liao et al., 2019; Rafińska et al., 2019). Se ha determinado que tanto las AgNPs como los iones Ag$^+$ liberados de las NPs ejercen toxicidad (Hsueh et al., 2015; Durán et al., 2016), y que la toxicidad de las AgNPs depende del modo de ingreso a la célula, ya sea por endocitosis (fagocitosis o pinocitosis) o difusión (Bartłomiejczyk et al., 2013). Además, se ha evidenciado que son múltiples los mecanismos de acción de las AgNPs en las células (Hsueh et al., 2015). Las AgNPs pueden acumularse en la pared celular y la membrana celular causando cambios morfológicos (Zhang et al., 2019). En un estudio realizado por Ramalingam et al. (2016) se determinó que las AgNPs cambian la carga superficial de las bacterias Gram –, a valores cercanos a la neutralización, −3.5 ± 0.8 mV para *E. coli* y −5.4 ± 0.5 mV para *P. aeruginosa*. La neutralización de la carga superficial produce una alteración de la permeabilidad de la membrana celular, corrugación de la superficie, grandes espacios en la membrana, lo que produce una gran cantidad de desechos celulares, lo que causa posteriormente la muerte celular. Por otra parte, se ha determinado que la unión de las AgNPs a las proteínas de membrana, también puede interferir en la permeabilidad de la membrana, alterar la cadena respiratoria, la división celular y el transporte de iones (Ivask et al., 2014). La adhesión de las AgNPs a las membranas también puede aumentar la rigidez de la membrana, producir la descomposición de los ácidos grasos, las proteínas y los carbohidratos de la membrana (Tang & Zheng, 2018). Además, se ha demostrado que pueden interactuar con receptores de membrana y receptores de factores de crecimiento (Shang et al., 2014).

Se ha observado que después de unirse a las membranas celulares, las AgNPs también pueden penetrar en las células microbianas e interactuar y producir un daño directo a las organelas celulares como las mitocondrias y el núcleo, lo que convella a la fragmentación del núcleo, arresto

del ciclo celular, apoptosis, etc (Shang et al., 2014). También, se ha propuesto que las AgNPs son capaces de unirse a las bases purina y pirimidina del ADN, además de que interrumpen el enlace de hidrógeno entre las cadenas helicoidales del ADN lo que causa un daño en su estructura (Baptista et al., 2018). Kang et al. (2010) determinaron que NPs localizadas en el núcleo inducen daño al ADN y arresto de la citocinesis. Por otra parte, mediante microscopía electrónica de transmisión (TEM) se ha determinado que las AgNPs pueden producir condensación del citoplasma (Ghosh et al., 2012). Además, las AgNPs pueden interactuar con otras biomoléculas como los lípidos y las proteínas, lo que conduce a la inhibición de los procesos de transcripción y traducción, lo que interfiere en la síntesis de proteínas y la interrupción del funcionamiento celular (Rinna et al., 2015).

Además del efecto de las AgNPs sobre las células, algunas evidencias indican que las AgNPs liberan iones de plata (Ag^+), que podrían generarse e introducirse en la celula por disolución oxidativa de los AgNPs en presencia de oxígeno, siendo también responsables de daños a nivel celular (Reidy et al., 2013). Los iones Ag^+ pueden interactuar con los grupos tiol, amino, imidazol, fosfato y carbonilo que están presentes en la membrana celular o proteínas (Hsueh et al., 2015). Al unirse a los grupos tiol (-SH) de las proteínas forman enlaces SH-Ag estables, que pueden alterar la estructura 3D de las proteínas y por lo tanto bloquear los sitios de activos (Holt & Bard, 2005). McShan et al. (2014) indicaron que la interacción de las AgNPs con proteínas u otras macromoléculas que contienen azufre es un mecanismo de toxicidad importante atribuible a la alta afinidad entre el ión Ag^+ y el azufre. Además, los iones Ag^+ pueden unirse con los ácidos nucleicos, afectando la división celular (Hsueh et al., 2015).

Otro mecanismo producido por las AgNPs y los iones Ag^+ es el aumento del estrés oxidativo, que está relacionado con la generación de especies de oxígeno reactivo (ROS). Los ROS son un grupo de oxidantes que son altamente reactivos, pero de corta duración e incluyen el peróxido de hidrógeno (H_2O_2), radicales superóxido (O_2 -), oxígeno singlete ($^1O_2$) y radical hidroxilo (•OH). Los ROS son productos de la respiración aeróbica y sus concentraciones intracelulares generalmente se mantienen en un nivel relativamente bajo, debido a moléculas como el glutatión reducido (Tang & Zheng, 2018). Autores como Gao et al. (2011) reportaron que las NPs causan acumulación de ROS por reducción del glutatión y por lo tanto activan la ruta de apoptosis mitocondrial. Zhang et al. (2019), midieron la producción de ROS después de la aplicación de diferentes concentraciones y tamaño de AgNPs (10 nm y 50 nm) sobre *Azotobacter vinelandii* y *Nitrosomonas europaea* y comprobaron que la generación de ROS estaba relacionada con el

tamaño y la concentración de las AgNPs, además, concluyeron que el colapso de la estructura celular se debe principalmente al daño oxidativo. Por lo tanto, debido a la naturaleza reactiva de los ROS, estos causan daño directo a los componentes celulares como el ADN, ARN, membranas mitocondriales, ribosomas, proteínas y también al peptidoglucano presente en la membrana celular (Sangave et al., 2020) (Fig. 3).

Por otra parte, algunos estudios han demostrado que la carga superficial de las AgNPs está asociada con su toxicidad. Por ejemplo, se ha indicado que la superficie positiva de las células de *Bacillus* sp las protege contra la toxicidad de las AgNPs cargadas positivamente, por un mecanismo de repulsión electroestática (El Badawy et al., 2011). Estudios realizados mediante microscopía electrónica de barrido y transmisión, utilizando la bacteria Gram- *Nitrosomonas europea* y *Escherichia coli* tratadas con AgNPs, sugieren que las AgNPs podrían ingresar al interior de la célula, comprometer las paredes celulares, ingresar al periplasma, condensar el ADN y causar fugas del citoplasma (Chen et al., 2011; Wang et al., 2017). Sin embargo, se determinó que la actividad antimicrobiana depende de la liberación de los iones Ag$^+$, ya que las células de *N. europaea* tratadas con 1 mg L^{-1} de Ag$^+$ tenían morfologías bastante diferentes, incluyendo membranas rotas y deprimidas que eran mucho más graves que las expuestas a 10 mg L^{-1} de AgNPs (Wang et al., 2017).

A pesar de la opinión común sobre la actividad antibacteriana de las AgNPs, en los últimos años se han reportado resistencias en bacterias y hongos (Rafińska et al., 2019). En un estudio realizado por Gambino et al. (2015) confirmaron que algunas especies de *Bacillus* aisladas del suelo son especialmente resistentes a las AgNPs. Recientemente se ha demostrado que *B. subtilis* exhibe una alta resistencia a las AgNPs y este fenómeno está asociado con los siguientes procesos: (I) iniciación de la formación de endosporas, (II) reducción del ión Ag$^+$ liberado de las NPs y (III) modificación de la superficie de AgNPs. Por otra parte, recientemente se ha estudiado si las AgNPs en el ambiente, pueden estimular la transferencia horizontal de genes de resistencia a antibióticos. Lu et al. (2020), demostró que las AgNPs y los iones Ag$^+$ podrían acelerar la transferencia horizontal de genes de resistencia a antibióticos cuando están presentes en concentraciones ambientalmente relevantes y subinhibitorias, y de ese modo facilitar la transmisión de la resistencia a los antibióticos. Este estudio reveló que tanto los iones Ag$^+$ como las AgNPs podrían promover la transferencia de genes conjugativos induciendo la sobreproducción de ROS, desencadenando la respuesta SOS y aumentando la permeabilidad de la membrana celular. Sin embargo, se necesita más investigación sobre los efectos de los

AgNPs en la transferencia horizontal de genes en las comunidades microbianas presentes en ecosistemas como los suelos.
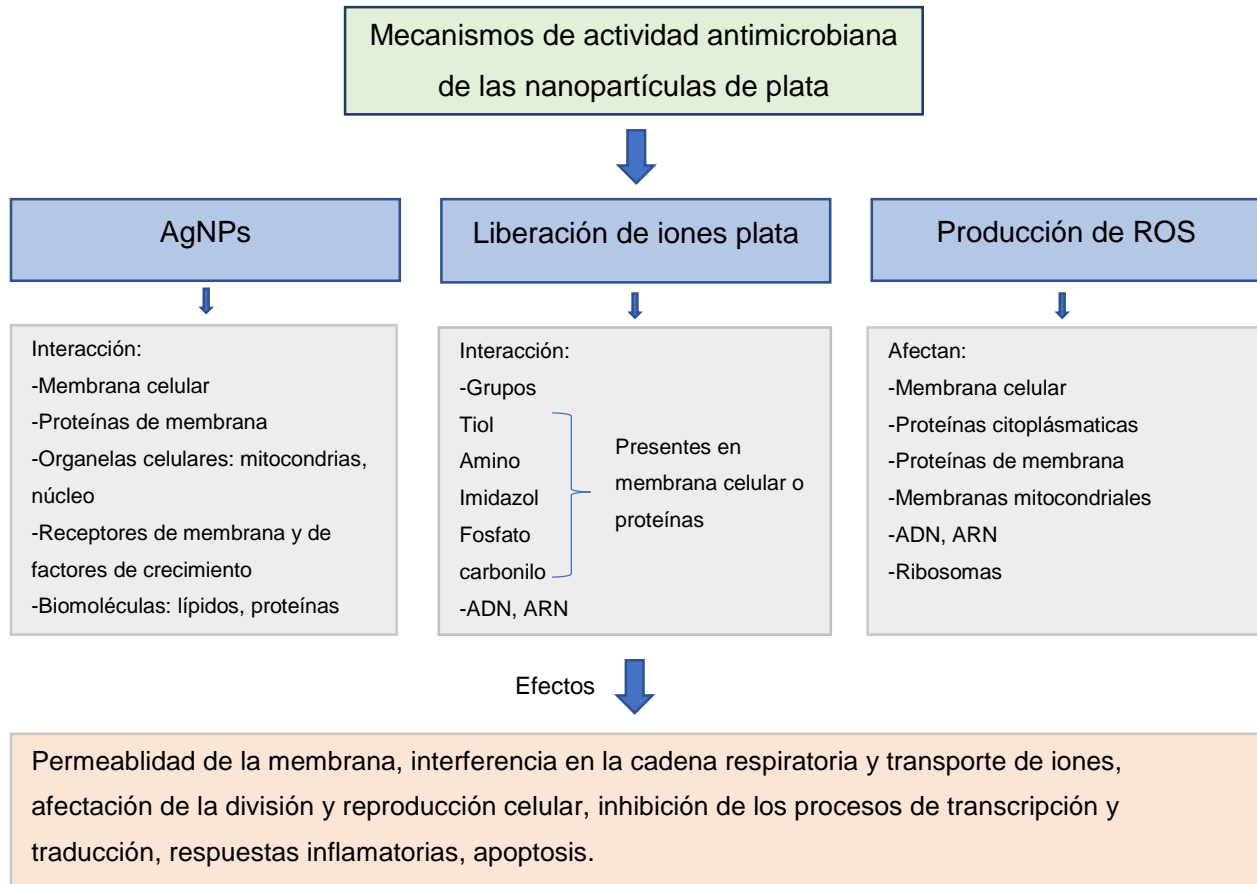
Fig. 3. Mecanismos antimicrobianos y efectos de las AgNPs a nivel celular.

2.6.   Destino y transporte de las nanopartículas de plata en el suelo

2.6.1. Incidencia de las nanopartículas de plata en el suelo

El rápido desarrollo de la nanotecnología ha permitido el uso de las AgNPs en numerosas aplicaciones industriales, biomédicas, alimenticias y agrícolas, lo que provoca que, desde diferentes fases de su ciclo de vida desde su producción, su uso y su disposición final, se esté llevando a su liberación al medio ambiente (Fig. 4). Debido a su uso extendido, las AgNPs pueden ingresar al suelo a través de la aplicación de biosólidos, pesticidas, fertilizantes, remediación del

suelo, desechos de productos o de aplicaciones biomédicas (Agtuca, 2014). Lo anterior plantea una preocupación con respecto a los posibles riesgos sobre la comunidad microbiana presente en los suelos, ya que éste es considerado el mayor reservorio de las NPs si lo comparamos con el agua y el aire (Mahapatra et al., 2015). Sin embargo, son muy pocos los estudios que han investigado los efectos de estas NPs con los microorganismos del medio ambiente (Nogueira et al., 2012; Sun et al., 2014; Mahapatra et al., 2015; Coll et al., 2016), y mucho menos en suelos agrícolas (Peyrot et al., 2014; Shah, 2014; Velicogna et al., 2016).
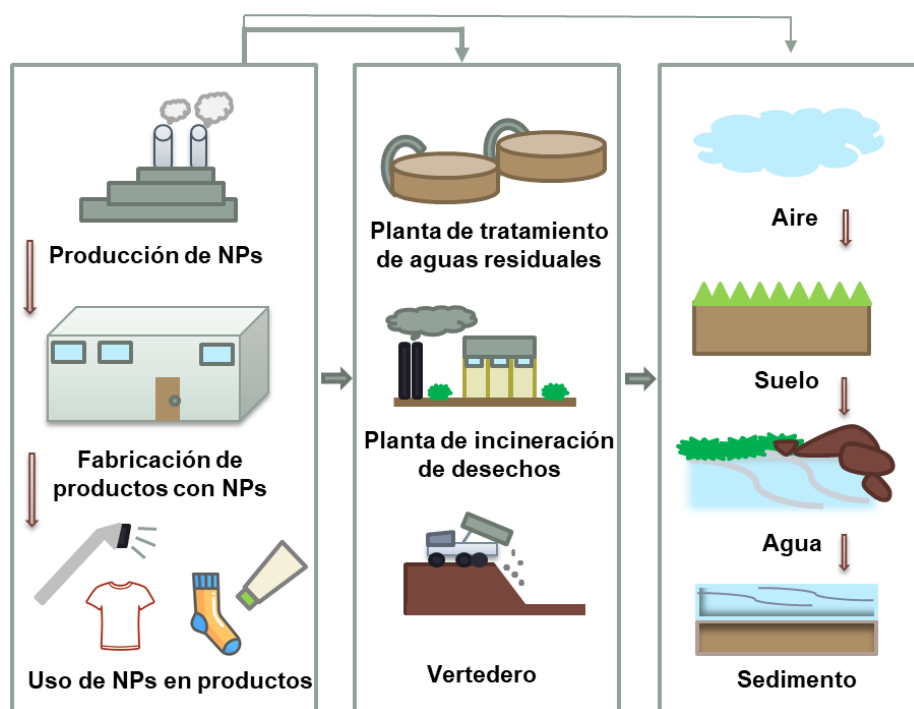
Fig. 4. Flujo de nanopartículas de plata desde su producción hasta la incidencia en el medio ambiente.

Algunos estudios recientes han cuantificado las concentraciones de AgNPs sintéticas principalmente en aguas superficiales y sedimentos, utilizando especialmente la técnica de espectrometría de masas con plasma acoplado inductivamente de partículas individuales (spICP-MS, por sus siglas en inglés) (Gondikas et al., 2018; Wang et al., 2020). Sin embargo, la cuantificación de NPs en suelos sigue siendo un desafío. En la mayoría de los casos, los métodos analíticos no pueden cuantificar concentraciones de NPs muy bajas, e incluso si se detectan, existen dificultades para diferenciar las NPs naturales de las sintéticas. Además, durante el proceso de extracción y separación de las NPs del suelo pueden ocurrir cambios en las propiedades físico-químicas de las NPs (Zhao et al., 2020). Por lo tanto, actualmente, una forma

utilizada para obtener información sobre los niveles existentes de NPs sintéticas en el suelo es a través de modelos probabilísticos (Gottschalk et al., 2013; Coll et al., 2016; Sun et al., 2016; Zhao et al., 2020).

Los modelos probabilísticos se han utilizado para predecir las concentraciones de AgNPs en el suelo (Sun et al., 2014; Coll et al., 2016; Giese et al., 2018). Según un estudio realizado por Giese et al. (2018), la concentración ambiental prevista de AgNPs en un suelo agrícola se encuentra en el rango de 0,0094 – 0,7922 µg Kg^{-1} en un escenario 100% persistente, con una concentración máxima de AgNPs de 1,52 µg kg^{-1} para 2017 y 10 µg kg^{-1} previsto para el 2050. Actualmente, la mayoría de las investigaciones que han determinado el impacto de las AgNPs sobre las comunidades microbianas del suelo, han utilizado concentraciones de NPs mucho más altas que las reportadas anteriormente (Peyrot et al., 2014; McKee and Filser, 2016; McGee et al., 2017; Samarajeewa et al., 2017), ya que no se han basado en modelos probabilísticos de concentraciones de NPs en el suelo, y además, pocos estudios han utilizado una combinación de técnicas para evaluar el impacto de las AgNPs sobre las comunidades microbianas, entre ellas, la genómica, las actividades enzimáticas, y la biomasa microbiana.

2.6.2. Transformaciones de las nanopartículas de plata en el suelo

Una vez que las NPs llegan al suelo sufren una serie de transformaciones físicas, químicas y biológicas (Fig. 4), que dependen tanto de las características físico-químicas y biológicas del suelo (Frenk et al., 2013; Dimkpa, 2018), como de las características de las NPs. Dentro de los principales factores físico-químicos del suelo que afectan a las NPs se encuentran el pH, el estrés iónico, la fuerza iónica, el contenido de materia orgánica y arcilla, la concentración de oxígeno, etc. (Schlich and Hund-Rinke 2015; Bundschuh et al. 2018; Grün et al. 2018; Yang et al., 2019). Dentro de las propiedades biológicas se encuentran, los metabolitos y macromoléculas producidas y secretadas por las raíces de plantas, y fauna del suelo (Badri et al., 2012; Hayashi et al., 2016).

Se ha reportado que las principales transformaciones físicas que sufren las NPs el suelo son la homoagregación, la heteroagregación, y la aglomeración. Por otra parte, las transformaciones químicas incluyen la disolución de especies iónicas, la adsorción de las AgNPs y los iones de Ag$^+$ sobre materia orgánica, la precipitación con cloruro, la oxidación y reducción, la adquisición de revestimiento superficial, el cambio en la carga superficial, y el cambio en la forma. Asimismo,

la principal transformación biológica que sufren las AgNPs constituye el recubrimiento con proteínas y otras biomoléculas que forman la "corona" (Pachapur et al., 2016; Dimkpa, 2018) (Fig. 5). Estas modificaciones, en consecuencia, afectan el destino, el transporte, y la toxicidad de las NPs.

Transformación de las AgNPs

Físicas
-Agregación
-Aglomeración
-Sedimentación

Químicas
-Disolución
-Redox
-Sulfidación
-Adsorpción: materia orgánica, arcillas
-Degradación del agente estabilizante

Biológicas
-Bio-modificación: corona
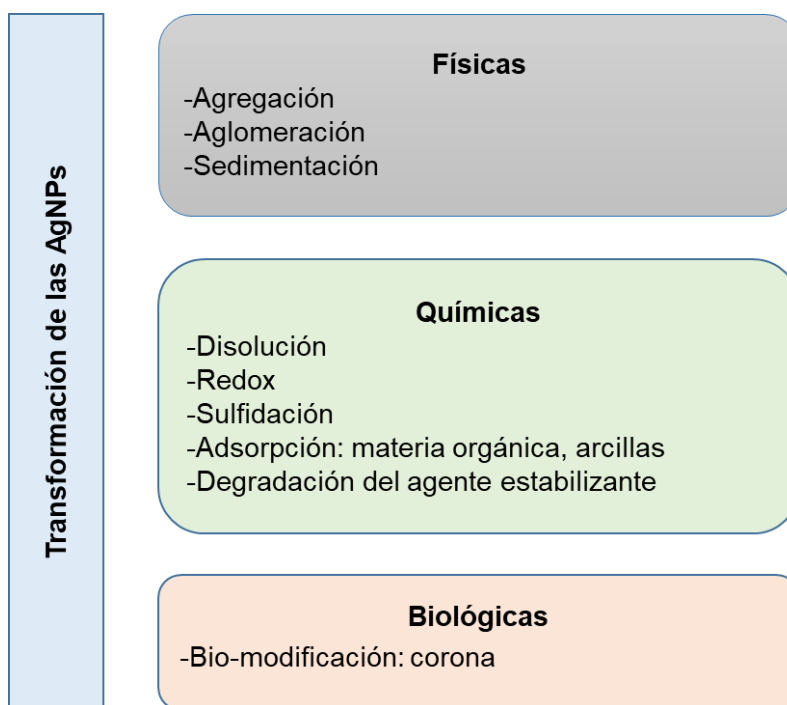
Fig. 5. Transformaciones físicas, químicas y biológicas que sufren las nanoparticulas de plata en el suelo.

Una de las principales transformaciones que sufren las AgNPs es la agregación. Así, la homoagregación se refiere a la agregación de NPs entre sí, y la heteroagregación se refiere a la agregación de NPs con partículas del suelo, como materiales arcillosos o la materia orgánica (Dwivedi et al., 2015). Por otra parte, las NPs pueden sufrir procesos de aglomeración que se dan por el movimiento browniano de las mismas. La aglomeración depende principalmente del número de NPs, el carbono orgánico disuelto y de la fuerza iónica (Soni et al., 2015). En este sentido, se ha demostrado, que tanto la agregación, como la aglomeración son procesos que dependen del tiempo (Casals et al., 2012). Por lo tanto, el proceso de agrupamiento de las NPs puede ocurrir mediante agregación irreversible o aglomeración reversible.

Dentro de los componentes del suelo, la materia orgánica, es uno de los factores que mayor afecta el comportamiento de las NPs. En el suelo las AgNPs interactúan con la materia orgánica, afectando su transporte, transformación y toxicidad (Pachapur et al., 2016; Makselon et al., 2017; Li et al., 2018; González-Fuenzalida et al., 2018; 2018; Sutton et al., 2019). Se ha determinado que la tasa de disolución de las AgNPs se ve afectada por su interacción con la materia orgánica. Por ejemplo, Klitzke et al. (2015) observaron una disminución en la liberación de los iones Ag^+ de las AgNPs, con el aumento en el contenido de materia orgánica, de manera similar a lo determinado por Wang et al. (2018), que comprobaron que la retención de iones Ag^+ se debió principalmente al contenido de materia orgánica del suelo, lo cual es un factor crítico que será estudiado en esta Tesis Doctoral. Asimismo, Peyrot et al. (2014), determinaron que la materia orgánica no aumentó el ión Ag^+ disuelto. Además, se ha encontrado que los ácidos húmicos disminuyen la agregación de las AgNPs y la adsorción al suelo (Bae et al., 2013), debido al aumento en la estabilización de las NPs. Por otra parte, Klitzke et al. (2015), evaluaron el impacto de un suelo inundado sobre el destino y la estabilidad de AgNPs, así como la interacción de estas NPs con las partículas naturales del suelo y determinaron que la adsorción de AgNPs en las partículas del suelo no es gobernado por la carga superficial de las mismas y que la exposición de las AgNPs a la solución del suelo disminuye la liberación del ión Ag^+, probablemente debido a recubrimientos por la materia orgánica. En un estudio realizado por Schlich & Hund-Rinke, (2015), se determinó que la toxicidad de las AgNPs en diferentes suelos, evaluada mediante la transformación de carbono y la evaluación de bacterias oxidantes de amonio, disminuía con el incremento del contenido de arcilla, y el incremento del pH del suelo.

Además, se ha determinado que, debido a la alta superficie específica de los coloides naturales presentes en el suelo, las NPs pueden adsorberse a estos coloides, lo cual facilita su transporte. La mayoría de estas superficies coloidales están cargadas negativamente y adsorben NPs positivas por medio de atracción electrostática e intercambio de ligandos (Pachapur et al., 2016). Makselon et al. (2018), determinaron que existe una asociación entre las AgNPs y los coloides del suelo, principalmente compuestos de Al, Fe, Si y materia orgánica, lo cual influye sobre la persistencia y toxicidad en estos ambientes.

Por otra parte, en un estudio realizado por Wang et al. (2015), se determinó que PVP-AgNPs tenían mayor movilidad con el incremento del pH y la capacidad de intercabio iónico, mientras que el oxido de hierro, el calcio y el potasio disminuían su movilidad. Mientras que Wang et al. (2018) estudiaron la retención de PVP-AgNPs y de los iones Ag^+, y determinaron que la

capacidad de retención de PVP-AgNPs en los suelos se correlacionó positivamente con la capacidad de intercabio catiónico, y el contenido de óxido de hierro, aluminio y manganeso, lo cual se debió pricipalmente a que las PVP-AgNPs cargadas negativamente se unen a los óxidos de hierro y aluminio cargados positivamente. Por otra parte, determinaron una correlación negativa con el pH y la conductividad eléctrica. En general, las superficies de los suelos tienen una carga más negativa a valores de pH más altos debido a la desprotonación, lo que desfavorece la unión de NPs cargadas negativamente. Por lo tanto, la capacidad de adsorción de las PVP-AgNPs con los componentes del suelo disminuye al aumentar el pH (Wang et al., 2018).

Además de las características físico-químicas de los suelos, las características de las AgNPs también pueden afectar significativamente su toxicidad en el medio ambiente. Algunas propiedades que afectan la toxicidad son su tamaño, concentración, forma, estabilidad, carga y agente estabilizante (Pachapur et al., 2016; Grün et al., 2019). Se ha demostrado que una vez liberadas en el medio ambiente, la concentración de AgNPs es crucial para los mecanismos de disolución y agregación (Lead et al., 2018). Estudios realizados por He et al. (2019), determinaron que la concentración, el tamaño y el agente estabilizante de las AgNPs afectan su movilidad en el suelo, habiendo mayor transporte a mayores concentraciones y tamaños menores de AgNPs, y que la presencia de agentes estabilizantes como el PVP y el citrato, promueven aún más el transporte. Además del tipo de suelo y las características de las AgNPs, varios estudios han reportado un impacto significativo del tiempo de exposición sobre la toxicidad de AgNPs en los suelos (Raas et al., 2017; Grün et al., 2018). Por otra parte, se ha determinado que las biomoléculas secretadas por las raíces de plantas y fauna del suelo pueden interactuar con las AgNPs y, en consecuencia, afectar su transporte, aglomeración, deposición, biodisponibilidad y toxicidad (Abbas et al., 2020). Estas biomoléculas pueden formar una corona en la superficie de las NPs (Digiacomo et al., 2019; Nasser et al., 2020), proceso que depende de las características de las NPs y las condiciones físico-químicas del suelo (Pulido-Reyes et al., 2017). Sin embargo, este es un tema que requiere más estudios.

2.7. Impacto de las nanopartículas de plata sobre las propiedades físico-químicas y comunidades microbianas del suelo

2.7.1. Impacto sobre las propiedades físico-químicas del suelo

Diversos parámetros físico-químicos se han utilizado para determinar la calidad del suelo (Xu et al., 2016). Entre ellos, se encuentran, el pH, el contenido de materia orgánica, el contenido de nitrógeno total, el contenido de fósforo total, entre otros (Xu et al., 2016). Sin embargo, hasta la fecha son pocos los estudios que se encuentran relacionados con el efecto de las NPs sobre las características físico-químicas del suelo. En un estudio realizado por Liu et al. (2019), en microcosmos de humedales, se determinaron efectos agudos y crónicos de las AgNPs sobre los procesos de transformación y los contenidos de las diversas especies del nitrógeno (TN, NH_4^+-N, NO_3^--N y NO_2^--N) en el suelo, en un experimento realizado a corto plazo (0–5 días) y a largo plazo (55-60 días). Se reportó que las tasas de eliminación de nitrógeno total y nitrógeno amoniacal se redujeron significativamente después de la exposición a corto plazo (5 días) utilizando 1 y 50 mg L^{-1} de AgNPs. Además, determinaron que la exposición de AgNPs a largo plazo (60 días) causó efectos inhibitorios acumulativos en la eliminación de nitrógeno total y amoniacal. Por otra parte, Hänsch & Emmerling (2010), realizaron un estudio a medio plazo (cuatro meses) para determinar el impacto de la aplicación de AgNPs sobre las propiedades químicas del suelo. Encontraron que el pH del suelo se mantuvo estable durante todo el período experimental. Lo mismo, ocurrió con el contenido de carbono orgánico del suelo. Por el contrario, el contenido total de nitrógeno se redujo de manera leve pero significativa en los tratamientos con 32 y 320 µg Kg^{-1} de AgNPs en relación con el tratamiento con 3,2 µg Kg^{-1} de AgNPs y el control y, por lo tanto, la relación C: N de esos tratamientos también aumentó.

En un estudio reciente, Zhang et al. (2020), añadieron una concentración de 100 mg kg^{-1} de AgNPs en suelos con ausencia o presencia de plantas de pepino (*Cucumis sativa*) y estudiaron su efecto sobre el contenido de materia orgánica disuelta (MOD) en el suelo, el pH y los elementos biodisponibles, después de 60 días de exposición a las AgNPs. Los resultados determinaron que la exposición a AgNPs aumentó significativamente el pH en ambos suelos, pero no cambió el contenido de MOD. Sin embargo, se encontró un aumento significativo en los macroelementos (K, Na, Mg, Si) y microelementos (Al, Cu, Cd) del suelo, concluyendo que el mecanismo para la movilización de estos elementos por AgNPs es actualmente desconocido, pero podría deberse a cambios en la comunidad microbiana del suelo y el metaboloma.

2.7.2. Impacto sobre las enzimas extracelulares

El estudio de las actividades enzimáticas se ha utilizado para determinar la función de las comunidades microbianas. Por ejemplo, autores como Peyrot et al. (2014), estudiaron el efecto de las enzimas fosfomonoesterasa, arilsulfatasa, β-D-glucosidasa, y leucina-aminopeptidasa, en suelos con diferente contenido de materia orgánica. En general determinaron que la adición de materia orgánica aumentaba las actividades enzimáticas. Con respecto al uso de la actividad enzimática para evaluar los efectos de las NPs, Chai et al. (2015), han utilizado la actividad de las enzimas ureasa y catalasa para evaluar el efecto de NPs óxido metálicas (ZnO, SiO_2, TiO_2 y CeO_2) en los microorganismos de suelos agrícolas.

Por otra parte, Rahmatpour et al. (2017), evaluaron el efecto de las AgNPs en suelos calcáreos, con diferente textura y salinidad y determinaron que las actividades de las enzimas ureasa y fosfatasa fueron inhibidas con las mayores concentraciones de AgNPs en el suelo con menor contenido de arcilla y fuerza iónica, concluyendo que la toxicidad depende de la concentración de las AgNPs y del tipo de suelo. Por su parte, McGee et al. (2018), en un experimento basado en microcosmos, determinaron que las enzimas de suelos de pastizales agrícolas se vieron afectadas por las AgNPs, ya que hubo una reducción en la actividad de la enzima deshidrogenasa con la concentración de 1 mg kg^{-1} de AgNPs. De manera contraria, se determinó que la actividad ureasa fue menos susceptible a la aplicación de las AgNPs, sin embargo, a la concentración más alta ≥ 10 mg kg^{-1} si hubo inhibición de esta enzima.

Asimismo, Samarajeewa et al. (2019), estudiaron los efectos de las AgNPs sobre las comunidades microbianas de un suelo franco arenoso al cual se le adicionó biosólidos que contenían PVP-AgNPs a concentraciones de 3, 48, 123, 290 y 706 mg kg^{-1}. Se determinó que hubo un incremento en la actividad de la enzima deshidrogenasa con el aumento en el tiempo de incubación a la concentración de 3 mg kg^{-1} para el día 49. Además, determinaron que la actividad de la fosfatasa ácida se redujo en un 27% a 290 mg kg^{-1} de PVP-AgNPs en el día 49 en comparación con el control. Por el contrario, la actividad de la β-glucosidasa varió sin una tendencia constante a lo largo del tiempo. En general, los resultados de este estudio demostraron que la toxicidad en las actividades enzimáticas del suelo fue dependiente de la concentración de AgNPs. En un estudio reciente, Yan et al. (2020), evaluaron los efectos de diferentes concentraciones de PVP-AgNPs (0, 1, 10 y 100 mg kg^{-1}) sobre las actividades de las enzimas ureasa y deshidrogenasa. Se observaron inhibiciones significativas lo cual fue dependiente de la

dosis de AgNPs, siendo la enzima deshidrogenasa la más susceptible en comparación con la ureasa. Además, las dos actividades enzimáticas en todos los tratamientos con AgNPs no se recuperaron al final del experimento, lo que indica que las inhibiciones enzimáticas por efecto de las AgNPs fueron persistentes. Resultados similares han sido reportados por McGee et al. (2017), con una disminución inmediata en la actividad de la deshidrogenasa a la concentración de 50 mg kg^{-1} de AgNPs, sin embargo, la actividad de la ureasa disminuyó significativamente en el día tres.

2.7.3. Impacto sobre la biomasa microbiana

La determinación de la biomasa microbiana es un indicador sensible de los cambios en la composición de la comunidad microbiana del suelo en respuesta al estrés ambiental como la contaminación por nanomateriales y metales pesados (Chandra et al., 2014). En un estudio realizado por Collins et al. (2012), se utilizó el análisis ácidos grasos fosfolipídicos (PLFAs) para evaluar el impacto de las NPs de Cu y ZnO sobre la abundancia de microorganismos de un suelo agrícola, y se determinó que no hubo un impacto significativo sobre la abundancia total de los microorganismos del suelo, aunque se observó una mayor proporción de bacterias Gram – que Gram +. Xu et al. (2015), estudiaron mediante PLFAs, la biomasa microbiana en un suelo cultivado con arroz inundado y tratado con diferentes concentraciones de NPs de TiO_2 y CuO. Los autores concluyeron que las NPs de CuO redujeron en mayor medida la biomasa microbiana con respecto a las de TiO_2. Otros autores, como Durenkamp et al. (2016), aplicaron a un suelo lodos de plantas de tratamiento de aguas residuales mezclados con AgNPs y ZnO, y observaron una reducción en la biomasa fúngica, después de la exposición a las NPs. Por otra parte, se ha estudiado el impacto de las NPs sobre la biomasa y estructura de las comunidades microbianas, utilizando el método EL-FAME. Por ejemplo, Vittori Antisari et al. (2016) investigaron el impacto de iones y NPs (Ag^+, Co^{2+}, AgNPs, CoNPs) sobre la comunidad microbiana del suelo y de las heces de la lombriz de tierra *Lumbricus rubellus*. Determinaron un aumento en la biomasa de las bacterias Gram –, en la proporción de bacterias / hongos y una disminución en la proporción de bacterias Gram + / Gram –, después de la aplicación de las AgNP.

2.7.4. Impacto sobre la diversidad microbiana

La secuenciación de amplicones se ha utilizado para determinar la diversidad de las comunidades de hongos y bacterias en diferentes tipos de suelos expuestos a AgNPs (Cao et al., 2017; McGee et al., 2017; Asadishad et al., 2018; Forstner et al., 2019; Zhang et al., 2020), y

se han encontrado resultados interesantes, ya que se ha determinado que algunos grupos microbianos se ven afectados negativamente, mientras que otros se estimulan, en un proceso que depende de diversos factores como las propiedades del suelo, las características de las AgNPs, y además, de la propia flora microbiana del suelo (McGee et al., 2018; Forstner et al., 2019; Zhang et al., 2020).

Por otra parte, se ha observado que cuando las AgNPs entran en el proceso de tratamiento de aguas residuales y se convierten en Ag_2S, el ión Ag^+ puede afectar a la composición de la comunidad bacteriana y fúngica del suelo (Forstner et al., 2019). Además, se concluyó que para las comunidades bacterianas estos efectos son influenciados por la concentración de cloruro en el suelo, ya que se ha demostrado que el cloruro aumenta la biodisponibilidad del ión Ag^+. En un estudio realizado por McGee et al. (2018), se examinaron los efectos dependientes de la concentración de AgNPs sobre las estructuras de comunidades bacterianas y fúngicas en un suelo de pastizales agrícolas en un experimento basado en microcosmos. Los autores determinaron que los filos de bacterias Acidobacterias y Verrucomicrobia fueron altamente sensibles. Además, se encontró una gran reducción en la abundancia relativa de géneros perteneciente al filo Acidobacteria, con la excepción del género *Geothrix* que aumentó en respuesta a las AgNPs. Por otra parte, se observó una gran tolerancia al ión Ag^+ entre los géneros pertenecientes al filo Bacteriodetes. Similarmente, la abundancia relativa de proteobacterias como *Dyella* aumentó después de la aplicación de AgNPs.

Por su parte, Liu et al. (2017) evaluaron la composición de la comunidad bacteriana del suelo cultivado con trigo después de la exposición a una concentración baja de AgNPs (1 mg kg^{-1}). Estos autores observaron que la estructura de la comunidad bacteriana se vio afectada por la exposición a AgNPs, más significativamente durante la etapa de transición de la plántula a la etapa vegetativa del trigo, sin embargo, hubo una recuperación después de 49 días de exposición a las NPs. Además, en un estudio realizado por Wang et al. (2017) se utilizó la NGS para investigar los efectos de diferentes concentraciones de AgNPs (10, 50 y 100 mg kg^{-1}) sobre la estructura de la comunidad microbiana del suelo después de 7 días de exposición. Determinaron una disminución en la abundancia relativa de los filos Acidobacteria, Actinobacteria, Cianobacteria y Nitrospirae con el aumento de la concentración de las AgNPs; mientras que los filos Proteobacteria y Planctomicetos aumentaron. Recientemente, Zhang et al. (2020) estudiaron el efecto de 100 mg Kg^{-1} de AgNPs en suelos con ausencia y presencia de plantas de pepino (*Cucumis sativa*) durante 60 días. La respuesta de la comunidad microbiana del suelo se

investigó mediante la secuenciación de fragmentos del gen ribosomal 16S rRNA. Los resultados indicaron que la estructura de la comunidad bacteriana del suelo se alteró con la exposición a los iones Ag^+ en ambos suelos, y pudieron determinar que varios grupos bacterianos asociados con el ciclo del carbono, el nitrógeno y el fósforo se vieron afectados por las AgNPs en ambos suelos.

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3. Objetivos

Objetivo general

El Objetivo General de esta Tesis Doctoral es evaluar el efecto de concentraciones ambientalmente relevantes de nanopartículas de plata en la comunidad microbiana de suelos con diferentes propiedades físico-químicas, bioquímicas y microbiológicas. Este objetivo general se alcanzará mediante diversos análisis físico-químicos, cuantificación de la biomasa microbiana, y la evaluación de la funcionalidad y biodiversidad de la comunidad de hongos y bacterias en suelos con condiciones contrastadas.

Objetivos Específicos

1. Analizar el efecto de las nanopartículas de plata sobre las características físico-químicas de suelos agrícolas tropicales cultivados con *Coffea arabica.*
2. Establecer el efecto de las nanopartículas de plata sobre la biomasa, composición y diversidad de las comunidades bacterianas y fúngicas en los suelos agrícolas tropicales y Mediterráneo-semiáridos mediante el análisis de ácidos grasos y la secuenciación de amplicones.
3. Determinar el efecto de las nanopartículas de plata sobre la actividad de las comunidades microbianas en los suelos agrícolas tropicales y Mediterráneo-semiáridos mediante el análisis de diferentes actividades enzimáticas relacionadas con la biogeoquímica edáfica y el ciclo de los nutrientes.

5. Artículos científicos

1. Montes de Oca-Vásquez, Gabriela; Solano-Campos, Frank; Vega-Baudrit, José; López-Mondéjar, Rubén; Odriozola, I; Vera, Alfonso; Moreno, José Luis; Bastida, Felipe. (2020). Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity. Journal of Hazardous Materials, 391, 122224. https://doi.org/10.1016/j.jhazmat.2020.122224

— Revista: Journal of Hazardous Materials

— Área: Ciencias ambientales

— Quartil: 1

— Factor de impacto: 9.038

2. Montes de Oca-Vásquez, Gabriela; Solano-Campos, Frank; Vega-Baudrit, José; López-Mondéjar, Rubén; Vera, Alfonso; Moreno, José Luis; Bastida, Felipe. (2020). Organic amendments exacerbate the effects of silver nanoparticles on microbial biomass and community composition of a semiarid soil. Science of the Total Environment, 744, 140919. https://doi.org/10.1016/j.scitotenv.2020.140919

— Revista**:** Science of the Total Environment

— Área: Ciencias ambientales

— Quartil: 1

— Factor de impacto: 6.551

Artículo 1

Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity[1]

[1]**Montes de Oca-Vásquez, Gabriela**; Solano-Campos, Frank; Vega-Baudrit, José; López-Mondéjar, Rubén; Odriozola, I; Vera, Alfonso; Moreno, José Luis; Bastida, Felipe. (2020). Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity. Journal of Hazardous Materials, 391, 122224. https://doi.org/10.1016/j.jhazmat.2020.122224

Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity

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Highlights

- The response of the soil microbiome to realistic AgNPs concentrations was determined.
- Soil physico-chemical properties were not affected after the AgNPs application.
- The toxicity of AgNPs on soil microbial biomass depended on exposure time.
- The biomass of Gram+ bacteria was more sensitive to AgNPs than fungal biomass.
- AgNPs had little impact on soil microbial diversity and enzyme activities.

Graphical abstract
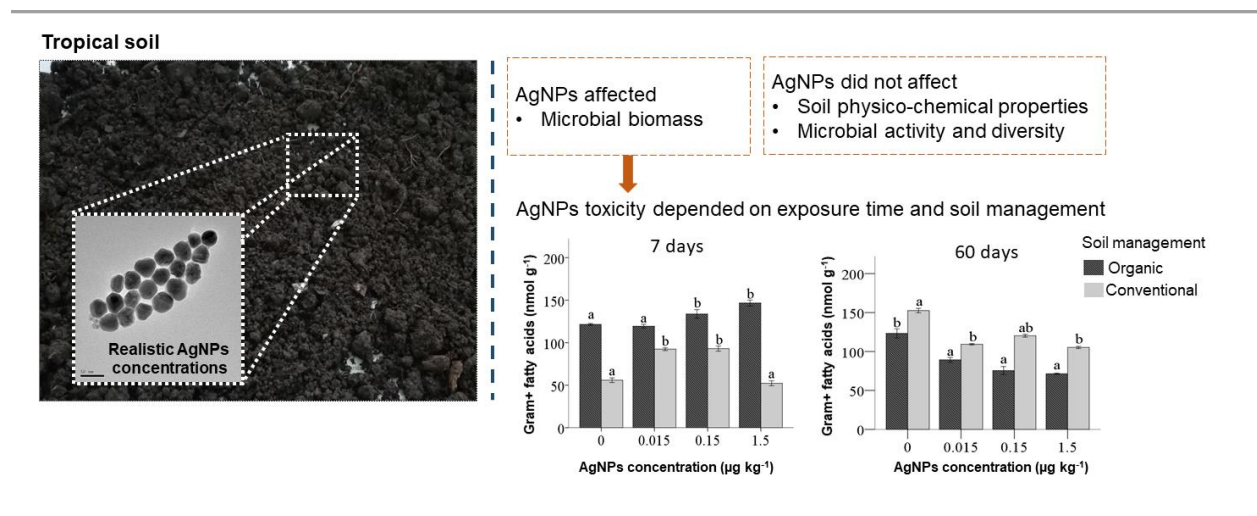
Abstract

The increasing use of silver nanoparticles (AgNPs) due to their well-known antimicrobial activity, has led to their accumulation in soil ecosystems. However, the impact of environmental realistic concentrations of AgNPs on the soil microbial community has been scarcely studied. In this work, we have assessed the impact of AgNPs, that mimic real concentrations in nature, on tropical soils cultivated with *Coffea arabica* under conventional and organic management systems. We evaluated the biomass, extracellular enzyme activities, and diversity of the soil microbial community, in a microcosm experiment as a function of time. After seven days of incubation, we found an increase in microbial biomass in an AgNPs-concentration-independent manner. In contrast, after 60-day-incubation, there was a decrease in Gram+ and actinobacteria biomass, in both soils and all AgNPs concentrations. Soil physico-chemical properties and enzyme activities were not affected overall by AgNPs. Regarding the microbial community composition, only some differences in the relative abundance at phylum and genus level in the fungal community were observed. Our results suggest that environmental concentrations of AgNPs affected microbial biomass but had little impact on microbial diversity and may have little effects on the soil biogeochemical cycles mediated by extracellular enzyme activities.

Keywords: Ag nanoparticles, soil microbial community, enzyme activities, biomass.

1. Introduction

The emergence of nanotechnology is concerning because there are now thousands of consumer products that contain nanoparticles (NPs), especially silver nanoparticles (AgNPs), one of the most commercialized nanomaterials worldwide, principally due to their antifungal and antibacterial properties (McGee et al., 2018). As such, nanoparticles are used in a wide variety of products, including textiles (Ballottin et al., 2017), cosmetics (Salvioni et al., 2017), medical devices (van Hengel et al., 2017), electronic devices (Matsuhisa et al., 2017), food (Kumar et al., 2018), and products for wastewater treatment (Bi et al., 2018). Owing to these uses, there are multiple points of entry of NPs into ecosystems, principally in soil, such as the use of biosolids as fertilizer (i.e., sludges and composts) (Wang et al., 2016; Kraas et al., 2017), application of AgNPs for plant growth promotion (Hänsch and Emmerling, 2010), and plant disease management (Haq and Ijaz, 2019).

Soil microbial communities play essential roles in ecosystem functioning, especially in the transformation and processing of nutrients in terrestrial ecosystems, soil fertility, and sustainability of the planet (Graham et al., 2016; Shen et al., 2016; Camenzind et al., 2018). For instance, soil microbial enzymes are deeply involved in the cycles of carbon (i.e. β-glucosidase), nitrogen (i.e. urease) and phosphorus (i.e. phosphatase), and are generally considered as indicators of soil quality (Bastida et al., 2008). It is known that soil microbial communities are sensitive to environmental stressors, potentially resulting in substantial impacts on biogeochemical processes and ecosystem function (Shen et al., 2016; McGee et al., 2017). However, in spite of their importance for the maintenance of ecosystem functions (Delgado-Baquerizo et al., 2016), little is known about the effect of AgNPs on the soil microbial community (Simonin and Richaume, 2015; Bundschuh et al., 2018; Grün et al., 2018). Recent studies suggest that AgNPs can accumulate in the soil environment and have toxic effects on the microbial communities (Beddow et al., 2017; Samarajeewa et al., 2017). Several authors have concluded that the toxic effects on microbial communities are highly dependent on the AgNPs concentration in the soil (Hänsch and Emmerling, 2010; Shin et al., 2012; Peyrot et al., 2014). However, most studies have evaluated AgNPs at higher levels than actually occur in nature (Rahmatpour et al., 2017; Storey and Doyle, 2017; Asadishad et al., 2018; Grün et al., 2018). Further, it has been demonstrated that the toxic effects on the soil microbial community are also highly dependent on both the NPs properties and the physico-chemical soil properties (Raffi and Husen, 2019; Grüm et al. 2019). The chemical nature of the NPs along with their concentration, size, shape, surface chemistry, charge, and other aspects seem to play an important role in the toxicity (Gottschalk et al., 2010; Schlich and

Hund-Rinke, 2015; Grüm et al. 2019). In soil, the texture, organic matter content, and pH have been suggested to play a role in the bioavailability of NPs (Schlich and Hund-Rinke, 2015; Rahmatpour et al., 2017; Grüm et al. 2019).

Nowadays, there are probabilistic analyses to predict the concentrations of engineered AgNPs in agricultural soil, based on modeling studies (Giese et al., 2018). While there are studies that have addressed the impact of NPs in soil, they have been carried out mainly with high, non-realistic concentrations of NPs (Simonin and Richaume, 2015; Storey and Doyle, 2017; Arvidsson, 2018; Grün et al., 2018) - in the range of 0.1 − 1815 mg kg^{-1} (Peyrot et al., 2014; McKee and Filser, 2016; McGee et al., 2017; Samarajeewa et al., 2017). In contrast, predicted environmental concentrations of AgNPs in agricultural soils are in the range of 0.24 − 792.23 ng kg^{-1}, with a maximum of 10 µg kg^{-1} predicted for 2050 (Giese et al., 2018). Since only a few studies have considered realistic concentrations of AgNPs (Bundschuh et al., 2018; Grün et al., 2018), more research should be carried out to address this matter more profoundly (Simonin and Richaume, 2015). Particularly, whether or not realistic NPs concentrations affect the diversity and functioning of soil microbial communities should be addressed (Bastida et al., 2008; Chung et al., 2011; Zornoza et al., 2015). Taking into consideration the importance of soil microbial communities for the maintenance of soil ecosystem services and the increasing release of NPs into the environment due to industrial development, the aim of this study was to reveal the effects of AgNPs, at realistic concentrations occurring in nature, on the biomass, activity, and diversity of the microbial community of a soil cultivated with *Coffea arabica*, grown for coffee consumption. In Costa Rica, coffee is one of the main export products with a cultivation area of 93,697 ha and a 2019−2020 harvest of more than 1.9 million bushels (Instituto del Café de Costa Rica, 2019).

To our knowledge, this is the first work evaluating the impact of environmental concentrations of AgNPs on a tropical agricultural soil, and under different management systems. Soil management can alter the diversity and composition of soil microbial communities (Smith et al., 2016; García-Delgado et al., 2019; Moreno et al., 2019) and soil microbial populations can differ in their resistance to AgNPs (Tlili et al., 2017; Asadishad et al., 2018; Grün et al., 2018). Hence, we hypothesized that soils subjected to different management (conventional vs organic) would have evolved different microbial communities (in terms of biomass, diversity, and activity) that differ in their sensitivity to silver nanoparticles at relevant concentrations found in nature.

2. Materials and methods

2.1. Characterization of silver nanoparticles

Citrate-coated AgNPs were purchased from Sigma-Aldrich (St. Louis, USA). According to the manufacturer, these AgNPs have a mean particle size of 40 nm in powder form, as measured by transmission electron microscopy (TEM). The particle size of an AgNPs suspension (1:3 dilution of commercial solution) was verified using TEM (JEOL, JEM2011), at an acceleration voltage of 120 kV (Fig. S1. Supporting Information), previously to prepare the homogenous AgNPs suspensions applied to the microcosm. Samples were prepared by placing 5 µl of the reaction mixtures on carbon-coated copper grids, followed by drying in a desiccator with silica for 16 h.

In addition, the hydrodynamic diameter, zeta potential, and polydispersity of the AgNPs were determined by dynamic light scattering (DLS), using a Zetasizer Nano Zs90 instrument (Malvern Instruments, UK). The analyses were carried out with a dispersion angle of 90°, at a temperature of 25 °C, using samples diluted (1:3) with Milli-Q water (Fig. S2). The hydrodynamic diameter and zeta potential of AgNPs was 33.09 ± 0.38 nm and -32.5 ± 3.34 mV, respectively.

2.2. Study area

Soils were collected at the Santa Lucía experimental farm – located in Santa Lucía, Barva, Heredia, Costa Rica (coordinates 10°01′19.5″N,84°06′43.5″W) – and managed by Universidad Nacional. The climate in this area is classified as Tropical Monsoon, according to the Köppen-Geiger climate classification, and the climax vegetation is characterized as a premontane wet forest, according to the Holdridge life zone system. The average annual temperature and precipitation are 21.5 °C and 2371 mm, respectively. The average elevation of the study area is 1159 m.a.s.l. The soil is principally of volcanic origin, and classified as Andisols (FAO, 1988).

Within this experimental farm, *Coffea arabica* cv. Caturra was cultivated under a conventional management system (CMS) in an area of 8000 m^2 in the three years prior collecting the samples, and under an organic management system (OMS) in an adjacent area of 8000 m^2 in the 6 years prior collecting the samples. Three plots of 2000 m^2 each for each type of management were established, giving a total of 6 plots. In this study, conventional and organic managements refer mainly to the fertilization, herbicides and fungicides utilized. Thus, the conventional management included fertilization with 780 kg ha^{-1} of N, 1066 kg ha^{-1} of P_2O_5, and 453 kg ha^{-1} of K_2O per year, in addition to bimonthly applications of herbicide (glyphosate) and fungicide (triazole compounds). The organic management consisted of alternated fertilization with green compost and chicken

manure, at 1.5 kg of each per plant thrice a year, and mechanical weed control. Overall, there were no differences between the two soil management regimes in the soil total N and organic matter contents, pH, electrical conductivity, or texture (Table S1). The microbial biomass, enzyme activities (β-glucosidase, urease and acid phosphatase), and microbial diversity of operational taxonomic units (OTUs) presents in CMS and OMS are shown in Table 1.

Table 1. Microbial biomass, enzyme activities and microbial diversity presents in CMS and OMS.

	CMS	OMS
Biomass (nmol g^{-1})		
Fungal	3.58 ± 1.37	17.46 ± 3.80
Bacterial	57.28 ± 26.10	143.04 ± 9.62
Gram +	44.73 ± 22.32	116.39 ± 10.93
Gram -	12.55 ± 3.79	26.66 ± 9.06
Actinobacterial	6.80 ± 3.66	18.83 ± 2.11
Enzyme activities		
β-Glucosidase (μmol PNP g^{-1} h^{-1})	1.04 ± 0.05	1.38 ± 0.03
Urease (μmol NH$_3$ g^{-1} h^{-1})	1.21 ± 0.09	1.57 ± 0.05
Acid phosphatase (μmol PNP g^{-1} h^{-1})	12.44 ± 0.64	11.87 ± 0.29
Microbial diversity		
Fungal OTUs	2623 (1996)*	2700 (2073)
Bacterial OTUs	5176 (2419)	6576 (3819)

*number in parenthesis indicate the soil type-specific OTUs.

2.3. Soil microcosms and experimental design

Twenty soil samples (0 − 20 cm soil cores) were collected in each plot, covering the whole area. A composite soil sample of approximately 10 kg was obtained by mixing the 20 samples per plot, and was utilized for further microcosm assays. Large woody litter, such as branches, was removed from the soil samples, and the soil was dried at room temperature and sieved (2-mm mesh). The treatments were established in replicates (n = 3). For each regime (OMS and CMS), three different stock solutions were made in order to add the same volume (55 mL) of the AgNPs suspension to all the soil samples in order to obtain final concentrations of 0.015, 0.15, and 1.5 μg kg^{-1}. Soils receiving the same volume of ultrapure water were used as controls. Three times of exposure to AgNPs (7, 30, and 60 days), were independently established. Homogeneous AgNPs suspension was added to 150 g of soil in 200-mL containers and then extensively mixed by stirring with a spoon to obtain a uniform distribution. The microcosms were placed in random positions in a temperature-controlled (28°C) chamber (LABNET 211 DS, Labnet International,

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US), in the dark. The water retention capacity was gravimetrically controlled at 60 % throughout the incubation period. This experimental design resulted in a total of 72 microcosms, including 3 replicates per treatment (2 soil managements × 3 concentrations of AgNPs × 3 sampling times × 3 replicates, plus controls). At the end of each incubation time, 3 g of soil from each microcosm were stored at -20 ºC for up to 4 weeks for DNA extraction and the remaining sample was kept at 4 °C for further analysis of soil physico-chemical properties, enzyme activities, and fatty acids.

2.4. Physico-chemical analysis of soil samples

After the AgNPs application, the electrical conductivity and pH were measured in a 1:5 (w:v) aqueous extract, with a conductivity meter and pH meter (Crison mod.2001, Barcelona, Spain), respectively. The total nitrogen content (N) and total organic carbon (TOC) were determined using an elemental analyzer (C/N Flash EA 1112 Series-Leco Truspec). Aqueous extracts of soil were prepared with deionized water (1:5, w:v) by shaking for 2 h, followed by centrifugation at 13.000 rpm for 15 min and filtration. The water-soluble C (WSC) and N (WSN) contents in these extracts were determined in an analyzer for liquid samples (Multi N/C 3100, Analytik Jena).

2.5. Microbial fatty acids and enzyme activities

Ester-linked microbial fatty acids were extracted from 1 g of soil by chloroform-methanol extraction, as described by Schutter and Dick (2000). Fatty acids were quantified by gas chromatography (Trace GC Ultra, Thermo Scientific) using a 60 m capillary GC column (SGE Analytical Science, BPX70, 60 m x 0.25 mm ID x 0.25 μm film) and helium as the carrier gas. The initial temperature was 120 °C for 0.5 min, then it was increased to 140 °C with a ramp of 1 °C/min, then to 170 °C with increments of 2 °C/min, and finally to 210 °C at 2 °C/min. The fatty acids i15:0, a15:0, 15:0, i16:0, i17:0, 18:1ω 9t, and cy19:0 were representative of the bacterial biomass (Frostegård et al., 1993; Dungait et al., 2011) and 18:2ω6 of the fungal biomass (Brant et al., 2006; Rinnan and Bååth, 2009). The Gram-positive (G+) representative fatty acids were i15:0, a15:0, i16:0, and i17:0, and the Gram-negative (G-) representative fatty acid was cy19:0 (Frostegård et al., 1993; Dungait et al., 2011).

The urease activity in the soil was determined by the buffered method of Kandeler and Gerber (1988). Briefly, 0.5 mL of a solution of urea (0.48 %) and 4 mL of borate buffer (pH 10) were added to 1 g of soil and then incubated for 2 h at 37 °C. The ammonium concentration of the centrifuged extracts was determined by a modifed indophenol-blue reaction and measured at 690 nm by spectrophotometry, and expressed in units of micromoles of ammonium-N produced per gram of

soil (dry weight) and hour (μmol NH$_4$-N g^{-1} h^{-1}). The acid phosphatase (PA) and β-glucosidase (BA) activities were determined according to Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977), respectively. Two mL of MUB (Modifed Universal Buffer), pH 6.5 for the β-glucosidase and acid phosphatase assay, and 0.5 mL of *p*-nitrophenyl substrate (*p*-nitrophenyl-β-d-glucopyranoside for β-glucosidase and *p*-nitrophenyl phosphate for acid phosphatase) were added to 0.5 g of soil. The mixtures were incubated at 37 °C for 1 h. Then, the *p*-nitrophenol released was measured at 400 nm by spectrophotometry. Both activities were expressed in units of micromoles of *p*-nitrophenol (PNP) produced per gram of soil (dry weight) and hour (μmol PNP g^{-1} h^{-1}).

2.6. DNA extraction and amplification

The DNA was extracted from 400 mg of freeze-dried soil sample using a Fast DNA Spin Kit for soil and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA). The V4 region of bacterial 16S ribosomal RNA (rRNA) gene was amplified using the barcoded primers 515F and 806R, as described previously (Caporaso et al., 2012). The PCR amplification of the fungal ITS2 region from DNA was performed using barcoded gITS7 and ITS4 (Ihrmark et al., 2012), in three PCR reactions per sample, as described previously (Žifčáková et al., 2016). The PCR products were cleaned using a MinElute Kit (Qiagen) and their concentrations measured by Qubit. After libraries were prepared, sequencing of fungal and bacterial amplicons was performed on Illumina MiSeq. The amplicon sequencing data were processed using the pipeline SEED2 (Větrovský et al., 2018), as previously described (Žifčáková et al., 2016). Briefly, pair-end reads were merged and whole amplicons of bacterial 16S rRNA gene or the ITS2 regions of fungal amplicons were cleaned of chimeras and clustered into operational taxonomic units (OTUs) at a 97 % similarity level. Consensus sequences were constructed for each cluster, and the closest hits at the genus or species level were identified using BLASTn against the Ribosomal Database Project (Cole et al., 2014) (for bacteria) or UNITE (Koljalg et al., 2014) (for fungi). Sequences identified as non-bacterial or non-fungal were discarded. Low-abundance OTUs, with less than 0.5 % of the sequences for fungal and bacterial analyses, were filtered. The pipeline SEED2 was also used for diversity calculations (Větrovský et al., 2018). The DNA sequences have been deposited in the MG-RAST public database with project ID mgp90907.

2.7. Data analysis

The normality and the homogeneity of variances were tested by the Ryan-Joiner and Levene tests, respectively. The variables were subjected to two-way ANOVA. The two factors included in this experimental design were: i) AgNPs concentration, which had 4 levels (0, 0.015, 0.15, and 1.5 µg kg^{-1}); and ii) soil management, which had two levels (organic soil management, OMS; and conventional soil management, CMS). Further, for each soil management regime, the data were submitted to one-way ANOVA, followed by post hoc analyses using Tukey's significant difference test. Differences at $P < 0.05$ were regarded as statistically significant. All results are reported as means ± standard deviation. These statistical analyses were performed using IBM SPSS (version 20.0; SPSS Inc, Chicago, IL, USA). Principal component analysis (PCA) of the relative abundance of fatty acids for each independent time was performed using PAST 3.22 (Hammer et al., 2001). The effect of the distinct treatments and types of management on the bacterial and fungal community composition was assessed using PERMANOVA (Anderson, 2001). The Euclidean distance for the Hellinger-transformed bacterial and fungal composition (i.e., a Hellinger distance matrix) (Legendre and Gallagher, 2001) was used as a response matrix and the fixed factors management, treatment, and their interaction were used as explanatory variables. PERMANOVA was performed with adonis function of *vegan* (Oksanen et al., 2018; R Core Team, 2018). To visualize the results, the Hellinger-transformed community compositions were submitted to PCA and plotted in a biplot that represents the Hellinger distances among sampling units (Legendre and Legendre, 2012). The PCA was performed with the function rda in the *vegan* package. The analysis was limited to the most abundant species.

3. Results

3.1. Physico-chemical analyses of soil samples

Relative to the control, we only found a significant reduction ($P < 0.05$) in the pH with the highest concentration of AgNPs in the CMS, and in the 0.15 and 1.5 µg kg^{-1} treatments in the OMS, at 7-day-incubation (Table S2). The physico-chemical properties that differ between OMS and CMS were electrical conductivity, pH and WSN (Table S3).

3.2. Microbial biomass and community structure estimated by fatty acids analysis

There were differences in microbial biomass, after incubation, between the two types of soil management (conventional and organic) (Table S4). Overall, after 7-day-incubation with AgNPs, we found a statistically significant increase in all the groups of microorganisms estimated by fatty

acids analysis in the OMS soil, and an increase in the fungal, bacterial, Gram+, and actinobacterial fatty acids contents in the CMS, in a concentration-independent manner (Fig. 1). At 30-day-incubation, bacterial and actinobacterial fatty acids did not change significantly at any AgNPs concentration and any type of soil. In the OMS and AgNPs concentrations of 0.15 and 1.5 µg kg^{-1}, we observed a significant decrease in the fungal and Gram+ fatty acids contents and a statistically significant increase in the Gram- fatty acid content. However, in the CMS we did not find significant differences in biomass regarding the control. Further, in OMS and CMS at 60-day-incubation, in all the AgNPs concentrations, there was a significant decrease in the bacterial fatty acid content, including Gram+ and actinobacterial representative fatty acids. In contrast, we found an increase in the fungal fatty acid content in the OMS treated with the lowest concentration of AgNPs, and at the concentrations of 0.015 and 1.5 µg kg^{-1} in the CMS. For Gram- representative fatty acids, an increase was found at the 0.015 and 1.5 µg kg^{-1} doses for the OMS, while a decrease at 0.015 and an increase at 1.5 µg kg^{-1} occurred for the CMS (Fig. 1).
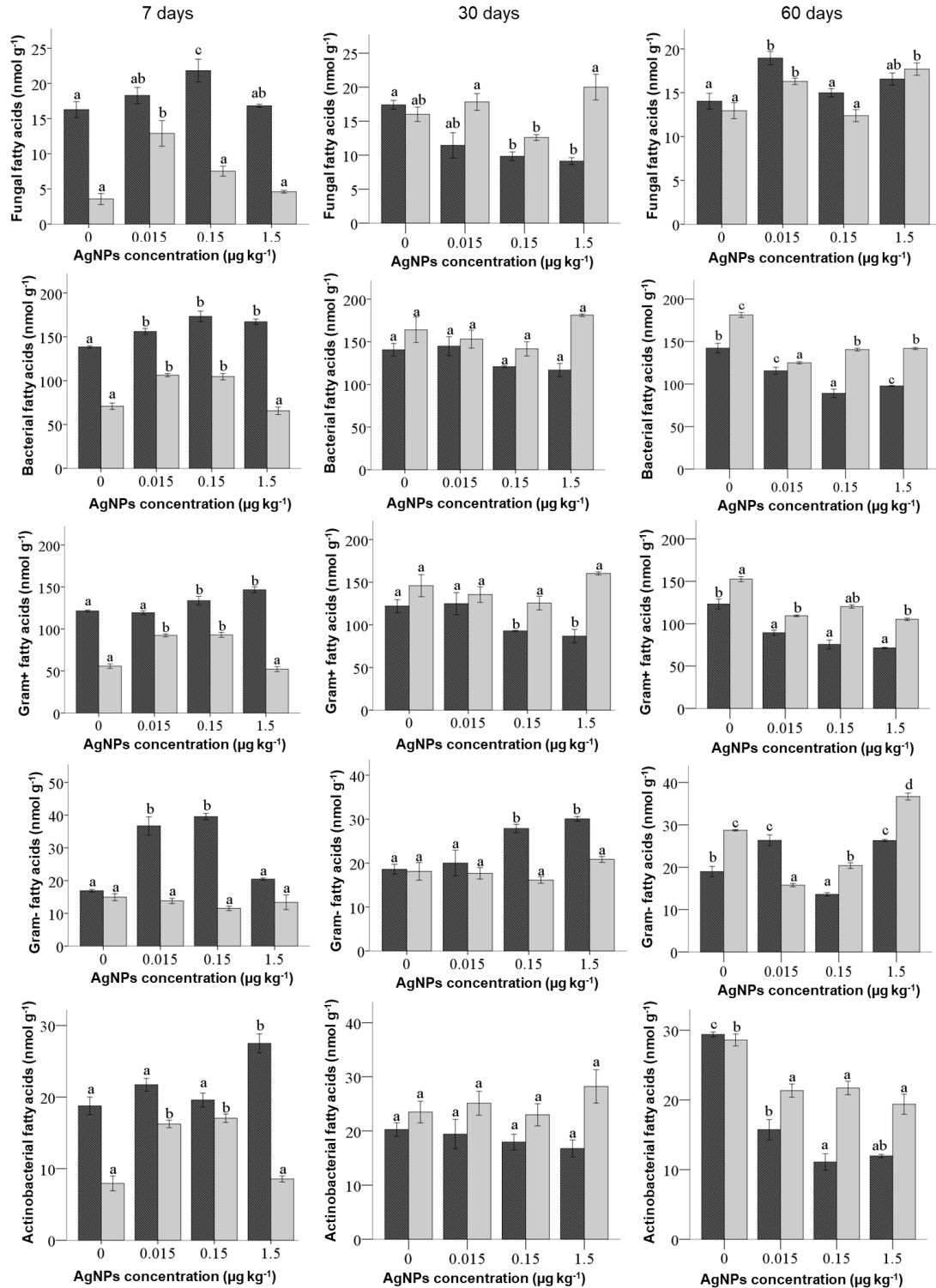
Fig. 1. Fatty acid content of organically managed soil (bars = dark gray) and conventionally managed soil (bars = gray) exposed to different concentrations of AgNPs (0; 0.015; 0.15; and 1.5 µg kg^{-1}), at 7, 30, and 60 days of incubation. Different letters indicate significant differences (P < 0.05) for treatments within the same soil management, according to one-way ANOVA followed by a post-hoc Tukey test carried out for multiple comparison among the mean values observed at the different AgNPs concentrations.

The PCA performed with the relative abundance (%) of fatty acids showed that the AgNPs concentration, exposure time, and soil management influenced the structure of the microbial community (Fig. 2). The first two principal components explained 69–85 % of the observed variance. For the CMS, component 1 clearly differentiated between the control and all AgNPs treatments at 7-day-incubation, and between the highest concentration and all the other treatments at 60-day-incubation. For the OMS, at 7-day-incubation, component 1 differentiated between the control and the low and medium doses; at 30-day-incubation, it differentiated between the medium and high doses; and at 60-day-incubation, the control was separated from all the other treatments.
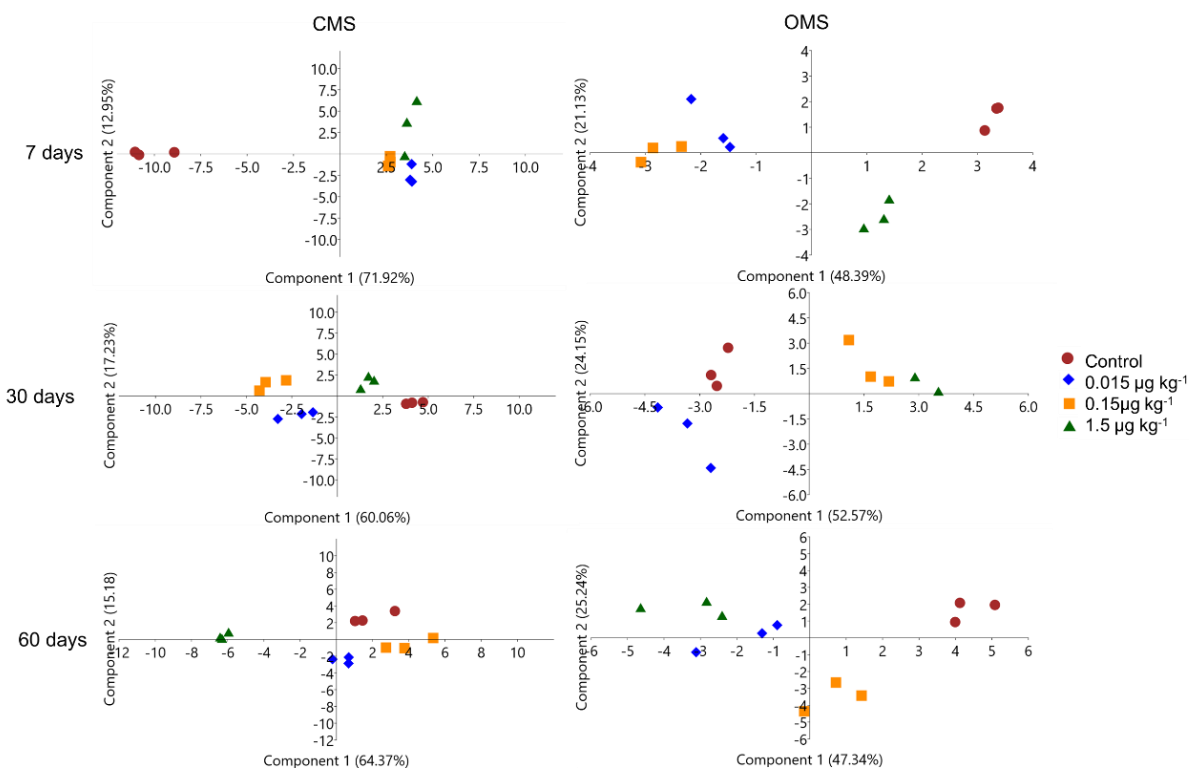

Fig. 2. The principal component analysis (PCA) performed with the relative abundance of fatty acids of organically managed soil (OMS) and conventionally managed soil (CMS) exposed to different concentrations of AgNPs (0; 0.015; 0.15; and 1.5 µg kg^{-1}), at 7, 30, and 60 days of incubation. Values in brackets denote the percentage of the data variation explained by a given component.

3.3.   Enzyme activities

Overall, the β-glucosidase and urease activities, for all incubation times, were affected significantly by the soil management ($P < 0.05$), but not by the concentration of AgNPs. The phosphatase activity was not affected by the soil management or the AgNPs concentration (Table S4). One-way ANOVA performed separately for each type of soil management confirmed the results of the two-way ANOVA, indicating no significant differences among the soil enzyme activities measured in the different treatments with AgNPs and the unpolluted controls (Table S5).

3.4.   The diversity and composition of the soil microbial community determined through amplicon sequencing

A PCA of the Hellinger-transformed distances of the bacterial and fungal communities studied through 16S rRNA gene and ITS sequencing, respectively, revealed clear differences in the composition of the microbial community between the CMS and OMS, for both the bacterial (PERMANOVA P-value = 0.001) and fungal (PERMANOVA P-value = 0.001) communities (Fig. 3A and B, respectively). However, the impact of the AgNPs on the composition of the soil microbial communities was not significant (PERMANOVA P-value > 0.05).
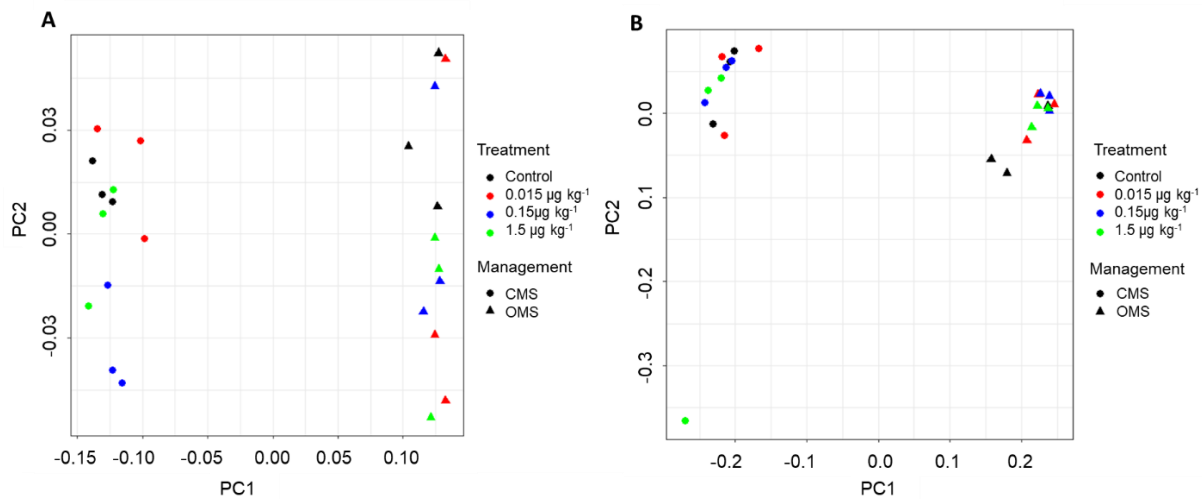

Fig. 3. Principal component analysis (PCA) biplots of the Hellinger-transformed distances of the (A) bacterial and (B) fungal community composition.

Independently of the AgNPs concentration and the soil management, the most abundant bacterial phyla were Proteobacteria, Actinobacteria, and Acidobacteria, with up to 39.1, 23.7, and 10.4 % of the sequences, respectively (Fig. 4A). At the genus level, the most abundant taxa were *Gaiella*, *Rhodoplanes*, "*Candidatus* Xiphinematobacter", *Conexibacter*, and *Aciditerrimonas*, with up to 7.1, 5.3, 3.6, 3.4, and 3.2 % of the sequences, respectively (Fig. 4B). Overall, differences in the relative abundance of bacterial phyla and genera were not observed among the AgNPs treatments within each soil management type.
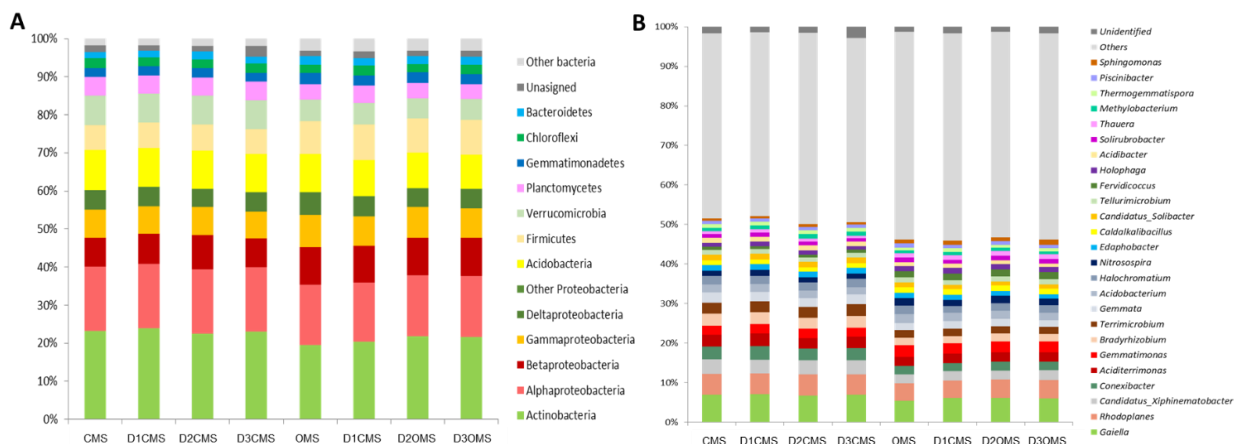

Fig. 4. The composition of the bacterial communities at the phylum/class (A) and genus (B) levels. CMS, D1CMS, D2CMS, and D3CMS are the soil samples with conventional management, corresponding to 0; 0.015; 0.15; and 1.5 µg kg^{-1} AgNPs, respectively. OMS, D1OMS, D2OMS, and D3OMS are the soil samples with organic management, corresponding to 0; 0.015; 0.15; and 1.5 µg kg^{-1} AgNPs, respectively, at 60 days of incubation.

The most abundant fungal phylum was Ascomycota, with up to 82.63 % of the sequences. At the genus level, the most abundant taxa in both the CMS and OMS were *Mortierella*, *Fusarium*, and *Penicillium*, with up to 13.99, 9.94, and 6.79 % of the sequences, respectively. The relative abundance of *Humicola*, *Saitozyma*, *Mortierella*, *Talaromyces*, *Staphylotrichum*, *Pyrenochaetopsis*, *Neurospora*, *Chaetosphaeria*, *Sagenomella*, and *Metarhizium* differed between the OMS and CMS ($P < 0.05$). Some significant differences, within the CMS and OMS, in the relative abundance of fungal populations were observed. In the OMS, the relative abundance of the phylum Mortierellomycota and the genus *Mortierella* was significantly higher in all the AgNPs treatments than in the control ($P < 0.01$) (Fig. 5A, 5B). In addition, the relative abundance of the genus *Fusarium* was significantly lower at the highest concentration of AgNPs compared to the control ($P = 0.038$), and the relative abundance of *Fusicolla* differed significantly between the lowest AgNPs concentration and the control ($P = 0.018$) (Fig. 5B). Among the samples of the CMS soil receiving AgNPs, the relative abundance of Mortierellomycota and

Mortierella was significantly lower at the highest AgNPs concentration than in the control ($P <$ 0.01) (Fig. 5A, 5B).
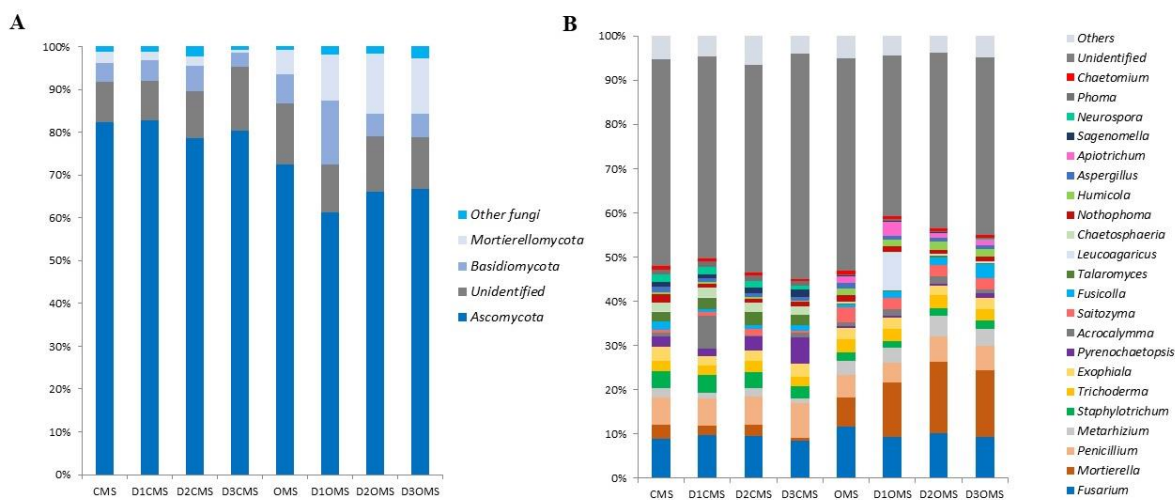

Fig. 5. The composition of the fungal communities at the phylum (A) and genus (B) levels. CMS, D1CMS, D2CMS, and D3CMS are the soil samples with conventional management, corresponding to 0; 0.015; 0.15; and 1.5 µg kg^{-1} AgNPs, respectively. OMS, D1OMS, D2OMS, and D3OMS are the soil samples with organic management, corresponding to 0; 0.015; 0.15; and 1.5 µg kg^{-1} AgNPs, respectively, at 60 days of incubation.

4. Discussion

4.1. Impact of AgNPs on soil physico-chemical properties and microbial biomass

Overall, the low - and realistic - AgNPs doses used here did not affect the soil physico-chemical (i.e. electrical conductivity) and chemical properties (i.e. water-soluble C and N) examined, unlike the changes reported in other studies using higher doses of AgNPs (Hänsch and Emmerling, 2010; Antisari et al., 2013). However, we did observe significant impacts of the AgNPs on the soil microbial biomass, which suggests that microbial soil properties might be more sensitive to these AgNPs than soil abiotic properties and that the impact of the AgNPs on the microbial biomass is likely due to their inherent toxicity and biocide effects rather than indirect effects on soil physico-chemical properties. In addition, the impacts of the AgNPs on the soil microbial biomass were dependent on the incubation time and the soil management. However, the impact of AgNPs concentration used here was nonlinear, under our experimental conditions. Other studies have found a similar trend, with the concentrations used being less significant in the toxicity of AgNPs than soil texture, silver species, or exposure time (Grün et al., 2018).

When comparing the different incubation times in both types of soil, a decrease in fungal and Gram+ biomass was detected at 30-day-incubation in OMS, while in CMS a decrease in bacterial, Gram-, Gram+, and actinobacterial fatty acid content was observed at 60-day-incubation. An enhancement of the toxicity with increasing exposure time has also been highlighted in soils subjected to much higher doses (Schlich and Hund-Rinke, 2015; Tlili et al., 2017; Grün et al., 2018). It was clear that the AgNPs impacts on the two types of soil samples were different in some microbial groups and at different incubation times. These differences could be attributed to the distinct microbial communities, as OMS had more biomass, enzyme activities and microbial diversity than CMS. Similarly, Sillen et al. (2015) reported that differences in the properties of the soil microbial community, such as biomass and diversity, might result in differences in the impact of AgNPs and the appearance of the effects. Furthermore, the negative impact of the AgNPs on the soil microbial biomass after 60-day-incubation contrasts with the expected protective effect of the high organic matter content in these tropical soils. In detail, it has been suggested that organic matter and clays can complex to AgNPs, reducing their mobility and toxicity (Rahmatpour et al., 2017; Schultz et al., 2018). However, while this protective mechanism could have operated at the beginning of the incubation, the high organic matter content did not protect the bacterial, Gram+ and actinobacterial groups, from the harmful impact of the AgNPs in the long term, even at the low and realistic doses assayed here. This lack of protective effect could be due to a slow release of the Ag^+ ions from the AgNPs complexed to the organic matter and clays, and to the higher toxicity of Ag^+ when compared to AgNPs, as reported by other authors (Yang et al., 2013; Rahmatpour et al., 2017; Schultz et al., 2018).

More specifically, our results revealed that, at 60-day-incubation, the bacterial biomass was more sensitive to the AgNPs than the fungal biomass, in OMS and CMS. Indeed, fungal and bacterial communities may display varied sensitivities to Ag^+ (Pshennikova et al., 2011), with eukaryotic cells being more tolerant of Ag^+ (Kathiresan et al., 2010) and heavy metals (Hiroki, 1992). Several studies have provided evidence for these differences in the responses of bacterial and fungal communities (Kumar and Mamidyala, 2011; Gryndler et al., 2012; Sillen et al., 2015). Further, we found that Gram+ were more sensitive than Gram- bacteria to the AgNPs. In agreement, Kumar et al. (2014) found a significant reduction in the Gram+ bacteria, but not in the Gram-, in an arctic tundra soil treated with 0.066 % and 6.6 % AgNPs. Similarly, Liu et al. (2017) found a decrease in the Gram+ bacteria, and an increase in the Gram-, during AgNPs (1 mg kg^{-1}) exposure. This greater resistance of Gram- bacteria can be attributed to the lipopolysaccharides of the outer

membrane, which may provide resistance against silver penetration into the cell (Marambio-Jones and Hoek, 2010; Jacobson et al., 2015).

In addition, we found that AgNPs treatments affected the biomass of some microbial groups only in OMS, at 7 and 30-day-incubation. Moreover, in the case of Gram- fatty acids, an opposite response was detected between OMS and CMS at 60 day-incubation. Likewise, other researchers have reported dissimilar responses of different microbial groups to AgNPs, to the point to being species specific (Hänsch and Emmerling 2010; Pallavi et al., 2016). Furthermore, it has been reported that soil microbial communities can differ in their resistance to AgNPs (Tlili et al., 2017; Asadishad et al., 2018; Grün et al., 2018), due to the presence of sensitive and resistant species, and because some microorganisms can generate tolerance (Samarajeewa et al. 2016). However, further work is needed to study in detail the changes in the microbial community, specially the diversity, and determine which genera and species are responsible for the resistance or tolerance to AgNPs in both soil types. Also, it is important to study the dissolution and aggregation rate of the AgNPs, the organic matter components, and the extracellular polymeric substances in both soils, which can affect the AgNPs toxicity to microorganism. This knowledge will allow a better understanding of how the changes in the microbial communities can modulate the impact of AgNPs.

4.2. Impact of AgNPs on enzyme activities

In general, there was no effect on the enzyme activities (β-glucosidase, urease, and acid phosphatase), in both soils, within the range of AgNPs concentrations used in this study. The lack of impact on enzyme activities has been reported previously in studies using higher doses (Colman et al., 2013; Peyrot et al., 2014; Asadishad et al., 2018; Grün et al., 2018). Our results imply that AgNPs affected preferentially the microbial biomass, probably due to the Ag ion released from AgNPs, rather than the extracellular environment (i.e., enzyme activities) (Nannipieri et al., 1990; Burns et al., 2013). It is possible that the high organic matter and clay content, in our soils, contributed to the complexation with AgNPs, and therefore, protected the extracellular enzymes (Burns et al., 2013; Peyrot et al., 2014; Elzobair et al., 2016; Asadishad et al., 2018). In addition, Peyrot et al., 2014 reported that the toxic effect on extracellular enzymes was exerted by the AgNPs instead of ionic Ag, at least at low Ag concentrations. Therefore, the absence of significant differences between AgNPs treatments and the controls, could be related to the stabilization of extracellular enzymes by complex with inorganic surfaces (clay, iron oxides

and hydroxides) and adsorption with organic colloids, protecting the enzymes from the AgNPs effects (Nannipieri, 2018).

4.3. Impact of the AgNPs on microbial diversity

Overall, for both types of soil management, the AgNPs had no significant effect on the composition of the microbial communities, in agreement with other studies that have investigated the effect of medium and high concentrations of AgNPs (Liu et al., 2017; Samarajeewa et al., 2017; Grün et al., 2018). Other researchers have found a similar outcome; for instance, Colman et al. (2013) found no significant differences in the composition of the bacterial community after 50 days of exposure to 0.14 mg kg^{-1} AgNPs in a forest soil - a dose within the range of our study -. Further, Asadishad et al. (2018) did not find significant differences in the microbial community of an agricultural soil when using 1 mg kg^{-1} of citrate coated-AgNPs and only found differences at a higher concentration, 100 mg kg^{-1}, much higher than the doses utilized here. Nevertheless, in our work, the AgNPs impacted the relative abundance of some fungal populations. For instance, we found that the AgNPs treatments increased the relative abundance of the phylum Mortierellomycota and the genus *Mortierella* in the OMS. It has been reported previously that this genus is abundant in natural soils after disturbance because its members may act as decomposers of freshly dead mycelia and bacterial biomass (Brabcová et al., 2018; López-Mondéjar et al., 2018). It can because Mortierella obtains C from simple organic compounds available in soil, like root or hyphal exudates (López-Mondéjar et al., 2018). AgNPs treatments could affect the fungal and bacterial biomass and this may increase the incidence of the opportunistic decomposer *Morteriella*. In the CMS, we observed a different trend, with a decrease in the Mortierellomycota and the genus *Mortierella* at the highest dose; this was probably due to differences in the soil management (conventional and organic), because the CMS used fungicides that may decrease the abundance of *Mortierella*. Also, in the OMS, a decrease in some fungal genera like *Fusarium* and *Fusicolla* was detected with the application of AgNPs. Different *Fusarium* spp. cause recurrent diseases affecting crops worldwide (wheat, barley, oats, rye, and triticale) (Tarazona et al., 2019) and some studies have demonstrated the toxic effect of NPs on different *Fusarium* spp. (Malandrakis et al., 2019; Tarazona et al., 2019).

5. Conclusions

Previous studies using higher doses have opened the door to investigate the impacts of AgNPs in soil, but there was a need to better understand the effects of AgNPs at lower concentrations, similar to those occurring in nature. Our study shows that environmentally relevant concentrations

of AgNPs have detrimental effects on the microbial biomass, in a way dependent on the time of exposure and soil management, but independently of the AgNPs concentration. The sensitivity of the biomass of soil microbial groups to AgNPs seems to vary, fungi being more resistant than bacteria and Gram- bacteria more resistant than Gram+. In comparison to their impacts on the soil microbial biomass, the AgNPs had little effect on microbial community composition and on the extracellular soil enzyme activities, which points to a limited impact of silver nanoparticles in soil biogeochemical cycles in a short-term. With this, we state that AgNPs had great impact on soil microbial biomass (studied through fatty acids), but that the effects on the relative abundance of the bacterial dominant organisms are not too selective (studied through DNA sequencing). The results of this research would give to the scientific community the baseline in the effect of realistic concentrations of AgNPs. Future research should address the responses of soil microbial communities and soil physico-chemical characteristics to realistic concentrations of AgNPs with different properties (i.e., size, shape, etc.), for a wider variety of soil types and land-uses.

CRediT authorship contribution statement

Gabriela Montes de Oca-Vásquez: Investigation, Data curation, Writing - original draft, Visualization, Funding acquisition. Frank Solano-Campos: Methodology, Investigation, Resources, Writing – review & editing, Funding acquisition. José R. Vega-Baudrit: Resources, Writing - review & editing, Funding acquisition. Rubén López-Mondéjar: Formal analysis, Resources, Data curation, Writing – review & editing. Iñaki Odriozola: Formal analysis, Resources, Data curation. Alfonso Vera: Investigation, Writing - review & editing. José L. Moreno: Writing - review & editing, Funding acquisition. Felipe Bastida: Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.122224.

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Artículo 2

Organic amendments exacerbate the effects of silver nanoparticles on microbial biomass and community composition of a semiarid soil[2]

[2]**Montes de Oca-Vásquez, Gabriela**; Solano-Campos, Frank; Vega-Baudrit, José; López-Mondéjar, Rubén; Vera, Alfonso; Moreno, José Luis; Bastida, Felipe. (2020). Organic amendments exacerbate the effects of silver nanoparticles on microbial biomass and community composition of a semiarid soil. Science of the Total Environment, 744, 140919. https://doi.org/10.1016/j.scitotenv.2020.140919

Organic amendments exacerbate the effects of silver nanoparticles on microbial biomass and community composition of a semiarid soil

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Highlights

• Organic amendment determined the toxicity of AgNPs in soil microorganisms.

• Bacterial biomass and community composition were sensitive to AgNPs in the amended soil.

• Fungal and Gram- biomass were more tolerant to AgNPs toxicity than Gram+.

• AgNPs did not affect the extracellular enzymes activities.

• AgNPs decreased the fungal richness in the unamended soil.

Graphical abstract
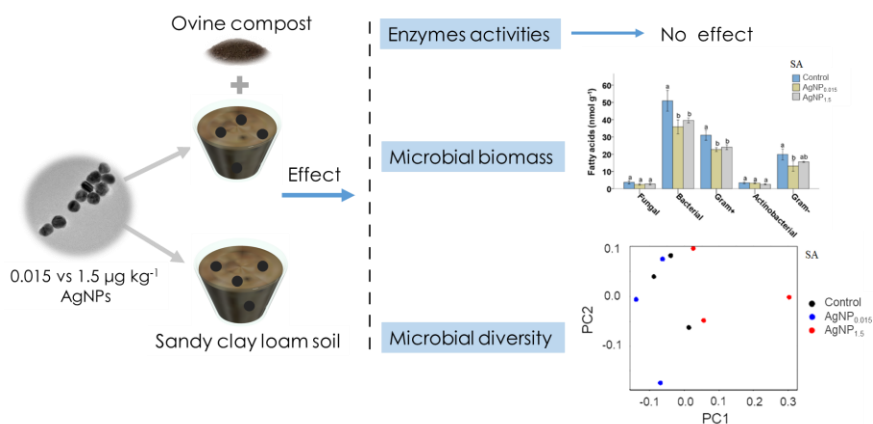
Abstract

Increased utilization of silver nanoparticles (AgNPs) can result in an accumulation of these particles in the environment. The potential detrimental effects of AgNPs in soil may be associated with the low fertility of soils in semiarid regions that are usually subjected to restoration through the application of organic amendments. Microbial communities are responsible for fundamental processes related to soil fertility, yet the potential impacts of low and realistic AgNPs concentrations on soil microorganisms are still unknown. We studied the effects of realistic citrate-stabilized AgNPs concentrations (0.015 and 1.5 µg kg^{-1}) at two exposure times (7 and 30 days) on a sandy clay loam Mediterranean soil unamended (SU) and amended with compost (SA). We assessed soil microbial biomass (microbial fatty acids), soil enzyme activities (urease, β-glucosidase, and alkaline phosphatase), and composition of the microbial community (bacterial 16S rRNA gene and fungal ITS2 sequencing) in a microcosm experiment. In the SA, the two concentrations of AgNPs significantly decreased the bacterial biomass after 7 days of incubation. At 30 days of incubation, only a significant decrease in the Gram+ was observed at the highest AgNPs concentration. In contrast, in the SU, there was a significant increase in bacterial biomass after 30 days of incubation at the lowest AgNPs concentration. Overall, we found that fungal biomass was more resistant to AgNPs than bacterial biomass, in both SA and SU. Further, the AgNPs changed the composition of the soil bacterial community in SA, the relative abundance of some bacterial taxa in SA and SU, and fungal richness in SU at 30 days of incubation. However, AgNPs did not affect the activity of extracellular enzymes. This study demonstrates that the exposure time and organic amendments modulate the effects of realistic concentrations of AgNPs in the biomass and composition of the microbial community of a Mediterranean soil.

Keywords: Engineered nanoparticles, Emergent contaminants, Soil microorganisms, Organic matter, Biodiversity, Microbial biomass

1. Introduction

Nanoparticles (NPs) are used as additives in a variety of commercial products due to their unique physico-chemical properties (Mohan et al., 2019). Among the NPs, silver nanoparticles (AgNPs) are widely utilized in various commercial products worldwide including agrochemicals, coatings, medical devices, textiles and food packaging, owing to their antimicrobial properties (Doolette et al., 2016; Li et al., 2017). AgNPs have been found to be toxic at different trophic levels, including bacteria (Saravanan et al., 2018), fungi (Bocate et al., 2019), algae (Kleiven et al., 2018), plants (Das et al., 2018), nematodes (Rossbach et al., 2019) and crustaceans (An et al., 2019). Because of the increased use of AgNPs in consumer products, there is a potential risk involved in releasing AgNPs into the environment during all stages of their life cycle, including production, use and disposal (Gottschalk et al., 2013; Mahapatra et al., 2015; McGillicuddy et al., 2017). Indeed, AgNPs have been found in various environments, including oceans, fresh water, wastewater, air and soils (Cervantes-Aviles et al., 2019; Zhang et al., 2019).

AgNPs enter the soil environment through several pathways, including direct applications of nanopesticides or nanofertilizers containing AgNPs (Ma et al., 2016; Pandey et al., 2018) and through biosolid amendments (Lead et al., 2018). Therefore, in the last few years there has been a worldwide effort to understand the impact, fate, transformations, and toxicity mechanisms of AgNPs in soil ecosystems (Li et al., 2017; Rahmatpour et al., 2017; Lead et al., 2018; McGee et al., 2018). In addition, some studies have predicted the concentrations of AgNPs in soil based on probabilistic models (Sun et al., 2014; Coll et al., 2015; Giese et al., 2018). According to Giese et al. (2018), the predicted environmental concentration of AgNPs in an agricultural soil is in the range of $0.0094–0.79223$ µg Kg^{-1} in a 100% persistent scenario, with a maximum concentration of AgNPs of 1.52 µg kg^{-1} for 2017 and 10 µg kg^{-1} predicted for 2050. However, studies regarding the impact of environmental and relevant concentrations of AgNPs on the biomass, activity and diversity of soil microbial communities are still scarce (Grün et al., 2019; Montes de Oca-Vásquez et al., 2020).

Soil microbial communities drive many ecosystem functions (Schloter et al., 2018; Delgado-Baquerizo et al., 2020) and contribute greatly to nutrient transformations and decomposition of organic matter (Kallenbach et al., 2016; Delgado-Baquerizo et al., 2018; Schloter et al., 2018), which are indispensable in maintaining healthy and fertile soils (McGee et al., 2018). Soil pollutants can affect microbial communities, as microbes are very sensitive to environmental perturbations (Bastida et al., 2008c; Zornoza et al., 2015; Bünemann et al., 2018).

Several studies have demonstrated that the toxicity of AgNPs in soil microbial communities is controlled by multiple soil physico-chemical properties, such as organic matter and clay content, pH, moisture, and ionic strength (Bundschuh et al., 2018; Grün and Emmerling, 2018; Makselon et al., 2018), as well as by the shape, size, surface charge, surface coating and concentration of the AgNPs themselves (Klitzke et al., 2015; Pachapur et al., 2016; Rahmatpour et al., 2017; Grün et al., 2019). Besides bacteria and fungi, other soil organisms can interact with NPs. For instance, a variety of metabolites and macromolecules produced and secreted by plant roots and soil fauna (Badri et al., 2012; Hayashi et al., 2016), can interact with the AgNPs and consequently affect their mobility, agglomeration, deposition, bioavailability and toxicity (Abbas et al., 2020).

In soil ecosystems AgNPs interact with organic matter (OM), affecting their aggregation, agglomeration and transformation (i.e., sulfidation and dissolution) (Pachapur et al., 2016; Li et al., 2017; Makselon et al., 2018; Sutton et al., 2019), which influence their mobility (González-Fuenzalida et al., 2018), bioavailability (Pachapur et al., 2016), and toxicity. The above-mentioned interactions depend on the OM molecular weight and the OM components (such as humic and fulvic acids), as well as the AgNPs coating (Coutris et al., 2012; Yang et al., 2014; Adrian et al., 2019). For instance, Klitzke et al. (2015) observed that the OM from soil solutions decreased the release of Ag^+ from bare AgNPs. However, Peyrot et al. (2014) determined that the OM did not increase the dissolved Ag^+ compared with the unamended soil. Besides, humic acids have been found to decrease the AgNPs aggregation and the soil adsorption (Bae et al., 2013).

In addition, previous research has found some impacts of AgNPs on soil ecosystems, including effects on enzyme activities, microbial biomass, microbial diversity, nitrification, and decomposition of organic matter (Eivazi et al., 2018; Grün et al., 2018). However, most of these studies have been performed with unrealistically high concentrations of AgNPs in comparison to their content in soils (Samarajeewa et al., 2017). Currently, there is a lack of research addressing the impact of realistic AgNPs concentrations that can potentially affect the composition, biomass and functioning of soil microorganisms (McGee et al., 2018).

The Mediterranean semiarid climates have fragile soils with low amounts of organic matter and microbial biomass, resulting from adverse climatic conditions or inadequate agricultural management (García et al., 1994; Bastida et al., 2006). Given the low microbial biomass of these soils, a potential harmful effect (i.e. nanoparticles) can be of particular concern in these soils. In these environments, the application of organic amendments (i.e. composts, sludges, etc.) is a common practice to slow down soil degradation and to promote soil fertility through increased soil

nutrient content, soil physical structure and the activity and biomass of microbial communities (Tejada et al., 2006; Bastida et al., 2008b; Tejada et al., 2010; Bastida et al., 2013). Therefore, the impacts of AgNPs can be of particular concern in agricultural Mediterranean soils, were the implications of the interaction between AgNPs and organic amendments (and related increases in OM) on soil microbial communities are scarcely known (Peyrot et al., 2014). In order to fill this gap, we carried out a study using a Mediterranean soil with and without organic amendment as a proxy to understand the interaction between real environmental concentrations of AgNPs and organic amendments in soil. In light of recent literature, we hypothesized that (1) there is a dose-dependent effect of NPs on the soil microbial community, and (2) soil chemical and microbial changes associated with organic amendments can influence the toxicity of AgNPs on bacterial and fungal communities. The knowledge provided by this type of investigation is fundamental if we wish to understand the real impacts of NPs on the environment and, in particular, on soils that need inputs of organic matter to sustain productivity in the face of global change (Huang et al., 2016).

2. Materials and methods

2.1. Silver nanoparticles and characterization

Spherical citrate-stabilized AgNPs were obtained froma commercial supplier (Sigma-Aldrich, St. Louis, USA), with a mean particle size of 40 nm ± 4 nm as determined by transmission electron microscopy (TEM), according to the manufacturer. In addition, the size and shape of the AgNPs were confirmed by TEM (Fig. S1, Supporting information). The hydrodynamic diameter, the polydispersity index (PDI) and the surface charge of the AgNPs (ζ-potential) were determined as described previously (Montes de Oca-Vásquez et al., 2020), prior to their use in the microcosms experiment. The hydrodynamic diameter of AgNPs was 32.99 ± 0.47 nm, the ζ-potential was −34.6 ± 2.39 mV and the PDI was 0.532 ± 0.006. The AgNPs suspension was stored in the dark at 4 °C.

2.2. Soil studied

A 1 ha plot in a lemon orchard with intensive agricultural use was selected in the municipality of Totana (37°44′14.5"N, 1°26′10.3"W), Murcia, Spain. The soil formed in limestone parent material, and was classified as a calcaric Fluvisol (IUSS Working Group WRB, 2015) with a sandy clay loam texture (clay content of 21.6%). The main soil physico-chemical properties are shown in Table 1. The climate is cold semi-arid according to the Köppen-Geiger climate classification. The

average annual temperature is 16.9 °C, and average precipitation is 311 mm/yr. The average elevation of the study area is 200 m above sea level (m.a.s.l).

Table 1. Main soil physico-chemical properties.

	SU	SA
Total N (g 100g^{-1})	0.09±0.01	0.16±0.04
Total C (g 100g^{-1})	3.75±0.08	4.33±0.45
Organic C (g 100g^{-1})	0.61±0.03	1.28±0.08
CaCO$_3$ (%)	26.12±0.47	25.70±4.25
pH (1:5)	8.78±0.20	8.32±0.19
Electrical conductivity (1:5) (dS/m)	0.41±0.07	1.67±0.02

Total N = total nitrogen, Total C = total carbon, Organic C = organic carbon. SU = unamended soil, SA = soil amended with compost. The values represent the average ± standard deviation of 3 replicate samples.

2.3. Experimental design

Twenty soil samples were collected from the study area to a depth of 15 cm, and were mixed to form a single composite sample. Branches and other large woody litter were removed and the soil was then air dried and sieved (2-mm mesh). One part of the soil received organic amendment by the addition of 5% (w/w) of compost (ovine manure certified for organic agriculture). The compost used had a pH (1:10) of 7.7, an electrical conductivity (EC) (1:10) of 13 dS/m, an organic carbon content of 33% and humic acids content of 4.6% on a dry weight basis, and its N-P-K ratio was 1.5–1.8–4.6. This amendment resulted in an increase in the soil organic matter to 2.20%. The relative abundance of the taxa at the genus level in the soil unamended (SU) and soil amended with compost (SA) are present in Table S1, and the unique and shared operational taxonomic units (OTUs) between the SU and SA are present in Fig. S2. The effects of different doses of AgNPs in soil microbial communities were evaluated through a microcosm experiment under controlled conditions. Given the relatively unknown effects of AgNPs, a controlled microcosm assay was required before scaling up future approaches in the field.

The microcosms were performed in plastic pots filled with 60 g of soil amended with compost and unamended. An aqueous commercial dispersion of AgNPs (0.02 mg ml^{-1}) was used to obtain two working solutions prepared in ultrapure water immediately before use. Then, a 1 ml aliquot of each AgNPs working solution was added in small drops onto the soils (SA and SU), to obtain final concentrations of 0.015 µg kg^{-1} (AgNP$_{0.015}$) and 1.5 µg kg^{-1} (AgNP$_{1.5}$). Negative control microcosms treated with the same volume of ultrapure water without AgNPs were prepared. The soils were manually mixed to obtain a uniform distribution. Two incubation periods to AgNPs, 7

and 30 days of incubation (DOI), were independently studied. Three replicates of each treatment were established. The microcosms were placed randomly in a chamber (Equitec, EGCS 501 3SHR model, Spain) and incubated in the dark at 28 °C. During the incubation period, soil water content was gravimetrically controlled at 50% of water holding capacity (WHC). Given the relatively low AgNPs concentrations, we did not expect an effect on the soil WHC (Gil-Díaz et al., 2014; Komendová et al., 2019). Before applying AgNPs, the microcosms were pre-incubated in the above-mentioned conditions for 15 days in order to adjust microbial communities to organic amendment, temperature and water content. This experimental design resulted in 36 microcosms, including three replicates per treatment. After each incubation period, the soil from each microcosm was stored at 4 °C for analysis of extracellular enzyme activities and fatty acids content. In addition, 3 g of soil from the 30 DOI were kept at −20 °C for DNA extraction and sequencing.

2.4. Physico-chemical and chemical analysis of soil samples

Total organic carbon (TOC) and total nitrogen (N) were analyzed using an Elemental Analyzer (C/N Flash EA 1112 Series-Leco Truspec). A soil to water ratio of 1:5 (w:v) was used for the determination of the EC and the pH of the two soil types using a pH meter and a conductivity meter (Crison 2001, Barcelona, Spain), respectively.

2.5. Microbial biomass and enzyme activities

Ester-linked fatty acid methyl esters (FAMEs), hereafter referred to as "fatty acids", were extracted from 3 g of soil according to Schutter and Dick (2000). Fatty acids were analyzed as described elsewhere (Montes de Oca-Vásquez et al., 2020). All the FAMEs mentioned in this study have been described following the standard nomenclature (Vestal and White, 1989). Therefore each fatty acid was named using the total number of C atoms:number of double bonds, followed by information about double bonds, if necessary, i.e. unsaturation positions (ω) in the molecule. Prefixes such as "a" and "i", for anteiso- and iso-, complete the nomenclature. The fatty acids i15:0, a15:0, i16:0, i17:0, 16:1ω9, cy17:0, cy19:0, 10Me16:0 and 10Me18:0 were indicators of the bacterial biomass (Frostegård et al., 1993; Dungait et al., 2011), and the fatty acid 18:2ω6 was considered to represent the fungal biomass (Brant et al., 2006; Rinnan and Baath, 2009). The sum of i15:0, a15:0, i16:0, i17:0, 10Me16:0 and 10Me18:0 were considered to represent Gram-positive (Gram+) fatty acids, and 16:1ω9, cy17:0 and cy19:0 were summarized as indicators of Gram-negative (Gram-) fatty acids (Frostegård et al., 1993; Dungait et al., 2011). The

actinobacterial representative fatty acids were 10Me16:0 and 10Me18:0 (Dungait et al., 2011). Urease activity was measured by colorimetric NH_4^+ determination according to the buffer method described by Kandeler and Gerber (1988). Alkaline phosphatase and β-glucosidase activities were assayed by the methods of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977), respectively, which involve the measurement of *p*-nitrophenol (PNP) released and expressed as µmol PNP g^{-1} h^{-1}.

2.6. DNA extraction, amplification and sequencing

The FastPrep Instrument and the FastDNA Spin Kit for soil (MP Biomedicals, Santa Ana, CA, USA) were used for DNA extraction from 0.5 g of freeze-dried soil sample. The barcoded primers 515F and 806R were used to amplify the V4 region of bacterial 16S ribosomal RNA (rRNA) genes (Caporaso et al., 2012). Barcoded primers gITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) were used to target the fungal internal transcribed spacer 2 (ITS2) region. Further details on PCR conditions have been described in detail elsewhere (Žifčáková et al., 2016). The MinElute PCR Purification kit (Qiagen) was used for post amplification cleanup and the DNA concentrations were quantified using a Qubit 4 fluorometer (ThermoFisher Scientific). After libraries were prepared (MiSeq Reagent Kit v3, Illumina), sequencing of fungal and bacterial amplicons was done on an Illumina MiSeq instrument using 2 × 300 bp paired-end reads. The SEED2 pipeline (Větrovský et al., 2018) was used for processing the raw reads. FASTQ-join (Aronesty, 2013) was used for merging pair-end reads. Before processing the ITS2 region was detected and extracted with ITS EXTRACTOR 1.0.8 software (Nilsson et al., 2010). Detection and filtering of chimeric sequences, and generation of clusters was done using USEARCH (Edgar, 2013) at a 97% similarity level. For each cluster a consensus sequence was constructed and the closest hits at the genus level were identified using BLASTn software against the Ribosomal Database Project for bacteria (Cole et al., 2014) or UNITE for fungi (Nilsson et al., 2019). Sequences identified as non-bacterial or non-fungal were discarded. For fungal and bacterial analyses, low-abundance OTUs (with less than 0.1% of the sequences) were filtered. The sequences of 16S rRNA genes and ITS2 regions have been deposited in the MG-RAST (ID mpg94756).

2.7. Data analysis

Shapiro-Wilk and Levene tests were used to verify the data normality and the homogeneity of variances, respectively. Further, the FAMEs content and enzyme activity data at each exposure time (7 and 30 DOI) and soil (SA and SU) were analyzed independently through one-way analysis

of variance (ANOVA) and post-hoc Tukey's significant difference test ($P < 0.05$). All results were reported as means ± standard deviation. Statistical analyses were conducted using IBM SPSS (version 20.0; SPSS Inc., Chicago, IL, USA). The relative abundance of fatty acids was subjected to principal component analysis (PCA) using PAST 3.26 (Hammer et al., 2001). The effect of the fixed factor AgNPs treatment on microbial community composition was assessed using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) with the Euclidean distance for the Hellinger-transformed bacterial and fungal composition (Legendre and Gallagher, 2001) used as a response matrix. PERMANOVA was performed with the Adonis function of the vegan package (Oksanen et al., 2019) in the R software environment (R Core Team, 2019). A PCA that represented the Hellinger distances among sampling units (Legendre and Legendre, 2012) was used to visualize the results of community composition. The rda function in the vegan package was used to perform the PCAs. Only the most abundant OTUs (> 0.1%) were used in the analysis. The relative abundances of the fungal and bacterial populations at the phylum/class and genus levels were analyzed independently through one-way ANOVA and post-hoc Tukey's significant difference test ($P < 0.05$). Diversity indices (richness, Shannon-Wiener and Chao-1) were obtained from the matrix of OTUs abundances using PAST 3.26 (Hammer et al., 2001). Venn diagrams were created using the program Venny (Oliveros, 2007) to visualize the fungal and bacterial OTUs that were unique and shared between SU and SA.

3. Results

3.1. Microbial biomass, community structure estimated by FAMEs analysis and enzyme activities

In the SA treatments, after 7 DOI, the AgNPs significantly decreased the bacterial and Gram+ fatty acids contents ($P < 0.05$), compared to the control in both AgNPs concentrations (AgNP$_{0.015}$ and AgNP$_{1.5}$). Otherwise, the fatty acid content of Gram- bacteria only decreased for AgNP$_{0.015}$. However, at 30 DOI, only AgNP$_{1.5}$ significantly reduced the Gram+ fatty acids content ($P < 0.05$) (Fig. 1). In contrast, in the SU treatments, we did not find a statistically significant effect of AgNPs on the fatty acids content of the different microbial groups at 7 DOI, while at 30 DOI, there was a significant increase in the bacterial and Gram- fatty acids content in AgNP$_{0.015}$ ($P < 0.05$) (Fig. 1).

Fig. 1. Microbial fatty acids content (nmol g^{-1}) of amended (SA) and unamended (SU) soils, treated with two AgNP concentrations (AgNP$_{0.015}$ = 0.015 µg kg^{-1}, AgNP$_{1.5}$ = 1.5 µg kg^{-1}), at 7 and 30 days of incubation. A) SA at 7 days, B) SA at 30 days, C) SU at 7 days, and D) SU at 30 days. Different letters indicate significant differences ($P < 0.05$) within the same microbial group and the same soil management.

We studied microbial community structure using PCA of the relative abundance of fatty acids (Fig. 2). The structure of the microbial community strongly differed between SA and SU (Fig. S3). We then separately performed PCA at 7 and 30 DOI for SA and SU. In general, the first two components explained between 58.14–69.00% and 14.38–29.72% of the variance, respectively. At 7 DOI, PC1 clearly separated AgNP$_{0.015}$ from the control and AgNP$_{1.5}$ in SA (Fig. 2A). At 30 DOI, PC1 differentiated AgNP$_{1.5}$ from the control and AgNP$_{0.015}$ (Fig. 2B). In the SU, at 7 DOI, there was no clear separation of the treatments (Fig. 2C); however, at 30 DOI, PC1 separated AgNP$_{0.015}$ from the control and AgNP$_{1.5}$ (Fig. 3D). Results of the one-way ANOVA performed separately for SU and SA revealed no significant differences ($P > 0.05$) in the soil enzyme activities between the two AgNPs concentrations and the control (Table S2).

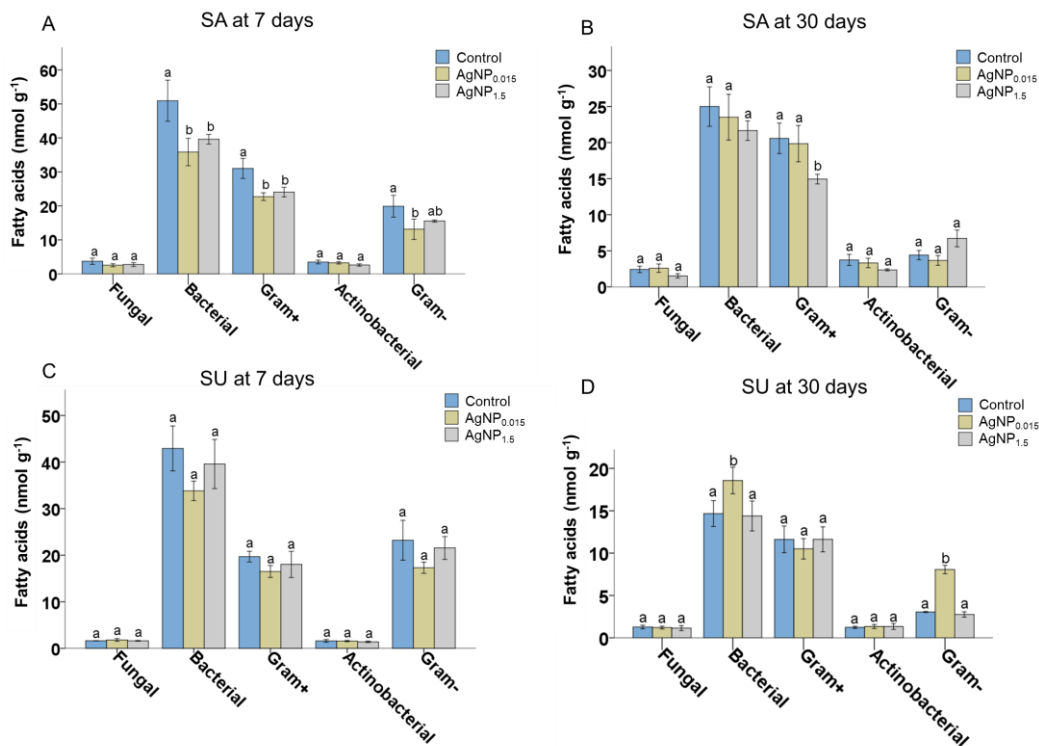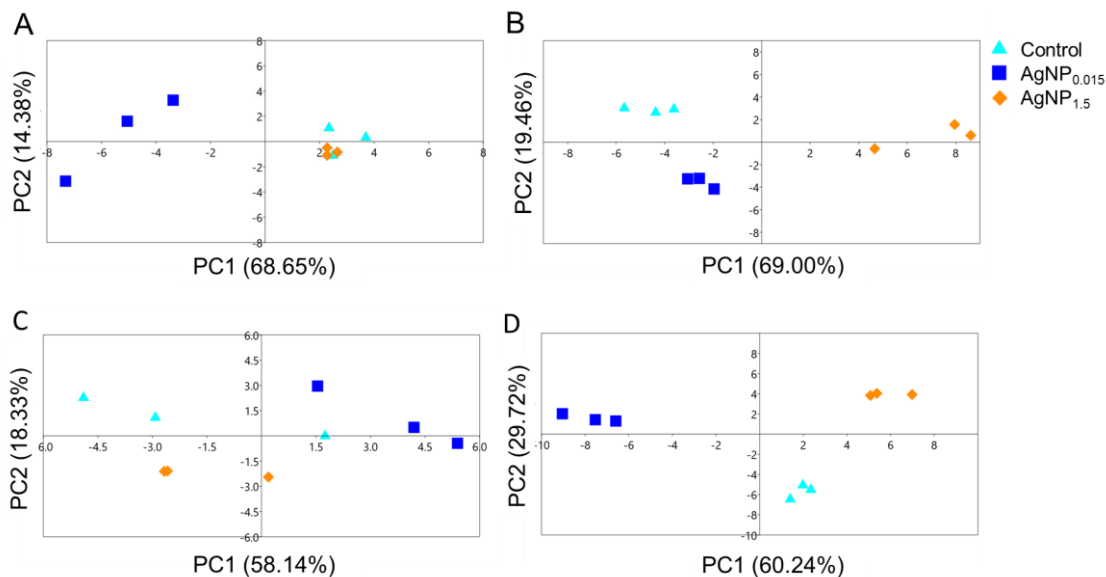Fig. 2. Principal component analysis (PCA) of the relative abundance of fatty acids in amended (SA) and unamended (SU) soils, treated with two AgNP concentrations (AgNP$_{0.015}$ = 0.015 µg kg^{-1}, AgNP$_{1.5}$ = 1.5 µg kg^{-1}), at 7 and 30 days of incubation. A) SA at 7 days, B) SA at 30 days, C) SU at 7 days, and D) SU at 30 days. Values in parenthesis denote the percentage of the data variation explained by a given component.

3.2.   Microbial diversity and composition estimated by DNA sequencing

The results of the PCA of Hellinger-transformed distances for fungal and bacterial communities revealed that the effect of organic amendment in the composition of the microbial community was much stronger than that of AgNPs dosage (Fig. S4). A PCA of the Hellinger transformed distances of fungal communities did not reveal significant changes in community composition between the control and the two AgNPs concentrations after 30 DOI either for SU (PERMANOVA P-value =0.638) (Fig. 3A), or for SA (PERMANOVA P-value = 0.475) (Fig. 3B). Furthermore, the composition of bacterial communities studied through 16S rRNA gene sequencing showed significant changes due to AgNPs in SA (PERMANOVA P-value = 0.03) (Fig. 3D), but not in SU (PERMANOVA P-value = 0.106) (Fig. 3C).
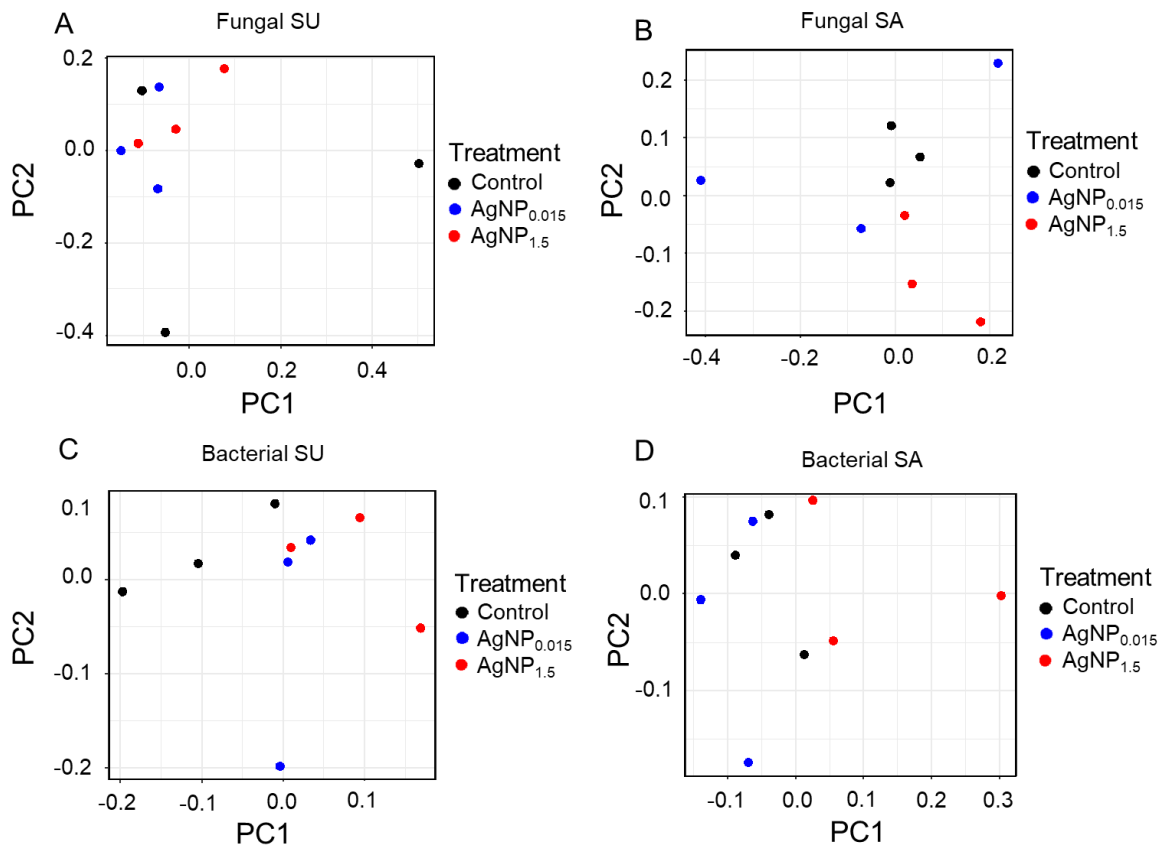
Fig. 3. Principal component analysis (PCA) of the Hellinger-transformed distances based on ITS2 (fungal community) and 16S rRNA gene (bacterial community) sequencing in amended (SA) and unamended (SU) soils, treated with two AgNP concentrations (AgNP$_{0.015}$ = 0.015 µg kg^{-1}, AgNP$_{1.5}$ = 1.5 µg kg^{-1}), at 30 days of incubation. A) Fungal in SU, B) Fungal in SA, C) Bacterial in SU, and D) Bacterial in SA.

The most abundant fungal phylum (in both SU and SA) was Ascomycota, which constituted more than 83% of the sequences (Fig. 4A). In the SU, the most abundant genera were *Fusarium*, *Cladosporium*, *Morteriella* and *Alternaria*, together accounting for more than 45% of the sequences (Fig. 4B). In addition, in the SA the most abundant genera were *Alternaria*, *Fusarium*, *Cladosporium*, *Penicillium* and *Aspergillus*, which together accounted for more than 59% of the sequences (Fig. 4B). The relative abundance of fungal phyla and genera did not change significantly between the AgNPs treatments in SA and SU. The most abundant bacterial phyla and classes were Alphaproteobacteria, Actinobacteria, Gammaproteobacteria and Firmicutes, which together accounted for more than 73% of the sequences in the SU, and more than 67% of the sequences in the SA (Fig. 4C). At the genus level, the most abundant taxa in the SU soils were *Lysobacter*, *Erythrobacter*, *Nocardioides* and *Sphingomonas*, which made up more than 37% of the sequences (Fig. 4D). In SU the phylum Acidobacteria was less abundant in AgNP$_{1.5}$

than in the control (P-value = 0.012), while the phylum Gemmatimonadetes was less abundant in AgNP$_{0.015}$ than in the control (P-value = 0.037). The genus *Bacillus* was more abundant in AgNP$_{1.5}$ than in the control (P-value = 0.043), while *Gemmatimonas* was less abundant in AgNP$_{0.015}$ (P-value = 0.014), and *Iamia* was less abundant in AgNP$_{1.5}$ (P-value = 0.003), than in the control. In addition, the most abundant genera in the SA soils were *Sphingomonas*, *Gemmatimonas*, *Nocardioides* and *Lysobacter*, together accounting for more than 27% of the sequences (Fig. 4D). The class Betaproteobacteria was significantly less abundant in the AgNP$_{1.5}$ than in the control (P-value = 0.044). At the genus level, a significant increase in the relative abundance of *Lysinibacillus* was found in AgNP$_{1.5}$ compared to the control (P-value = 0.024).
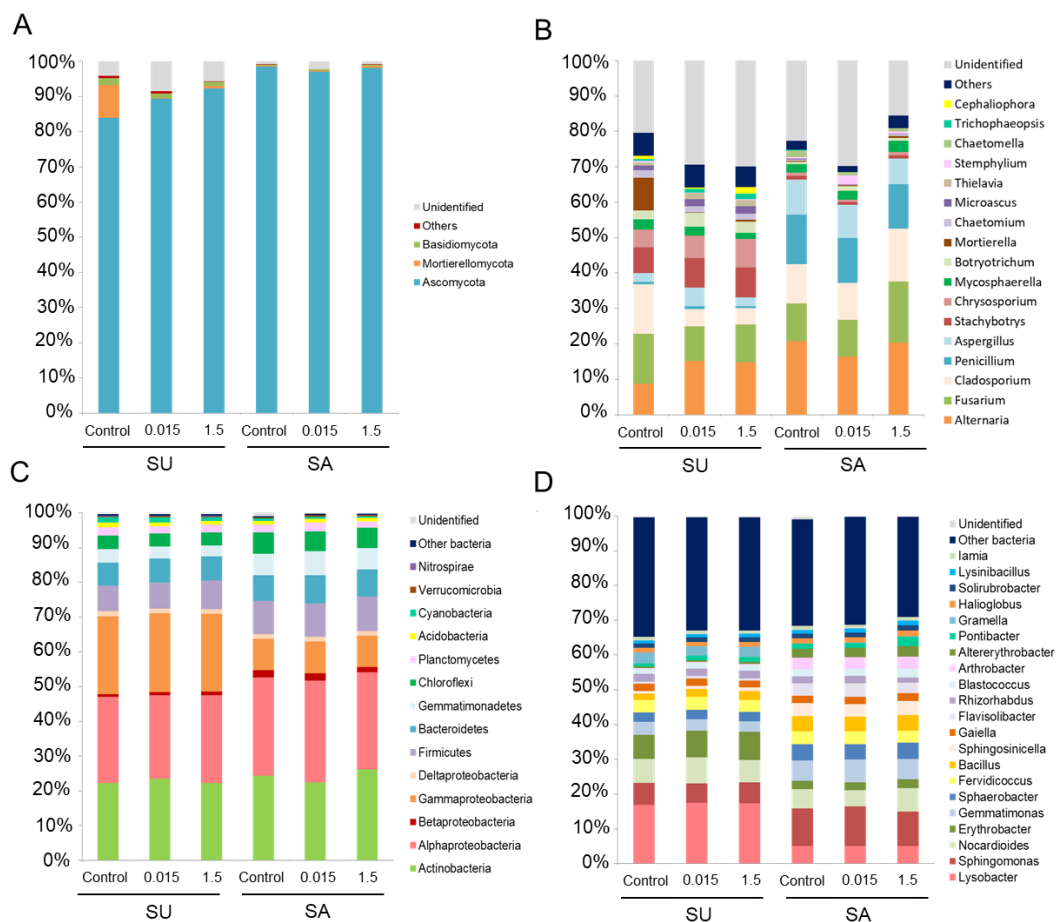
Fig. 4. Relative abundance of fungal and bacterial populations at the phylum/class and genus levels in amended (SA) and unamended (SU) soils, treated with two AgNP concentrations (0.015 = 0.015 µg kg^{-1}, 1.5 = 1.5 µg kg^{-1}), at 30 days of incubation. A) Fungal populations at the phylum level, B) Fungal populations at the genus level, C) Bacterial populations at the phylum/class level, and D) Bacterial populations at the genus level. The figures include taxa with relative abundance ≥ 0.1% for both fungi and bacteria.

Furthermore, in SU, the richness of the fungal community significantly decreased in $AgNP_{1.5}$ compared to the control ($P < 0.05$). In SA, the richness of bacteria decreased in $AgNP_{1.5}$ compared to $AgNP_{0.015}$ ($P < 0.05$) (Table 2). Neither Chao-1 nor Shannon-Wiener indices showed significant differences between AgNPs treatments and the control, in both SA and SU (Table S3).

Table 2. Bacterial and fungal richness in control and AgNP- treated soils.

Treatments	Bacteria	Fungi
SU		
Control	1989±674a	322±29.9a
$AgNP_{0.015}$	1622±192a	256±147ab
$AgNP_{1.5}$	1526±255a	225±12.5b
SA		
Control	1617±350ab	228±46.2a
$AgNP_{0.015}$	1877±210a	259±92.0a
$AgNP_{1.5}$	1263±4.72b	238±23.5a

Data are shown as average ± standard deviation. Different letters indicate significant differences ($P < 0.05$) within the same microbial group and the same soil management. SU = unamended soil, SA = soil amended with compost. $AgNP_{0.015}$ and $AgNP_{1.5}$ represent two AgNPs concentrations ($AgNP_{0.015}$ = 0.015 µg kg^{-1}, $AgNP_{1.5}$ = 1.5 µg kg^{-1}, respectively).

4. Discussion

4.1. Microbial biomass and enzyme activities

Biological effects of AgNPs in soil are strongly dependent on physico-chemical soil properties (Cornelis et al., 2010; Bundschuh et al., 2018; Grün et al., 2018) and AgNPs characteristics (Gwin et al., 2018), and it has been reported that organic amendments have a strong physico-chemical impact on soil (Bastida et al., 2008a; Bastida et al., 2008b). We found that the organic amendment enhanced the negative effect of AgNPs on soil microbial biomass (as estimated by fatty acids content), particularly in the Gram+ and Gram- bacterial biomass at 7 DOI and in Gram+ biomass at 30 DOI. On the contrary, we found that the bacterial biomass, including Gram-, increased in SU after 30 days of AgNPs application at the lowest concentration. The negative effects found in the SA could be attributed to the exchange and/or the adhesion to the citrate anion by the humic acids, which are more abundant in the SA due to the compost addition. This could be explained by the fact that the carboxylic acids of the citrate are weakly associated to the AgNPs surface, and provide a negatively charged surface that could be displaced by many other molecules, such as humic acids. This assumption has been supported by others, that reported that humic acids can bind to AgNPs inducing an increase in their negative surface charge, increasing the electrostatic repulsion between AgNPs, as well as between AgNPs and the soil matrix, conferring stability from aggregation, thus decreasing its precipitation (Diegoli et al., 2008; Bae et al., 2013;

Yecheskel et al., 2018). The increase in the AgNPs stability by organic amendment could be associated with a higher toxicity, because the stabilized AgNPs can be more bioavailable and be responsible for the rapid toxic effects on the microbial biomass observed at 7 DOI. Nevertheless, in the SU, an increase in particle size by a greater aggregation of AgNPs (Choi and Hu, 2008; Calder et al., 2012; McGee et al., 2017; Bollyn et al., 2018), could result in lower toxicity towards the soil microbial community. Moreover, in the SA, when the incubation time increased to 30 DOI, the impact of AgNPs on microbial biomass was not noticeable, except in the Gram+ biomass at the highest AgNPs concentration. Similarly, Grün et al. (2019) reported that as exposure time increased, damage to the microbial biomass decreased. This finding could indicate that the microbial community could have experienced an adaptive rebound that enhanced growth after long-term exposure to NPs (Bao et al., 2016; Durenkamp et al., 2016; Tang et al., 2018). In addition, in SA, we found that the impacts of AgNPs were dependent on the microbial group, with the fungal and Gram- biomasses being more tolerant than Gram+ bacteria, as also reported elsewhere (Kumar et al., 2012; Kumar et al., 2014; Liu et al., 2017; Gómez-Sagasti et al., 2019). This differential sensitivity could be explained by differences in the structure of cell membranes and walls that could determine the degree of penetration of Ag^+ into the cell. Fungal cell walls are thicker than those of bacteria (Gow et al., 2017; Xie et al., 2017), and the Gram- outer membrane lipopolysaccharides have been shown to confer resistance against Ag^+ penetration into the cell (Marambio-Jones and Hoek, 2010; Jacobson et al., 2015), providing a major protective effect in these microbial groups compared to Gram+ bacteria. Furthermore, it has been reported that Gram- bacteria are more dependent on labile carbon compounds derived from plant biomass, whereas Gram+ bacteria use more complex carbon compounds derived from soil organic matter, although this depends on availability (Kramer and Gleixner, 2008; Fanin et al., 2019). Thus, Gram- bacteria could take advantage of the citrate coating from the AgNPs, an easily assimilable carbon source, when the AgNPs concentration is low ($AgNP_{0.015}$) and therefore has not affected the microbial biomass. This capacity of the Gram- bacteria might explain the stimulating effect detected in the SU at 30 DOI. However, in the SA, these citrated-mediated effects on Gram- bacteria were very slight because the effect of the small amount of citrate is probably not comparable with that of the available C in organic amendments. Nevertheless, further studies using a citrate control are necessary to elucidate this assumption.

The PCA of relative abundance of fatty acids indicated a AgNPs induced change in microbial community structure in both SA and SU at 30 DOI, and also in SA at 7 DOI. These changes are consistent with the above-mentioned results, suggesting that compost enhanced the negative

impact of AgNPs in soil. Furthermore, the change in microbial community structure in SU at 30 DOI may be explained by the previously noted increase in Gram- biomass. Other authors have found shifts in the structure of microbial communities after AgNPs application at much higher concentrations than those used in this study (Durenkamp et al., 2016; Chen et al., 2017). While the effects of AgNPs on microbial biomass can be significant depending on the microbial group, we did not find a significant effect in the extracellular enzyme activities evaluated. In this regard, the results reported in the literature are contradictory, with authors finding either increases (Cao et al., 2017), decreases (Liu et al., 2017; McGee et al., 2017; Rahmatpour et al., 2017; Eivazi et al., 2018), or no changes in enzyme activities after AgNPs treatments (Peyrot et al., 2014; Asadishad et al., 2018; Grün et al., 2018). This disagreement about the effects of AgNPs on soil extracellular enzymes may be because toxicity depends on several properties like AgNPs size, type, and concentration, type of enzyme, exposure time (Li et al., 2017; Asadishad et al., 2018) and physico-chemical properties of the soil (Grün et al., 2019). Further, our results suggest that the impacts of low and realistic AgNPs concentrations in the extracellular environment are negligible, while the impacts at the level of microbial biomass are stronger. This negative effect on microbial biomass could have an impact on plant nutrient availability and therefore in crop productivity, as microbial biomass plays a fundamental role in controlling ecosystem functioning by constituting a sink and a source of nutrients, such as carbon, nitrogen, phosphorous, and sulfur (Aponte et al., 2010; Kuzyakov, 2010; Singh and Gupta, 2018).

4.2. *Microbial community composition and diversity*

The application of organic amendments had a strong influence on the composition of soil fungal and bacterial communities (Fig. S2), as noted elsewhere (Bastida et al., 2013; Bastida et al., 2015; Cozzolino et al., 2016; Chen et al., 2017; Federici et al., 2017; Sadet-Bourgeteau et al., 2019). Similar to previous investigations using higher concentrations of AgNPs (Colman et al., 2013; Liu et al., 2017; Tlili et al., 2017; Yan et al., 2020; Zhang et al., 2020), our findings demonstrate that environmentally realistic concentrations of AgNPs affected bacterial community composition to a greater degree than they did fungal communities. These results suggest that fungal communities are more resistant to AgNPs than bacterial communities (Kumar et al., 2014; Sillen et al., 2015). Fungi are known to cope with metal-stress more effectively than bacteria (Sillen et al., 2015), yet there is little information about the effects of AgNPs on soil fungal community composition (Sweet and Singleton, 2015; McGee et al., 2018), specifically using high-throughput DNA sequencing (Kumar et al., 2014; Vitali et al., 2019; Montes de Oca-Vásquez et

al., 2020). Overall, the effects of AgNPs on bacterial community composition were evident in the SA, suggesting that organic amendment enhances the impacts of AgNPs on the microbial composition. The microbial community composition could influence key ecosystems services, including the decomposition and turnover of organic matter in soil (Schimel and Schaeffer, 2012). While some authors have reported that changes in the microbial community composition affects soil organic carbon turnover (Don et al., 2017; Han et al., 2017), and nitrogen cycling process (Zheng et al., 2019), others suggested that due to the functional redundancy of microbial communities, changes in the soil microbial community composition do not play such as significant role (Graham et al., 2016; Dai et al., 2017). However, the link between microbial community composition and ecosystem services is still poorly understood (Dai et al., 2017; Zheng et al., 2019), and needs further research.

Otherwise, in this study the relative abundance of *Sphingomonas* and *Arthrobacter* were higher in SA than SU. Previous studies demonstrated that compost amended can change the diversity and composition of soil bacterial communities (Liang et al., 2018; Sigmund et al., 2018; Xiang et al., 2018), due to the increase in nutrient availability and the introduction of additional microorganisms into the native microbial communities (Zhen et al., 2014; Sigmund et al., 2018). However, most of the compost endogenous microflora fail to invade the soil environment (Saison et al., 2006; Mallon et al., 2018). Similar to our results, *Arthrobacter* increased in compost treated soil compared to the soil without compost, as observed by other authors (Liang et al., 2018). Moreover, *Sphingomonas* has been reported to increase in the composted pig manure amended rhizosphere soil (Xiang et al., 2018). Furthermore, Le et al. (2017), found that the addition of humic acid stimulated the growth of *Sphingomonas* in soil. Likewise, as reported by Wu et al. (2016), the relative abundance of *Lysobacter* decreased in the soil amended with compost.

Furthermore, AgNPs impacted different bacterial taxa in SA and SU. In agreement with other studies, we observed decreases in the relative abundance of Betaproteobacteria in SA (Grün and Emmerling, 2018), as well as Acidobacteria (Wang et al., 2017; Grün and Emmerling, 2018) and Gemmatimonadetes in SU (Huang et al., 2017; Cao et al., 2018), after AgNPs exposure. These microbial populations are generally associated with essential functions related to soil fertility. For instance, Betaproteobacteria plays a major role in organotrophic denitrification (Coyotzi et al., 2017; Bellini et al., 2018), Gemmatimonadetes in nitrogen fixation (Wang et al., 2019) and Acidobacteria is related to cellulose degradation (Štursová et al., 2012).

Additionally, fungal richness decreased with the highest concentration of AgNPs in the SU, similar to the results reported by Vitali et al. (2019). The reduction in fungal richness in the absence of changes in fungal biomass suggests that certain tolerant fungal populations can occupy the niche left by more sensitive populations that were affected by AgNPs (Rath and Rousk, 2015; Samarajeewa et al., 2017). This could be the case for *Alternaria* and *Chrysosporium*, both reported as metal tolerant microorganisms, whose abundance seems to increase after AgNPs exposure (Ali-Shtayeh and Jamous, 2000; Požárová et al., 2001; Mohammadian et al., 2017). Although most studies demonstrating the effects of AgNPs to soil microbial communities have been carried out in microcosm experiments, similar negative impacts have been reported in field mesocosms studies (Hänsch and Emmerling, 2010; Colman et al., 2013; Shah et al., 2014; Liu et al., 2017). Given that the effects of AgNPs in soil-systems are still not sufficiently known, microcosm studies are needed to understand the effects of AgNPs on microbial communities under controlled settings.

5. Conclusions

Our study reveals the effects of environmentally realistic concentrations of AgNPs on the microbial community of a Mediterranean soil and highlights the interactive role of organic amendments in the impacts of AgNPs. We conclude that organic amendment in semiarid Mediterranean soils could increase the toxicity of AgNPs in terms of soil microbial biomass and community composition. However, AgNPs did not affect extracellular enzyme activities that mediate biogeochemical transformations. In addition, we found that bacteria were more sensitive to AgNPs than fungi, and Gram- bacteria more tolerant than Gram+ bacteria. According to our data, care must be taken when applying organic amendments to soils contaminated with AgNPs, or when using amendments derived from wastewater biosolids/sewage sludge that may contain high concentrations of AgNPs or Ag_2S in semiarid Mediterranean soils. Nevertheless, it is important to take into consideration that toxicity depends of many factors, including AgNPs properties such as surface charge and coating, as well as soil type and exposure time, the interaction of the AgNPs with biomolecules, and the transformations and modifications of the pristine AgNPs that take place during aging on the toxicity of AgNPs. Lastly, these results reflect the importance of using different biochemical and molecular techniques to elucidate, in a holistic way, the effects of environmentally realistic concentrations of AgNPs on soil microbial communities.

CRediT authorship contribution statement

Gabriela Montes de Oca-Vásquez: Investigation, Data curation, Writing- original draft, Visualization, Funding acquisition. Frank Solano-Campos: Formal analysis, Resources, Writing - review & editing, Funding acquisition, Visualization. José R. Vega-Baudrit: Resources, Writing – review & editing, Funding acquisition. Rubén López-Mondéjar: Formal analysis, Data curation, Writing - review & editing. Alfonso Vera: Investigation, Writing - review & editing. José L. Moreno: Writing - review & editing, Funding acquisition. Felipe Bastida: Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140919.

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Discusión global

Los suelos están continuamente expuestos a AgNPs que entran a través de varias vías como la aplicación de fertilizantes y pesticidas (Batley et al., 2013), biosólidos (Benn & Westerhoff, 2008), o través del riego con aguas residuales (Gottschalk et al., 2009), entre otros. Una vez que las AgNPs entran al suelo pueden afectar la actividad, la biomasa y la diversidad de las comunidades microbianas (Vittori Antisari et al., 2016; McGee et al., 2017; Samarajeewa et al., 2019; Forstner et al., 2019; Zhang et al., 2020), así como a las características fisico-químicas del suelo (Hänsch and Emmerling, 2010; Antisari et al., 2013). Por lo tanto, y debido a la importancia de la comunidad microbiana para las funciones que desempeña el suelo, la evaluación de estas propiedades es indispensable para determinar cambios importantes en el ecosistema del suelo bajo la influencia de las nanopartículas.

En esta tesis Doctoral evaluamos el efecto de distintas concentraciones ambientalmente relevantes de AgNPs en las comunidades microbianas de suelos agrícolas tropicales con alto contenido en materia orgánica, en comparación con suelos Mediterráneos-semiáridos que poseen una menor biomasa, actividad y diversidad microbiana. En el caso del suelo agrícola tropical, se evaluaron suelos sometidos a dos manejos diferentes (convencional frente a orgánico), con características físico-químicas similares (pH, conductividad eléctrica, nitrógeno total, carbono orgánico total), pero con características microbiológicas distintas en términos de biomasa, diversidad y actividad microbiana, que difirieron en su sensibilidad a las AgNPs. Por otra parte, evaluamos el efecto de las AgNPs en un suelo Mediterráneo-semiárido sin y con la aplicación de enmiendas orgánicas (compost ovino). Se determinó que hubo cambios físico-químicos y microbianos del suelo asociados con la aplicación de enmiendas orgánicas, lo cual influyó en la toxicidad de las AgNPs sobre las comunidades bacterianas y fúngicas. Se ha reportado que diferencias en el manejo agronómico, como la adición de compost, biosólidos o diferencias en la fertilización (van der Bom et al., 2018; Dignam et al., 2018; Guo et al., 2020), así como diferencias en las propiedades físicas del suelo, como la densidad aparente, la porosidad, la aireación, la infiltración y la capacidad de retención de agua (Morais Pereira et al., 2013; Wakelin 2016; Singh et al., 2018; Yang et al., 2019), y diferencias en las condiciones microclimáticas, como la vegetación, la luminosidad, la humedad, pueden afectar las comunidades microbianas del suelo (Schlatter et al. 2015; Hortal et al., 2017; Eisenhauer et al. 2017). Por tanto, las comunidades microbianas de estos suelos de regiones climáticas tan distintas, y, además, con manejos diferentes, pueden responder de manera distinta a las AgNPs

(Tlili et al., 2017; Asadishad et al., 2018; Grün et al., 2018). Debido a lo anterior, el estudio de estos suelos formados en condiciones climáticas contrastadas, y con diferentes propiedades físico-químicas y microbiológicas nos permitió obtener conclusiones más holísticas sobre los efectos de las AgNPs en el suelo.

En general, en los dos tipos de suelos estudiados, agrícola tropical (andisol) y Mediterráneo-semiárido (fluvisol calcárico), no se encontró un efecto significativo de las AgNPs sobre las actividades enzimáticas evaluadas, lo cual ha sido una tendencia reportada por otros autores, aún utilizando concentraciones más altas (Asadishad et al., 2018; Colman et al., 2013; Grün & Emmerling, 2018; Peyrot et al., 2014). Esta ausencia de efecto en la actividad enzimática de los suelos podría estar relacionado con la estabilización de las enzimas extracelulares, debido a su unión con superficies inorgánicas, como arcillas, óxidos e hidróxidos de hierro y la adsorción con coloides orgánicos, lo cual podría dar protección a las enzimas de los efectos tóxicos de las AgNPs (Nannipieri et al., 2018). Por lo tanto, nuestros resultados sugieren que los impactos de concentraciones bajas y realistas de AgNPs en el entorno extracelular no fueron significativos. Asimismo, las AgNPs no provocaron un impacto sobre las propiedades físico-químicas evaluadas (conductividad eléctrica, carbono y nitrógeno solubles en agua), en el suelo Andisol. Similarmente Shah & Belozerova. (2008) encontraron que el contenido de pH, carbono orgánico total y nitrógeno total del suelo se mantuvo constante durante todo el experimento y no fueron influenciados por la presencia de NPs de sílice, paladio, oro y cobre a las concentraciones de 0.013% o 0.066%, utilizando suelo disponible comercialmente. En contraste con lo reportado por Hänsch y Emmerling. (2010) que determinaron en un suelo franco arenoso que las cantidades totales de N se redujeron significativamente con las concentraciones de 32 y 320 µg Ag Kg^{-1} suelo y por lo tanto, también hubo un aumento en la relación C:N en esos tratamientos. Sin embargo, debido a las diferencias en las condiciones experimentales que existen en la literatura, principalmente en términos del tipo de suelo, las concentraciones y el tipo de NPs, es difícil comparar los resultados con otros estudios.

Por otro lado, y contrario a lo encontrado para las actividades extracelulares, los impactos de las AgNPs a nivel de biomasa microbiana fueron notables, ya que en los dos tipos de suelos estudiados y en los tratamientos dentro de ambos tipos de suelos, se encontraron diferencias significativas en el efecto de las AgNPs sobre la biomasa microbiana. De manera general se determinó que los impactos de las AgNPs en la biomasa microbiana, en los dos tipos de suelo, fueron dependientes del tiempo de incubación y de los grupos microbianos, no encontrándose

un efecto dosis-dependiente. La falta de efecto dosis-dependente sobre la biomasa microbiana (analizada mediante ácidos grasos) observada en ambos tipos de suelos, podría deberse a las bajas concentraciones de NPs utilizadas, más en consonancia con las concentraciones estimadas existentes en el medioambiente. Sin embargo, existe un amplio vacío en la literatura científica con respecto a este tema y, además, debido a la novedad de la investigación presentada en esta Tesis Doctoral, la discusión de estos resultados y su comparación con otras investigaciones es complicada. Así, los cambios de las NPs, a nivel químico, físico o físicoquímico en el suelo son ampliamente desconocidos, y no son el objetivo de este trabajo de investigación. No obstante, cabe recalcar que los impactos de las AgNPs, y los iones Ag^+ liberados en el suelo están sujetos a diversos cambios físico-químicos, como heteroagregación, homoagregación, disolución, sulfidación, sorción, además de que pueden sufrir cambios en su estabilidad coloidal y biodisponibilidad, en un marco espacio-tiempo complejo (Levard et al., 2012; Cornelis et al., 2013; Grün et al., 2018). A pesar de esto, en el suelo Andisol, se determinó un aumento en la toxicidad con el aumento del tiempo de exposición, lo cual ha sido una tendencia observada por otros autores (Schlich & Hund-Rinke, 2015; Tlili et al., 2017; Grün & Emmerling, 2018), pero, por el contrario, en el suelo Mediterráneo-semiárido (Fluvisol calcáreo), se encontró una disminución en la toxicidad con el aumento del tiempo de exposición. Estas discrepancias podrían atribuirse a las diferencias entre las comunidades microbianas en términos de biomasa, actividades enzimáticas y diversidad microbiana, y a las distintas características físico-químicas de ambos suelos, ya que ha sido reportado que los efectos de las AgNPs son altamente dependientes de dichas propiedades (Cornelis et al., 2010; Sillen et al., 2015; Bundschuh et al., 2018; Elhaj Baddar et al., 2019; Grün & Emmerling, 2018). Dentro de las características físico-químicas que fueron distintas entre ambos tipos de suelos, destacan el pH, el contenido de materia orgánica, el contenido de nitrógeno, y la conductividad eléctrica. Es conocido que las AgNPs interactúan con la materia orgánica, modificando la agregación, aglomeración, disolución y transformación de las AgNPs (Pachapur et al., 2016; Li et al., 2018; Makselon et al., 2018, 2017; Sutton et al., 2019), lo que influye en su movilidad (González-Fuenzalida et al., 2018), biodisponibilidad y toxicidad (Pachapur et al., 2016). Además, se han reportado resultados contradictorios a este respecto. Así, mientras que algunos estudios han determinado que existe una menor toxicidad de las AgNPs en suelos con un alto contenido de arcilla y materia orgánica, debido a la inmovilización de las AgNPs por heteroagregación (Schlich & Hund-Rinke, 2015; Rahmatpour et al., 2017), otras investigaciones han determinado una mayor toxicidad, debido a la mayor estabilización de las AgNPs (Diegoli et al., 2008; Bae et al., 2013; Yecheskel et al., 2018). Por ejemplo, Coutris et al. (2012), determinaron que las AgNPs

estabilizadas con citrato fueron más móviles en los suelos ricos en materia orgánica que en el suelo mineral, similar al aumento de movilidad de las AgNPs-PVP con el aumento de la materia orgánica determinado por Wang et al. (2015). Probablemente, en los suelos Andisoles de Costa Rica y con un alto contenido de materia orgánica, se pudo haber dado una tasa de disolución más lenta del ión Ag^+, debido a la unión de las AgNPs con la materia orgánica, que en un inicio pudo proteger de los efectos tóxicos a la comunidad microbiana. Sin embargo, en los suelos Fluvisoles calcáreos del sureste español, la tendencia es contraria, con una mayor toxicidad a los 7 días de incubación en el suelo con mayor contenido de materia orgánica (SA), debido a la adición de compost rico en ácidos húmicos, la cual podría deberse a la unión de las AgNPs con la materia orgánica. Esta unión podría haber favorecido la movilidad de las AgNPs, debido a su estabilización por un aumento en la carga negativa de las AgNPs, lo que aumenta su biodisponibilidad y toxicidad (Diegoli et al., 2008; Coutris et al., 2012; Bae et al., 2013; Wang et al., 2015; Yecheskel et al., 2018).

Por lo tanto, estos resultados diferentes entre ambos tipos de suelos podrían deberse a que se ha determinado que las interacciones entre las AgNPs y la materia orgánica dependen de las características de la materia orgánica, como el peso molecular, y de los componentes de la materia orgánica, como los ácidos húmicos y fúlvicos (Adrian et al., 2019; Coutris et al., 2012; Yang et al., 2014).

Por otra parte, se observaron diferencias en el efecto de las AgNPs sobre las comunidades microbianas entre ambos tipos de suelos, en términos de composición microbiana. Con respecto a la composición de la comunidad microbiana, en los suelos Andisoles, y para ambos tipos de manejo del suelo (orgánica o convencional), las diferentes concentraciones de AgNPs no tuvieron un efecto significativo en la composición de las comunidades fúngicas y bacterianas. Resultados similares han sido reportados por otros investigadores, utilizando concentraciones más altas que las empleadas en esta investigación (Samarajeewa et al., 2017; Grün & Emmerling, 2018; Liu et al., 2017). Sin embargo, en los suelos Fluvisoles calcáreos con la adición de enmienda orgánica, se determinó que las AgNPs sí afectaron la composición de la comunidad bacteriana. Con respecto a la abundancia relativa de los grupos taxonómicos a nivel de filo/clase o género, las AgNPs tuvieron un efecto dependiente del tipo de suelo y del manejo, lo cual podría estar relacionado a las características microbiológicas. Lo anterior concuerda con estudios previos, los cuales han sugerido que el impacto de las AgNPs es dependiente del grupo microbiano, inclusive a nivel de especie (Hänsch & Emmerling, 2010; Pallavi et al., 2016), ya que algunas especies

han mostrado sensibilidad, resistencia o tolerancia a las AgNPs (Samarajeewa et al., 2017; Samarajeewa et al., 2019). Nuestros resultados respaldan lo anterior, ya que se encontraron diferencias a nivel de filo, clases y géneros bacterianos y fúngicos, entre los dos tipos de suelos. Por ejemplo, a nivel bacteriano, en los suelos Andisoles, los filos más abundantes fueron Proteobacteria, Actinobacteria y Acidobacteria, respectivamente, y los de géneros más abundantes fueron *Gaiella*, *Rhodoplanes*, "*Candidatus* Xiphinematobacter", *Conexibacter* y *Aciditerrimonas*. En los suelos Fluvisoles, los filos y clases más abundantes fueron Alphaproteobacteria, Actinobacteria, Gammaproteobacteria y Firmicutes, y los géneros más abundantes fueron *Lysobacter*, *Erythrobacter*, *Nocardioides* y *Sphingomonas*. De manera similar, a nivel de géneros fúngicos, se encontraron diferencias entre ambos tipos de suelos, siendo *Mortierella*, *Fusarium* y *Penicillium* los más abundantes en los suelos Andisoles; y *Fusarium*, *Cladosporium*, *Morteriella*, *Alternaria*, *Penicillium* y *Aspergillus* los más abundantes en los suelos Mediterráneos estudiados.

A pesar de este resultado, la tendencia, a nivel de biomasa de algunos grupos microbianos se mantuvo relativamente similar en ambos tipos de suelo. Por ejemplo, se determinó de manera general que la biomasa de bacterias Gram – y la de hongos fue más resistente a la toxicidad de las AgNPs, al compararlas con la biomasa de bacterias Gram +. Varios estudios han proporcionado respaldo científico a estas diferencias en las respuestas de las comunidades bacterianas y fúngicas (Kumar & Mamidyala, 2011; Gryndler et al., 2012; Sillen et al., 2015), lo cual sugiere que las células eucariotas son más tolerantes al ión Ag^+ (Kathiresan et al., 2010). Además, otros autores también han determinado una mayor tolerancia a la acción de las AgNPs de las bacterias Gram – con respecto a las Gram + (Kumar et al., 2014; Liu et al., 2017). Esta mayor resistencia de las bacterias Gram – se puede atribuir a la diferencia en la composición de la membrana celular externa, ya que las bacterias Gram – poseen una membrana externa compuesta fundamentalmente de fosfolípidos y con un cierto contenido de lipopolisacáridos, que pueden proporcionar resistencia contra la penetración de las AgNPs y el ión Ag^+ en la célula (Marambio-Jones & Hoek, 2010; Jacobson et al., 2015).

Finalmente, cabe recalcar que esta investigación es pionera en su campo, y puede ser empleada como base para el desarrollo de nuevos estudios nanotoxicológicos. Además, ayudaría al desarrollo de normativas o políticas de gestión ambiental, para la producción, uso, manejo y descarte de las AgNPs y otros nanomateriales similares; en coordinación con las respectivas autoridades nacionales en la materia. En relación al uso y descarte de las AgNPs presentes en

diversos productos comerciales, es necesario crear conciencia sobre su posible efecto nocivo sobre el medio ambiente.

Conclusiones generales

La presente Tesis Doctoral tuvo como objetivo evaluar el efecto de concentraciones ambientalmente relevantes de AgNPs, sobre la comunidad microbiana de Andisoles (suelos agrícolas tropicales) y Fluvisoles Calcáreos (suelos Mediterráneos semiáridos) con diferentes propiedades físico-quimicas, bioquímicas y microbiológicas. En particular se evaluó el efecto de diferentes concentraciones de AgNPs en el contenido de la biomasa microbiana, las actividades enzimáticas y la composición, abundancia y diversidad de las comunidades de hongos y bacterias de los suelos. Para ello, nos basamos en que el efecto tóxico de las AgNPs depende de las características físico-químicas y microbiológicas de los suelos, además de las propiedades de las AgNPs.

Para lograr cumplir este objetivo general, se realizaron experimentos en microcosmos, en donde se adicionaron diferentes concentraciones de AgNPs y se incubaron a distintos tiempos. La utilización de distintas técnicas químicas, bioquímicas y moleculares nos permitió elucidar de una manera más holística los efectos de las AgNPs en las comunidades microbianas del suelo.

Como conclusión general, se determinó que, aún a concentraciones ambientalmente relevantes, las AgNPs tienen efectos perjudiciales sobre la biomasa, composición y diversidad microbiana, de una manera dependiente del tiempo de exposición y del tipo del suelo, pero independientemente de la concentración de AgNPs, ya que no se encontró una relación dosis-dependiente. De manera notable, las concentraciones ambientalmente relevantes de AgNPs utilizadas no afectaron otros parámetros como las características físico-químicas del suelo, entre estas el pH, el contenido de carbono, nitrógeno y la conductividad eléctrica; y tampoco afectaron a las actividades de enzimas extracelulares como la β-glucosidasa, la fosfatasa y la ureasa, que catalizan las transformaciones biogeoquímicas del C, P y N.

La importancia ecológica de estos resultados radica en que una disminución en la biomasa microbiana podría tener un efecto en la fertilidad del suelo y en los ciclos de nutrientes y su disponibilidad para las plantas, y, con ello, potencialmente afectar a la productividad de los cultivos, ya que la biomasa microbiana constituye un sumidero y una fuente de nutrientes (carbono, nitrógeno, fósforo y azufre). Por otra parte, cambios en la composición de la comunidad microbiana podría influir en los servicios del ecosistema, como la descomposición y la renovación

de la materia orgánica en el suelo que es fundamental para el mantenimiento de la calidad edáfica.

Como conclusiones específicas se indica lo siguiente:

— Las bajas concentraciones de AgNPs tuvieron un gran impacto en la biomasa microbiana de los suelos Andisoles y Fluvisoles Cálcáricos, sin embargo, la sensibilidad de los grupos microbianos del suelo a las AgNPs varió, determinándose que los hongos fueron más resistentes que las bacterias, y que las bacterias Gram – fueron más resistentes que las Gram +. Este hecho podría estar relacionado a que los hongos poseen una pared celular más gruesa comparada con las bacterias, y porque las bacterias Gram – poseen una membrana externa compuesta de lipopolisacáridos, que pueden proporcionar resistencia contra la penetración de las AgNPs en la célula.

— En los suelos Andisoles, las características microbiológicas relacionadas con el tipo de manejo del suelo (orgánico o convencional) y el alto contenido de materia orgánica del suelo, influyeron sobre la respuesta microbiana a las AgNPs. Así, se observó una tendencia al aumento de la toxicidad con el aumento en el tiempo de incubación con las NPs. Además, el efecto en la biomasa microbiana mostró ser dependiente de los diferentes grupos taxonómicos que difieren en sus sensibilidades o tolerancias a las AgNPs.

— En los suelos Andisoles, las concentraciones de AgNPs tuvieron poco efecto en la composición de la comunidad microbiana. Sin embargo, en los suelos Fluvisoles, si se determinó un efecto notable en la composición de la comunidad bacteriana. Las diferencias en las características físico-químicas, como el contenido de materia orgánica, el contenido de nitrógeno, el pH, y la conductividad eléctrica, así como en las características microbiológicas del suelo, como la biomasa, las actividades enzimáticas y la diversidad microbiana, podrían estar determinando la respuesta de la comunidad microbiana de estos suelos a las AgNPs.

— Las concentraciones de AgNPs adicionadas al suelo no tuvieron un efecto tóxico dosis-dependiente sobre la biomasa y la diversidad microbiana, lo cual fue independiente del tipo de suelo y de su manejo.

— La enmienda orgánica añadida a los suelos Mediterráneos influyó sobre el impacto de las AgNPs, debido a que podrían aumentar la toxicidad de las AgNPs sobre la biomasa microbiana del suelo y la composición de la comunidad bacteriana a corto plazo, lo que podría influir sobre los servicios ecosistémicos claves, como la descomposición de la materia orgánica en el suelo. Sin embargo, la toxicidad disminuyó con el aumento en el tiempo de incubación, lo cual podría estar relacionado con mecanismos de adaptación microbianos al efecto tóxico de las AgNPs.

— La abundancia relativa de diferentes grupos taxonómicos se vio estimulada o disminuida después de la aplicación de las AgNPs, lo cual dependió del tipo y del manejo del suelo. Estas poblaciones microbianas se asocian generalmente con funciones esenciales relacionadas con la fertilidad del suelo, como la desnitrificación organotrófica, la fijación de nitrógeno y la degradación de celulosa, etc.

— Las AgNPs disminuyeron la riqueza de hongos en el suelo Fluvisol Calcáreo, sin la adición de compost, pero no disminuyeron la biomasa fúngica. Estos resultados sugieren que ciertas poblaciones fúngicas tolerantes podrían ocupar el nicho dejado por poblaciones más sensibles que fueron afectadas por las AgNPs.

Recomendaciones

Es importante tener en cuenta que la toxicidad de las AgNPs sobre las comunidades microbianas y el efecto de este tipo de NPs en las características físico-químicas del suelo, depende de muchos factores, entre los que se encuentran las propiedades intrínsecas de las AgNPs, como la carga superficial, el tipo de agente estabilizante, el tamaño, y la forma; así como de las características físico-químicas del suelo, como el pH, la conductividad eléctrica, la capacidad de intercambio catiónico, el contenido de materia orgánica y el contenido de arcilla. Asimismo, otros factores como el tiempo de exposición y las biomoléculas secretadas por los microorganismos o exudadas por las plantas pueden tener un impacto sobre la toxicidad de las AgNPs. Por lo tanto, debido a que son múltiples los factores que influyen en la toxicidad de las AgNPs, en futuras investigaciones se recomienda tener en cuenta lo siguientes aspectos:

— Ampliar el conocimiento en las respuestas de las comunidades microbianas del suelo a concentraciones realistas de AgNPs, estudiando diferentes propiedades de las AgNPs (tamaño, forma, carga superficial y agente estabilizante) en una mayor variedad de tipos de suelo, con diferentes características físico-químicas y microbiológicas, y en una mayor cantidad de usos de la tierra.

— Estudiar la interacción de las AgNPs con las biomoléculas que son producidas y secretadas por la fauna del suelo y exudadas por las raíces de las plantas, ya que su interacción con las AgNPs puede afectar el transporte, transformación, y la toxicidad.

— Estudiar con mayor resolución los cambios en la comunidad microbiana empleando técnicas de secuenciación de tercera generación de lecturas largas, que nos permitan determinar con mayor confianza y resolución los cambios en la composición de la comunidad y discernir qué géneros y especies son resistentes o tolerantes a las AgNPs en ambos tipos de suelo.

— Utilizar otras técnicas moleculares tales como la metagenómica funcional o metaproteómica para entender con mayor resolución los cambios en la funcionalidad del suelo sometido al efecto de nanoparticulas, y determinar cuales genes, rutas metabólicas y proteínas específicas se expresan de manera diferencial entre los tratamientos control

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y los que tienen AgNPs, para explicar los posibles mecanismos de resistencia de los microorganismos a la exposición de las AgNPs.

— Realizar estudios de la tasa de disolución y transformación de las AgNPs, como la homoagregación y la heteroagregación, en distintos tipos de suelos con características físico-químicas y microbiológicas contrastantes. Este conocimiento permitirá comprender mejor cómo las AgNPs afectan a las comunidades microbianas.

— Determinar las características de la materia orgánica, tales como su composición y el peso molecular, que pueden influir en la transformación, movilidad y toxicidad de las NPs en en el suelo.

— Emplear $AgNO_3$ como control positivo para esclarecer si los efectos sobre las comunidades microbianas se deben a las NPs o a la liberación de los iones Ag^+. Así, como un control con NO_3 para determinar su efecto como fuente de N en los microorganismos.

— Realizar estudios a nivel de mesocosmos y de campo para contrastar los resultados obtenidos a nivel de microcosmos.

— Estudiar otras formas de aplicación de AgNPs en los suelos, como sus mezclas con lodos de depuradora y biosólidos, e investigar la pre-incubación de las AgNPs en estos sistemas, para determinar la transformación que sufren las AgNPs, antes de aplicarse en el suelo.

— Determinar el impacto de las AgNPs en las comunidades microbianas del suelo, en estudios a largo plazo, para determinar cambios en la respuesta a través del tiempo.

— Estudiar el efecto de las AgNPs en otros organismos del suelo, como arqueas, algas, protozoos y nematodos, los cuales a la fecha han sido poco estudiados y que cumplen funciones ecológicas muy importantes.

— Establecer estrategias de biorremediación en suelos contaminados con altas concentraciones de NPs.

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ANEXO 1

Información complementaria del artículo 1: Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity.
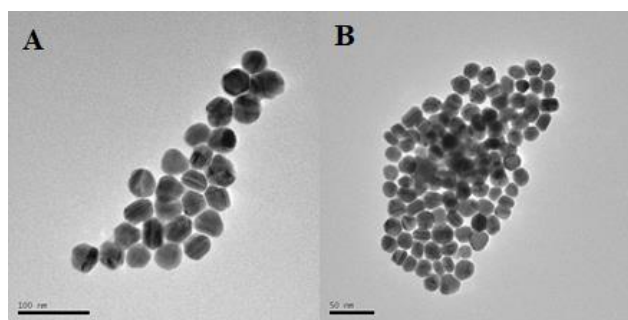
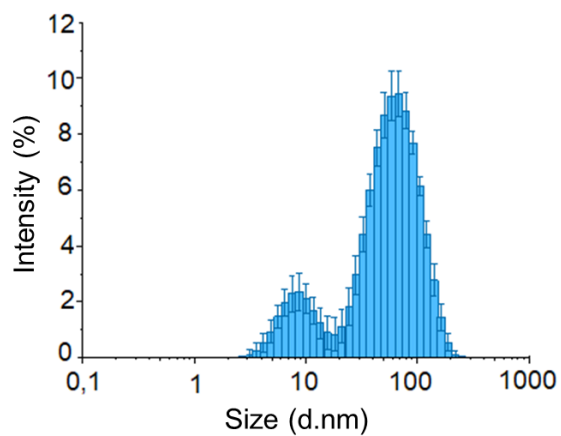**Fig. A1.1.** Transmission electron micrography of silver nanoparticles. A, AgNPs scale = 100 nm. B, AgNPs scale = 50 nm.

Fig. A1.2. Size distribution by AgNPs intensity at 25 °C.

Table A1.1. Main soil physico-chemical properties.

	CMS	OMS
Total N (g 100 g^{-1})	0.84 ± 0.02	0.73 ± 0.03
OM (g 100 g^{-1})	14.76 ± 1.65	15.72± 1.96
pH	5.65 ± 0.07	5.4 ± 0.28
Acidity saturation (%)	2.5 ± 0.71	3.5 ± 0.71
Electric conductivity (mS cm^{-1})	0.06 ± 0.03	0.08 ± 0.03
Texture	Clayey	Sandy loam

Total N = total nitrogen, OM = total organic matter. CMS (conventionally managed soil); OMS (organically managed soil).

Table A1.2. Physico-chemical properties of the CMS and OMS soils, treated with different concentrations of AgNPs (0.015, 0.15, and 1.5 µg kg^{-1}) for different times.

	CMS				OMS			
Parameters	pH	EC (µS cm^{-1})	WSC (mg kg^{-1})	WSN (mg kg^{-1})	pH	EC (µS cm^{-1})	WSC (mg kg^{-1})	WSN (mg kg^{-1})
7 days								
0	5.98 ± 0.04^b	118.33 ± 3.21^a	1236.82 ± 17.50^b	77.85 ± 6.49^b	6.18 ± 0.01^c	133.00 ± 2.00^{ab}	1224.37 ± 27.01^a	104.81 ± 16.00^a
0.015	5.94 ± 0.01^b	123.67 ± 3.79^a	1200.21 ± 1.67^a	86.04 ± 7.11^a	6.16 ± 0.03^{bc}	134.33 ± 1.53^b	1214.09 ± 19.25^a	113.20 ± 14.46^a
0.15	5.87 ± 0.07^b	121.67 ± 3.06^a	1211.24 ± 14.50^{ab}	79.84 ± 8.08^{ab}	6.10 ± 0.02^b	127.67 ± 2.89^a	1205.48 ± 7.38^a	101.10 ± 5.25^a
1.5	5.81 ± 0.01^a	115.33 ± 2.52^a	1205.35 ± 9.20^{ab}	83.05 ± 4.74^{ab}	6.05 ± 0.01^a	129.67 ± 1.53^a	1220.95 ± 4.19^a	111.60 ± 14.81^a
30 days								
0	5.81 ± 0.02^a	135.33 ± 2.08 ^{a}	162.77 ± 25.05^a	71.90 ± 1.37^a	6.01 ± 0.05^b	150.00 ± 7.21^a	190.17 ± 2.49^a	94.87 ± 2.22^a
0.015	5.77 ± 0.02^a	130.67 ± 4.16 ^{a}	163.63 ± 6.51^a	69.80 ± 4.16 ^{a}	5.90 ± 0.02^a	156.67 ± 5.03^a	192.37 ± 13.90^a	87.57 ± 3.63^a
0.15	5.75 ± 0.02^a	131.67 ± 8.14 ^{a}	163.70 ± 30.55^a	71.20 ± 3.90 ^{a}	5.83 ± 0.03^a	150.67 ± 3.79^a	207.17 ± 12.67^a	88.50 ± 3.44^a
1.5	5.76 ± 0.02^a	131.00 ± 8.49 ^{a}	175.60 ± 5.46^a	69.23 ± 2.38 ^{a}	5.82 ± 0.02^a	141.33 ± 6.11^a	200.93 ± 11.26^a	89.6 ± 4.99^a
60 days								
0	5.54 ± 0.00 ^{a}	151.33 ± 7.37^a	417.43 ± 40.72^a	87.75 ± 4.27^a	5.79 ± 0.04 ^{a}	187.00 ± 2.65^a	433.72 ± 60.53^a	117.69 ± 0.93^a
0.015	5.58 ± 0.02^a	145.00 ± 4.83^a	428.48 ± 84.74^a	85.47 ± 0.32^a	5.73 ± 0.15^a	175.00 ± 1.73^a	468.82 ± 51.61^a	115.49 ± 5.23^a
0.15	5.52 ± 0.06^a	146.50 ± 3.54^a	431.81 ± 71.44^a	89.41 ± 3.45^a	5.68 ± 0.03^a	180.33 ± 7.09^a	448.47 ± 42.51^a	121.36 ± 8.81^a
1.5	5.60 ± 0.05^a	147.00 ± 0.00^a	425.81 ± 19.77^a	85.16 ± 1.45^a	5.68 ± 0.02^a	177.67 ± 7.09^a	444.48 ± 16.83^a	117.71 ± 4.65^a

EC, Electrical conductivity, WSC, water-soluble carbon, WSN, water-soluble nitrogen. CMS (conventionally managed soil); OMS (organically managed soil). Different letters indicate significant differences ($P < 0.05$) within each type of soil management, according to one-way ANOVA followed by a post-hoc Tukey test carried out for multiple comparison among the mean values observed at the different AgNPs concentrations.

Table A1.3. Two-factors ANOVA for soil physico-chemical parameters.

Parameter	pH 7		pH 30		pH 60		EC 7		EC 30		EC 60	
	F^c	P^d	F	P	F	P	F	P	F	P	F	P
Factors[b]												
M	477.280	0.000	17.953	0.001	16.054	0.001	109.122	0.000	69.616	0.000	265.361	0.000
T	49.361	0.000	5.288	0.010	1.741	0.199	6.130	0.006	1.809	0.186	3.576	0.038
M X T	3.179	0.053	4.544	0.017	0.976	0.428	3.417	0.043	4.194	0.023	1.042	0.401

	WSC 7		WSC 30		WSC 60		WSN 7		WSN 30		WSN 60	
Factors[b]	F	P	F	P	F	P	F	P	F	P	F	P
M	0.215	0.649	22.078	0.000	1.115	0.307	44610.261	0.000	194.630	0.000	314.541	0.000
T	3.174	0.053	0.737	0.545	0.196	0.897	1.746	0.198	2.198	0.128	0.045	0.987
M X T	1.334	0.298	0.387	0.764	0.071	0.975	3.662	0.035	0.872	0.476	0.027	0.994

7, 30, and 60 indicate the incubation days.
[a] EC, Electrical conductivity, WSC, water-soluble carbon, WSN, water-soluble nitrogen.
[b] Factors: management (M), AgNPs treatment (T).
[c] F-ratio
[d] *P*-values

Table A1.4. Two-factors ANOVA for fatty acids.

Parameter	Fungal 7		Fungal 30		Fungal 60	
	F^c	Pd	F	P	F	P
Factors[b]						
M	203.439	0.000	31.769	0.000	6.834	0.019
T	13.016	0.000	7.555	0.002	19.255	0.000
M X T	6.353	0.005	9.976	0.001	3.175	0.053
	Bacterial 7		Bacterial 30		Bacterial 60	
Factors[b]	F	P	F	P	F	P
M	757.215	0.000	21.210	0.000	230.720	0.000
T	34.263	0.000	2.270	0.120	85.070	0.000
M X T	16.977	0.000	3.672	0.035	15.147	0.000
	Gram+ 7		Gram+ 30		Gram+ 60	
Factors[b]	F	P	F	P	F	P
M	718.714	0.000	32.498	0.000	190.873	0.000
T	24.313	0.000	3.112	0.056	90.044	0.000
M X T	48.939	0.000	4.839	0.014	4.920	0.013
	Gram- 7		Gram- 30		Gram- 60	
Factors[b]	F	P	F	P	F	P
M	220.897	0.000	31.935	0.000	57.509	0.000
T	26.542	0.000	9.739	0.001	129.190	0.000
M X T	38.054	0.000	6.578	0.004	84.690	0.000
	Actinobacterial 7		Actinobacterial 30		Actinobacterial 60	
Factors[b]	F	P	F	P	F	P
M	207.811	0.000	17.918	0.001	62.617	0.000
T	15.443	0.000	0.364	0.780	73.486	0.000
M X T	30.113	0.000	1.398	0.280	11.128	0.000

[b] Factors: management (M), AgNPs treatment (T).
[c] F-ratio
[d] *P*-values

Table A1.5. Two-factors ANOVA for enzyme activities.

Parameter	Glucosidase 7		Glucosidase 30		Glucosidase 60	
	F	P	F	P	F	P
Factors[a]						
M	175.840	0.000	468.035	0.000	355.627	0.000
T	1.081	0.385	1.141	0.362	3.144	0.054
M X T	1.037	0.403	0.734	0.547	0.367	0.778
	Urease 7		Urease 30		Urease 60	
Factors[a]	F	P	F	P	F	P
M	278.053	0.000	562.350	0.000	176.992	0.000
T	2.635	0.085	0.905	0.460	1.024	0.408
M X T	0.398	0.756	0.518	0.676	1.930	0.165
	Phosphatase 7		Phosphatase 30		Phosphatase 60	
Factors[a]	F	P	F	P	F	P
M	0.001	0.977	2.412	0.140	0.683	0.421
T	2.166	0.132	2.329	0.113	0.724	0.552
M X T	1.944	0.163	1.199	0.342	0.429	0.735

[b] Factors: management (M), AgNPs treatment (T).
[c] F-ratio
[d] *P*-values

Table A1.6. Mean values and standard deviations of the activities of β-glucosidase, urease, and phosphatase, in soils under conventional (CMS) and organic management (OMS).

	CMS			OMS		
	β-Glucosidase	Urease	Phosphatase	β-Glucosidase	Urease	Phosphatase
	(μmol PNF g^{-1} h^{-1})	(μmol NH$_3$ g^{-1} h^{-1})	(μmol PNP g^{-1} h^{-1})	(μmol PNF g^{-1} h^{-1})	(μmol NH$_3$ g^{-1} h^{-1})	(μmol PNP g^{-1} h^{-1})
7 days						
0	1.00 ± 0.11[a]	1.11 ± 0.11[a]	12.50 ± 0.74 [a]	1.36 ± 0.05[a]	1.56 ± 0.08[a]	11.93 ± 0.32 [a]
0.015	0.91 ± 0.06[a]	0.99 ± 0.03[a]	12.39 ± 0.48 [a]	1.33 ± 0.05[a]	1.46 ± 0.03[a]	13.21 ± 0.54[b]
0.15	0.92 ± 0.06[a]	1.03 ± 0.01[a]	12.30 ± 0.42[a]	1.32 ± 0.07[a]	1.55 ± 0.08[a]	12.54 ± 0.49[ab]
1.5	1.00 ± 0.09[a]	0.99 ± 0.09[a]	12.22 ± 0.96[a]	1.28 ± 0.02[a]	1.51 ± 0.08[a]	11.77 ± 0.27[a]
30 days						
0	0.73 ± 0.03[a]	0.94 ± 0.04[a]	11.93 ± 0.65[a]	1.50 ± 0.10[a]	2.01 ± 0.05 [a]	10.71 ± 0.23[a]
0.015	0.83 ± 0.02[b]	0.95 ± 0.03[a]	12.05 ± 0.49[a]	1.47 ± 0.03[a]	2.10 ± 0.20 [a]	11.80 ± 0.95[a]
0.15	0.85 ± 0.04[b]	0.93 ± 0.13[a]	11.90 ± 0.66[a]	1.54 ± 0.17[a]	1.94 ± 0.16 [a]	11.65 ± 1.01[a]
1.5	0.79 ± 0.03[a]	0.87 ± 0.11[a]	12.23 ± 0.26[a]	1.47 ± 0.05[a]	2.03 ± 0.05 [a]	12.34 ± 0.34[a]
60 days						
0	0.88 ± 0.03[a]	1.53 ± 0.02[a]	12.41 ± 0.48[a]	1.32 ± 0.05[a]	2.15 ± 0.19 [a]	12.06 ± 0.77[a]
0.015	0.95 ± 0.06[a]	1.47 ± 0.11[a]	12.63 ± 1.08[a]	1.44 ± 0.07[a]	2.02 ± 0.12 [a]	12.83 ± 1.14[a]
0.15	0.93 ± 0.04[a]	1.42 ± 0.18[a]	11.37 ± 1.76[a]	1.39 ± 0.09[a]	2.27 ± 0.23 [a]	12.36 ± 1.34[a]
1.5	0.92 ± 0.04[a]	1.45 ± 0.02[a]	12.16 ± 0.13[a]	1.34 ± 0.06[a]	2.29 ± 0.10 [a]	12.74 ± 0.86[a]

Different letters indicate significant differences (*P* < 0.05) within each type of soil management, according to the one-way ANOVA followed by a post-hoc Tukey test carried out for multiple comparison among the mean values observed at the different AgNPs concentrations.

Tabla A1.7. Análisis de los elementos químicos de los suelos cultivados con café que posee manejo convencional y manejo orgánico

Elementos químicos	CMS	OMS
Al (g/100g)	5,03	4,66
As (mg/Kg)	<0,01	<0,01
Be (mg/Kg)	0,75	0,68
Bi (mg/Kg)	17,67	17,41
B (mg/Kg)	10,99	7,64
Ca (g/100g)	0,27	0,30
Cd (mg/Kg)	0,53	0,54
Co (mg/Kg)	13,67	13,98
Cr (mg/Kg)	55,73	54,31
Cu (mg/Kg)	66,50	62,55
Fe (mg/Kg)	35397,06	34614,48
K (g/100g)	0,04	0,05
La (mg/Kg)	<0,01	<0,01
Li (mg/Kg)	2,11	3,17
Mg (g/100g)	0,28	0,36
Mn (mg/Kg)	388,21	498,45
Mo (mg/Kg)	0,82	0,64
Na (g/100g)	0,05	0,04
Ni (mg/Kg)	26,10	27,59
Pb (mg/Kg)	16,11	14,75
P (g/100g)	0,12	0,11
Rb (mg/Kg)	1,87	2,61
Sb (mg/Kg)	0,68	0,52
Se (mg/Kg)	4,74	4,83
Si (mg/Kg)	106,60	55,08
S (g/100g)	0,09	0,08
Sr (mg/Kg)	52,20	62,42
Ti (mg/Kg)	789,69	572,22
Tl (mg/Kg)	196,14	237,81
V (mg/Kg)	96,72	89,38
Zn (mg/Kg)	42,58	44,36

Tabla A1.7. Composición de la comunidad de plantas, del sitio de estudio cultivado con café que posee manejo orgánico.

Género y especie	Familia	Cantidad
Montanoa ribiscifolia	Asteraceae	90
Inga spectabilis	Fabaceae	3
Jungla neotropica	Jungladaceae	2
Senna spectabilis	Fabaceae	1
Codia alliodora	Baraginaceae	8
Codia alliodora	Baraginaceae	2
Diphysa americana	Fabaceae	1
Inga vera	Fabaceae	3
Inga spectabilis	Fabaceae	1
Tabebuia rosea	Bigaoniaceae	1
Cedrela odorata	Meliaceae	12
Psidium guajava	Myrtaceae	31
Jungla neotropica	Jungladaceae	1
Acnistus arborescens	Solanaceae	5
Persea americana	Laureaceae	1
Inga punctata	Fabaceae	1
Jungla neotropica	Jungladaceae	2
Cecropia obtusifolia	Urticaceae	3
Cojoba arborea	Fabaceae	1
Cinnamomum triplenerve	Laureaceae	1
Croton draco	Euphorbiaceae	1
Annona cherimola	Annonaceae	1
Hura crepitans	Euphorbiaceae	1
Cordia alliodora	Boraginaceae	2
Cordia alliodora	Boraginaceae	2
Trichilia havanensis	Meliaceae	1
Zanthoxylum rohoifolium	Rutaceae	1
Eucalyptus sp	Myrtaceae	1

ANEXO 2

Información complementaria del artículo 2: Organic amendments exacerbate the effects of silver nanoparticles on microbial biomass and community composition of a semiarid soil.
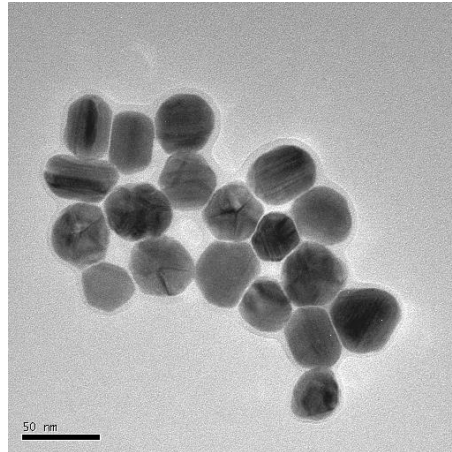
Fig. A2.1. Transmission electron micrography of silver nanoparticles. AgNPs scale = 50 nm.
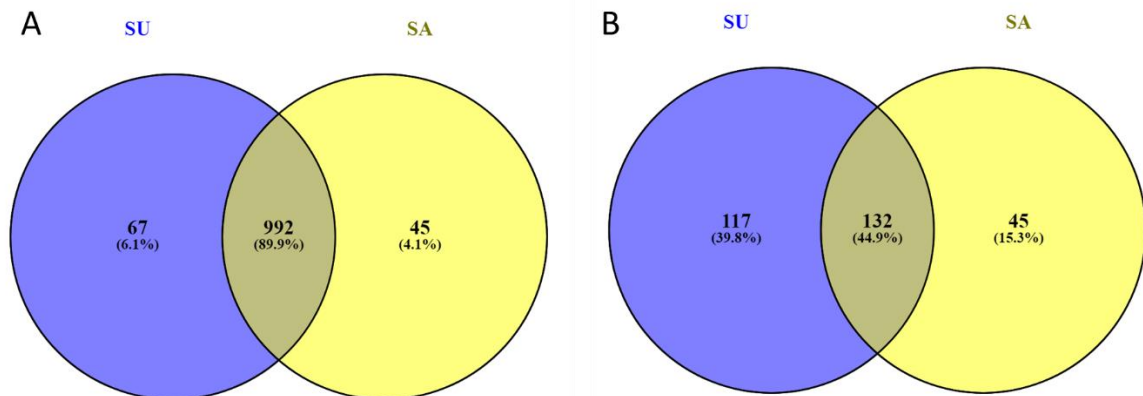

Fig A2.2. Venn diagram of the number of shared and unique OTUs in unamended soils (SU), and soils amended with compost (SA). (A) bacterial and (B) fungal OTUs. The data represents the OTUs sequences with > 0.1% mean relative abundance.
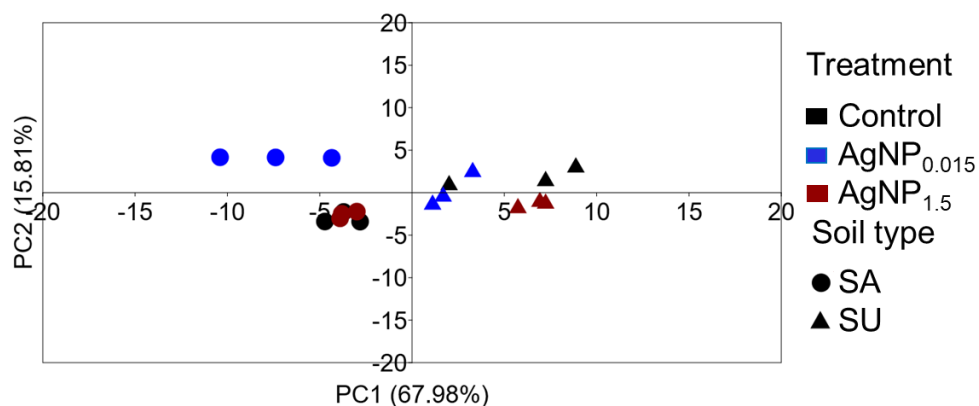
Fig. A2.3. Principal component analysis (PCA) of the relative abundance of fatty acids in amended (SA) and unamended (SU) soils, treated with two AgNPs concentrations (AgNP$_{0.015}$ = 0.015 µg kg^{-1}, AgNP$_{1.5}$ = 1.5 µg kg^{-1}), at 7 days of incubation. Values in parenthesis denote the percentage of the data variation explained by a given component.
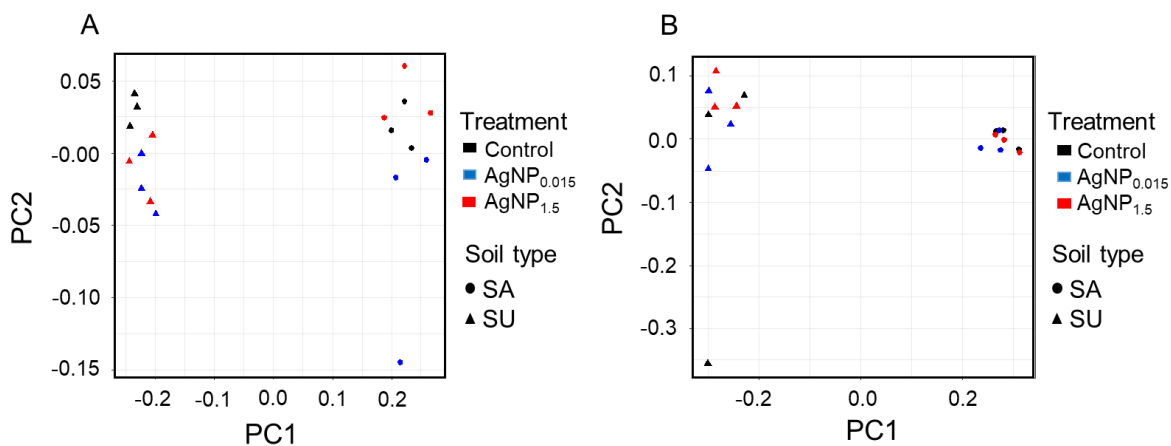

Fig. A2.4. Principal component analysis (PCA) of the Hellinger-transformed distances of the bacterial (A) and fungal community composition (B) in unamended soils (SU), and soils amended with compost (SA), treated with two AgNP concentrations (AgNP$_{0.015}$ = 0.015 µg kg^{-1}, AgNP$_{1.5}$ = 1.5 µg kg^{-1}), at 30 days of incubation. PERMANOVA *P* value = 0.001, for fungal and bacterial communities. Fixed factor soil type was used as the explanatory variable.

Table A2.1. The composition of the fungal and bacterial communities at the genus level, in unamended soils (SU), and soils amended with compost (SA). Percentage of sequences with > 0.1% mean relative abundance.

Fungal genus	SU	SA	Bacterial genus	SU	SA
Acremonium	0.16±0.09	0.08±0.04	*Aciditerrimonas*	0.96±0.05	0.35±0.06
Alternaria	8.93±1.61	20.87±5.40	*Altererythrobacter*	0.50±0.05	2.61±0.02
Arachnomyces	0.16±0.12	0.01±0.01	*Arthrobacter*	0.19±0.04	3.53±0.45
Arthrographis	0.05±0.01	0.07±0.03	*Bacillus*	1.77±0.12	4.59±0.19
Ascobolus	0.17±0.18	0.00±0.00	*Blastococcus*	1.62±0.06	2.29±0.13
Aspergillus	2.59±0.60	10.01±1.90	*Clostridium*	0.56±0.13	0.46±0.03
Botryotrichum	2.49±0.86	0.68±0.47	*Conexibacter*	0.39±0.03	0.54±0.13
Cephaliophora	1.00±0.91	0.00±0.00	*Erythrobacter*	7.39±1.08	2.49±0.04
Chaetomella	0.00±0.00	1.86±1.69	*Fervidicoccus*	3.88±0.27	3.88±0.21
Chaetomium	2.19±0.63	0.41±0.05	*Flavisolibacter*	0.58±0.09	3.79±0.53
Chrysosporium	5.09±0.25	0.95±0.16	*Flexibacter*	0.44±0.08	0.77±0.07
Cladosporium	14.21±19.67	11.21±1.72	*Fodinicurvata*	1.13±0.42	0.10±0.01
Coprinopsis	1.46±2.53	0.00±0.00	*Gaiella*	2.04±0.10	2.15±0.31
Curvularia	0.00±0.00	0.80±0.04	*Geminicoccus*	0.84±0.10	0.75±0.08
Fusarium	14.35±12.39	10.82±2.15	*Gemmatimonas*	3.84±0.11	6.09±0.16
Gymnoascus	0.25±0.04	0.08±0.08	*Gramella*	3.39±0.70	0.08±0.04
Humicola	0.09±0.12	0.16±0.14	*Halioglobus*	1.45±0.18	1.58±0.23
Ilyonectria	0.26±0.09	0.01±0.01	*Holophaga*	0.71±0.08	0.24±0.04
Microascus	1.17±0.34	0.15±0.02	*Iamia*	1.10±0.02	1.13±0.23
Mortierella	9.40±15.66	0.30±0.08	*Lysinibacillus*	1.00±0.05	1.19±0.03
Mycosphaerella	2.95±1.36	2.40±0.79	*Lysobacter*	18.16±0.53	5.31±1.59
Penicillium	0.66±0.51	14.03±3.31	*Marmoricola*	0.72±0.07	0.49±0.13
Pithoascus	0.09±0.06	0.03±0.03	*Nocardioides*	7.37±0.60	5.89±0.15
Preussia	0.40±0.43	0.10±0.12	*Planifilum*	1.15±0.12	0.61±0.07
Rhizopus	0.15±0.06	0.03±0.04	*Pontibacter*	0.92±0.12	1.54±0.56
Solicoccozyma	0.19±0.10	0.08±0.04	*Porphyrobacter*	0.71±0.10	0.32±0.13
Spiromastix	0.10±0.10	0.03±0.05	*Ramlibacter*	0.05±0.01	1.25±0.38
Spizellomyces	0.44±0.76	0.00±0.00	*Rhizorhabdus*	2.39±0.28	1.94±0.17
Stachybotrys	7.26±0.68	0.96±0.30	*Rubrobacter*	1.05±0.04	0.94±0.09
Stagonosporopsis	0.00±0.01	0.19±0.18	*Solirubrobacter*	1.20±0.06	1.35±0.17
Stemphylium	0.48±0.82	0.47±0.10	*Sphaerobacter*	2.82±0.15	4.79±0.13
Subramaniula	0.12±0.21	0.00±0.00	*Sphingomonas*	6.69±0.85	11.40±0.71
Thermomyces	0.25±0.23	0.07±0.01	*Sphingosinicella*	0.85±0.07	4.02±0.28
Thielavia	0.91±0.26	0.02±0.02	*Streptomyces*	0.79±0.03	0.89±0.02
Trichophaeopsis	0.51±0.66	0.20±0.30	*Tepidisphaera*	0.73±0.10	0.85±0.12
Others	1.43±0.39	0.39±0.06	Others	20.57±0.82	19.62±1.13
Unassigned	20.02±4.33	22.53±1.84	Unassigned	0.05±0.02	0.17±0.27

Values are means ± standard deviations of three replications.

Table A2.2. Mean values and standard deviations of the activities of β-glucosidase, urease, and alkaline phosphatase, in unamended soils (SU), and soils amended with compost (SA).

	SU			SA		
	β-Glucosidase (μmol PNP g^{-1} h^{-1})	Urease (μmol NH$_3$ g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})	β-Glucosidase (μmol PNP g^{-1} h^{-1})	Urease (μmol NH$_3$ g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})
7 DOI*						
Control	0.12 ± 0.01[a]	0.27 ± 0.03[a]	0.80 ± 0.05 [a]	0.17 ± 0.01[a]	0.41 ± 0.02[a]	1.31 ± 0.02 [a]
AgNP0.015	0.10 ± 0.005[a]	0.23 ± 0.02[a]	0.74 ± 0.05 [a]	0.16 ± 0.005[a]	0.34 ± 0.03[a]	1.34 ± 0.004[a]
AgNP1.5	0.11 ± 0.01[a]	0.27 ± 0.01[a]	0.79 ± 0.01[a]	0.15 ± 0.006[a]	0.34 ± 0.03[a]	1.26 ± 0.13[a]
30 DOI						
Control	0.10 ± 0.006[a]	0.14 ± 0.01[a]	0.46 ± 0.04[a]	0.16 ± 0.006[a]	0.26 ± 0.03[a]	0.66 ± 0.02[a]
AgNP0.015	0.10 ± 0.006[a]	0.15 ± 0.03[a]	0.44 ± 0.01[a]	0.15 ± 0.006[a]	0.21 ± 0.005[a]	0.69 ± 0.06[a]
AgNP0.015	0.09 ± 0.01[a]	0.12 ± 0.04[a]	0.38 ± 0.05[a]	0.15 ± 0.01[a]	0.16 ± 0.01[a]	0.60 ± 0.06[a]

Different letters indicate significant differences ($P < 0.05$) within each type of soil management.

*DOI = days of incubation

Table A2.3. Bacterial and fungal diversity indices in control and AgNPs- treated soils.

Treatments	Bacteria		Fungi	
	Shannon-Wiener	Chao-1	Shannon-Wiener	Chao-1
SU				
control	5.24±0.11a	3726±1227.94a	3.38±0.42a	510±112a
AgNP$_{0.015}$	5.15±0.08a	3247±377.80a	3.80±0.45a	518±255a
AgNP$_{1.5}$	5.15±0.03a	3100±352.58a	3.68±0.37a	261±1.81a
SA				
Control	5.55±0.08a	3158±871.13a	2.89±0.13a	378±125.6a
AgNP$_{0.015}$	5.56±0.06a	3370±238.05a	2.93±0.16a	438±184a
AgNP$_{1.5}$	5.40±0.13a	2338±64.52a	2.87±0.00a	371±65.5a

Data are shown as average ± standard deviation. Different letters indicate significant differences ($P < 0.05$) within the same microbial group and the same soil management. SA = soil amended with compost, SU = unamended soil, treated with two AgNPs concentrations (AgNP$_{0.015}$ = 0.015 μg kg^{-1}, AgNP$_{1.5}$ = 1.5 μg kg^{-1}), at 30 days of incubation.

ANEXO 3

Producción científica

Publicaciones científicas relacionadas al tema de tesis

Montes de Oca-Vásquez, Gabriela; Solano-Campos, Frank; Vega-Baudrit, José; López-Mondéjar, Rubén; Odriozola, I; Vera, Alfonso; Moreno, José Luis; Bastida, Felipe. (2020). Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity. *Journal of Hazardous Materials*, 391, 122224. https://doi.org/10.1016/j.jhazmat.2020.122224

Montes de Oca-Vásquez, Gabriela; Solano-Campos, Frank; Vega-Baudrit, José; López-Mondéjar, Rubén; Vera, Alfonso; Moreno, José Luis; Bastida, Felipe. (2020). Organic amendments exacerbate the effects of silver nanoparticles on microbial biomass and community composition of a semiarid soil. *Science of the Total Environment*, 744, 140919. https://doi.org/10.1016/j.scitotenv.2020.140919

Gabriela Montes de Oca-Vásquez, Frank Solano-Campos, Bernal Azofeifa-Bolaños, Amelia Paniagua-Vasquez, José Vega-Baudrit, Antonio Ruiz-Navarro, Rubén López-Mondéjar, Felipe Bastida. (2020). Microhabitat heterogeneity associated with Vanilla spp. And its influences on the microbial community of leaf litter and soil. *Soil Ecology Letter*, 2, 195-208. https://doi.org/10.1007/s42832-020-0041-7

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