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FERTILIZANTES Y HERBICIDAS FOSFATADOS EN EL SUELO: EFECTO SOBRE LA COMUNIDAD MICROBIANA Y LA DINÁMICA DE NUTRIENTES EN SUELOS AGRÍCOLAS DEL VALLE DE CUATRO CIÉNEGAS, COAHUILA

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RESUMEN

La agricultura es una de las actividades más importantes para los seres humanos, sin embargo, la superficie agrícola ha tenido que ser ampliada a zonas poco aptas, como son los ecosistemas desérticos, debido al continuo crecimiento poblacional y la necesidad de incrementar la productividad agrícola. Para aumentar la productividad agrícola, se emplean insumos externos, como fertilizantes minerales y plaguicidas, lo cual trae consecuencias como la degradación y pérdida de fertilidad de suelos, causada por diversos factores incluidos los cambios en la estructura y actividad de las comunidades microbianas del suelo. Por lo anterior, es necesario determinar los efectos de diferentes fertilizantes y plaguicidas, así como buscar estrategias que ayuden a reducir el grado de degradación de los suelos agrícolas. Una alternativa de manejo es la adición de materia orgánica, pues es una importante fuente de energía para que los microorganismos se mantengan activos y produzcan enzimas despolimerizadoras y mineralizadoras, aumentando la disponibilidad de N y P para las plantas. Sin embargo, es importante tomar en cuenta la composición de esta materia orgánica añadida para mejorar la disponibilidad de nutrientes. Además de los fertilizantes, los herbicidas son otro de los insumos agrícolas más utilizados. Este tipo de compuestos han generado diversos efectos nocivos en el ambiente, influyendo en la degradación de los suelos, y en particular, en los organismos que lo habitan. Uno de los herbicidas con mayor demanda es el glifosato, una molécula con un grupo fosfonato que se caracteriza por su enlace directo carbono-fósforo (C-P), el cual es más estable que el de otras moléculas orgánicas que contienen P y le confiere más resistencia a la degradación.

En México, las zonas áridas abarcan cerca del 50% de la superficie agrícola de nuestro país. Las zonas áridas y semiáridas en México se encuentran principalmente en los desiertos Chihuahuense, Sonorense, y en la región del complejo del valle de Tehuacán-Cuicatlán. Dentro del desierto Chihuahuense se encuentra el Valle de Cuatro Ciénegas, Coahuila (VCC), un sitio caracterizado por su baja disponibilidad de nutrientes, destacando su bajo contenido de P en suelo. A pesar de la escasez de agua y de la poca disponibilidad de nutrientes, los suelos del VCC han sido ampliamente utilizados para actividades agrícolas,

principalmente para producir alfalfa, lo que ha contribuido a la degradación del suelo debido al uso excesivo de agroquímicos y a los grandes volúmenes de agua requeridos.

Debido a lo anterior, la presente tesis tuvo como objetivo analizar el efecto de fertilizaciones de compuestos orgánicos e inorgánicos fosfatados y de un herbicida fosfatado (glifosato) en la dinámica de C, N, P y en la actividad microbiana de suelos agrícolas del VCC. Como resultados importantes se obtuvo que las fertilizaciones de compuestos orgánicos lábiles con fósforo, como ácido ribonucleico (ARN) y adenosina monofosfato (AMP), aumentan la mineralización potencial de C llevada a cabo por los microorganismos del suelo, además, los compuestos orgánicos fosfatados, independientemente de su estructura, estimulan la actividad de la enzima fosfodiesterasa en comparación con la adición de fertilizantes inorgánicos, como fosfato de calcio y fosfato de amonio. La adición de fertilizantes con fósforo influye en otros ciclos de nutrientes, como el C y el N, pues las moléculas orgánicas lábiles desencadenan un "efecto primming", permitiendo que los microorganismos degraden el C orgánico restante, y al no suministrarlo de manera continua, existe menor competencia por parte de los microorganismos heterótrofos, permitiendo el crecimiento y aumento de actividad de los microorganismos quimioautótrofos capaces de realizar el proceso de nitrificación.

Además de los fertilizantes fosfatados, el glifosato y una formulación comercial de este herbicida, también alteran aspectos de la dinámica de C y P, ya que ambos compuestos disminuyen la mineralización potencial de C de los microorganismos del suelo, además, la formulación comercial induce un aumento en la actividad de fosfodiesterasas y fosfonatasas e influye en la sorción no biológica del P. El herbicida produce cambios en la dominancia de algunos ácidos grasos, disminuyendo la dominancia de ácidos grasos pertenecientes a hongos y actinobacterias y de algunos relacionados a bacterias Gram negativas. Los efectos colaterales más fuertes, se presentaron al aplicar la formulación comercial del herbicida y en un sitio donde el glifosato no había sido aplicado previamente, en comparación con un sitio con historial de 5 años de uso continuo de glifosato. Aun así, se obtuvieron aislados bacterianos capaces de utilizar, tanto el glifosato puro como su formulación comercial como

única fuente de fósforo, los cuales podrían ser estudiados en un futuro para biorremediar sitios contaminados con el herbicida.

ABSTRACT

Agriculture is one of the most important activities for human beings, however, the agricultural area has been expanded to unsuitable areas, such as desert ecosystems, due to continuous population growth and the need to increase agricultural productivity. To increase agricultural productivity, external inputs are used, such as mineral fertilizers and pesticides, which has consequences such as soil degradation and loss of fertility, caused by various factors including changes in the structure and activity of soil microbial communities. Therefore, it is necessary to determine the effects of different fertilizers and pesticides, as well as seek strategies that help reduce the degree of degradation of agricultural soils. A management alternative is the addition of organic matter, since it is an important source of energy for microorganisms to remain active and produce depolymerizing and mineralizing enzymes, increasing the availability of N and P for plants. However, it is important to consider the composition of this added organic matter to improve nutrient availability. In addition to fertilizers, herbicides are another of the most widely used agricultural inputs. These kinds of compounds have generated several effects on the environment, influencing the degradation of soils, and in particular, the organisms that inhabit it. One of the most demanded herbicides is glyphosate, a molecule with a phosphonate group, characterized by its direct carbon-phosphorus (C-P) bond, which is more stable than that of other organic molecules that contain P and confers more resistance to degradation

In Mexico, drylands cover about 50% of the agricultural area of our country. Desert areas in Mexico are found primarily in the Chihuahuan, Sonoran, and Tehuacán-Cuicatlán valley complex region. Within the Chihuahuan desert is the Cuatro Ciénegas Basin, in Coahuila (CCB), a site characterized by its scarcity of nutrients, highlighting its low content of P in the soil. Despite the scarcity of water and the limited availability of nutrients, the soils of the CCB have been widely used for agricultural activities, mainly to produce alfalfa, which has contributed to soil degradation due to the excessive use of agrochemicals and large volumes of water required.

Due to the above, this thesis aims to analyze the effect of fertilization of organic and inorganic phosphate compounds and a phosphate herbicide (glyphosate) on the dynamics of C, N, P and on the microbial activity of agricultural soils of the CCB. As important results, we obtained that the fertilizations of labile organic compounds with phosphorus, such as ribonucleic acid (RNA) and adenosine monophosphate (AMP), increase the C mineralization performed by soil microorganisms, in addition, phosphate organic compounds, regardless of their structure, stimulate the phosphodiesterase enzyme activity compared to the addition of inorganic fertilizers such as calcium phosphate and ammonium phosphate. The addition of fertilizers with phosphorus influences other nutrient cycles, such as C and N, since labile organic molecules triggered a "priming effect", allowing microorganisms to degrade the remaining organic C, and when C is not supplied continuously, there is less competition from heterotrophic microorganisms, allowing the growth and increased activity of autotrophic microorganisms capable of carrying out the nitrification process.

In addition to phosphate fertilizers, glyphosate and a commercial formulation of this herbicide also alter aspects of C and P cycling since both compounds decreased soil microbial C mineralization. In addition, the commercial formulation induces an increase in the activity of phosphodiesterases and phosphonatases and influences the non-biological sorption of P. The herbicide produces changes in the dominance of some microbial fatty acids, decreasing the dominance of fungi and actinobacteria and of some fatty acids related to Gram negative bacteria. The strongest side effects occurred with the application of the commercial formulation of the herbicide and at a site where glyphosate had not been previously applied, compared to a site with a 5-year history of glyphosate use. Even so, bacterial isolates capable of using both pure glyphosate and its commercial formulation as the only source of phosphorus were obtained, which could be studied in the future to bioremediate sites contaminated with the herbicide.

INTRODUCCIÓN GENERAL

La agricultura es una de las actividades más importantes para los seres humanos, sin embargo, la superficie agrícola ha tenido que ser ampliada, muchas veces a zonas poco aptas para esta actividad como son los ecosistemas desérticos, debido al continuo crecimiento poblacional y la necesidad de incrementar la productividad agrícola (SEMARNAT, 2012). Los ecosistemas desérticos cubren aproximadamente 45% de la superficie terrestre de nuestro planeta (Prăvălie et al., 2019) y se estima que en estos ecosistemas habitan más de 2 mil millones de personas (Prăvălie, 2016). A pesar de que en estos ecosistemas existe poca disponibilidad de agua, energía y nutrientes en el suelo, el 25% de la superficie de estos sitios está destinado a la agricultura (Prăvălie, 2016).

La alta demanda de los productos agrícolas ha conllevado a la intensificación de métodos para realizar esta actividad, los cuales implican utilizar mayores volúmenes de agua, energía, fertilizantes, plaguicidas, herbicidas y especies no nativas (Rey Benayas & Bullock, 2012). Los ecosistemas áridos y semiáridos tienen condiciones climáticas y edáficas difíciles para llevar a cabo las prácticas agrícolas, debido a las cortas y variables temporadas de lluvia, la degradación del suelo, el bajo contenido de materia orgánica del suelo y la alta salinidad (García-Palacios et al., 2019; Kassam et al., 2012); sin embargo, después de la revolución verde durante la segunda mitad del siglo 20, la utilización de fertilizantes sintéticos y plaguicidas ayudaron a mejorar el rendimiento de los cultivos, aunque esto ha tenido diversos impactos ambientales (García-Palacios et al., 2019). Los cultivos agrícolas no son 100% eficientes para utilizar los nutrientes proporcionados por fertilizantes minerales; se estima que la adquisición de nitrógeno (N) por las plantas es de un 30 a 50%, mientras que la adquisición de fósforo es de 10 a 25% del aplicado (Lubkowski, 2016). Los nutrientes que no son absorbidos por las plantas pasan por procesos que llevan a disminuir su disponibilidad, como la lixiviación, volatilización, precipitación y transformaciones microbianas. Algunos de estos procesos, como la lixiviación y la volatilización, producen la dispersión ambiental del exceso de fertilizantes, ocasionando la contaminación de otros ecosistemas, como los acuáticos, provocando problemas como la eutrofización (Lubkowski, 2016). Además, las

cantidades excesivas de fertilizantes en el suelo, pueden cambiar tanto el nivel de salinidad, como el pH del suelo, conduciendo a su degradación gradual (Lubkowski, 2016).

El fósforo, es uno de los principales nutrientes limitantes para la productividad primaria en los ecosistemas desérticos (Cross & Schlesinger, 2001). La fertilización mineral con fósforo, por lo general resulta en la acumulación de este elemento en formas no disponibles o en su pérdida por lixiviación. En ecosistemas desérticos dominan tipos de suelo ricos en calcio, como los Calcisols y Gipsisols (Koohafkan & Stewart, 2008), lo que propicia que ocurran reacciones de precipitación de fósforo con calcio, formando compuestos insolubles (Chapin III et al., 2011; Cross & Schlesinger, 1995, 2001). El P también es susceptible a procesos de adsorción y oclusión en partículas del suelo, convirtiéndose en un nutriente no disponible para las plantas de cultivo. Sin embargo, las plantas necesitan el P para producir compuestos esenciales para la vida, como los fosfolípidos de membrana, el ATP, y los ácidos nucleicos (White & Metcalf, 2007).

Los insumos agrícolas, como fertilizantes minerales y plaguicidas, no solo tienen efectos en las características físicas y químicas del suelo o por su dispersión a otros ecosistemas, también pueden tener efectos en los microorganismos del suelo, los cuales a menudo son pasados por alto. Estos efectos pueden medirse como cambios en la riqueza y abundancia de microorganismos, en la biomasa microbiana del suelo, o en la actividad biológica, como la respiración del suelo y las actividades enzimáticas (E. K. Bünemann et al., 2006). Los efectos de la aplicación de fertilizantes minerales sobre los microorganismos suelen variar entre diferentes estudios y estas variaciones están relacionadas con la disminución del pH del suelo por fertilizaciones de N o S, y con cambios en la concentración de C orgánico, que puede llegar a aumentar en experimentos en campo y en macetas debido al aumento de biomasa de los cultivos lo que propicia la liberación de C orgánico al suelo (E. K. Bünemann et al., 2006; Moore et al., 2000). También se ha determinado que fertilizaciones con P pueden cambiar la estructura de la comunidad microbiana del suelo, aumentando la abundancia de bacterias copiótrofas (de rápido crecimiento) debido a la facilidad de adquisición del P inorgánico, y a que al no tener limitación por P, la producción de enzimas para obtención de carbono orgánico (energía) es más eficiente (Fanin et al., 2015).

Es importante también estudiar el efecto que puede tener la aplicación de fertilizantes sobre las funciones de los microorganismos del suelo. Los microorganismos son esenciales en el reciclaje de nutrientes en el ecosistema y en la disponibilidad de nutrientes, debido a que realizan procesos como la fijación de N, la mineralización de P orgánico y la solubilización de P (Prashar et al. 2014). Debido a que las plantas necesitan obtener nutrientes como el P de forma inorgánica y soluble, es importante mantener la abundancia y actividad de los microorganismos capaces de realizar procesos como la mineralización de fósforo orgánico (Mącik et al., 2020). La mineralización de P es realizada por la acción de enzimas hidrolíticas, como las fosfatasas, fosfodiesterasas, fitasas y fosfonatasas (Turner et al., 2003; Cade-Menun 2005). La producción de fosfatasas está regulada por el regulón Pho que además expresa genes para transportar y asimilar P; sin embargo, se conoce que muchos genes pertenecientes a este regulón pueden inhibir su expresión cuando hay altas concentraciones de P en el ambiente (Vershinina & Znamenskaya, 2002), por lo que la aplicación continua de fertilizantes fosfatados en suelos agrícolas podría resultar en la pérdida de funciones microbianas. También se ha demostrado la disminución de copias del gen phoD encargado de la producción de fosfatasas debido a fertilizaciones con P inorgánico (Ikoyi et al., 2018), así como el cambio en la estructura de comunidades fúngicas, bacterianas y micorrícicas (Ikoyi et al., 2018; Mącik et al., 2020).

Debido a los efectos descritos que tienen las fertilizaciones minerales en las prácticas de agricultura intensiva, es necesario buscar alternativas que ayuden a realizar una agricultura más sustentable, en el contexto de alcanzar los objetivos de producción sin comprometer el futuro en términos de degradación o agotamiento de los recursos naturales (Matson et al., 1997). Una de estas alternativas son las prácticas de agricultura orgánica, que en vez de utilizar fertilizantes inorgánicos, promueven el uso de fertilizantes y enmiendas orgánicas como compostas y abonos (Garibaldi et al., 2017).

El uso de enmiendas orgánicas puede proporcionar al suelo características deseables como mayor capacidad de retención de agua y capacidad de intercambio catiónico, además de promover el crecimiento de organismos benéficos (Bulluck et al., 2002), aumentar el

contenido de biomasa microbiana del suelo, la actividad de los microorganismos (Bastida et al., 2008; Fernández-Getino et al. 2012; Ros, Hernandez, and García 2003; Scotti et al. 2015), además de la protección de los nutrientes como N y P, por el aumento de P y N en biomasa microbiana, evitando pérdidas por lixiviación (Bulluck et al., 2002; Mäder et al., 2002), mejorando de esta manera la fertilidad y calidad del suelo.

La adición de materia orgánica es también un método para recuperar suelos degradados en zonas áridas y semiáridas que han perdido su fertilidad debido a prácticas agrícolas, que ayuda a mejorar las características del suelo, en especial el ciclaje de nutrientes (Ros et al., 2003). Diversos estudios han demostrado que la utilización de enmiendas orgánicas, como la utilización de abonos y abonos verdes pueden reducir las condiciones salinas del suelo; sin embargo, la disminución de sodicidad edáfica es variable (Seenivasan, Prasath, y Mohanraj 2016; Badia 2000), además han demostrado que la adición de materia orgánica proporciona un suministro de energía y nutrientes a los microorganismos resistentes a la salinidad, y ayuda a la activación de su metabolismo que suele ser bajo, debido a las condiciones de bajo contenido de C orgánico que tienen por lo general los suelos salinos (Wong, Dalal, y Greene 2009).

No obstante, la materia orgánica es altamente compleja y heterogénea, contiene compuestos orgánicos que varían desde biomasa microbiana lábil y material vegetal fresco hasta compuestos que pueden permanecer en el suelo por miles de años (Townsend et al. 1995). Es importante conocer el contenido de nutrientes de las enmiendas orgánicas, pero la concentración total de un nutriente (C, N o P) en una enmienda no garantiza que este se encuentre en una forma química disponible para ser utilizado por el cultivo en un tiempo determinado. Por lo tanto, la concentración total de nutrientes no es un indicador adecuado de la calidad de abonos o enmiendas orgánicas (Wang et al., 2004). La influencia de la materia orgánica en las propiedades del suelo y en los microorganismos depende de la cantidad, calidad y del tipo de los materiales añadidos (Barzegar et al., 2002).

Además de los fertilizantes inorgánicos, los plaguicidas también son insumos agrícolas ampliamente utilizados, ya que se estima que a nivel mundial se utilizan 2 millones de

toneladas de plaguicidas anualmente (Sharma et al., 2019). Los herbicidas representan cerca del 60% del tipo de plaguicidas más usados (Duke & Dayan, 2018). El glifosato, es el herbicida más utilizado globalmente, en términos de kilogramos por año (Duke, 2020). El glifosato (N-fosfonometil glicina) es un compuesto químico cuya estructura molecular contiene un grupo fosfonato, un átomo de carbono unido directamente a un átomo de fósforo por medio de un enlace covalente, que a diferencia de los esteres de fosfato, tiene mayor resistencia a la degradación (Hayes et al., 2000). El grupo fosfonato le da al glifosato características respecto a su movilidad en el suelo, ya que tiene la capacidad de formar enlaces con minerales del suelo, de forma similar a las moléculas de ortofosfato (PO_4^{3-}) compitiendo por los mismos sitios de adsorción (De Jonge et al., 2001), por lo que las fertilizaciones con fósforo inorgánico en campos agrícolas pueden influenciar la biodisponibilidad y transporte del glifosato en el suelo (Gimsing & Borggaard, 2002). El glifosato ha sido estudiado en cuanto a su ecotoxicidad en organismos acuáticos, macrofauna del suelo, aves y humanos (Blake & Pallett, 2018; Duke, 2020); sin embargo, los efectos en los microorganismos del suelo son menos conocidos y varían dependiendo de características como el pH del suelo y las dosis de glifosato aplicadas (Nguyen et al., 2016). Los microorganismos del suelo son además la clave para la degradación del glifosato, ya que algunas bacterias, producen enzimas como la fosfonatasa y el complejo enzimático C-P liasa que son capaces de romper el enlace directo C-P de los fosfonatos con el fin de utilizar estas moléculas como fuente de fósforo (Shushkova et al., 2012; Sviridov et al., 2015), por lo que el uso de microorganismos se ha considerado una opción viable y eficiente para la restauración de ecosistemas contaminados con herbicidas organofosforados (Singh & Walker, 2006).

Algunos de estos microorganismos útiles para la biorremediacicón de compuestos organofosforados, pueden encontrarse en sitios con oligotrofía de P. Las concentraciones más bajas de P total en suelos, han sido reportadas en ecosistemas áridos (Tapia-Torres & García-Oliva, 2013). En México, las zonas áridas abarcan cerca del 50% de la superficie agrícola de nuestro país (SEMARNAT, 2012). Las zonas áridas y semiáridas en México se encuentran principalmente en los desiertos Chihuahuense y Sonorense, en la región del complejo del valle de Tehuacán-Cuicatlán, ubicado en los estados de Puebla y Oaxaca y en el semidesierto

Queretano-Hidalguense. Dentro del desierto Chihuahuense, se encuentra el valle de Cuatro Ciénegas, Coahuila (VCC), un sitio caracterizado por su bajo contenido de P en el suelo, donde se presentan concentraciones hasta 5 veces menores del P total a las reportadas en otros desiertos norteamericanos (Tapia-Torres & García-Oliva, 2013). Aunado a la limitación por P, el N también es un elemento limitante debido a la baja productividad de este ecosistema por las condiciones de aridez, ya que la disponibilidad de este nutriente en el suelo depende en gran medida de los microorganismos que llevan a cabo su fijación biológica y la descomposición de la materia orgánica (Augusto et al., 2017). Sin embargo, a pesar de la escasez de agua y de la poca disponibilidad de nutrientes, los suelos del VCC han sido ampliamente utilizados para actividades agrícolas, principalmente para la producción de alfalfa, la cual ocupa más del 71% de la superficie sembrada en esta región (Beltrán, 2017). El cultivo de alfalfa es uno de los factores que más ha contribuido a la degradación del suelo, por el uso de fertilizantes químicos, plaguicidas (Challenger, 1998) y los grandes volúmenes de agua requeridos por este cultivo (aproximadamente 219 L kg^{-1} de alfalfa; Ríos-Flores et al., 2011).

Entre los principales cambios de la dinámica de nutrientes del suelo debido a la actividad agrícola en el VCC se encuentra el aumento de nitrificación (Hernández-Becerra et al. 2016; Martínez-Piedragil 2013), que aumenta la susceptibilidad del suelo a la pérdida del N por lixiviación y desnitrificación. Las actividades agrícolas también han modificado la composición de la comunidad de microorganismos edáficos; por ejemplo, se ha reportado la desaparición del phyla Cyanobacteria en un cultivo de alfalfa de Cuatro Ciénegas, y reducido la abundancia de phyla Proteobacterias y de Actinobacterias (Hernández-Becerra et al., 2016). Estos tres phyla contienen grupos de bacterias con distintas funciones importantes en el ecosistema, como la fotosíntesis (Cyanobacteria), la fijación de nitrógeno, nitrificación, desnitrificación y solubilización de fósforo (algunas bacterias del phyla Proteobacteria), la degradación de compuestos orgánicos complejos como celulosa o quitina y la producción de antibióticos (algunas bacterias del phyla Actinobacteria; Prescott, Harley, y Klein 2004; Paul 2014).

Por todo lo anterior, es necesario buscar estrategias que ayuden a reducir el grado de degradación de los suelos agrícolas en el VCC, como la utilización de enmiendas orgánicas y la búsqueda de microorganismos capaces de degradar compuestos contaminantes, como los herbicidas. De los trabajos previos, se sabe que en condiciones naturales del VCC, existen microorganismos capaces de favorecer la disponibilidad de nutrientes, pues tienen diversas estrategias para liberar el P de diferentes moléculas orgánicas y de moléculas inorgánicas estables como los fosfatos de calcio (Tapia-Torres et al., 2016) en las condiciones químicas que presentan sus suelos (pH alcalino, superior a 8; Tapia-Torres et al. 2015) y al aplicar materia orgánica en suelos agrícolas reduciendo las fertilizaciones minerales, estos microorganismos podrían disponer de energía para mantenerse activos, pero es necesario conocer las necesidades energéticas y nutricionales de estos que permiten facilitar el suministro de nutrientes a las plantas de cultivos agrícolas. Además, trabajos previos han demostrado que los microorganismos del VCC tienen la capacidad de obtener el fósforo a partir de fosfonatos utilizando este tipo de compuestos para crecer, que existe actividad de la enzima fosfonatasa en suelos, y que hay presencia de bacterias con los genes *phn*, que codifican para importadores de fosfonatos, permeasas y C-P liasas (Alcaraz et al., 2010; Tapia-Torres et al., 2016), por lo que los microorganismos de Cuatro Ciénegas, podrían degradar compuestos con grupos fosfonato como lo es el glifosato.

Pregunta de investigación

¿Cómo afecta la adición de fertilizantes orgánicos e inorgánicos con fósforo y de herbicidas organofosforados a la actividad microbiana y dinámica de nutrientes de suelos agrícolas del valle de Cuatro Ciénegas?

Objetivo General

Analizar el efecto de la adición de fertilizaciones de compuestos orgánicos e inorgánicos fosfatados y de un herbicida fosfatado (glifosato) en la dinámica de C, N y P y en la actividad microbiana de suelos agrícolas del valle de Cuatro Ciénegas, Coahuila.

Objetivos específicos

1. Realizar una revisión bibliográfica sobre el papel de los microorganismos y sus enzimas en la descomposición de la materia orgánica del suelo.
2. Determinar el efecto de fertilizaciones con moléculas de fósforo orgánicas e inorgánicas en la actividad microbiana (respiración y actividad enzimática) de suelo agrícola del VCC, en la dinámica de los nutrientes C, N y P, y en las relaciones estequiométricas entre los microorganismos del suelo y sus recursos.
3. Determinar el efecto de un herbicida organofosforado (glifosato) en la actividad microbiana de suelo agrícola del VCC, la dinámica de los nutrientes C, N y P y la capacidad de las bacterias del suelo para degradar el glifosato y una formulación comercial de este herbicida.

Estructura de la tesis

Para cumplir con los objetivos descritos, la presente tesis se compone de 3 capítulos, los cuales se resumen a continuación:

Capítulo I. El papel de los microorganismos en la producción de exoenzimas clave para la liberación de nutrientes (N y P) a partir de la materia orgánica del suelo:

Este capítulo consiste en una revisión de la literatura que reúne información sobre el papel que juegan los microorganismos en la descomposición de la materia orgánica del suelo, las enzimas involucradas y los distintos procesos que realizan para promover el reciclado de nutrientes orgánicos en suelos agrícolas.

Capítulo II. How inorganic and organic phosphorus molecules modify microbial activity, stoichiometry, and nutrient dynamics in an agricultural soil:

Este capítulo tuvo como objetivo probar experimentalmente los efectos de la incorporación de compuestos orgánicos e inorgánicos fosfatados en la transformación de nutrientes y en la actividad microbiana de suelos agrícolas del VCC. Se utilizaron los compuestos orgánicos que más comúnmente están presentes en el suelo y en la materia orgánica, los cuales son ácido fítico, diésteres de fosfato (el compuesto utilizado fue ARN) y monoésteres de fosfato (el compuesto utilizado fue adenosín monofosfato); en cuanto a los compuestos inorgánicos se utilizaron dos fertilizantes de uso agrícola, el fosfato de amonio (MAP) y el fosfato de calcio. Se realizó un experimento de incubaciones del suelo obtenido de una parcela de alfalfa del VCC para aplicar los conceptos de estequiometría ecológica y determinar cómo las diferentes fuentes de P modifican las limitaciones de nutrientes para los microorganismos del suelo y su eficiencia de uso de carbono; además se midió la actividad microbiana a partir del potencial mineralización de C y las actividades de exoenzimas despolimerizadoras y mineralizadoras.

Capítulo III. Glyphosate-based herbicides alter soil carbon and phosphorus dynamics and microbial activity:

Este capítulo se enfocó en investigar el efecto del glifosato, un herbicida organofosforado, en su forma pura y comercial, en la dinámica de nutrientes y actividad microbiana de suelos agrícolas con uso previo de glifosato y suelos abandonados sin uso previo de glifosato. En este capítulo específicamente se buscaron los posibles efectos del glifosato y de su formulación comercial en la adsorción de fósforo en el suelo y en la disponibilidad de nutrientes, tomando en cuenta los almacenes de nutrientes orgánicos, nutrientes

inmovilizados en biomasa microbiana, la actividad enzimática, los análisis estequiométricos de adquisición de nutrientes y la mineralización potencial del C, además se midieron los cambios en la estructura de la comunidad microbiana del suelo por medio de un perfil de ácidos grasos y el potencial de las bacterias del suelo para degradar el herbicida comercial y en su forma pura para utilizarlo como fuente de fósforo.

CAPÍTULO I: El papel de los microorganismos en la producción de exoenzimas clave para la liberación de nutrientes (N y P) a partir de la materia orgánica del suelo

Pamela Chávez-Ortiz, Felipe García-Oliva

El papel de los microorganismos en la producción de exoenzimas clave para la liberación de nutrientes (N y P) a partir de la materia orgánica del suelo

The role of microorganisms in the production of key exoenzymes for nutrient release (N and P) from soil organic matter

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RESUMEN

La materia orgánica del suelo es un almacén importante de energía y nutrientes, por esto, su manejo puede ser una herramienta importante para el aumento de la fertilidad de suelos agrícolas, por lo tanto es necesario conocer los factores que afectan a la descomposición de la MO y que promueven la liberación de nutrientes esenciales como el nitrógeno (N) y el fósforo (P). La presente revisión está enfocada en uno de los factores bióticos que afecta la descomposición de la MO, que es la diversidad, actividad y función de la comunidad microbiana del suelo. Los microorganismos del suelo, para cumplir sus demandas nutricionales, tienen la capacidad de producir enzimas extracelulares (también conocidas como exoenzimas o ecoenzimas) que realizan procesos de despolimerización de las macromoléculas que forman la materia orgánica del suelo y procesos de mineralización, donde se liberan nutrientes en forma inorgánica como NH_4^+, y HPO_4^{2-}. Este artículo, se centró principalmente en las enzimas β-Glucosidasa, lacasa, N-acetil glucosidasa, fosfodiesterasa, fosfomonoesterasa, fitasa y fosfonatasa, en las reacciones que realizan estas enzimas, en la información actual sobre su distribución en los diferentes grupos microbianos del suelo, y en cómo su producción es regulada a partir de la estequiometría entre nutrientes disponibles del

suelo y nutrientes en la biomasa microbiana (C, N y P). La revisión realizada para este artículo hizo notar además, la falta de estudio sobre enzimas encargadas de la descomposición de moléculas orgánicas con P. A pesar de que este es un nutriente comunmente limitante en el suelo y por lo tanto, se necesitan conocer los distintos mecanismos que existen para su reciclaje a partir de la MO, diversos estudios se enfocan en el estudio de fosfatasas en general sin abordar otras enzimas encargadas de la obtención de P, como fosfodiesterasas, fitasas alcalinas y fosfonatasas.

Palabras clave: *bacterias; disponibilidad de nutrientes; estequiometría; fósforo; nitrógeno*

SUMMARY

Soil organic matter is an important pool of nutrients, and its management can be an important tool for increasing the fertility of agricultural soils. For this reason, it is necessary to know the factors that affect its decomposition and promote the release of essential nutrients such as nitrogen and phosphorus. This review is focused on one of the biotic factors that affects the decomposition of organic matter, which is the activity and function of the soil microbial community. Soil microorganisms, to meet their nutritional demands, produce extracellular enzymes (exoenzymes) that carry out processes of depolymerization of macromolecules that form the organic matter of the soil and mineralization processes, which leave free nutrients in inorganic form such as NH_4^+, and HPO_4^{2-}. In this article, we focus mainly on the enzymes β-Glucosidase, laccase, N-acetyl glucosidase, phosphodiesterase, phosphomonoesterase, phytase and phosphonatase, in the reactions that these enzymes carry out, in what is currently known about their distribution in the different groups soil microbes, and how their production is regulated from the stoichiometry between available nutrients in the soil and nutrients in the microbial biomass (C, N and P).

Index words: *bacteria; nitrogen; nutrient availability; phosphorus; stoichiometry*

INTRODUCCIÓN

La materia orgánica del suelo (MOS) es uno de los componentes principales que define la calidad del suelo, ya que regula su humedad, estructura, suministro de nutrientes disponibles y la actividad microbiana (McLauchlan, 2006). En particular, la MOS es la principal fuente de energía para los microorganismos heterótrofos, así como también de nutrientes contenidos en las moléculas orgánicas, tales como el N y el P (Lehmann y Kleber, 2015). Sin embargo, los sistemas agrícolas intensivos han utilizado los fertilizantes inorgánicos para satisfacer las grandes cantidades de nutrientes que demandan, reduciendo de esta manera la cantidad y modificando la composición química de la MOS (Voroney *et al.,* 1981). Algunos autores consideran que aumentar la cantidad de MOS es una alternativa para mejorar la calidad de los suelos agrícolas (Tiessen *et al.,* 1992; Badia, 2000; Ding *et al.*, 2013).

Sin embargo, la MO utilizada como abonos y enmiendas orgánicas, tiene una gran variabilidad en su composición química, en su estabilización en el suelo y en el tipo y cantidad de nutrientes que puede liberar en el suelo al ser degradada (Sikora y Szmidth, 2001). Para poder hacer un manejo adecuado de la MOS en sistemas agrícolas, es por lo tanto importante conocer los mecanismos de su descomposición y su estabilización en el suelo, así como los principales factores que determinan estos dos procesos. Los microorganismos del suelo, su diversidad y su capacidad metabólica, son los principales actores bióticos que controlan la transformación de la MOS (Schimel y Weintraub, 2003). Por esto mismo, el presente trabajo tuvo como objetivo realizar una revisión de la literatura sobre el papel que juegan los microorganismos en la descomposición de la materia orgánica del suelo, y los procesos microbianos que favorecen el reciclado de nutrientes en suelos a partir de la degradación de la MOS, por medio de la producción de enzimas extracelulares. En este trabajo se abordan los procesos de descomposición y estabilización de la MOS, los procesos realizados por microorganismos para degradar los compuestos más abundantes en la MO, como celulosa, lignina, quitina, ésteres y diésteres de fosfato por medio de la producción de enzimas extracelulares (exo-enzimas), la distribución de las principales exo-enzimas encargadas del reciclaje de nutrientes en los distintos phyla microbianos edáficos y finalmente se aborda el tema de estequiometría, donde se intenta entender cómo la síntesis

de exo-enzimas está relacionada con el mantenimiento de la estequiometría entre los cocientes de nutrientes en la biomasa microbiana y los cocientes de nutrientes en la MOS.

DESARROLLO DEL TEMA

Fracciones de la materia orgánica, procesos de estabilización y descomposición

La materia orgánica del suelo es altamente compleja y heterogénea, contiene compuestos orgánicos que varían desde la biomasa microbiana lábil y material vegetal fresco hasta compuestos que pueden permanecer en el suelo por miles de años (Townsend *et al.*, 1995). Por lo anterior, la MOS se divide en diferentes fracciones o almacenes. Las fracciones físicas en las que la MOS se divide para su estudio en laboratorio, son la materia orgánica particulada (MOP) y la materia orgánica unida a minerales (MOM), dentro de la cual se encuentra la materia orgánica disuelta (MOD). Cada una de estas fracciones tiene caracaterísticas distintas, mientras que la MOP consiste en fragmentos pequeños de MO y forma parte de la MO que no está protegida contra la descomposición (Six et al., 2002), la MOM consiste en moléculas o fragmentos microscópicos de material orgánico que ha sido transformado, los cuales están más protegidos contra la descomposición debido a su asociación con minerales, convirtiéndose en una fracción de la MOS más persistente (Lavallee et al., 2020). La MOD, forma parte de esta última fracción y es una fracción en la MOS altamente dinámica (Lavallee et al., 2020).

La MOD se define como la fracción soluble de la MO que puede pasar a través de filtros de 0.45 um (Bolan et al., 2011). A pesar de que la MOD, representa solo una pequeña fracción, es altamente móvil y activa, por lo que tiene gran influencia en los ciclos biogeoquímicos, además de que las concentraciones de MOD son altamente susceptibles a cambios inducidos por humanos, como la agricultura (Bolan et al., 2011).

Sin embargo, la MOS tambien se puede dividir en distintas fracciones de acuerdo a su tiempo de recambio: una fracción lábil o activa (5%) que contiene microorganismos vivos y productos microbianos, además de residuos y exudados vegetales y animales con un tiempo de recambio entre 1 y 5 años; una fracción intermedia (60-85% del total) donde la materia orgánica se encuentra físicamente protegida y/o en formas químicas con más resistencia a la descomposición, con un tiempo de descomposición que va de años a décadas; y una fracción

pasiva (10-40% del total), la cual es químicamente recalcitrante y puede estar también protegida físicamente, de la cual su tiempo de descomposición puede ser de cientos a miles de años (Townsend *et al.,* 1995; Parton *et al.,* 1987).

La formación de los almacenes intermedios y pasivos se atribuye principalmente a la preservación selectiva de sustancias recalcitrantes que se obtienen de la descomposición del material vegetal (Lützow *et al.,* 2008), además de la interacción de la materia orgánica con minerales y de sus propiedades hidrofóbicas; que forman parte de los mecanismos de estabilización de la MOS (Sollins *et al.,* 1996).

La estabilización de la MOS se define como la disminución de la MOS que puede potencialmente perderse por respiración (Sollins *et al.,* 1996) y es efecto de tres principales mecanismos: El aumento en la recalcitrancia de las moléculas, las interacciones organominerales y la disminución de su accesibilidad (protección física).

Se consideran moléculas recalcitrantes a aquellas que presentan mayor resistencia a la degradación (Lützow *et al.,* 2008). Las moléculas recalcitrantes pueden ser de origen vegetal (residuos de las plantas y rizodepósitos; Lützow *et al.,* 2006), de origen microbiano, como mureina, quitina, melanina y algunos lípidos o de origen animal, como la quitina y la queratina (Lützow *et al.,* 2008). Algunos de estos compuestos son menos degradables debido a su composición estructural, por factores como tamaño de la molécula, polaridad, enlaces ester, átomos de C cuaternarios, grupos nitrogenados fenil- y heterocíclicos e hidrocarbonos de cadenas largas (hidrofóbicos; Golchin *et al.,* 1997). Los compuestos macromoleculares más fácilmente degradables son aquellos que tienen enlaces hidrolizables, que pueden romperse por medio de enzimas hidrolasas (Lützow *et al.,* 2008), mientras que los más recalcitrantes contienen anillos aromáticos en su estructura, como la lignina; y moléculas ricas en C alquilo, como los lípidos, ceras, cutina y suberina (Kögel-Knabner, 2002). Así mismo, son recalcitrantes los compuestos derivados del proceso de humificación. Los polímeros húmicos se forman por la descomposición parcial de polímeros, seguido de reacciones de condensación y polimerización, las cuales son extracelulares y espontáneas, posiblemente catalizadas por exoenzimas o enzimas que provienen de la autólisis celular

(Sollins, *et al.,* 1996). La recalcitrancia inherente o adquirida de las moléculas orgánicas, es un mecanismo de protección bioquímica contra la descomposición y estas moléculas pueden considerarse parte del almacén pasivo de la MOS (Six et al., 2002)

A pesar de que la estructura química es un factor importante en las primeras etapas de la descomposición de la MO, a largo plazo pueden tomar más importancia otros mecanismos de estabilización. La MOS interactúa con superficies minerales del suelo, como las arcillas, y puede someterse a procesos de precipitación, sorción y formación de complejos, lo cual disminuye su disponibilidad para ser utilizada por microorganismos (Sollins *et al.,* 1996), a pesar de que las moléculas sean pequeñas y fácilmente degradables (Lützow *et al.,* 2008), constituyendo un mecanismo de estabilización química (Six et al., 2002). La disminución en la accesibilidad a la MOS, es ocasionado por procesos de agregación de la MOS formando un almacen de MO físicamente protegida (Six et al., 2002). Los procesos de precipitación, sorción y formación de complejos anteriormente explicados también influyen en los procesos de agregación. Los macroagregados del suelo (mayores a 250 micras) pueden ocluir compuestos macromoleculares como la lignina, quitina, melanina y polisacáridos (Lützow *et al.,* 2008), sin embargo, los microagregados protegen fisicamente a la MOS de forma más estable y por un mayor periodo de tiempo contra la descomposición, y pueden formar parte del almacen intermedio/lento de la MOS (Six et al., 2002). Los procesos de estabilización debido a la falta de accesibilidad de la MO y las interacciones organominerales, dominan en las fases posteriores de la descomposición y operan a escalas mayores de tiempo, en comparación con la estabilización relacionada a la recalcitrancia química (Sollins *et al.,* 1996).

La descomposición de la materia orgánica está altamente relacionada a los procesos de estabilización, y se puede dividir este proceso en tres etapas (Berg, 2000):

Etapa 1. Depende de la calidad del sustrato (Berg, 2000) y consiste en la descomposición de sustancias solubles y holocelulosa no lignificada. En esta fase, la tasa de degradación aumenta cuando existe una mayor concentración de nitrógeno (N), fósforo (P) y azufre (S;

Berg 2014). En esta etapa existe nula o poca degradación de compuestos lignificados (Berg, 2000). Esta etapa está relacionada a la descomposición de la fracción activa o lábil de la MO.

Etapa 2. Domina la degradación de tejidos lignificados y lignina (Berg, 2014); durante la degradación de estos compuestos aumenta las concentración de N y P en el suelo (Berg, 2000). Las altas concentraciones de N pueden llegar a inhibir la producción de enzimas lignolíticas, pero el Mn tiene un efecto estimulante en la degradación de lignina, ya que este elemento es esencial para la actividad de la enzima Mn-peroxidasa, y también está involucrado en la regulación de otras enzimas como la lacasa y la lignina peroxidasa que se encarga de oxidar compuestos de lignina (Berg, 2000; Perez y Jeffries, 1992). Los procesos de estabilización de la MOS relacionados a sorción, oclusión y agregación de la MO empiezan a tomar mayor importancia en esta etapa y a disminuir la disponibilidad de gran cantidad de MO que no fue degradada en las primeras etapas (Sollins *et al.,* 1996).

Etapa 3. Es la etapa final, donde hay un aumento de la concentración de N y por consecuencia, la inhibición de enzimas lignolíticas; ademas, la MO más vieja se encuentra estabilizada en agregados o superficies organominerales, por lo que la tasa de descomposición de la MOS es cercana a cero (Sollins *et al.,* 1996; Berg, 2014) y los niveles de lignina llegan a mantenerse constantes debido a que llegan a un valor límite de descomposición (Berg, 2000).

La descomposición de la MO está controlada por factores bióticos y abióticos (Fig 1). Dentro de los factores abióticos se encuentran la textura del suelo y el pH, que influyen en los procesos de oclusion, adsorción, agregación, precipitación, y por lo tanto, la estabilización de la MO (Sollins *et al.,* 1996), y los factores climáticos, como temperatura, precipitación, y radiación solar (Austin y Vivanco, 2006; Parton *et al.,* 1987; Sierra *et al.,* 2015). La temperatura es un factor de interés, ya que la sensibilidad de la MO a la temperatura puede aumentar cuando aumenta la recalcitrancia química de la MO (Davidsson *et al.*, 2006; von Lützow y Kögel-Knabner, 2009; Plante *et al.,* 2010; Craine et al., 2010), obteniendo valores de Q_{10} de 1 para la MOP lábil y de 7.46 para la fracción recalcitrante de la MO (Jia et al., 2020). Esto sucede debido a que las reacciones enzimáticas necesarias para descomponer

MO químicamente compleja, requieren de una mayor energía de activación, por lo que se favorecerían con el aumento de temperatura (Fierer et al., 2005). Un controlador biótico muy importante de la descomposición de la MO es la actividad de los microorganismos del suelo (Fig. 1) con la produción de exoenzimas (Schimel y Weintraub, 2003).

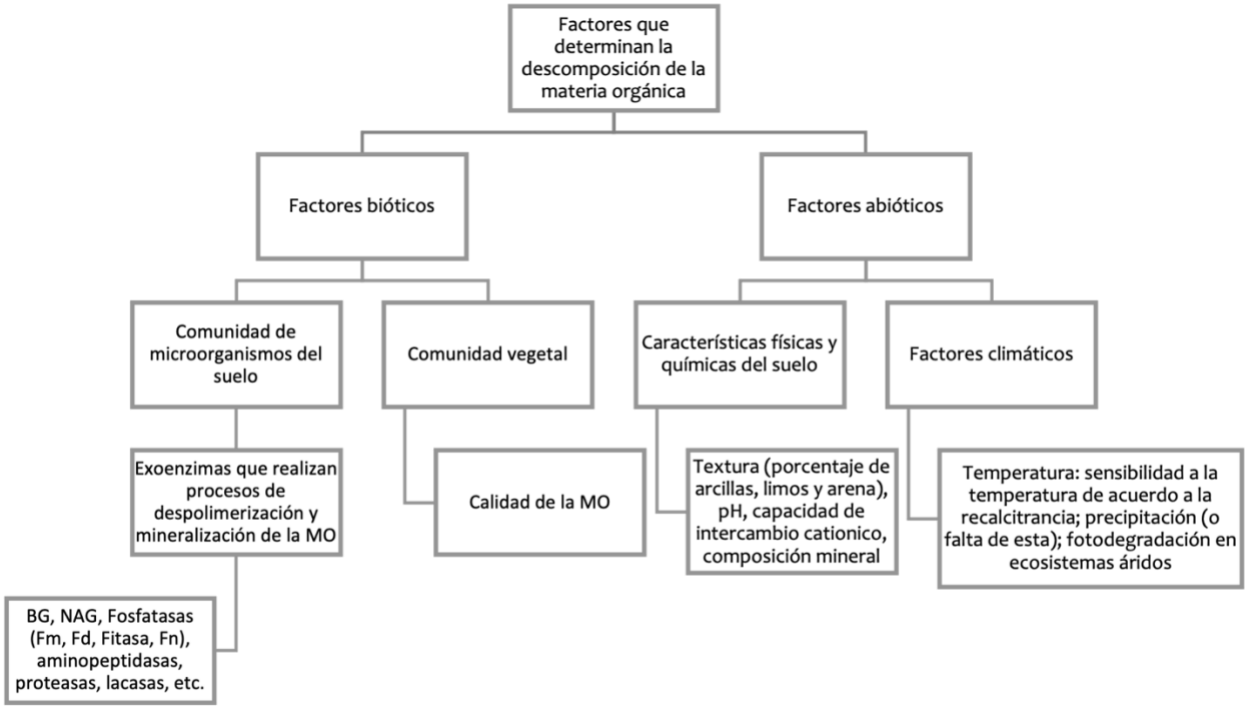

Figura 1. Factores de la descomposición de la MOS. Los principales factores que controlan la degradación de la materia orgánica se pueden clasificar en bióticos y abióticos. Las abreviaciones de las enzimas en esta figura corresponden a BG: β-glucosidasa, NAG: N-acetil glucosaminidasa, Fm: fosfomonoesterasa, Fd: Fosfodiesterasa, Fn: Fosfonatasa.

Figure 1. MOS decomposition factors. The main factors that control the degradation of organic matter can be classified in biotic and abiotic factors. The abbreviations for the enzymes in this figure correspond to BG: β -glucosidase, NAG: N-acetyl glucosaminidase, Fm: phosphomonoesterase, Fd: Phosphodiesterase, Fn: Phosphonatase.

Diversidad y función de los microorganismos del suelo

Debido a la importancia de los microorganismos en los procesos de descomposición de la MO; es importante conocer la diversidad taxonómica y funcional de estos. En el suelo y la rizósfera se pueden encontrar diversos organismos, como bacterias, hongos, oomicetos, nemátodos, protozoos, algas, virus, archaeas y artrópodos (Mendes *et al.*, 2013) y se estima que se pueden registrar hasta 1011 células microbianas por gramo de raíz (Gams, 2007) y hasta mil millones de células (10^9) por gramo de suelo (Roesch *et al.*, 2007).

Roesch *et al.* (2007), en un muestreo de diferentes suelos a lo largo del continente americano, estimaron que en 1 g de suelo se pueden encontrar desde 10,000 hasta 21,000 OTUS bacterianos. Sin embargo, la diversidad bacteriana varía a través de diferentes grupos de suelo y ecosistemas. Delgado-Baquerizo y Eldridge (2019) encontraron que los predictores más fuertes de la diversidad bacteriana a escala mundial, dentro y entre diferentes biomas, fueron el pH (relación positiva), el C del suelo (relación negativa, a excepción de climas áridos), la radiación ultravioleta (relación negativa) y tipo de vegetación, con mayor diversidad en pastizales que en bosques, debido a un efecto negativo indirecto relacionado a menor pH en suelos de bosques y mayor contenido de C en el suelo. Un estudio realizado por Rousk *et al.* (2010) determinó que diferentes grupos bacterianos dominaban de acuerdo a diferentes valores de pH del suelo, por ejemplo, las Acidobacterias disminuían cuando el pH aumentaba, mientras que las Gamma-Proteobacteria aumentaban con el aumento de pH.

De acuerdo a Fierer *et al.* (2012), en un estudio realizado en suelo de 16 sitios de biomas distintos, los phyla bacterianos que dominan en suelos y se consideran ubícuos de suelo son: Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria y Verrucomicrobia; coincidiendo con Roesch *et al.* (2007), que encontró como phyla dominantes Bacteroidetes, Betaproteobacteria y Alphaproteobacteria presenetes en 4 sitios diferentes analizados (Brasil, Florida, Iliniois y Canadá), seguidos por otros phyla tambien abundantes pero en menores proporciones que fueron Acidobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, Nitrospira y Verrucomicrobia. Algunos microorganismos predominan en ciertos biomas, por ejemplo, en los desiertos cálidos, también se encontraron como phyla dominates a Chloroflexi, Cianobacteria, Firmicutes y Gemmatimonadetes (Fierer *et al.,* 2012). En cuanto a las Archaeas, Thaumarchaeota fue el grupo más dominante encontrado por Fierer *et al.* (2012). Particularmente, en sitios agrícolas, dominan el grupo Crenarcheota, dentro de las cuales se destacan las archeas oxidadoras de amonio, que llevan a cabo el proceso de nitrificación (Fulthorpe *et al.,* 2008); este último grupo, se caracteriza por estar presente en mayor medida en muestras de rizósfera en comparación con muestras de suelo libre de vegetación (Bomberg *et al.,* 2003). A pesar de que en los suelos dominan phyla determinados, esto no quiere decir que entre diferentes tipos de suelos se comparta la mayoría de especies

o de OTUS. Fulthorpe *et al.* (2008) determinaron que menos del 5% de OTUS bacterianos se comparten entre suelos de sitios separados por más de 9000 km.

Los hongos también son componentes importantes del suelo y la rizósfera. Un muestreo global de suelos, reveló que los phyla mas dominantes de hongos son Basidiomycota (dentro de los cuales domina el grupo taxonómico Agaricomycetes), Ascomycota, Mortierellomycotina y Mucoromycotina; sin embargo, los phyla de hongos con más riqueza de OTUS son Ascomycota, Basidiomycota, Chytridiomycota y Criptomycota (Tedersoo *et al.*, 2014). En este mismo estudio, se determinó que existen factores que determinan la riqueza global de hongos en el suelo, los cuales son la precipitación media anual y la concentración de calcio, pero existen diferentes predictores de riqueza de acuerdo a los grupos filogenéticos y funcionales de hongos. El pH, por ejemplo, afectó a las comunidades de hongos de forma variable, la riqueza de hongos ectomicorrícicos era mayor en suelos neutros a ligeramente ácidos, los hongos micorrícicos arbusculares también aumentaron su riqueza con el aumento de pH. Sin embargo, los hongos saprótrofos fueron más diversos conforme el pH del suelo disminuía (Tedersoo *et al.*, 2014).

Funciones relacionadas a la descomposición de la MOS

Los microorganismos que se encuentran en el suelo juegan un papel importante, debido a que estos realizan funciones benéficas en las plantas y aumentan la calidad y fertilidad de suelos. Los microorganismos pueden promover el crecimiento y desarrollo de las plantas por medio del aumento de disponibilidad de nutrientes, protección contra patógenos, estimulación de la respuesta inmune, producción de hormonas que promueven el crecimiento vegetal y mecanismos que ayudan a la planta a tolerar estrés abiótico (Mendes *et al.*, 2013). Una de las funciones más importantes de los microorganismos en los suelos agrícolas, es la producción de exoenzimas para descomponer la MO, con el objetivo de obtener C, N y P para su propio crecimiento, pero que a su vez aumentan el almacén disponible de N y P para los cultivos.

La disponibilidad de N y P en el suelo, se da por medio de procesos realizados por microorganismos como la fijación de nitrógeno, la solubilización de P (Prashar *et al.*, 2014)

y la descomposición de la materia orgánica del suelo, que incluye los procesos de despolimerización y mineralización que generan N y P inorgánicos a partir de moléculas orgánicas. Las plantas requieren que los nutrientes, como N y P, se encuentren de forma disuelta y principalmente inorgánica en el suelo para poder obtenerlos (Richardson *et al.*, 2009). Sin embargo, anualmente, la mayoría de nutrientes requeridos por las plantas son suministrados por los procesos de descomposición de la materia orgánica del suelo realizados por microorganismos (Schlesinger y Bernhardt, 2013).

La mayor parte del nitrógeno en el suelo se encuentra en moléculas complejas, como proteínas, ácidos nucleicos y quitina, que no pueden pasar directamente por las membranas celulares de plantas y microorganismos. Los microorganismos entonces secretan exoenzimas, como proteasas, ribonucleasas y quitinasas para descomponer estos polímeros en sus subunidades, como aminoácidos, nucleótidos y monómeros de N-acetil glucosamina, que son monómeros orgánicos solubles en agua (Nitrógeno orgánico disuelto) y pueden pasar por las membranas celulares, tanto de plantas (algunas especies, con la ayuda de ectomicorrizas) como de microorganismos (Chapin *et al.,* 2011). Este proceso es conocido como despolimerización y es uno de los pasos clave en el ciclo del N, ya que regula la transformación de los polímeros con N contenidos en la materia orgánica hasta una forma de N biodisponible (Schimel y Bennett, 2004). El nitrógeno orgánico disuelto (NOD) puede ser posteriormente inmovilizado por microorganismos o puede ser mineralizado en amonio (NH_4^+), el cual posteriormente se puede nitrificar, pero tanto el amonio como el nitrato, son una fuente de N disponible para las plantas (Richardson *et al.,* 2009). Cuando el sistema se encuentra limitado por C, los microorganismos pueden inmovilizar moléculas de NOD para usarlas como fuente de C, y liberar el N inorgánico en exceso en forma de NH_4^+, dejándolo disponible para las plantas, en caso contrario, los microorganismos inmovilizan el N en sus células (Chapin *et al.,* 2011).

En el caso del fósforo (P), el anión ortofosfato (PO_4^{3-}) procedente del intemperismo de la apatita es la forma principal de P inorgánico disponible para la biota (plantas y microorganismos); sin embargo, esta forma química de P es poco abundante en el suelo, ya que es muy reactiva, y puede pasar por procesos de precipitación y oclusión que disminuyen

su disponibilidad (Frossard *et al.,* 2000). En las etapas del desarrollo del suelo posteriores al interperismo, estos procesos dan lugar a que dominen formas de fósforo ocluido y fósforo orgánico (Po) en el sistema, siendo este último, la principal fuente de P disponible (Walker y Syers, 1976).

El Po en los suelos se encuentra principalmente en formas de monoésteres, dentro de los cuales esta el fosfato de inositol, y en forma de diésteres de fosfato (Turner *et al.,* 2003; Cade-Menun, 2005). No obstante, también se pueden encontrar otros compuestos como los fosfonatos, los que pueden representar una fuente alternativa de P disponible en condiciones de deficiencia de P inorgánico (Kolowith *et al.,* 2001).

La obtención de ortofosfatos inorgánicos a partir de moléculas orgánicas ocurre por el proceso de mineralización, llevado a cabo por exo-enzimas hidrolíticas producidas por bacterias, plantas y hongos (Paul, 2014). Sin embargo, al igual que ocurre con las moléculas nitrogenadas, muchos compuestos orgánicos fosfatados necesitan ser degradados o despolimerizados antes de poder mineralizarse. Los diésteres de fosfato son compuestos en los que el grupo ortofosfato (PO_4^{3-}) está unido a dos átomos de carbono por medio de dos enlaces ester (RO-(PO_2^-)-OR; McMurry, 2008). Ejemplos de diésteres son los fosfolípidos y ácidos nucleicos, que consitituyen una gran proporción de las entradas de P orgánico al suelo, y su degradación es un proceso secuencial en el que debe de actuar una fosfodiesterasa seguida de una fosfomonoesterasa (Turner y Haygarth, 2005). Los ácidos nucleicos deben ser hidrolizados por fosfodiesterasas que liberan nucleótidos (Nannipieri *et al.,* 2011), mientras que los fosfolípidos como la lecitina, se degradan por un tipo de fosfodiesterasas llamadas fosfolipasas para producir diacil glicéridos y fosforilcolina (dependiendo del tipo de fosfolípido), que necesita posteriormente de una fosfomonoesterasa para ser mineralizado (Kuroshima y Hayano, 1982). La producción de fosfodiesterasas se considera un paso clave para el reciclaje de Po (Nannipieri *et al.,* 2011; Turner y Haygarth, 2005).

A diferencia de los diésteres de fosfato, los monoésteres de fosfato son moléculas en las que el grupo ortofosfato unido únicamente a un grupo orgánico por medio de un enlace ester (C-O-P) y pueden ser mineralizadas directamente por la acción de exoenzimas como

fosfomonoesterasas. Dentro de las enzimas fofomonoesterasas, podemos encontrar fosfatasas ácidas y alcalinas, de acuerdo al pH del suelo de donde provienen o a su pH óptimo. Las fosfatasas ácidas son producidas por plantas y microorganismos, pero no se ha detectado que las plantas produzcan fosfatasas alcalinas, por lo la actividad de esta enzima se le atribuye principalmente a los microorganismos del suelo (Nannipieri *et al.,* 2011).

El hexakifosfato de myo-inositol (ácido fítico) es un tipo de fosfato de inositol, el cual puede representar alrededor de 80% del del almacén de fósforo orgánico del suelo y prevalece debido a su estabilidad (Greaves y Webley, 1969; Quiquampoix y Mousain, 2004). La hidrólisis de ácido fítico o fitato es un proceso que responde a la deficiencia de fósforo (Konietzny y Greiner, 2004) a pesar de que algunas fosfomonoesterasas poco específicas pueden degradar fosfatos de inositol más simples, el ácido fítico es una molécula donde la hidrólisis de un grupo fosfato es más específica para las enzimas fitasa (Quiquampoix y Mousain, 2004).

Además de los ésteres de fosfato, otro grupo de compuestos organofosforados que podemos encontrar en el suelo y que son utilizados como fuentes de P por los microorganismos, son los fosfonatos, estos son compuestos con un enlace directo carbono-fósforo y que pueden encontrarse de forma natural en el suelo o debido a la adición de organofosfonatos sintéticos como insecticidas, herbicidas, y retardantes de flamas (Cook *et al.*, 1978). En estos compuestos, el enlace C-P les confiere mayor estabilidad en comparación con el enlace C-O-P de los ésteres de fosfato y por lo tanto, son más resistentes a la degradación por factores químicos, térmicos y fotolíticos (Hayes *et al.,* 2000) por lo que son degradados por enzimas específicas como las fosfonatasas (fosfonoacetaldehído hidrolasa), fosfonoacetato hidrolasas, fosfonopiruvato hidrolasas y el complejo enzimático menos específico, la C-P liasa (Kononova y Nesmeyanova, 2002; Obojska *et al.,* 1999). La hidrólisis del enlace C-P de algunos fosfonatos puede requerir pasos previos, como la acción de una aminotransferasa en el 2-aminoetil fosfonato y la acción de mutasas en la degradación del fosfoenolpiruvato (Kononova y Nesmeyanova, 2002).

Distribución de las principales enzimas despolimerizadoras y mineralizadoras en los distintos grupos microbianos

Las enzimas encargadas de los procesos de despolimerización de compuestos orgánicos para obtener moléculas orgánicas de bajo peso molecular con C, N y P y de mineralización para obtener Pi, se encuentran ampliamente distribuidas entre los microorganismos del suelo. A continuación, se menciona la distribución y diversidad filogenética de los microorganismos que contienen las enzimas β-glucosidasa (BG), N-acetil glucosaminidasa (NAG), fosfodiesterasa (Fd), fosfomonoesterasa (Fm), fitasa, fosfonatasa y Polifenol oxidasa o lacasa.

La Figura 2 muestra la distribución de las enzimas NAG, Fm, Fd, fitasa y BG en los phyla bacterianos (Lim *et al.,* 2007; Berlemont y Martiny, 2013; Zimmerman, Martiny y Allison, 2013; Ragot, Kertesz y Bünemann, 2015; Cotta *et al.,* 2016).Todas las enzimas están presentes en miembros de los phyla Proteobacteria, Actinobacteria, y firmicutes.

β-Glucosidasas. Las enzimas BG forman parte de la superfamilia de enzimas glicosido hidrolasas (GH), que participan en la degradación de la celulosa. Las familias GH contienen al menos 3 tipos de proteínas que actúan sobre enlaces 1,4-glucosídicos, dentro de las cuales se encuentran las β-glucosidasas, que participan en el paso final de la despolimerización de celulosa, y producen glucosa a partir de la hidrólisis de β-glucósidos (BRENDA: EC3.2.1.21; Hayano y Tubaki, 1985). Berlemont y Martiny (2013), encontraron que las β-glucosidasas eran encontradas principalmente en las familias de genes GH1 y GH3 las cuales estaban ampliamente distribuidas filogenéticamente, ya que se encontraron en un 79% de 5123 genomas bacterianos analizados provenientes de la base de datos PATRIC (Pathosystems Resource Integration Center). De este porcentaje, un 80% tenía genes para β-glucosidasa distribuidos principalmente en 10 phyla bacterianos (Fig. 2). Berlemont y Martiny (2013) clasificaron a las bacterias como oportunistas cuando solamente tenian el gen para la enzima BG, pero no para la enzima celulasa, y estos organismos constituyeron el 70%; y a las bacterias que contenían genes tanto de la enzima celulasa y la enzima BG las clasificaron como verdaderos degradadores de celulosa, los cuales constituyeron el 30%.

N-acetil glucosaminidasa. De acuerdo a análisis bioinformáticos realizados por Zimmerman *et al.* (2013) identificando secuencias de aminoácidos procariontes, la enzima NAG (EC 3.2.1.52) estuvo presente en el 36.9% de un total de 3058 genomas analizados; y en 19 de 30 phyla (Fig. 2). Esta enzima además de estar presente en bacterias, también se encuentra en organismos Archaea, en los phyla Euryarchaeota y Crenarchaeota.

Fosfomonoesterasa. La enzima fosfatasa alcalina Fm (EC 3.1.3.1), analizada por Zimmerman *et al.* (2013) por medio de la búsqueda de la secuencia de aminoácidos, y se encontró en el 31.9% de los genomas analizados, correspondientes a 22 phyla de 30 (Fig. 2). También se reportaron en el phyla de Archaeas Euryarchaeota. Tanto para la enzima Fm como para la enzima NAG, su presencia en el genoma era consistente para algunos géneros bacterianos como *Bulkholderia* (NAG y Fm) y *Vibrio* (NAG); sin embargo, para otros géneros bacterianos, como Escherichia y Enterococcus hay variación entre especies en cuanto al potencial de producción de enzimas. La fosfomonoesterasa también ha sido estudiada a partir del gen PhoA por Siles et al., (2022), quienes por medio del estudio de metagenomas y genomas de la base de datos de Integrated Microbial Genomes and microbiomes (IMG), separando los datos por uso de suelo (forestal, agrícola, pastizal), encontraron un número mayor de copias de este gen en los phyla bacterianos Verrucomicrobia, Proteobacteria, Planctomycetes, Firmicutes y Acidobacteria; en los phyla de archeas Thermoproteota y Euryarcheota y en los phyla fúngicoas Ascomycota y Basiodiomicota, con una mayor abundancia de este gen en pastizales comparado con los otros dos tipos de uso de suelo.

Fosfodiesterasa. Las familias de genes PhoX y PhoD codifican para fosfodiesterasas no específicas, sin embargo los genes PhoX se han encontrado principalmente distribuídos en bacterias marinas (Sebastian & Ammerman, 2009). Las proteínas codificadas por los genes PhoD son proteínas unidas a periplasma o secretadas extracelularmente y han demostrado tener actividad tanto de fosfomonoesterasa como fosfodiesterasa, con especificidad similar para cada sustrato (km= 3.38 para fosfomonoesterasa y km= 3.13 para fosfodiesterasa; Kageyama *et al.,* 2011), por lo que es dificil estudiar de forma aislada la distribución bacteriana de fosfodiesterasas por medio de la presencia de estos genes, sin embargo aun así,

la distribución de la enzima Fd en suelo ha sido estudiada principalmente a partir del gen PhoD. Ragot *et al.* (2015) encontraron este gen en 63 secuencias pertenecientes al dominio Archaea, mientras que en el dominio Bacteria se encontraron 6469 secuencias y en el dominio Eucaria (Fungi) fueron encontradas 73 secuencias. En Archaea, el gen phoD fue encontrado en Euryarchaeota, mientras que en Bacteria, el gen se distribuyó en 20 phyla (Figura 2), dentro de los cuales hubo mayor presencia de estos genes en los phyla Proteobacteria (11522 copias de los genes), Firmicutes (9353), Actinobacteria (3475), y Bacteroidetes (1034). Se encontraron homólogos phoD en el reino Fungi en los phyla Ascomycota (68), Basidiomycota (14) y Blastocladiomycota (20). Las fosfodiesterasas incluyen un amplio conjunto de enzimas dentro de las cuales se encuentran las nucleasas, ampliamente distribuidas, ya que son esenciales para degradar ácidos nucleicos y las fosfolipasas, encargadas de la degradación de fosfolípidos (Nannipieri *et al.,* 2011). Estas últimas, sin embargo, suelen estar más relacionadas con microorganismos considerados patógenos, pues tienen la capacidad de romper membranas celulares de organismos vivos (Kameyama, 1996; Ko y Hora, 1970).

Fitasa. Esta enzima se encarga de mineralizar el ácido fítico (myo-inositol 1,2,3,4,5,6-hexakis dihidrógeno fosfato) y es una de las enzimas que presenta menor distribución entre los phyla bacterianos presentes en el suelo (Figura 2), debido a que a pesar de que existen diferentes tipos de fitasas, solo las enzimas conocidas como fitasas hélice- β (FHB; Genes BPP) están activas en pH neutros a alcalinos (Cotta *et al.,* 2016; Gontia-Mishra y Tiwari, 2013), y se ha reportado que son el único tipo de fitasa producido por microorganismos del suelo (Lim *et al.,* 2007). Los otros tipos de fitasas operan en pH ácidos que van desde 4 hasta 6.5 (Gontia-Mishra y Tiwari, 2013) y están presentes en bacterias y hongos. Sin embargo las FHB o fitasas alcalinas, son producida principalmente por bacterias pertenecientes a las Gammaproteobacterias, y bacterias del género *Bacillus* (Gontia-Mishra y Tiwari, 2013; Jorquera *et al.,* 2008; Lim *et al.,* 2007). Además de estos grupos bacterianos, Cotta *et al* (2016) encontraron en la rizósfera del maíz, bacterias con genes BPP pertenecientes a los géneros *Pseudomonas, Caulobacter, Idiomarina* y *Maricaulis*; y relacionado a la rizósfera de otras plantas, se han encontrado los genes BPP en microorganismos rizosfericos aislados

de los géneros *Klebsiella, Rahnella, Pseudomonas, Enterobacter* y *Serratia* (Meena *et al.*, 2017).

Fosfonatasa y C-P liasa: A pesar de que hay una amplia presencia de microorganismos capaces de degradar fosfonatos, no hay un grupo taxonómico específico de microorganismos capaz de hacerlo; la degradación de fosfonatos es más específica para ciertas cepas de especies de microorganismos (Kononova y Nesmeyanova, 2002). En un estudio realizado en suelos de bosque, se detectó que el gen phnX se encontraba en dos phyla (Proteobacteria y Firmicutes) y 13 órdenes distintos (Bergkemper *et al.*, 2016). En cuanto a la C-P liasa, existe un conjunto de genes principales o core (phnGIJK) para producir las proteínas necesarias de dicho complejo enzimático, pero se cree que la proteína codificada por el gen phnJ, podría ser la proteína clave para el complejo C-P liasa (Morales *et al.*, 2020). De acuerdo a Morales *et al* (2020), en las bases de datos Gen-Bank, EMBL-EBI y egg-Nog, este gen está distribuido en los phyla Actinobacteria, Proteobacteria, Firmicutes, Chloroflexi y Cianobacteria (Fig. 2). Huang, Su y Xu (2005) proponen que los genes phnX y el cluster de genes para la C-P liasas están ampliamente distribuidos en grupos bacterianos poco relacionados, debido a que han estado sometidos a la transferencia horizontal de genes.

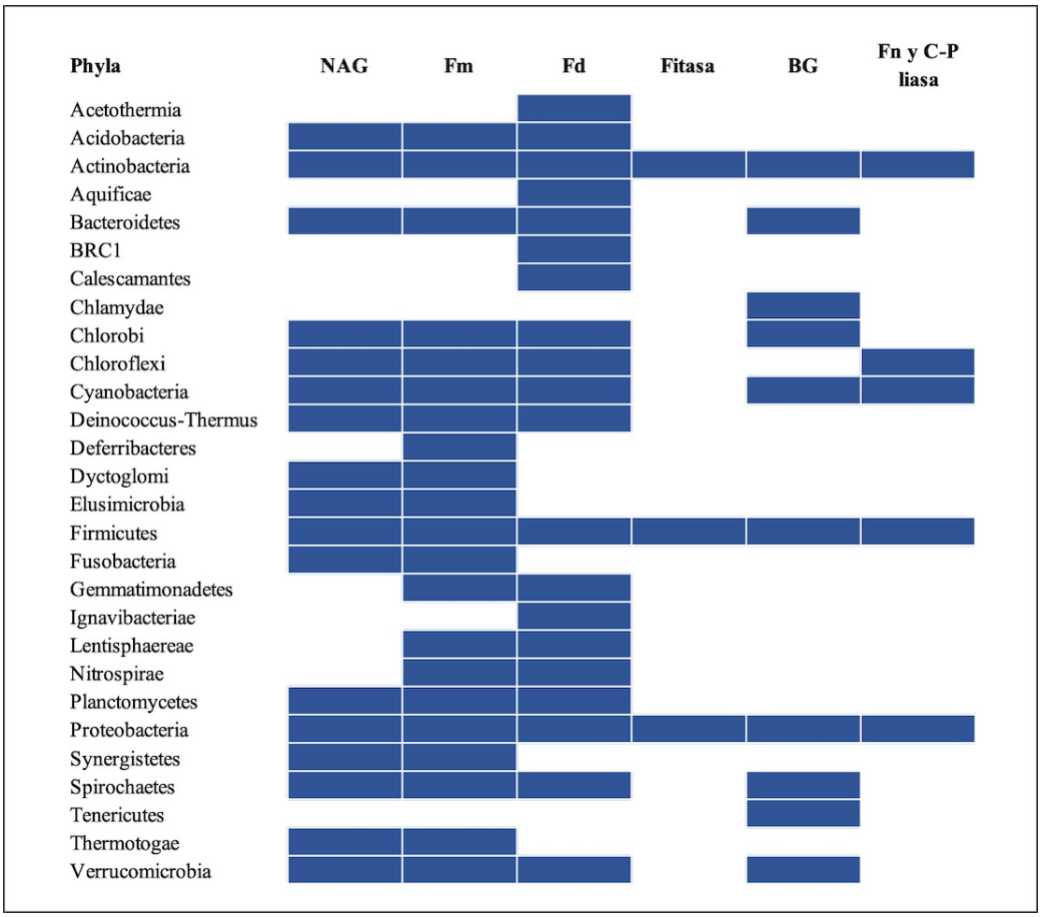

Figura 2. Distribución de enzimas en grupos bacterianos. Distribución de las NAG, Fm, Fd, fitasa alcalina (fitasa hélice β) y BG en los distintos phyla bacterianos. Los rectángulos azules indican la producción de enzimas por el grupo bacteriano. Las abreviaciones de las enzimas en esta figura corresponden a BG: β-glucosidasa, NAG: N-acetil glucosaminidasa, Fm: fosfomonoesterasa, Fd: Fosfodiesterasa, Fn: Fosfonatasa

Figure 2. Enzyme distribution in bacterial groups. Distribution of NAG, Fm, Fd, alkaline phytase (β propeller phytase) and BG in the different bacterial phyla. Blue rectangles indicate enzyme production by the bacterial group. The abbreviations for the enzymes in this figure correspond to BG: β-glucosidase, NAG: N-acetyl glucosaminidase, Fm: phosphomonoesterase, Fd: Phosphodiesterase, Fn: Fosfonatasa.

Lacasas. Las enzimas denominadas lacasas (polifenolexidasa), han sido descritas en hongos, plantas y bacterias (Valderrama *et al.*, 2003). Estas enzimas participan en la despolimerización de la lignina y son inducidas por compuestos fenólicos relacionados o derivados de la lignina, pero no están presentes en la Figura 2, debido a que las "lacasas verdaderas" son encontradas principalmente en hongos (Gianfreda *et al.*, 1999). Las lacasas son enzimas que forman un amplio subgrupo dentro de las enzimas denominadas Multicobre Oxidasas (MCOs; Hoegger *et al.*, 2006), estas se encuentran distribuidas en hongos de los

grupos Basidiomycetes, Ascomycetes y Deuteromycetes (Gianfreda *et al.,* 1999). Valderrama *et al.* (2003) encontraron estas enzimas distribuidas en los géneros de hongos *Agaricus, Ceriporiopsis, Coprinus, Coriolopsis, Coriolus, Filobasidiella, Lentinula, Marasmius, Phlebia, Pleurotus, Polyporus, Pycnoporus, Rhizoctonia, Schizophyllum* y *Trametes*, pertenecientes a la clase Basidiomycota; y en *Aspergillus, Colletotrichum, Myceliophthora, Neurospora* y *Podospora* pertenecientes a la clase Ascomycota. Hoegger *et al.* (2006) encontraron de 361 secuencias de aminoácidos de MCOs, 259 pertenecientes a hongos. La construcción de árboles filogenéticos dio como resultado la separación de organismos con diferentes tipos de proteínas MCOs, que se agruparon únicamente en los Basidiomicetos y Ascomicetos con las enzimas pertenecientes a las *Laccasas sensu stricto*. Hoegger *et al.* (2006) encontraron que las secuencias bacterianas se separaron claramente de las secuencias de proteínas eucarioticas. Se cree que las lacasas encontradas en algunas bacterias como *Azospirillum lipoferum* y *Bacillus subtillis* tienen funciones distintas a las lacasas fúngicas, y participan en procesos de melanización y la formación de pigmentos de las esporas; también se han encontrado enzimas denominadas "pseudolacasas" en las bacterias Pseudomonas syringae y Xantomonas campestris (Hoegger *et al.,* 2006).

Estequiometría de las necesidades nutricionales de los microorganismos y su relación con la producción de enzimas

Los microorganismos producen las enzimas antes mencionadas cuando requieren obtener nutrientes del suelo y estos no están disponibles de la manera en que los requieren. Las necesidades nutricionales de los microorganismos se pueden definir por el cociente estequiométrico de su biomasa. Cleveland y Liptzin (2007) reportaron que el promedio global de este cociente para microorganismos del suelo era 60:7:1 (C:N:P). El requerimiento de nutrientes y la producción de enzimas está determinada por esta estequiometria (Sinsabaugh *et al.,* 2009) es decir, los microorganismos heterótrofos no tienden a adquirir la composición estequiométrica de los recursos de donde obtienen nutrientes, si no intentan mantener su estequiometria regulando la producción de enzimas, manteniendo su composición química celular constante y a esto se le llama homeostasis (Sterner y Elser 2002). Cuando cambia la estequiometría de un organismo al cambiar la estequiometría del recurso, se considera que los organismos no son homeostáticos (Tapia-Torres *et al.,* 2015). A pesar de que el grado de

homeostasis puede variar entre autótrofos y heterótrofos, para los organismos como individuos es esencial mantener la homeostasis (Sterner y Elser 2002), sin embargo, al evaluar una comunidad microbiana, se pueden obtener aparentemente comunidades no-homeostáticas (que cambien su cociente estequiométrico de biomasa microbiana al cambiar los cocientes estequiométricos de los recursos), y esto no es resultado del desbalance en la homeostasis de organismos aislados, sino el reflejo de cambios en la estructura de la comunidad microbiana (Fanin *et al.,* 2013). Por ejemplo, al aumentar la dominancia de estrategas R en una comunidad microbiana, los cocientes C:N:P de biomasa microbiana disminuyen (Heuck et al., 2015), o al modificar la dominancia de bacterias (caracterizadas por cocientes estequimétricos bajos en biomasa microbiana) y hongos (cocientes estequiométricos más altos) dentro de una comunidad microbiana (Fanin et al., 2013). Los microorganismos homeostáticos regulan su crecimiento por el nutriente más limitante, y responden a la disponibilidad de nutrientes con cambios en la producción de ecoenzimas (Fig. 3), lo cual contribuye a la homeostasis (Tapia-Torres *et al.,* 2015). Si en el suelo existen concentraciones bajas de N o P, o los cocientes estequiométricos del suelo son muy altos, los microorganismos tienen que producir enzimas como NAG, Fm, Fd, Fn, fitasas, proteasas y aminopeptidasas (Fig. 3b). Sin embargo, al momento que los microorganismos invierten recursos para obtener estos nutrientes, disminuyen los recursos disponibles para C, por lo que los microorganismos comienzan a invertir en enzimas para obtener monómeros de glucosa, como las enzimas BG o lacasas, en caso de que los compuestos orgánicos del suelo se encuentren en formas complejas. Por esto, las actividades enzimáticas también están correlacionadas entre sí y las pendientes que se obtienen en las relaciones de actividades enzimáticas de los ecosistemas (lnBG/lnNAG+lnLAP (leucin aminopeptidasa) para C:N y lnBG/lnFm para C:P) tienen una relación BG:NAG+LAP:Fm 1:1:1 (Sinsabaugh *et al.,* 2009; Tapia-Torres *et al.,* 2015).

La estequiometría de la actividad enzimática, a diferencia de la estequiometría de la biomasa microbiana, si está relacionada con los recursos del suelo, ya que se ha determinado que las actividades de BG, NAG, celobiohidrolasa, aminopeptidasa y Fm aumentan con la concentración de C orgánico del suelo en una relación cercana a 1.0 (Sinsabaugh *et al.,* 2014).

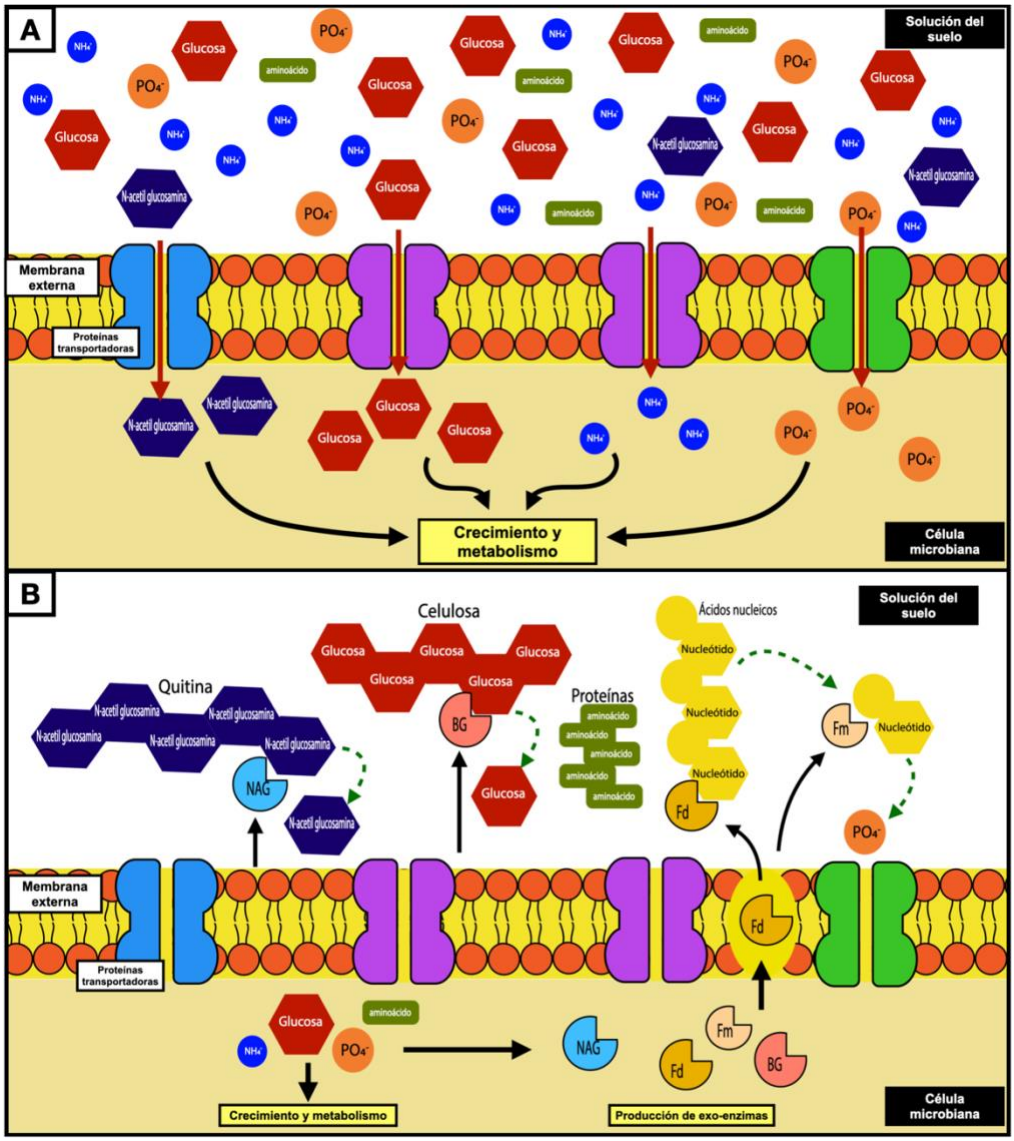

Figura 3. Los microorganismos regulan la producción de exoenzimas para aumentar la disponibilidad de los nutrientes C, N y P. En un escenario de disponibilidad de nutrientes (A) con C, N y P de formas lábiles (por ejemplo, glucosa para C, n-acetil glucosamina, aminoácidos y NH_4^+ para N, y iones ortofosfato para P), estas moléculas pueden ser transportadas al interior de las células con ayuda de transportadores de membrana, sin necesidad de producir enzimas extracelulares. Sin embargo, cuando hay limitación de nutrientes (B), los microorganismos producen exo-enzimas, como β-glucosidasa, para la obtención de glucosa a partir de celulosa, N-acetil glucosaminidasa para la obtención de N-acetil glucosamina a partir de quitina, fosfodiesterasa, para la obtención de monoésteres de fosfato a partir de diésteres y fosfomonoesterasa para la obtención de ortofosfatos inorgánicos, utilizando la materia orgánica del suelo como fuente de estos elementos. Estas moléculas pueden entrar a la célula y ser utilizadas para crecimiento y metabolismo, pero también parte de estos nutrientes son utilizados para la producción de más exoenzimas que se requieren para satisfacer las demandas nutricionales. La regulación de la producción de estas exoenzimas por los microorganismos es esencial para mantener su estequiometría elemental y regular la homeostasis.

Figure 3. Microorganisms regulate exoenzymes production to increase C, N and P availability. In a scenario of nutrient availability (A) with C, N and P labile forms (for example glucose for C, n-acetyl glucosamine, amino acids and NH_4+ for N, and orthophosphate ions for P), these molecules can be transported inside the cells with the help of membrane transporters, without the need of the production of extracellular enzymes. However, when there is nutrient limitation (B), microorganisms produce exo-enzymes, such as β-glucosidase, to obtain glucose from cellulose, N-acetyl glucosaminidase to obtain N-acetyl glucosamine from chitin, phosphodiesterase, to obtain phosphate monoesters from diesters and phosphomonoesterase to obtain inorganic ortophosphates, using soil organic matter as a source of these elements. These molecules can enter the cell and be used for growth and metabolism, but also part of these nutrients is used for the production of more exoenzymes that are required to satisfy nutritional demands. The regulation of these exoenzymes production by microbes is essential to maintain their elemental stoichiometry and for homeostasis regulation.

CONCLUSIONES

La composición de la materia orgánica del suelo depende de su origen, y es un factor importante en etapas tempranas de la descomposición. La descomposición de la MOS está controlada por factores bióticos y abióticos, de los cuales un controlador biótico muy importante es la actividad de los microorganismos del suelo con la producción de exoenzimas. La producción de estas enzimas modifica la disponibilidad de N y P en el suelo, que son requeridos por las plantas y microorganismos en forma de moléculas solubles. La disponibilidad del nitrógeno está regulada principalmente por procesos de despolimerización, puesto que los monómeros de N se consideran moléculas biodisponibles para los microorganismos, por esto es importante la acción de enzimas como la N-acetil glucosaminidasa, que se encarga de hidrolizar polímeros de Quitina. En cuanto al fósforo, su disponibilidad esta mediada tanto por la acción de enzimas despolimerizadoras, como las fosfodiesterasas, como por la acción de enzimas mineralizadoras, como las fosfomonoesterasas, fitasas y fosfonatasas, pues los microorganismos y las plantas, requieren de este nutriente en forma inorgánica y soluble. Además de estas enzimas, otras enzimas como la β-glucosidasa y la lacasa son requeridas, ya que despolimerizan celulosa y lignina, generando moléculas que son fuente de energía y carbono para los microorganismos heterótrofos del suelo, y son esenciales para que estos puedan producir otras enzimas. La diversidad microbiana del suelo es esencial para mantener los procesos de descomposición de la MOS, ya que a pesar de que la mayoría de las enzimas estudiadas en el presente artículo están ampliamente distribuidas entre distintos phyla microbianos, es difícil determinar esto a

nivel de especie, y a pesar de que entre distintos suelos se pueden encontrar grupos microbianos en común a nivel Phyla, menos del 5% de los OTUs bacterianos son compartidos. En este trabajo se identificó la falta de más estudios que se enfoquen en la distribución y presencia de las enzimas fosfomonoesterasa y fosfodiesterasa en microorganismos del suelo, relacionando la presencia de estos genes a la actividad enzimática debido a la baja especificidad de las fosfatasas y fosfodiesterasas codificadas por los genes PhoD. Esto es importante debido a que el P es un elemento comúnmente limitante en los ecosistemas, pero puede ser obtenido promoviendo los procesos de despolimerización y mineralización de la MOS. Los diésteres de fosfato, a pesar de ser poco estudiados, son una forma importante de P en la materia orgánica, presentes en dos tipos de biomoléculas; los fosfolípidos y los ácidos nucleicos, por lo que son un regulador clave de la disponibilidad de P. Además de esto, es importante tomar en cuenta que aunque los microorganismos tengan los genes de las enzimas mencionadas, la estequiometría entre su biomasa y los recursos disponibles, es un regulador importante para la producción de estas, pues son el regulador para el mantenimiento de la homeostasis, utilizando las enzimas para obtener nutrientes cuando los cocientes nutricionales del suelo (C:N:P) son muy diferentes a sus necesidades.

DECLARACIÓN DE ÉTICA

No aplicable

CONSENTIMIENTO PARA PUBLICACIÓN

No aplicable

DISPONIBILIDAD DE DATOS

No aplicable

CONFLICTO DE INTERESES

Los autores declaran que no tienen intereses en competencia.

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CAPÍTULO II: How inorganic and organic phosphorus molecules modify microbial activity, stoichiometry, and nutrient dynamics in an agricultural soil

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How inorganic and organic phosphorus molecules modify microbial activity, stoichiometry, and nutrient dynamics in an agricultural soil

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Abstract

The dynamics of C, N and P in soils determine their fertility and the growth of agricultural crop plants. These dynamics are highly dependent on microbial metabolism that in turn depends on the nutrients present in the soil. Farmers typically carry out fertilizations with either mineral or organic matter to increase the availability of nutrients in soils. One of the most applied nutrients in fertilizations is phosphorous, usually a limiting nutrient for plant growth. To degrade different substrates and increase nutrient availability, microorganisms require a combination of C, N and P. However, our knowledge is limited on how different forms of phosphorus molecules added as organic or inorganic fertilizations impact the activity of soil microorganisms, including their ability to produce the enzymes required to release nutrients from the different substrates. A soil incubation experiment was carried out adding 5 different phosphate molecules as treatments: three organic molecules (RNA, adenine monophosphate (AMP) and phytate), two inorganic molecules (calcium phosphate and ammonium phosphate) and a control, to which no phosphorus was added. Nutrient dynamics and soil microbial activity were measured at 19 days of incubation. Potential

microbial C mineralization (CO_2-C) was affected by the different P molecules added. Specific enzyme activities were also affected, specifically by organic treatments. P remained immobilized in the microbial biomass regardless of the source of P added (Pmic fraction). This suggested that soil microorganisms were clearly limited by phosphorus. Higher mineralization rates with organic compounds depleted available sources of C, such as dissolved organic carbon, which resulted on an increase of nitrification. C:N:P stoichiometry of microbial biomass denoted a change in the microbial community. These differences affected carbon use efficiency (CUE), threshold elemental ratio (TER), and homeostasis. We conclude that different organic and inorganic sources of P affect soil microorganisms differently modifying the dynamics of soil N and C. Labile organic phosphorus increases microbial activity because it is also a carbon source for heterotrophic microorganisms; however, in the course of incubation, this energy source decreases. The different sources of P notably impact microbial metabolism from the production of enzymes and influences the immobilization of P and N. These results denote that it is important to consider the phosphate compounds used in agriculture, since by affecting the microbial activity of the soil, this can affect plant productivity.

Keywords: *Phosphorus, microbial activity, enzyme activity, nutrients dynamics, C dynamic, N dynamic, microbial stoichiometry, soil microcosms, fertilization*

1. Introduction

Phosphorus is an essential element for the metabolism of living organisms (Tapia-Torres & García-Oliva, 2013). It is a fundamental element of biomolecules such as ATP, nucleic acids and phospholipids (Ashley et al., 2011). In the soil, phosphorus (P) originates from the

weathering of minerals that contain phosphates, mainly from apatite, which contains calcium phosphate minerals (Paul 2014; Schlesinger, 1991). The orthophosphate anion (HPO_4^{2-}) from the weathering of apatite is the main source of inorganic P available to biota. However, this chemical P form is not very abundant in soil since it is very reactive and can generate different types of molecules by precipitation, dissolution, and sorption processes (Doolette and Smernik, 2011). Another important source of P in the soil is organic P (Turner et al., 2003). The main organic P compounds found in soil are usually inositol phosphates, that can amount to one third to one half of the total organic P in the soil, followed in abundance by nucleic acids and phospholipids (Dalai, 1977; Stewart & Tiessen, 1987). Organic phosphate molecules must be made available to plants and soil microorganisms, through action of secreted enzymes (exoenzymes) produced by soil microorganisms. For example, macromolecules such as nucleic acids can be depolymerized by the action enzymes such as phosphodiesterases, or mineralized by phosphomonoesterases, phytases, and phosphonatases (Paul, 2014).

Through the production of different enzymes, microorganisms can regulate their phosphorus demand in response to the availability of nutrients in the soil (Tapia-Torres, Elser, et al., 2015). However, the production of enzymes involved in the acquisition of P not only depends on the availability of the organophosphate substrate and inorganic phosphorus (PO_4^{3-}), but it is also linked to the availability of carbon or energy (Luo et al., 2019), and of nutrients such as N and other elements (Olander & Vitousek, 2000). Enzymes that serve for the acquisition of C and N (ß-glucosidases and N-acetyl glucosaminidases, respectively) are therefore also

produced by microorganisms (Sinsabaugh et al., 2010), to balance the requirement of all nutrients.

Therefore, for microorganisms the allocation of energy and nutrients for the production of enzymes and for growth depends on the relative quantities of the different available elements, that is, the stoichiometry of elements in the microbial biomass and the availability of nutrients in the soil; that is, the relationships between the essential elements C:N:P (Sinsabaugh et al., 2002; Elser and Sterner 2002). The parameter *Threshold Elemental Ratio* (TER) helps us find the C:N or C:P ratios at which microbial metabolism changes from being controlled by the supply of energy (C) to being controlled by the supply of nutrients such as N and P (Sterner and Elser, 2002; Sinsabaugh and Follstad Shah, 2011). TER analyzes have been reported for natural terrestrial ecosystems in studies that address nutrient limitations (Tapia-Torres et al., 2015; Montiel-González et al., 2017; Cui et al. 2018a; Cui et al., 2018b). Other studies have analyzed TER in managed ecosystems to determine the effect that deforestation and change in land use have on microorganisms (Jiaoyang Zhang et al., 2020). Ecological stoichiometric analysis in agricultural systems is important to better understand the effect of fertilizers on soil microbial communities and the coupling of nutrient cycles. This is valuable information to perform sustainable food production practices that avoid the loss of diversity of soil microorganisms and therefore the loss of ecosystem services (Van de Waal et al., 2018).

The efficiency with which bacterial populations convert organic carbon substrates into biomass is generally termed Carbon Use Efficiency (CUE) and is quantified as carbon

accumulation in biomass (biomass production or sequestration) relative to carbon released from organic matter. CUE is an important physiological measure that ultimately determines the rate at which whole microbial communities decompose organic matter and release CO_2 (Manzoni et al., 2012; Moorhead et al., 2012; Sinsabaugh and Follstad Shah, 2012).

Sinsabaugh and Follstad Shah (2012) suggest that CUE is a function of the microbial community's ability to regulate enzyme expression and biomass composition to reduce the difference between nutrients in environmental resources and growth requirements and be able to maximize growth rate. Microbial CUE varies with environmental conditions such as resource stoichiometry and availability and it therefore depends to a great extent on the composition of the organic matter (OM) that serves as a carbon and nutrient resource for soil microorganisms, decreasing when OM is made up of recalcitrant compounds, because it increases the cost of cell catabolism (Sinsabaugh et al., 2013). Understanding how CUE is influenced by the organic molecules' complexity present in soil amendments is crucial, since depending on their chemical composition different fertilizers can affect the microbial community differently. Studies about the composition of composts and other organic amendments have been approached by nuclear magnetic resonance (NMR) of C^{13} principally to assess which chemical groups in OM predict its decomposition rates and nitrogen mineralization (Flavel and Murphy, 2006; Rowell et al., 2001). Rowel et al. (2001) found that the alkyl group was highly correlated to N mineralization, probably reflecting the presence of proteins, which are a particularly labile fraction of the organic pool, while the phenolic index, representing lignin or phenolic acids, was a factor which reduced N mineralization. However, Flavel and Murphy (2006) did not find a correlation with a specific

group of the ^{13}C-NMR spectra but found that N mineralization was related positively to initial total C and N of the amendments, as well as cellulose and lignin content, while C mineralization was correlated positively to total C, cellulose and NH_4^+ concentrations. Other studies have focused on C:N ratios of organic amendments as an indicator for quality and complexity, and asses their effect on soil fertility (Agrawal and Ghoshal, 2016; Mohanty et al., 2013; R. Scotti et al., 2015;Scotti et al., 2015; Riccardo Scotti et al., 2016), as a predictor of C and N mineralization rates and N immobilization by microorganisms. Hodge et al. (2000) and Scotti et al. (2015) reported that microbial growth can be limited by C:N ratio between 25-30 of organic amendments, promoting temporal N immobilization and impairing crop growth.

There are few studies that characterize phosphorus compounds in fertilizers. Most studies do not take this nutrient into account and do not assess how the organic and inorganic phosphorus molecules contained in the OM effect organic matter decomposition, its relationships with other nutrients such as C and N mineralization or immobilization processes, or in soil microbial activity.

Since P is an important fertilizer applied in agricultural fields and an essential element for soil microorganisms, but also dependent on C and N for its acquisition, in this work we analyzed how different inorganic and organic phosphate compounds with different complexity modify the stoichiometry and microbial activity in soil. We used an agricultural soil from the Cuatro Cienegas Basin in Coahuila, Mexico (CCB), a desert characterized by its low phosphorus availability in soil (Tapia-Torres et al., 2016; Tapia-Torres & García-

Oliva, 2013). In these soils microorganisms develop various adaptive strategies to obtain phosphorous, related to the production of ecoenzymes (Tapia-Torres et al., 2016).

In this work we evaluated using soil mesocosms, the effects of the incorporation of some of the most common organic compounds found in organic matter (OM) on the transformation of nutrients and microbial activity in soil. We evaluated inositol phosphates (phytic acid), nucleic acids in their macromolecular form (RNA) and a monophosphate ester such as adenosine monophosphate (AMP), as well as the effects of inorganic P molecules commonly used in mineral fertilizers, such as monoammonium phosphate (MAP) and calcium phosphate. Applying the concepts of ecological stoichiometry (CUE and TER), we also determined how the different sources of P modify the nutrients limitations for microorganisms and the efficiency of carbon use. We hypothesize that labile organic P molecules (monoester phosphate AMP and diester phosphate RNA) can improve nutrient availability by stimulating microbial community activity since these molecules are a source of C, N and P. On the other hand, since phytic acid molecule can be a source of carbon but not a source of N, its effect on microbial community will depend on the capacity of microbes to produce enzymes (phytases) to degrade it. Application of inorganic P (MAP and calcium phosphate) was expected to benefit microbial community growth and activity only to the point they become limited by energy or by nitrogen (in the case of calcium phosphate).

2. Materials and Methods

2.1 Study site

This study was carried out with samples obtained from an alfalfa farming plot located in the western side of the Cuatro Cienegas Basin (26°58'57" N, 102°5'10" W). The climate at Cuatro Cienegas (CCB) is hot and arid, with an average yearly temperature of 21.9°C and an average annual precipitation of 253 mm (Montiel-González et al., 2021). The dominant parent material in the west of CCB is calcium carbonate (Lehmann et al., 1999) and the dominant soil groups are Calcisols (García-Oliva et al., 2018).

The management at the farming plots consist of fertilization every 25 days, principally with MAP (monoammonium phosphate) technical grade, NPK 20-20-20 fertilizers, or with NPK 11-42-0 fertilizers. Vermicompost leachate is often applied at a dose of 100 L ha^{-1}. Insecticides are used based on the current problems and herbicides with the active compound clethodim are used for grasses.

2.2 Soil sampling

Soil sampling of an alfalfa crop was carried out in August 2018. We established 50 × 50 m plot within the alfalfa crop. Soil samples were taken in 5 transects chosen randomly in one side of 50 m. A subsample was taken each 10 m inside each transect, obtaining 5 subsamples that were mixed homogeneously, obtaining a composite soil sample per transect. At the end, 5 composite samples were obtained. Soil samples were taken from the top 15 cm of mineral soil with a soil core sampler, placed in black plastic bags and stored at 4°C until laboratory analyses.

2.3 Experimental design and incubation

An incubation experiment was done with soil amended with different phosphorus compounds as fertilizers (Fig 1). The experimental design consisted of one factor, which included six different treatments: five phosphate compounds and one control. For each treatment, we included five replicates, corresponding to each composite soil sampled obtained from the field. Soil incubations were carried out in a period of 19 days, at 28 °C (time defined by the obtained C mineralization rate data). A soil sample of 100 g was added into sterilized PVC tubes with an extreme closed with a mesh (pore diameter <0.05mm). Water was added to samples until 90% water holding capacity, determined according to Montiel-González (2018). During the incubation period, water in samples were maintained by weight measurements. PVC tubes were placed into 1 L glass flasks and sealed during the incubation, as shown in Fig. 1.

The added phosphorus compounds were chosen on the basis of the most common organic P compounds found in farming soils, like phosphate monoesters, phosphate diesters and phytic acid. As a phosphate monoester, we used adenosine monophosphate (AMP), and as a phosphate diester we used torula yeast RNA (Sigma-Aldrich). We used also two inorganic phosphate compounds: monoammonium phosphate (MAP; used at the study site as fertilizer) and monobasic calcium phosphate ($Ca(H_2PO_4)_2$), known as triple superphosphate and used commonly for fertilizer production. Phosphate compounds concentrations added to soil, were calculated according to the maximum P concentration used as a fertilizer in the sample site (16.5 kgP ha^{-1}). A concentration of 27.8 μg P g^{-1} of

soil was added, corresponding to 89.87 μmol P (Table 1), calculated based on the PVC area of 0.0018 m^2. Phosphorus sources were added in the water used to adjust samples to water holding capacity.

2.4 Potential C mineralization

Carbon mineralization was measured periodically along the 19 days of incubation. For this analysis, CO_2 traps were placed inside the glass flasks. These traps consisted of a vial with 10 ml of NaOH 1N, which was titrated periodically each third day with HCl 1N and $BaCl_2$, and replaced with new NaOH solution. The HCl used for titration was used to calculate C mineralization rates (Coleman et al., 1978). The metabolic quotient for CO_2 (qCO_2) was determined according to Anderson y Domsch (1993) dividing the accumulated CO_2-C by microbial biomass C after incubation (Cmic).

2.5 Biogeochemical and enzymatic activity analyses

Before and after the incubation, biogeochemical analysis and enzymatic activities were performed. Soil moisture content was determined by gravimetric analysis, drying the samples at 100°C at constant weight. The active soil pH in deionized water (1:10 w / v) was measured using a digital potentiometer (Thermo Scientific Orion 3star Plus). The weight of the samples in all the analyses was corrected with the fraction of dry soil obtained with the moisture content determination.

Total C, N and P were quantified (TC, TN and TP) using dry soil grounded in an agate mortar. The total C (TC) and total inorganic C (TIC) were determined by coulometric

detection (Huffman, 1977) in a total Carbon Analyzer (UIC model CM5012). The total organic C (COT) was calculated with the difference between TC and TIC. The TN and TP were determined after acid digestion, the TN was determined by the Kjeldahl macro method (Bremmer, 1996) and the TP by the reduction of molybdate with ascorbic acid method (Murphy and Riley, 1962). Both nutrients were measured by colorimetry in Braun-Lubbe Auto Analyzer 3 (Norderstedt, Germany).

The available forms of nitrogen (NH_4^+ and NO_3^-) were extracted with 2 M KCl according to the method of Robertson et al. (1999) and were determined by the phenol-hypochlorite method, while the available inorganic P (HPO_4^{2-}) was quantified following the methodology of Tiessen and Moir (1993) of P fractionation, using as extractant a solution of $NaHCO_3$ 0.5M and adjusted at pH 8.5 and was determined colorimetrically (Murphy and Riley, 1962).

The dissolved organic nutrients were determined by the difference between the total dissolved nutrient (C, N or P) and the dissolved inorganic nutrient. Dissolved organic C, N and P (DOC, DON, and DOP) were extracted with deionized water (1:4 w/v) according to Jones and Willett (2006) and filtered through a Millipore 0.45 μm filter. Filtrate was used directly to measure inorganic dissolved N and P, and total and inorganic dissolved C. For total dissolved N and P, the filtrate was acid digested. Total and inorganic forms of dissolved N and P were quantified in a Bran-Luebbe Auto analyzer 3 (Norderstedt, Germany). For the determination of the DOC, the total dissolved C (TDC) and dissolved inorganic C (DIC) were measured in a Carbon Autoanalyzer (TOC CM 5012).

Nutrients C, N and P within microbial biomass (Cmic, Nmic and Pmic) were obtained by the method of fumigation with chloroform and incubation for 24 h at 27 °C (Vance et al., 1987). Cmic and Nmic were extracted using 0.5 M K_2SO_4 according to Brookes et al. (1985) and filtered with Whatman No. 42 and No. 1, respectively. Cmic was quantified using a Carbon Auto Analyzer (TOC CM 5012). C concentration was measured from each extract as total carbon (TCmic) using the module for liquids (UIC-COULOMETRICS), and as inorganic carbon (ICmic) determined by the acidification module CM 5130. For Nmic, the filtrate was acid digested and determined as TN by the Macro-Kjeldahl method (Brookes et al., 1985). The Pmic was extracted according to Cole et al. (1977), using a solution of $NaHCO_3$ 0.5M and adjusted at pH 8.5, shaken for 16 h and filtered through Whatman No. 42 filters. Filtrates were digested using 11 N H_2SO_4 and a 50% w/v solution of ammonium persulfate and neutralized after the acid digestion. Microbial P was determined colorimetrically by the molybdate-ascorbic acid method using an Evolution 201 Thermo Scientific Inc. spectrophotometer, at a wavelength of 880 nm (Murphy and Riley, 1962).

Nutrients in microbial biomass were calculated subtracting data of the no-fumigated samples to fumigated samples data, and then, divided by its corresponding conversion factor. kEC (0.45) and kEN (0.54) determined by Joergensen (1996) and Joergensen and Mueller (1996) were used to calculate Cmic and Nmic, respectively, and *Kp* correction factor of 0.4 (Hedley et al., 1982; Lajtha & Jarrell, 1999) was used for Pmic calculations.

Differences (Δ) between biogeochemical variables before and after the incubation was calculated by a subtraction of the values at the beginning of the incubation, from the values at the end of the incubation. Therefore, net nitrification was calculated subtracting the values of available NO_3^- after incubation minus available NO_3^- before incubation.

Enzymatic activities of phosphomonoesterase (Phm), phosphodiestherase (Phd), Phytase (Phy) Beta-glucosidase (BG), N-acetyl glucosaminidase (NAG), polyphenol oxidase (POX) were quantified. For this analysis, 2 g of fresh soil and 30 ml of modified universal buffer (MUB) at pH 8 were used for the ecoenzymes extraction. Three replicates and one control (sample without substrate) were prepared per sample. Three substrate controls (substrate without sample) were also included per assay, and all were incubated at 30 ºC. The tubes were centrifugated after the incubation period and then 750 µl of supernatant were diluted in 2 ml of deionized water and 75 µl NaOH 1N.

The measurements of the enzymatic activity of Phm, Phd, BG and NAG are based on spectrophotometric determination of p-nitrophenol (pNP) released from substrates linked to pNP, per time unit (µmol pNP $[g\ SDW]^{-1}\ h^{-1}$; Tabatabai and Bremner, 1969; Verchot and Borelli, 2005; Fioretto et al., 2009), measured at 410 nm on an Evolution 201 spectrophotometer (Thermo Scientific, Inc.). The POX activity was determined by oxidation of the substrate 2,2′-azinobis-(-3 ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS), and it was measured directly at a wavelength of 460 nm. Phy was quantified according to the method of phosphonatase enzymatic activity measurement described by Tapia-Torres et al. (2016), using phytic acid as substrate and

quantifying released Pi by the ascorbic acid reduction method (Murphy and Riley, 1962) measured at a wavelength of 882 nm. Phy activity was expressed as micromoles of inorganic P released per gram of soil dry weight per hour (μmol Pi [g SDW]$^{-1}$ h^{-1}).

Specific enzyme activities (SEA) were calculated to determine how much enzyme is synthetized per concentration of nutrient immobilized in microbial biomass. The SEA was calculated according to Chávez-Vergara et al. (2016) and Waldrop et al. (2000)

(1) SEA= Enzymatic activity / Carbon in microbial biomass

Where enzymatic activity was in units of μmol g SDW^{-1} h^{-1} and C in microbial biomass was on units of mg C g SDW^{-1}.

2.6 Homeostasis and the threshold element ratio (TER)

With the biogeochemical and enzymatic results obtained from the incubation experiment where different phosphate compounds were applied to agricultural soil samples from the CCB, a homeostasis analysis was performed, performing simple linear regressions between the natural logarithm of DOC:DOP and the natural logarithm of Cmic:Pmic for C:P and between the natural logarithm of DOC:DON and the natural logarithm of Cmic:Nmic for C:N. Taking the linear regression, it was tested if the slope was different from 0, which would mean a non-homeostatic microbial community. The elemental ratio thresholds (TER) were calculated in relation to the elements C:P (TER) and C:N ($TER_{C:N}$) according to Sinsabaugh et al. (2009) using the following equations :

Ec. 1)

$TER_{C:P} = ((BG/(Phm+Phd))B_{C:P})/\rho_o$

Ec. 2)

$TER_{C:N} = ((BG/NAG)B_{C:N})/n_o$

Where $TER_{C:P}$ is the threshold elemental quotient for elements C and P; BG / (Phm + Phd) is the quotient of enzymatic activity for B-1,4-glucosidase (BG) and the sum of phosphomonoesterase plus phosphodiesterase (Phm + Phd); BC: P is the C: P ratio for microbial biomass (Cmic / Pmic) and ρo is a normalization constant. For elements C and N, the $TER_{C:N}$ (Ec. 2) is the threshold elemental ratio (dimensionless), (BG / (NAG)) is the ratio of enzymatic activity for β-glucosidase (BG) and N-acetyl glucosaminidase (NAG) , $B_{C:N}$ is the C: N ratio for microbial biomass (Cmic:Nmic), and n_o is a normalization constant. The normalization constants are the intercept calculated with a standardized major axis regression type II (SMATR). For the constant ρo, the regression is performed between the natural logarithm of the BG enzyme and the natural logarithm of the sum of the enzymes Phm and Phd. In contrast, for the constant n_o, the regression is calculated between the natural logarithm of the BG enzyme and the natural logarithm of the NAG enzyme. Equation 1 is a modified equation from Sinsabaugh et al. (2009), since only the Phm enzyme is used in the original equation, however the Phd enzyme has been included due to its importance and high activity in the soils of Cuatro Cienegas (Tapia-Torres et al., 2016). The $TER_{C:P}$ and $TER_{C:N}$ results, converted to natural logarithms, were compared with the resource ratios (soil nutrients, DOC:DOP and DOC:DON) using a Student´s t-test. This can tell us if the soil microorganisms are limited by energy (carbon) or by nutrients (N or P).

2.7 Carbon Use Efficiency

Carbon use efficiency (CUE) can be measured from ecological stoichiometry. In this work, we perform the calculation of the efficiency of carbon use in relation to N and P ($CUE_{C:N}$ and $CUE_{C:P}$) using the formulas developed in Sinsabaugh et al. (2013) and Sinsabaugh et al. (2016):

Ec. 3)

$$CUE_{C:X} = CUE_{MAX}(S_{C:X}/(S_{C:X}+K_x))$$

Where X represents element N or P; Kx is the mean saturation constant, which has a value of 0.5; CUE_{MAX} is the upper limit for the efficiency of microbial growth, which has a value of 0.6 based on thermodynamic constraints, and $S_{C:X}$ is calculated as follows:

Ec. 4)

$$S_{C:X} = (1/EEA_{C:X})(B_{C:X}/L_{C:X})$$

Where $EEA_{C:X}$ is the quotient of the enzymatic activities related to the nutrients C:X; $L_{C:X}$ is the quotient of the substrates consumed, which in this case were the dissolved organic nutrients of the soil and $B_{C:X}$ the quotient of elements in microbial biomass. From the data obtained from $CUE_{C:P}$ and $CUE_{C:N}$, the CUE calculation was performed, using the formula suggested by Sinsabaugh and Follstad Shah (2012) and Sinsabaugh *et al.*, (2016) as a best estimate for the CUE of the microbial community:

Ec. 5)

$$CUE = \sqrt{CUE_{C:N} \times CUE_{C:P}}$$

2.8 Statistical analysis

A one-way ANOVA was performed to determine the effect of the treatment on C mineralization, and on the biogeochemical and enzymatic variables, as well as on the differences between the beginning and the end of the incubation for enzymes activities and DOC, DON, DOP, NO_3^-, PO_4^-, NH_4^+, Cmic, Pmic and Nmic. Residual frequency distribution was assessed with Kruskal-Wallis test, to probe a normal distribution (García-Oliva & Maass, 1998). A Tukey HSD test was performed after the ANOVA to identify differences between treatments. An ANOVA was also made for the results of SEA, and LSD test was performed after the ANOVA for SEA obtained with Cmic.

A Pearson correlation was performed between biogeochemical post incubation variables, enzymatic activities (post incubation), accumulated C mineralization, qCO_2, and nitrification.

A Principal Component Analysis (PCA) was carried out to determine which variables explained result variance, and to see the grouping of the different treatments. The data matrix was made using all biogeochemical and enzymatic data from all the samples, excluding SEA and qCO_2. The analysis was carried out using the function "prcomp" on R software. All statistical analysis were performed using R software (R core team, 2020). One-way ANOVAs were performed to compare the results of $TER_{C:P}$, $TER_{C:N}$ (using the natural logarithm of TER), $CUE_{C:P}$, $CUE_{C:N}$ and CUE between treatments. Residual frequency distribution was assessed with Kruskal-Wallis test, to probe a normal distribution (García-Oliva & Maass, 1998). The Tukey HSD test was performed to

identify the treatments with significant differences, with the exception of the analysis done for the CUE in which no results were obtained with the Tukey HSD test and an LSD analysis was performed. To identify differences between the TER values and the ratios between dissolved organic nutrients, Student's t-tests were performed. For the TER calculation, type II linear regressions were performed between enzyme activities using the SMATR package. All statistical analyzes were performed using R software (R Core Team, 2019).

3. Results

3.1 Incubation experiment and metabolic quotient for CO_2 (qCO_2)

In order to obtain data on the potential C mineralization performed by soil microorganisms we measured the accumulated CO_2-C by microbial biomass (Cmic) after incubation with different substrates. All soil parameters measured before incubation are shown in table 2; they were descriptive of the studied site and used further to compare changes between before and after the fertilization experiment. After 19 days of incubation, it was observed that adenosine monophosphate (AMP) and RNA additions of P organic treatments had the highest C mineralization (950 and 863 µg CO_2-C g^{-1}, respectively), while the $Ca(H_2PO_4)_2$ addition and the control treatments had the lowest C mineralization values (781 and 739 µg CO_2-C g^{-1}, respectively; Table 3.). Cmic was lowest for the phytic acid treatment and therefore the qCO_2 calculated was higher for this phosphate ester treatment (1.9 ± 0.56) compared with the control (0.59 ± 0.03), suggesting a lower metabolic efficiency of the soil microbial community fertilized with phytate (Table 3).

3.2 Post-incubation biogeochemical analysis: Changes in C, N and P organic and inorganic pools and microbial P immobilization

In order to determine the effect of organic and inorganic P amendments, we evaluated dissolved organic and inorganic available C, N and P concentrations, and nutrients immobilized in microbial biomass in soil after 19 days. Nutrient availability analyses demonstrated that the AMP and RNA additions on P organic treatments resulted in higher NO_3^- concentrations than the other treatments, as well as nitrification (Table 3). In contrast, NH_4^+ and HPO_4^{2-} had no significant differences among treatments (Table 3). Dissolved organic C (DOC) was significantly greater for the treatment with $Ca(H_2PO_4)_2$ than for the RNA and AMP treatments (Table 3). Additionally, the $Ca(H_2PO_4)_2$ and RNA treatments had higher dissolved organic N (DON) concentration than the other treatments (Table 3).

Dissolved organic P (DOP) had greater concentration in the samples of $Ca(H_2PO_4)_2$, AMP and RNA treatments in comparison with the control samples (Table 3). Therefore, the control samples had higher DOC:DON and DOC:DOP ratios than the AMP and RNA treatments (Table 4); as well as the control had higher DON:DOP ratio than the monoammonium phosphate (MAP), AMP, RNA and phytic acid treatments (Table 4). The control, MAP, and $Ca(H_2PO_4)_2$ treatments had higher Cmic concentration than in the AMP, RNA and phytic acid treatments (Table 3). In contrast, treatments with RNA, AMP and $Ca(H_2PO_4)_2$ immobilized significantly more P compared to control (Table 3).

Additionally, organic treatments favored N immobilization in microbial biomass given that the Cmic:Nmic ratio was lower in these P organic treatments (RNA, AMP and phytic acid) than in the control treatment (Table 4). These results suggest that P organic treatments favored P and N immobilization in microbial biomass (Pmic) and high dissolved organic P (DOP) as well as higher available nitrate.

3.3 Enzyme activity and specific enzyme activity

Given that C, N and P dynamics depends on microbial enzymatic activity, therefore we measured the activities of β-glucosidase (BG), n-acetyl glucosaminidase (NAG), polyphenol oxidase (POX), phosphomonoesterase (Phm), phosphodiesterase (Phd) and phytase (Phy) under different organic and inorganic P amendments; this activity was normalized by dividing it between Cmic, obtaining specific enzyme activity (SEA), but non normalized enzyme activities are shown in Table S1. After 19 days of incubation, significant differences were only observed in the specific activity of POX, NAG and Phd (Table 5).

Our experiments showed that RNA and phytic acid treatments had higher POX SEA than the inorganic P treatments (MAP and $Ca(H_2PO_4)_2$). Some organisms use extracellular phenol oxidases to degrade lignin and humus to gain carbon and other nutrients and to mitigate the toxicity of phenolic molecules and metal ions. Organic treatments also resulted in higher Phd SEA than the inorganic P treatments and the control (Table 5) suggesting that microbes used phosphodiesterase to obtain P from such substrates.

Regarding NAG SEA values, the phytic acid treated samples had the highest values while the RNA, AMP and control treatments had the the lowest (Table 5). N-acetyl glucosaminidase is one of three enzymes that catalyze the hydrolysis of chitin, important in carbon (C) and nitrogen (N) cycling in soils. It participates in chitin conversion to amino sugars, which are major sources of mineralizable N in soils. The fact that in the presence of either of these two organic molecules that contain N, NAG was not produced, suggests that no more N was required.

3.4 Increases of DOC, DOP and Pmic after incubation regarding to soil samples before the incubation experiment

In order to evaluate differences after 19 days of incubation, we subtracted the final data with the data at the start of the experiment. The differences for organic dissolved nutrients and microbial nutrients post and pre incubation (Δ) are shown in Table 6. The $Ca(H_2PO_4)_2$ and the AMP treatments had the highest and lowest increase of DOC concentration after incubation, respectively. Similarly, the $Ca(H_2PO_4)_2$ treatment had the highest DON increment, but the lowest increment was in the MAP treatment. In contrast, the $Ca(H_2PO_4)_2$, RNA and AMP treatments had higher increment of DOP than the control, which had negative values.

Among microbial nutrients, only Pmic had a significant increment after incubation, among treatments the RNA and the Control had the highest and lowest values, respectively (Table 6).

3.5 A complex dynamics observed from the application of inorganic and organic P fertilization

In order to visualize the impact that the inorganic and organic sources of P had on the overall dynamics of the incubated soils, we carried out a principal component analysis of the obtained data. The first and second component explained 26 % and 17 % of variance, respectively (Table S1). The NO_3^- and NAG enzyme were the variables with higher weight in the first component, while the HPO_4^{2-} and POX enzyme were explained better the variance of second component (Fig. 2). The treatments were observed to cluster in three groups: only control in the left side of the first component and negative values of the second component, the $Ca(H_2PO_4)_2$, MAP and phytic acid treatments in the middle of the figure, and the AMP and RNA organic treatments in the right side of the first component (Fig. 2).

Given that three separate groups were observed in the PCA, Pearson correlation tests (Fig. 3) were done separately for each group. For the control samples, our results indicated that the microbial community seemed to require more energy to acquire phosphorus as shown by the positive correlation between microbial P and the enzymes BG ($r= 0.88$, $p= 0.046$), POX ($r= 0.89$, $p= 0.044$) and Phy ($r= 0.97$, $p= 0.007$; Fig 2a). These correlations were no longer observed in the other groups of treatments (the MAP, $Ca(H_2PO_4)_2$, and phytic acid group and the AMP and RNA group) (Fig. 3b, c); however, POX activity was correlated with the Phm activity in the AMP and RNA group ($r= 0.89$, $p= 0.004$,Fig 3c).

The correlations of the cluster with treatments with $Ca(H_2PO_4)_2$, MAP and phytic acid are showed in Fig 3b. Samples show greater microbial growth when they were able to immobilize more phosphorus, as shown by the positive correlation of Cmic with Pmic (r=0.58, p= 0.037). Pmic also correlated negatively with qCO_2 (r=-0.58, p= 0.046), which is an indicator of less metabolic efficiency of microorganisms when there is not enough phosphorus in their biomass. However, for this cluster of treatments, a phosphorus acquiring enzyme, the Phd, correlated negatively with Cmic (r= -0.61, p= 0.017).

Finally in the third group, negative correlations between qCO_2 and Pmic were also significant for the AMP and RNA group samples (r=-0.63, p= 0.041 Fig. 3c); also, Nmic and qCO_2 presented the same correlation for these treatments (r=-0.72, p= 0.017). For the same treatment group, a negative correlation was found between DOC and NO_3^- (r=-0.85, p= 0.0035) and a positive correlation between NO_3^- and CO_2-C (r=0.8, p= 0.04). The later also correlated negatively with NAG enzyme (r=-0.61, p= 0.04) and with DOC (r= -0.73, p= 0.018; Fig. 3c).

3.6 Homeostasis, Threshold Element Ratio and Carbon Use Efficiency

The microbial community, in most treatments, is a homeostatic community estimated by a standarized linear regression. This was suggested by a slope not different from 0 ($p>0.05$) according to the standarized linear regression performed for the control treatments, monobasic ammonium phosphate (MAP), calcium phosphate ($Ca(H_2PO_4)_2$), RNA and adenosine monophosphate (AMP) treatments as shown in figures 4 and 5. In contrast, the

samples treated with phytic acid (phytate) as a source of P turned out to be a non-homeostatic community, given that in the regressions carried out, it exhibits a slope different from zero (Fig. 4E and Fig. 5E). The microbial community of these soil samples has a tendency to decrease the C:P and C:N ratio of its microbial biomass (immobilization of nutrients) while increasing the C:P and C:N ratio of the resource.

The $TER_{C:P}$ analysis showed significant differences in this value between treatments. The $TER_{C:P}$ was higher for the samples with control and MAP treatments, followed by the treatments with $Ca(H_2PO_4)_2$ and RNA, while the TER was lower for the samples with AMP and phytic acid treatments (Fig. 6). Compared with the dissolved nutrient ratios (DOC:DOP), the $TER_{C:P}$ turned out to be less than this ratio for the control treatments, $Ca(H_2PO_4)_2$, AMP, RNA and phytic acid, but it turned out to be the same in the treatment with MAP.

The $TER_{C:N}$ was higher for the control, followed in equal measure by the samples treated with AMP, $Ca(H_2PO_4)_2$, MAP, phytic acid, and lower for the samples treated with RNA (Figure 7). Compared to the dissolved nutrient ratios, the $TER_{C:N}$ was less than the DOC:DON ratio for MAP- and RNA-treated soil while the $TER_{C:N}$ was higher for the control treatment. For the other treatments (AMP, $Ca(H_2PO_4)_2$ and phytate), the $TER_{C:N}$ was the same as the DOC:DON ratio.

The value of carbon use efficiency in relation to phosphorus ($CUE_{C:P}$) turned out to be statistically the same for all treatments (Figure 8A). However, the value of carbon use efficiency in relation to nitrogen ($CUE_{C:N}$) turned out to be different between treatments

(Figure 8B). The $CUE_{C:N}$ was higher for the samples treated with $Ca(H_2PO_4)_2$, it was intermediate for the samples treated with MAP and phytate, and lower for the samples treated with AMP, RNA and for the control samples (Fig. 8B). The total CUE (Figure 9) showed a similar trend to $CUE_{C:N}$.

4. Discussion

In this work, we seek to evaluate the effects of the incorporation of different organic compounds found in organic matter, such as inositol phosphates (phytic acid) and phosphate monoesters (AMP), and diesters (RNA), as well as the effects of inorganic P molecules commonly used in mineral fertilizers, such as monoammonium phosphate (MAP) and calcium phosphate on nutrient transformation and microbial activity in the soil. We also aimed to determine how different sources of P modify nutrients limitations for microorganisms applying concepts of ecological stoichiometry, such as the Threshold Element Ratio (TER) and Carbon Use efficiency (CUE).

4.1 Phosphorus sources effect on soil C and N dynamics

The soil that was chosen for the experiment, was a soil used for agriculture with conventional management, located at Cuatro Cienegas Basin (CCB), a desert characterized by its low concentrations of phosphorus in soils. In this work, we found that all of the evaluated phosphorus sources stimulated microbial C mineralization compared to the control, suggesting that the P was in fact the main limiting element for the activity and growth of microbial communities in the selected soil, as reported in previous studies for the study site (Perroni et al., 2014; Tapia-Torres, Elser, et al., 2015). As hypothesized, labile organic

treatments, such as adenosine monophosphate (AMP) and RNA promoted microbial C mineralization. It has been reported previously that soil bacteria from CCB prefer DNA as phosphorus substrate over inorganic phosphorus such as potassium phosphate and calcium phosphate when isolates are growth in culture media (Tapia-Torres et al., 2016). The degradation of DNA requires phosphodiesterase enzymes, as well as our substrate RNA, while the substrate AMP can be seen as a monomer from decomposition of nucleic acids and requires phosphomonoesterase (Lehninger et al., 2005). Both treatments contain not only P but also C and N, which suggest phosphorus colimitations with C and N in soil, and when these nutrients are added, microbial activity is promoted. However, the other organic treatment, phytic acid, did not have the same expected effect.

The microbial community and nutrient dynamic response to phytic acid, it was similar to inorganic substrates, as shown with the principal component analysis and with the accumulated C mineralization results. Moreover, the phytic acid treatment had the highest metabolic quotient (qCO_2) value, which can suggest that the microbial community is undergoing metabolic stress (Anderson & Domsch, 1993) or a microbial community with high energy requirements (Carpenter-Boggs et al., 2010). The metabolic quotient (qCO_2) also shown a negative correlation with Pmic in the principal component group of inorganic treatments and AMP and RNA group, suggesting that metabolic stress has an inverse relation with the amount of P immobilized in microbial biomass. These results suggest phytic acid is not a readily available source of P and C for soil microorganisms. Higher energy requirements may be due to phytic acid interactions with soil, as it is strongly bound to soil clays, soil organic matter and can react with soil minerals, such as calcium, favoring

precipitation and adsorption reactions (Dalai, 1977; McKercher & Anderson, 1989; Stewart & Tiessen, 1987; Wan et al., 2016), becoming less susceptible to microbial attack. This is important to CCB soils due to their high sorption capacity, which is greater when higher concentrations of organic compounds are found (Perroni et al., 2014), which tend to be higher in agricultural fields than in natural soils, because the continuous water and nutrient inputs increase total organic carbon and organic dissolved phosphorus in soils, compared to native grasslands (Hernández-Becerra et al., 2016). In consequence, phosphorus acquisition from phytic acid molecules is a two-step process, which demands more energy for soil microorganisms. First, insoluble and mineral-bounded phytate compounds need to be solubilized by bacteria or fungi capable of synthesizing organic acids and chelates (Hill & Richardson, 2007). Then free and soluble phytic acid can be hydrolyzed by phytases (Lim et al., 2007); specifically, B-propeller phytase, the active kind of phytase in neutral and alkaline soils, which break down each monoester bound to release inorganic phosphate (Cotta et al., 2016; Gontia-Mishra & Tiwari, 2013). Only after the complete dephosphorylation of the molecule, phytic acid is transformed into *myo*-inositol, which can be used as a carbon source by soil microbes (Cosgrove et al., 1970). These results suggest that the molecular structure play an important role in its decomposition, rather than only the concentration of C, N or P.

However, the addition of labile organic molecules treatments (AMP and RNA) can also affect soil N dynamics, promoting the nitrification rate, and therefore, increasing the susceptibility of soil N losses (Tapia-Torres, López-Lozano, et al., 2015). Two processes can explain this result. The first processes could be due to an apparent priming effect by the addition of these

labile organic molecules. A priming effect is caused when fresh organic matter (OM) inputs activate soil microorganisms and promote the degradation of soil organic matter (Garcia et al., 2017), even the more stable organic C fractions (Scotti, Bonanomi, et al., 2015). In our case, the organic treatments AMP and RNA acted as these OM inputs and served as an initial energy source to those microorganisms capable of mineralizing soil OM. Therefore, the measured DOC concentration was lower in the AMP and RNA treatments at the end of experiment, probably because of a high rate of depletion of C sources, as well as Cmic for both treatments; while the treatment $Ca(H_2PO_4)_2$, contained the greater concentration of DOC at the end of the experiment. We hypothesize that lower DOC results from higher C mineralization rates since it is correlated negatively, resulting in the depletion of labile organic matter in the soil. As a consequence, the carbon use efficiency in relation with nitrogen ($CUE_{C:N}$) was lower in the AMP and RNA treatments than in monobasic calcium phosphate ($Ca(H_2PO_4)_2$), These CUE results can be explained by the biogeochemical analysis performed at the end of the incubation period, when the DOC was consumed by microbial community. $CUE_{C:N}$ is expected to decrease when C is a limiting resource and the remaining organic matter to decompose has higher recalcitrance (Robert L. Sinsabaugh et al., 2013a).

The second process is related with the increment the activity of nitrifiers in the AMP and RNA treatments as a result of the DOC decrease at the end of incubation. Our results showed that the DOC concentration correlated inversely with nitrate in these both treatments, which point to enhanced activity of nitrifiers. This kind of microorganisms consist of quimioautotrophic bacteria and archaea; which obtain energy from NH_4, oxidating it to NO_2^- and then take the energy from NO_2^-, yielding NO_3^- (Fenchel et al., 2012); besides, these

bacteria have optimum activity in neutral to alkaline pH (Prosser, 1990), which is coincident with our soils. A decrease of COD at the end of the incubation in these soils could make these bacteria rise and be competitive among the heterotrophic bacteria. In a pulse of carbon, such as that created by the application of organic treatments AMP and RNA, rapid growing heterotrophic microbes (r strategist) immobilize nutrients and grow faster, which may be happening during the first days of the incubation. However, enhanced growth of these organisms may induce a rapid depletion of labile carbon sources, giving place to a reduction of r strategist, an increase of k strategist and quimioautotrophic bacteria (Montaño and Sánchez-Yañez, 2014), explaining also reductions in microbial C. A decrease of NOD in the AMP treatment while NO_3^- increase, is an indicator that heterotrophic bacteria are mineralizing organic matter containing N, and yielding NH_4^-, due to organic C limitation (Chapin III et al., 2011); subsequently, the NH_4^+ is rapidly used as a substrate for nitrifiers. These two processes suggest that while organic labile substrates such as ARN and AMP may benefit microbial respiration, it might be important to consider a constant supply of organic amendments in agricultural practices to avoid the soil N losses.

4.2 Effect of phosphorus addition on Phosphorus availability

In this work, we hypothesized that AMP and RNA treatments would promote soil nutrient availability, mainly phosphorus. However, we did not find a rise in available HPO_4^{-3} concentrations at the end of incubation, but we found higher DOP and Pmic concentrations in both labile organic treatments (RNA and AMP). The increases on Pmic are crucial because the microbial community is retaining labile forms of P in actively cycling biological pools, and lowering the rate at which labile inorganic P would be otherwise permanently lost via

adsorption into soil particles or leaching (Cleveland et al., 2002). On the other hand, organic phosphorus compounds are an essential fraction of soil total phosphorus, since in CCB grasslands, it can represent about 50% of total P (Perroni et al., 2014); and dissolved organic phosphorus is composed principally of products of microbial metabolism (Cleveland et al., 2002).

Besides the changes in organic and microbial P pools, the specific enzyme activity (SEA) of phosphorus enzymes differed among treatments. Organic treatments, either AMP, RNA or phytic acid, stimulated phosphodiesterase activity per unit of microbial biomass, as shown with the SEA of Phd, but phosphomonoesterase enzyme was not affected. Both phosphomonoesterases and phosphodiesterases are part of the Phosphate Regulon (Pho) in bacteria, responsible for phosphorus uptake, and responding to P starvation (Santos-Beneit, 2015). The lower concentration of inorganic phosphate but higher availability of organic P in organic treatments at the beginning of the experiment may have enabled the production of Phd enzyme, and as it is known, extracellular enzymes can persist in soil, associate with clay and organic matter particles and remain active (Nannipieri et al., 2011). Therefore, Phd may have persisted until the end of the experiment and have been measured.

Nucleic acids such as RNA and DNA are released by dead cells in the environment and are an important labile source of nutrients such as C, N and P (Tani & Nasu, 2010), particularly for bacteria from oligotrophic environments (Tapia-Torres et al., 2016). It had been shown in other studies in CCB soils, Phd activity tended to be higher than Phm (Montiel-González et al., 2017; Tapia-Torres et al., 2016), demonstrating that phosphodiester uptake plays a

prominent role in phosphorus cycling in these soils (Tapia-Torres et al., 2016). These studies agree with Turner and Haygarth (2005), who determined that in pasture soils, phosphodiesterase activity is the rate-limiting step that regulates P turnover because P availability depends on the degradation of fresh organic materials, which are abundant in phospholipids and nucleic acids, cellular components that are phosphate diesters.

Phosphorus turnover is highly important in agricultural systems, because inorganic phosphorus tend to be lost or unavailable to crops due to lixiviation or occlusion processes. Although inorganic P is the immediate phosphorus source for vegetation, it is necessary to promote the increase of labile organic P molecules and microbial P pools to avoid these losses, and the increase of enzymes that hydrolyze organic P compounds, such as phopomonoesterases, phosphodiesterases, phytases and phosphonatases, to allow a slow but constant release of inorganic P.

4.3 Effect of phosphorus addition on C, N and P stoichiometry

The microbial community, in most treatments, is homeostatic; that is, the C:N:P ratios in microbial biomass remains constant despite changes in these ratios of the resources (Elser and Sterner, 2002). Nevertheless, the microbial community in the phytic acid treatment resulted to be non-homeostatic. A known premise broadly used in ecological stoichiometry studies is that heterotrophs organisms are strictly homeostatic, while autotrophs can have a changing stoichiometry (Fanin et al., 2013; Persson et al., 2010) although, there are some scenarios in which a microbial community can change their stoichiometry according to the stoichiometry of their resource, becoming non homeostatic. A non-homeostatic behavior is a

mechanism to reduce stochiometric imbalances between the resources and the microbial biomass (Mooshammer et al., 2014), because it can occur through the microbial storage of nutrients in excess or by shifts in microbial community structure and therefore shifts in biomass stoichiometry (Mooshammer et al., 2014). In this work, we reported a lower value of Cmic:Pmic ratio compared to the control, suggesting more phosphorus immobilization with P addition; however, this difference was present in all phosphorus treatments, not only in phytic acid treatment. Fanin et al. (2013) suggested that non-homeostatic behaviors are due to changes in microbial community composition instead of shifts on microbial biomass of individual microbes, since they found the ratios bacteria:fungi and gram positive:gram negative change along with changes in homeostasis. For example, the reported C:N:P ratio for fungi is 250:16:1 (Ji Zhang & Elser, 2017), while the bacterial C:N:P ratio is 46:7:1 (Cleveland & Liptzin, 2007). In the phytic acid treatment, the average C:N:P ratio was 58:5:1, closest to bacterial biomass ratio, or to the average soil microbial biomass ratio (60:7:1), suggested by Cleveland and Liptzin (2007) but in the pre-incubation samples, microbial biomass stoichiometry was closest to fungal biomass stoichiometry (246:16:1, Table 1), as well as in the control samples (310:10:1; Table 4). This can lead us to hypothesize that homeostasis imbalances are due to a changing microbial community to different microbial groups when fertilizers are added. Regarding to phytic acid treatment, B-propeller phytase, the active kind of phytase in neutral and alkaline soils, is mainly produced by bacteria, while acid phytases are produced by fungi (Jain et al., 2016).

The threshold element ratio (TER) is the elemental proportion corresponding to a balanced microbial growth, without limitation by C or nutrients (Robert L. Sinsabaugh et al., 2016);

representing the critical ratio when organisms transition from net nutrient immobilization to net nutrient mineralization (Mooshammer et al., 2014), and it defines if the community is limited by nutrients (N or P) or by energy (C). When resource ratio C:N or C:P are greater than the TER, the system is limited by nutrients and immobilization processes dominate; when the resource ratio are lower than the TER, then the system is limited by energy, and nutrient mineralization occurs (Robert L. Sinsabaugh et al., 2013b). At this work, the treatment MAP, which was selected because it was used as a fertilizer in the agricultural plots where the soil was obtained, did not show differences between DOC: DOP ratio and TER; therefore it can be considered that the soil microbial community is co-limited by phosphorus and energy (Sterner & Elser, 2002). In contrast, for the $Ca(H_2PO_4)_2$, AMP, RNA, and phytic acid treatments, there is a limitation by P for the soil microbial community, because TER is lower than their soil DOC:DOP ratios; here, the microbial community is inclined to immobilize available phosphorus. This coincides with previous studies made at non managed soils from CCB, at the eastern side of the valley (Pozas Azules), where low concentrations of DOC were found as well as a trend for phosphorus limitation, and low values of $TER_{C:P}$ (Tapia-Torres, Elser, et al., 2015).

Regarding nitrogen, the $TER_{C:N}$ was lower than the DOC:DON ratio for MAP and RNA treated soil, suggesting that the microbial community is limited by N, which suggests a tendency to immobilize N, although it is not shown in microbial biomass. In CCB, it was also found that a site from the western side of the valley (Churince) with higher DOC values, as well as our MAP treatment, was limited by N (Tapia-Torres, Elser, et al., 2015). On the other hand, the $TER_{C:N}$ was higher for the control treatment, implying a

limitation by C or energy and a preference for mineralizing organic nitrogen compounds to obtain C and release NH_4^+, while immobilizing more C and lowering its losses by mineralization. This carbon limitation was also reflected in the $CUE_{C:N}$ previously discussed (section 5.1), because the AMP and RNA treatments had the lowest CUE. All these results, suggest that the addition of labile organic molecules with P (MAP and RNA) increase microbial N limitation, probably by the increment of N demand by the growing microbial community as a consequence of primming effect discussed previously (section 5.1).

The results of this work show that the soil microbial community responds differently to different phosphorous molecules; these effects show differences both between organic and inorganic molecules, and between the same groups of molecules with different chemical composition. This can have implications when carrying out fertilizations with organic matter in crop fields since the chemical structure of the molecules that make up composts and manures are usually unknown. Although the most labile organic compounds (AMP and RNA) favored C mineralization, they also showed a rapid decrease in DOC, which implied a reduction in microbial biomass and an increase in chemoautotrophic microorganisms such as nitrifying, indicating that when fertilizing with labile organic sources, the periodicity of application must be taken care of for avoid soil N losses.

5. Conclusions

Despite having carried out this experiment using soil from an agricultural field with conventional management, the soil microorganisms show a limitation by phosphorus, and by

carbon, which is shown in the control samples. These carbon limitations and low CUE levels are indicators of highly soil recalcitrant C compounds, and this is also reflected in microbial biomass ratios, which are similar to soil fungi biomass ratios. Carbon limitations were overcome with phosphorus fertilizations; besides, P treatments promoted the immobilization of this nutrient in microbial biomass and in some treatments (AMP, RNA and $Ca(H_2PO_4)_2$) it promoted the increase in DOP. All fertilizations reduced the soil microbial biomass ratios which can be an indicator of a changing microbial community, and an increase of bacterial biomass over fungal biomass. Although P in microbial biomass might not be available to crop plants temporarily, it is an organic phosphorus pool that is quickly recycled and can protect P from losses due to leaching and adsorption in soil minerals. The labile organic treatments (AMP and RNA) increased the availability of N; however, this nutrient was quickly nitrified. Even so, nitrate is an available form of N to plants, but it is vulnerable to be lost from the soil. This work shows that organic matter composition of diverse composts and manures added to crop is highly important, because not all organic phosphorus compounds have the same effect on microbial community and nutrient cycling; here we exemplify that phytic acid despite being an organic molecule of P, showed an inorganic molecule behavior and microorganisms that grew in this substrate were less efficient in terms of the mineralization of C (qCO_2).

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TABLES

Table 1. Phosphorus content and concentration of each chemical compound added to the fertilization experiment

	P content g mol^{-1} on dry basis	**Grams of reactant added per sample (25% water weight)**	**P added per sample (g)**	**μmol P per sample**
Phytic acid sodium salt hydrate	185.82	0.013	0.0028	90
Adenosine monophosphate	30.97	0.033	0.0028	90
RNA from torula yeast	9.8%	0.035	0.0028	90
Monoammonium phosphate	30.97	0.0103	0.0028	90
Calcium phosphate monobasic	61.94	0.011	0.0028	90

Table 2. Soil physic and chemical, biogeochemical and enzymatic activity values obtained from the alfalfa crop soil before the incubation fertilization experiment.

Variable	Mean (Stándar Error)	
Soil moisture (%)	29	(±0.006)
pH in water	8.1	(±0.017)
TOC (mg g^{-1})	26	(±1.2)
TN (mg g^{-1})	2.6	(±0.15)
TP (mg g^{-1})	0.6	(±0.045)
DOC (μg g^{-1})	28	(±2.9)
DON (μg g^{-1})	1.64	(±0.51)
DOP (μg g^{-1})	0.28	(±0.084)
NH_4 (μg g^{-1})	0.22	(±0.216)
NO_3 (μg g^{-1})	15	(±1.5)
HPO_4 (μg g^{-1})	3.4	(±1.479)
Cmic (μg g^{-1})	1190	(±192)
Nmic (μg g^{-1})	70	(±5.4)
Pmic (μg g^{-1})	5	(±0.86)
Cmic: Nmic	17	(±3)
Cmic: Pmic	246	(±33)
Nmic: Pmic	16	(±3)
Phm (μmolPNP h^{-1} g^{-1})	0.030	(±0.015)
Phd (μmolPNP h^{-1} g^{-1})	0.157	(±0.041)
Phy (μmolPi h^{-1} g^{-1})	0.056	(±0.056)
NAG (μmolPNP h^{-1} g^{-1})	0.008	(±0.003)
BG (μmolPNP h^{-1} g^{-1})	0.005	(±0.002)
POX (μmol ABTS h^{-1} g^{-1})	0.196	(±0.046)

TOC: Total organic carbon, TN: Total nitrogen, TP: Total phosphorus, DOC: Dissolved organic carbon, DON: Dissolved Organic Nitrogen, DOP: Dissolved organic phosphorus, NH_4: Available ammonium, NO_3: Available nitrate, HPO_4: Available inorganic phosphate, Cmic: Carbon immobilized in microbial biomass, Nmic: Nitrogen immobilized in microbial biomass, Pmic: Phosphorus immobilized in microbial biomass Phm: Phosphomonoesterase enzyme activity, Phd: Phosphodiesterase enzyme activity, Phy: Phytase enzyme activity, NAG: N-acetyl glucosaminidase enzyme activity, BG: β-glucosidase enzyme activity, POX: Polyphenol oxidase (laccase) enzyme activity. Standard error values are shown inside parenthesis.

Table 3. Data obtained for the different treatments and the control after 19 days incubation

Variable	Control	MAP (Pi)	$Ca(H_2PO_4)_2$ (Pi)	RNA (Po)	AMP (Po)	Phytic acid (Po)	F
pH (H_2O 1:5)	8.1 (±0.12)	8.1 (±0.060)	8.1 (±0.050)	8.2 (±0.040)	8.1 (±0.030)	8.1 (±0.060)	0.66
Cmic (μg g^{-1})	1283 (±76) ^{A}	1027 (±69) ^{AB}	1057 (±78) ^{AB}	673 (±99) ^{BC}	690 (±93) ^{BC}	611 (±134) ^{C}	8.3***
Nmic (μg g^{-1})	45 (±9)	39 (±4)	43 (±6.000)	58 (±6)	46 (±5)	50 (±12)	0.82
Pmic (μg g^{-1})	4.56 (±0.79) ^{D}	15.44 (±3) ^{BCD}	20.01 (±4.470) ^{BC}	36.95 (±5) ^{A}	27.22 (±1) ^{AB}	10 (±2) ^{CD}	13.5***
DOC (μg g^{-1})	164 (±24) ^{AB}	95 (±12) ^{ABC}	189 (±67) ^{A}	50 (±6) ^{BC}	30 (±4) ^{C}	64 (±5) ^{ABC}	4.7***
DON (μg g^{-1})	0.74 (±0.15) ^{C}	0.57 (±0.120) ^{C}	2.81 (±0.080) ^{A}	2.17 (±0.160) ^{B}	0.68 (±0.160) ^{C}	0.69 (±0.060) ^{C}	57.2***
DOP (μg g^{-1})	0.13 (±0.02) ^{B}	0.38 (±0.060) ^{AB}	0.57 (±0.110) ^{A}	0.72 (±0.070) ^{A}	0.7 (±0.080) ^{A}	0.5 (±0.120) ^{AB}	7.1 ***
NH_4 (μg g^{-1})	0 (±0)	0.004 (±0.004)	0 (±0)	0.062 (±0.062)	0 (±0)	0 (±0)	0.97
NO_3 (μg g^{-1})	57 (±6) ^{C}	69 (±9) ^{C}	51 (±6) ^{C}	102 (±7) ^{B}	135 (±7) ^{A}	58 (±7) ^{C}	22.4***
HPO_4 (μg g^{-1})	6.3 (±0.96)	15.2 (±2)	17.3 (±5)	10.6 (±1.43)	11.9 (±1.35)	10.8 (±1)	2.3
Nitrification (μgNO_3 g^{-1})	37 (±6) ^{C}	54 (±8) ^{C}	36 (±5) ^{C}	87 (±7) ^{B}	120 (±6) ^{A}	43 (±6) ^{C}	29.4***
CO_2-C (μgCO_2-C g^{-1})	739 (±6) ^{D}	831 (±11) ^{BC}	781 (±17) ^{CD}	863 (±9) ^{B}	950 (±17) ^{A}	802 (±4) ^{C}	39.2***
qCO_2	0.59 (±0.032) ^{B}	0.82 (±0.049) ^{AB}	0.83 (±0.084) ^{AB}	1.52 (±0.244) ^{AB}	1.60 (±0.216) ^{AB}	1.9 (±0.557) ^{A}	4**

Data are means of pH and biogeochemical variables after incubation per treatment. AMP: Adenosine monophosphate, MAP: Monoammonium phosphate, DOC: Dissolved organic carbon, DON: Dissolved Organic Nitrogen, DOP: Dissolved organic phosphorus, NH_4: Available ammonium, NO_3: Available nitrate, HPO_4: Available inorganic phosphate, Cmic: Carbon immobilized in microbial biomass, Nmic: Nitrogen immobilized in microbial biomass, CO_2-C: Carbon from CO_2 produced in mineralization, q-CO_2: Metabolic quotient; Pi: Inorganic phosphorus source; Po: Organic phosphorus source. Standard error in parentheses. Letters (A, B, C, D) show significant differences between treatments. p: *<0.05, **<0.01, ***<0.001.

Table 4. Data for nutrient immobilization obtained from dissolved nutrients and biomass ratios

Variable	C	MAP (Pi)	$Ca(H_2PO_4)_2$ (Pi)	RNA (Po)	AMP (Po)	Phytic acid (Po)	F
DOC:DON	295^{A} (±99)	225^{AB} (±84)	68^{AB} (±25)	24^{B} (±3.80)	52^{B} (±10)	95^{AB} (±6.0)	3.4**
DOC:DOP	1792^{A} (±720)	312^{AB} (±94)	367^{AB} (±126)	74^{B} (±12)	43^{B} (±6.8)	193^{B} (±82)	4.1**
DON:DOP	8.1^{A} (±3.5)	1.5^{B} (±0.14)	5.7^{AB} (±1.20)	3.1^{B} (±0.31)	0.816^{B} (±0.16)	1.9^{B} (±0.66)	2.7*
Cmic:Pmic	310^{A} (±44)	82^{B} (±18)	68^{B} (±20)	18^{B} (±1.20)	25^{B} (±3.3)	58^{B} (±3.8)	25.8***
Cmic:Nmic	34^{A} (±7.4)	28^{AB} (±4.3)	26^{ABC} (±2.9)	11^{C} (±0.99)	15^{BC} (±0.93)	14^{BC} (±2.1)	5.6***
Nmic:Pmic	10^{A} (±2.7)	2.9^{B} (±0.55)	2.5^{B} (±0.41)	1.66^{B} (±0.20)	1.7^{B} (±0.16)	4.6^{B} (±0.48)	8.7***

Means of dissolved nutrient ratios and nutrient within microbial biomass ratios, which denotes higher nutrient immobilization in microbial biomass the lower the value of the ratio Cmic:Nmic and Cmic:Pmic. Standard error is shown in parenthesis. Letters (A, B, C, D) indicate significant differences between treatments with the Tukey test. AMP: adenosine monophosphate, and MAP: monoammonium phosphate. p: *<0.05, **<0.01, ***<0.001.

Table 5. Specific Enzyme activities (SEA) after 19 days incubation

Variable	Control	MAP (Pi)	$Ca(H_2PO_4)_2$ (Pi)	RNA (Po)	AMP (Po)	Phytic acid (Po)	F
SEA BG (μmol pNP $mgCmic^{-1}$ h^{-1})	0.069 (±0.02)	0.06 (±0.014)	0.049 (±0.012)	0.102 (±0.038)	0.139 (±0.057)	0.132 (±0.074)	0.8
SEA POX (μmol tyr $mgCmic^{-1}$ h^{-1})	0.244 (±0.04) ^{AB}	0.171 (±0.050) ^{B}	0.082 (±0.052) ^{B}	0.493 (±0.082) ^{A}	0.371 (±0.195) ^{AB}	0.540 (±0.134) ^{A}	2.9*
SEA NAG (μmol pNP $mgCmic^{-1}$ h^{-1})	0.014 (±0.006) ^{B}	0.027 (±0.006) ^{AB}	0.030 (±0.007) ^{AB}	0.004 (±0.001) ^{B}	0.001 (±0.001) ^{B}	0.066 (±0.033) ^{A}	2.8*
SEA Phm (μmol pNP $mgCmic^{-1}$ h^{-1})	0.013(±0.007)	0.025 (±0.015)	0.16 (±0.134)	0.068 (±0.017)	0.097 (±0.054)	0.052 (±0.017)	0.8
SEA Phd (μmol pNP $mgCmic^{-1}$ h^{-1})	0.164 (±0.01) ^{B}	0.171 (±0.018) ^{B}	0.167 (±0.015) ^{B}	0.429 (±0.123) ^{A}	0.408 (±0.078) ^{A}	0.480 (±0.13) ^{A}	3.5*
SEA Phy (μmol Pi $mgCmic^{-1}$ h^{-1})	0.896 (±0.31)	0.403 (±0.207)	0.793 (±0.415)	0.696 (±0.373)	1.213 (±0.61)	0.497 (±0.32)	0.55

Means of specific enzyme activities per treatment, obtained with the division between enzymatic activity and Cmic. The standard error is indicated between parentheses. Letters (A, B, C, D) indicate significant differences between treatments as obtained from the Tukey test. AMP: adenosine monophosphate, and MAP: monoammonium phosphate. BG: β-glucosidase, NAG: N-acetyl glucosaminidase, POX: Polyphenol oxidase (laccase, Phm: Phosphomonoesterase, Phd: Phosphodiesterase, Phy: Phytase. p: *<0.05, **<0.01, ***<0.001.

Table 6. Dissolved organic nutrients (DOC, DON and DOP) and nutrients immobilized in microbial biomass (Cmic, Nmic and Pmic) after organic or inorganic P fertilization treatment.

Variable	control	MAP (Pi)	$Ca(H_2PO_4)_2$ (Pi)	RNA (Po)	AMP (Po)	Phytic acid (Po)	F
ΔDOC (μg g^{-1})	136 (±24) ^{AB}	67 (±13) ^{ABC}	160 (±67) ^{A}	22 (±4.5) ^{BC}	1.5 (±2.1) ^{C}	36 (±7.7) ^{ABC}	4.7 **
ΔDON (μg g^{-1})	-0.90 (±0.45) ^{BC}	-1.07 (±0.59) ^{C}	1.17 (±0.54) ^{A}	0.53 (±0.48) ^{AB}	-0.96 (±0.64) ^{BC}	-0.95 (±0.55) ^{BC}	3.12 *
ΔDOP (μg g^{-1})	-0.15 (±0.07) ^{B}	0.06 (±0.14) ^{AB}	0.30 (±0.12) ^{A}	0.45 (±0.08) ^{A}	0.41 (±0.06) ^{A}	0.23 (±0.14) ^{AB}	5**
ΔCmic (μg g^{-1})	94 (±218)	-163 (±217)	-133 (±154)	-517 (±254)	-500 (±228)	32 (±278)	1.4
ΔNmic (μg g^{-1})	-26 (±12)	-32 (±6.4)	-27 (±7.3)	-12 (±10)	-25 (±7.1)	-0.15 (±16)	0.43
ΔPmic (μg g^{-1})	-0.48 (±1.4) ^{D}	10 (±3.5) ^{BCD}	15 (±4.9) ^{BC}	32 (±5.2) ^{A}	22 (±1.1) ^{AB}	5.5 (±2.1) ^{CD}	11.6***

Means of the differences (Δ) between post and pre incubation values for dissolved organic nutrients (DOC, DON and DOP) and nutrients immobilized in microbial biomass (Cmic, Nmic and Pmic). The standard error is indicated between parentheses. AMP: adenosine monophosphate, and MAP: monoammonium phosphate.

FIGURES

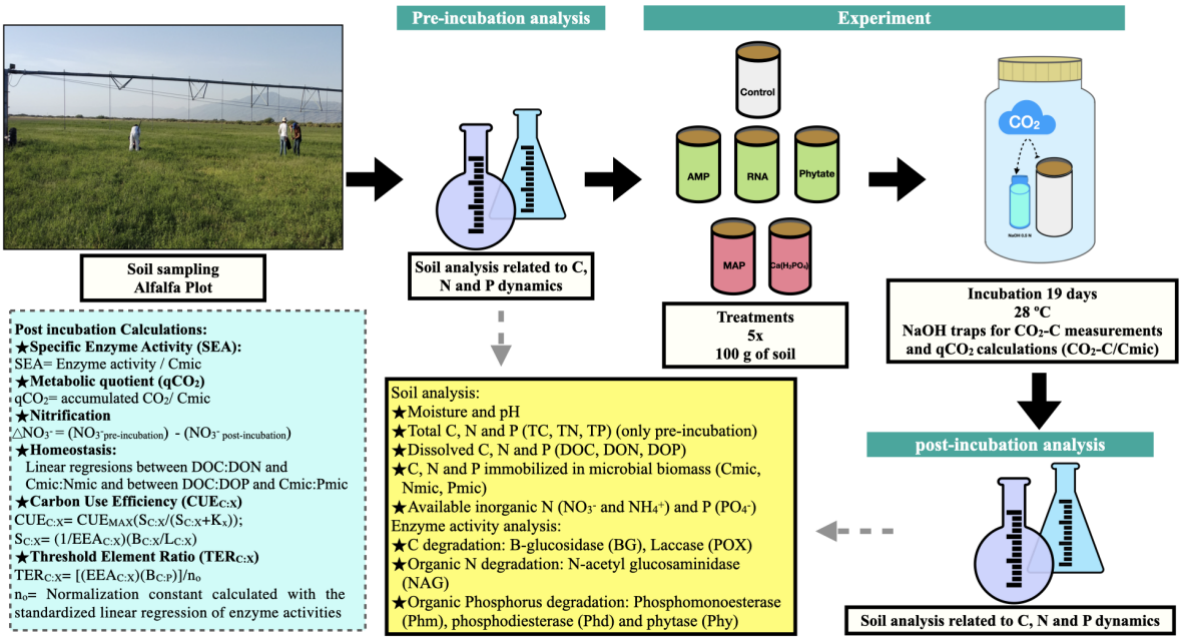

Figure 1. Methods summary. Soil laboratory analysis are described in the yellow box, which is related to pre-incubation and post-incubation analysis. Calculations made from chemical and enzymatic variables are specified in the blue box.

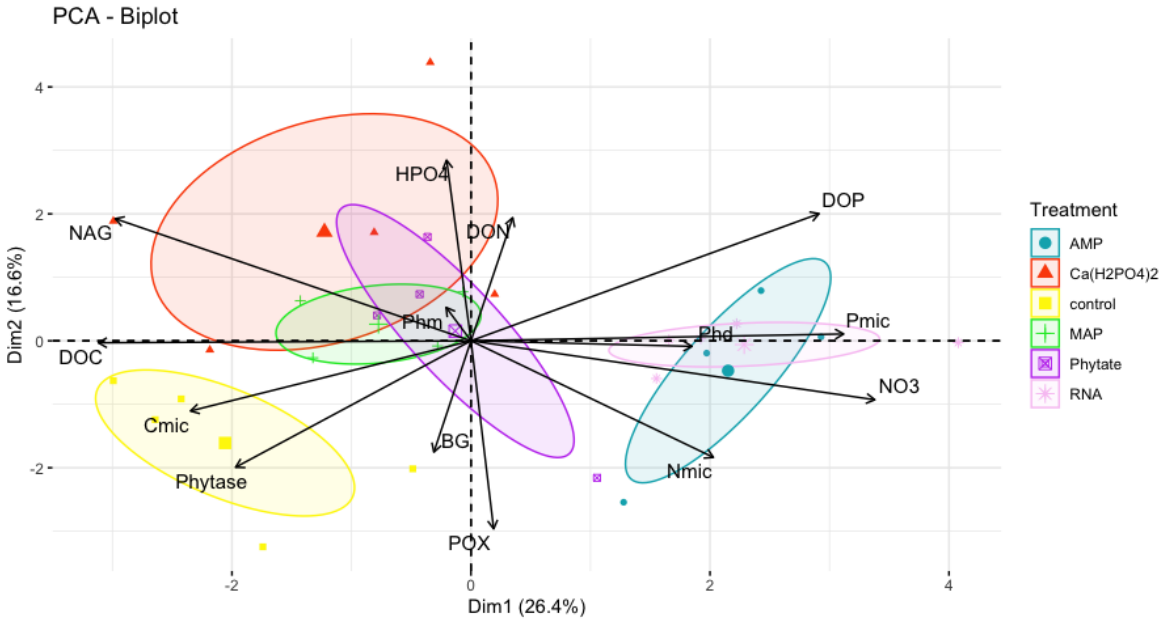

Figure 2. PCA analysis for biogeochemical and enzymatic variables obtained after the fertilization incubation experiment. Each color represents a treatment: Blue for AMP, yellow

for $Ca(H_2PO4)_2$, green for phytic acid, purple for MAP, pink for RNA. The control is shown in red. AMP: adenosine monophosphate, and MAP: monoammonium phosphate. This figure was made using using the "factoextra" package (Kassambara and Mundt, 2020) with R software (R core team, 2020).

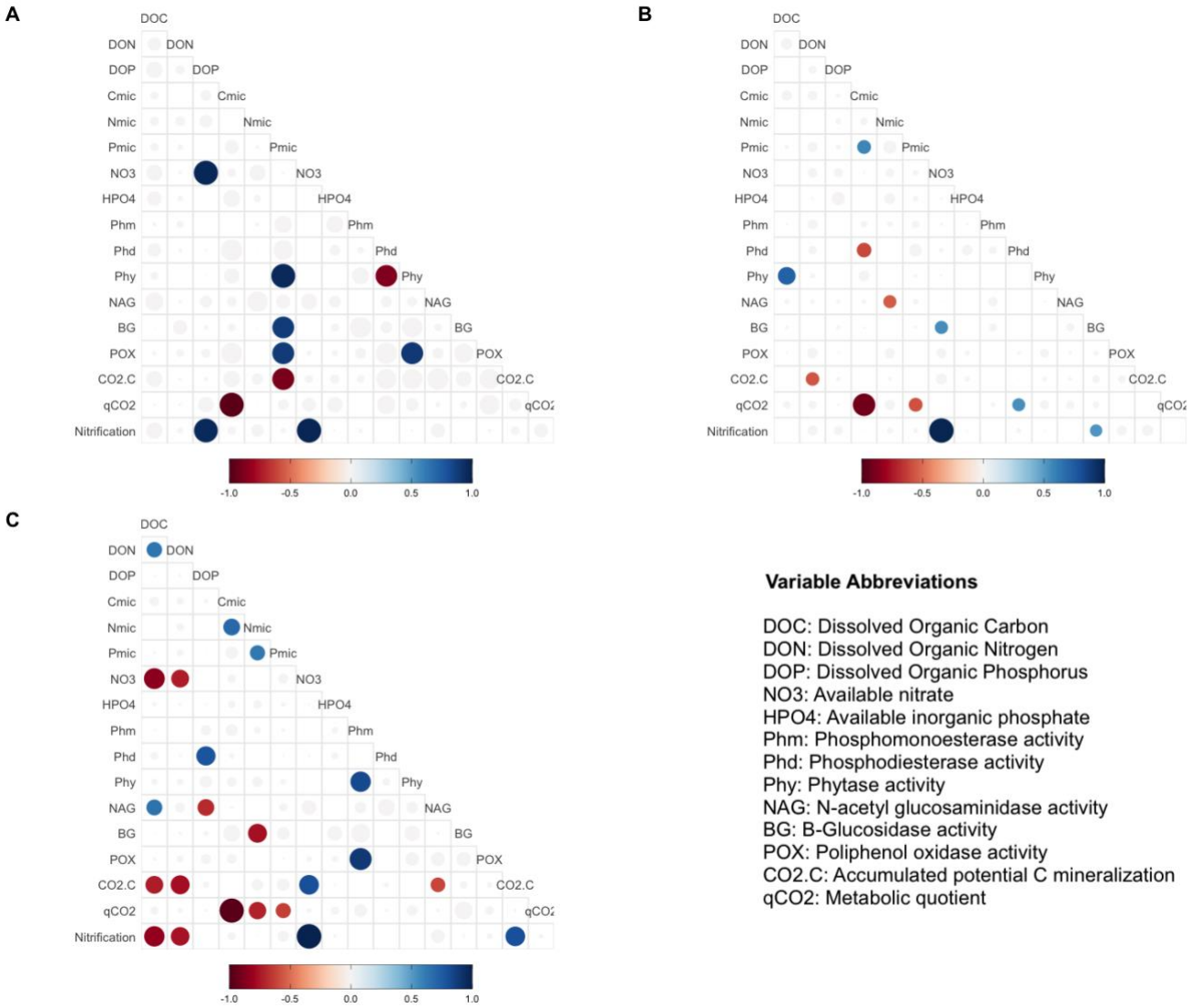

Figure 3. Pearson Correlation Test in different treatments group. Pearson correlation test was performed using biogeochemical and enzymatic variables, C mineralization, qCO_2 and nitrification (ΔNO_3) measured after incubation fertilization experiment. The circles represent significant correlations ($p<0.05$). The color scale indicates the correlation coefficient, and whether the correlation is positive (blue) or negative (red). The correlation analyses are divided by treatment groups according to principal component analyses: A) Control, B) MAP, $Ca(H_2PO_4)_3$ and phytic acid group, C) AMP and RNA group. AMP: adenosine

monophosphate, and MAP: monoammonium phosphate. This figure was made using the "ggcorrplot2" package (Cai and Matheson, 2021) in R software (R core team, 2020).

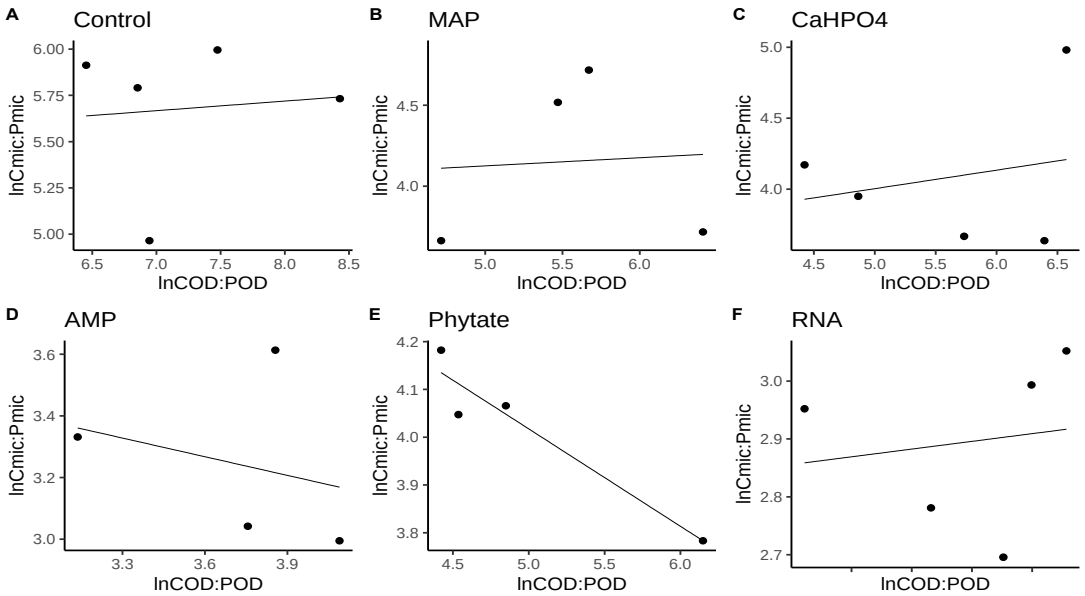

Figure 4. Soil microbial community homeostasis related to P acquisition estimated by an standardized linear regression. a) control, treatments: b) ammonium phosphate (MAP), c) Calcium phosphate ($CaHPO_4$), d) Adenosine monophosphate (AMP), e) Phytic acid (phytate) and f) Ribonucleic acid (RNA). These values represent strong homeostasis for all treatments because the slope is not different from 0, and there is not a relationship between the microbial biomass quotient and the substrate quotient (DOC:DOP) except for phytate (p=0.04). The equations for each figure are A) y=0.05x+5.3, R^2=-0.32. B) y=0.05x+3.87, R^2=-0.5. C) y=0.13x+3.35, R^2=-0.27. D) y=-0.2+3.99, R^2= -0.37. E) y=-0.2x+5.04, R^2=0.88. F) y=0.05x+2.67, R^2=-0.3.

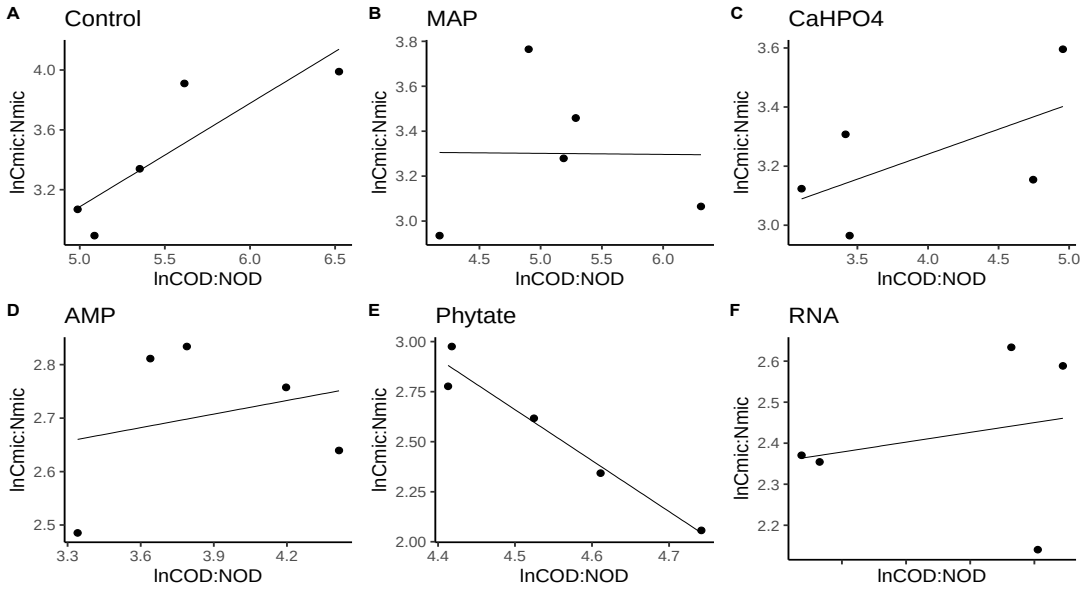

Figure 5. Soil microbial community homeostasis related with N acquisition estimated by an standardized linear regression.for the treatments a) control, b) ammonium phosphate (MAP), c) Calcium phosphate (CaHPO4), d) Adenosine monophosphate (AMP), e) Phytic acid (phytate) and f) Ribonucleic acid (RNA). These values represent strong homeostasis for all treatments because the slope is not different from 0, and there is not a relationship between the microbial biomass quotient and the substrate quotient (DOC:DON), except for phytate ($p=0.04$). The equations for each figure are A) $y=0.7x-0.37$, $R^2=0.66$. B) $y=-0.005x+3.3$, $R^2=-0.33$. C) $y=0.17x+2.6$, $R^2=0.15$. D) $y=0.085+2.4$, $R^2= -0.25$. E) $y=-2.5x+14.11$, $R^2=0.9$. F) $y=0.12x+2.05$, $R^2=-0.26$.

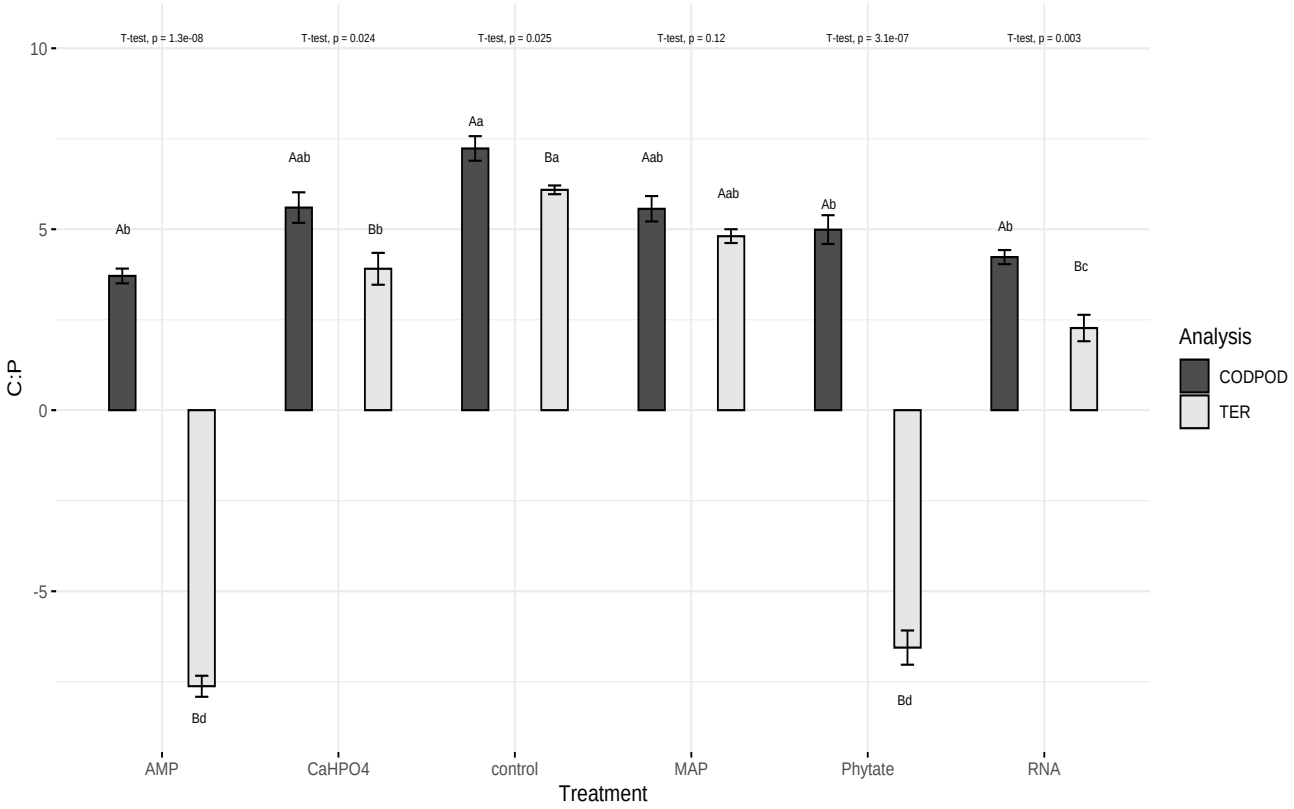

Figure 6. Mean of natural logarithms of DOC:DOP ratio and $TER_{C:P}$ of all treatments. Significant differences for the comparisons between DOC:DOP ratio (CODPOD, black bars) and the $TER_{C:P}$ (gray bars) values of each treatment are marked with uppercase letters, while the significant differences of the $TER_{C:P}$ or DOC:DOP values between treatments are marked with lowercase letters.

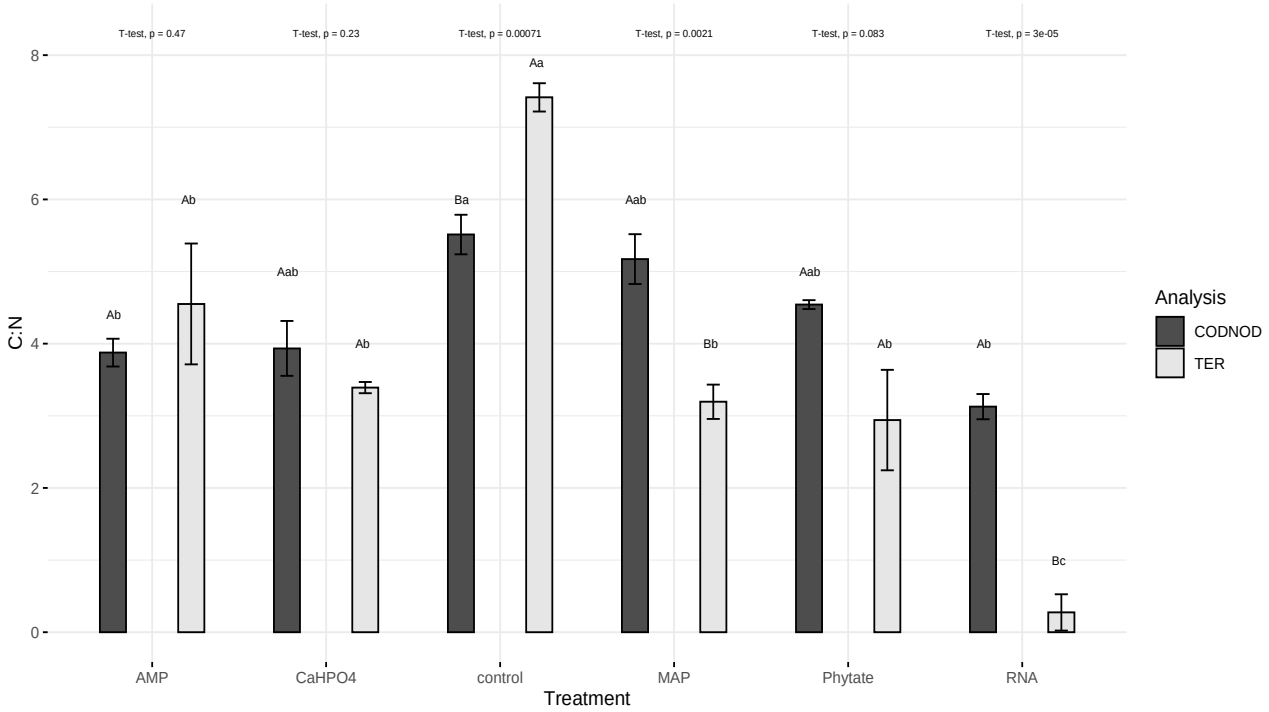

Figure 7. Means of natural logarithms of DOC:DON ratio and $TER_{C:N}$ of all treatments. Significant differences for the comparisons between DOC:DON ratio (CODNOD, black bars) and the $TER_{C:N}$ (gray bars) values of each treatment are marked with uppercase letters, while the significant differences of the $TER_{C:N}$ or DOC:DON values between treatments are marked with lowercase letters.

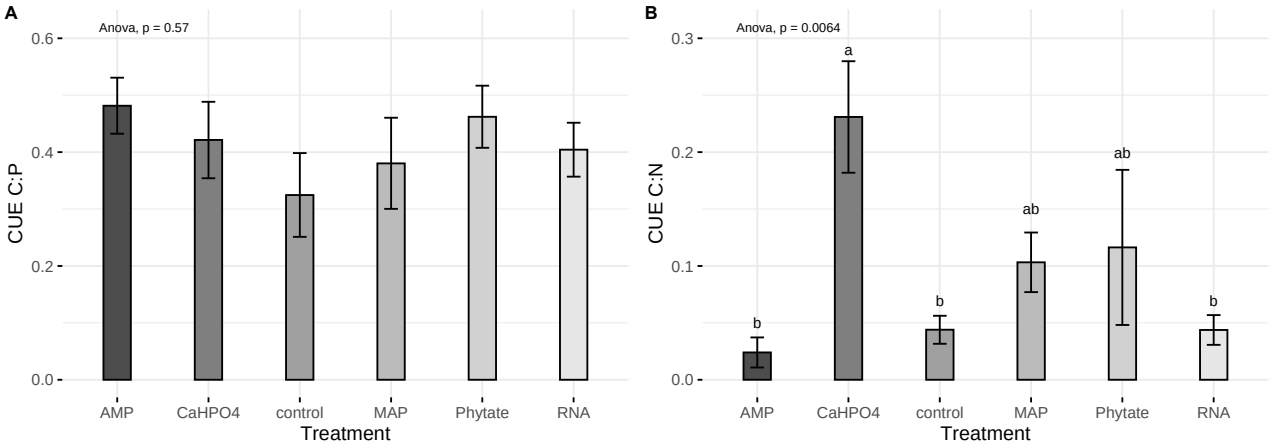

Figure 8. Means for A) $CUE_{C:P}$ y B) $CUE_{C:N.}$ Letters show significant differences between treatments obtained with the Tukey HSD test. The p value from the ANOVA analysis is shown on top of the figures.

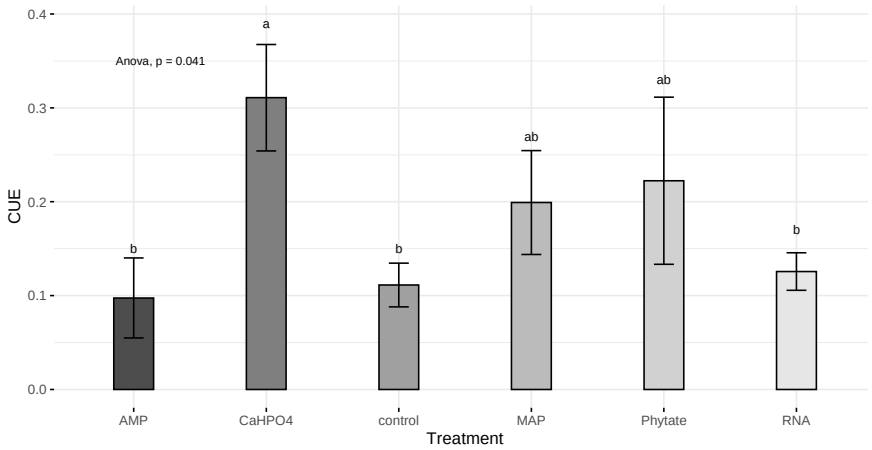

Figure 9. Means of CUE calculated with equation 5. Letters show significant differences between treatments obtained with an LSD test. The p value from the ANOVA analysis is shown on top of the bars.

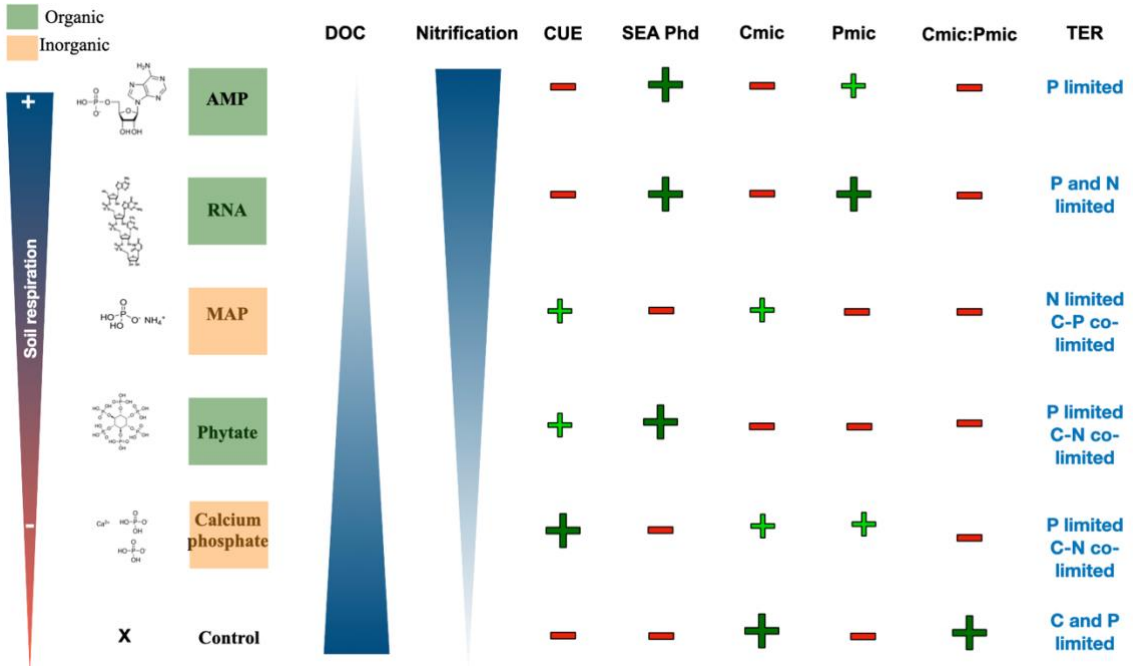

Figure 10. Main effects on soil microbial activity and nutrient dynamics caused by fertilization with different compounds containing phosphorus. DOC is dissolved organic carbon, CUE is carbon use efficiency, SEA Phd is specific enzyme activity of phosphodiesterase, Cmic and Pmic are carbon and phosphorus immobilized in microbial biomass respectively and TER is the threshold element ratio.

SUPPLEMENTARY MATERIAL

Table S1. Means and standard error of enzyme activities measured for each treatment after the 19 days of the incubation experiment.

Variable	Control	MAP (Pi)	$Ca(H_2PO_4)_2$ (Pi)	RNA (Po)	AMP (Po)	Phytic acid (Po)	p
BG (µmol pnp h^{-1} g^{-1})	0.086 (±0.02)	0.059 (±0.012)	0.053 (±0.015)	0.056 (±0.015)	0.08 (±0.028)	0.051 (±0.018)	0.673
POX (µmol tyr h^{-1} g^{-1})	0.3(±0.031)	0.181 (±0.061)	0.093 (±0.06)	0.306 (±0.028)	0.206 (±0.098)	0.302 (±0.072)	0.129
NAG (µmol pnp h^{-1} g^{-1})	0.02 (±0.008) ^{AB}	0.027 (±0.004) ^{AB}	0.032 (±0.008) ^{A}	0.003 (±0.001) ^{B}	0.001 (±0.001) ^{B}	0.023 (±0.01) ^{AB}	0.0079**
Phm (µmol pnp h^{-1} g^{-1})	0.016(±0.008)	0.023 (±0.013)	0.186 (±0.157)	0.041 (±0.006)	0.052 (±0.028)	0.04 (±0.017)	0.486
Phd (µmol pnp h^{-1} g^{-1})	0.212(±0.021)	0.172 (±0.013)	0.174 (±0.014)	0.262 (±0.091)	0.258 (±0.029)	0.224 (±0.009)	0.477
Phy (µmolpi h^{-1} g^{-1})	1.085(±0.32)	0.464 (±0.251)	0.87 (±0.476)	0.418 (±0.182)	0.665 (±0.311)	0.361 (±0.225)	0.521

Means of enzyme activities per treatment. The standard error is indicated between parentheses. Letters (A, B, C, D) indicate significant differences between treatments as obtained from the Tukey test. AMP: adenosine monophosphate, and MAP: monoammonium phosphate. BG: β-glucosidase, NAG: N-acetyl glucosaminidase, POX: Polyphenol oxidase (laccase, Phm: Phosphomonoesterase, Phd: Phosphodiesterase, Phy: Phytase.

Table S2. Percent of variance explained by the first two components (Eigenvalue) and weight of analized variables in the two first components (Eigenvector).

	PC 1	PC 2
Eigenvalue	3.69	2.32
Percent (%)	26.36	16.57
Accumulated percent (%)	26.36	42.93
	Eigenvector of the variables	
DOC	-0.74	-0.0074
DON	0.082	0.46
DOP	-0.69	0.476
Cmic	-0.557	-0.263
Nmic	0.482	-0.436
Pmic	0.74	0.026
NO_3	0.802	-0.22
PO_4	-0.048	0.676
Phm	-0.049	0.125
Phd	0.439	-0.021

Phy	-0.468	-0.473
NAG	-0.707	0.457
BG	-0.074	-0.416
POX	0.045	-0.704

CAPÍTULO III: Glyphosate-based herbicides alter soil carbon and phosphorus dynamics and microbial activity

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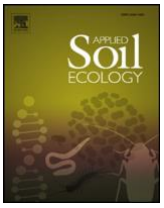

Glyphosate-based herbicides alter soil carbon and phosphorus dynamics and microbial activity

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ABSTRACT

Glyphosate is among the most used herbicides worldwide. However, both the active ingredient and additives of the commercial formulations may adversely affect chemical and biological processes in soil. Therefore, the aim of this work was to evaluate the effect of glyphosate and the commercial formulation (CH) on soil C, N and P dynamics, and microbial enzyme activity in two soil management conditions: a nopal plot (NP) with an history of 5 years of glyphosate application, and in an abandoned plot (AP) with a history of previous agricultural management without glyphosate applications. We performed three experiments: a) glyphosate and CH effects on phosphorus sorption by performing a non-biological P fixation experiment; b) glyphosate and CH effects on microbial activity, C, N and P dynamic, and the biodiversity of soil microorganisms in terms of biomarker fatty acids after a 27 day incubation experiment; and c) glyphosate and CH degradation was tested isolating bacteria from the incubated soil samples and growing in a defined media with potassium phosphate, glyphosate or CH as sole source of P. Our results suggested that other compounds from CH may be affecting P sorption, because when the herbicide was applied, P sorption was reduced in the NP, but in AP soil no significant effects were observed. In contrast, in the AP soil both glyphosate and the CH reduced C mineralization. Besides, CH application reduced Pmic concentration and laccase activity, but increased the specific activity of phosphonatase and phosphodiesterase in the AP soil. Additionally, the microbial community in NP soil revealed higher diversity index than the AP soil, but major changes in microbial groups were shown only when CH was applied. In vitro assay with native bacterial isolates confirmed their susceptibility to the commercial herbicide in both NP and AP soils. In conclusion, our results demonstrated changes on some processes related to soil C and P dynamics when pure glyphosate and a commercial glyphosate were applied, along with changes in microbial activity and community structure, being stronger the effects with the commercial formulation used. Further research should be done for test the effects of glyphosate on microbial stoichiometry and enzyme stoichiometry.

1. Introduction

Pesticides belonging to the group of organophosphates are among the most used pesticides worldwide. Phosphorus in these compounds sometimes occurs as molecules of phosphonates (Singh and Walker, 2006). Among the pesticides composed of phosphonates, glyphosate (*N*-phosphonomethyl glycine) stands out, a compound characterized by being one of the most used herbicides worldwide for about two decades (Duke et al., 2018). Fortunately, glyphosate can be degraded by different microbial enzymes (Kononova and Nesmeyanova, 2002). When phosphonate is used as a microbial phosphorus source, the C—P bond can be cleaved by the enzymatic complex C—P lyase, producing sarcosine and phosphate (HPO_4^{2-}), an available form of inorganic phosphorus used not only by microorganisms, but also by plants (Pipke et al., 1987; Sviridov et al., 2015).

However, glyphosate degradation in soils is highly variable. Several studies report that glyphosate is being rapidly inactivated in soils (Sprankle et al., 1975a, 1975b; Hensley et al., 1978), while other studies have also shown that biodegradation of glyphosate depend on soil physicochemical properties as soil mineral composition, soil texture and soil pH (Gimsing et al., 2004a; Gimsing et al., 2004b; Borggaard and Gimsing, 2008). This can be because organophosphorus pesticides

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widely used in agriculture such as glyphosate can be adsorbed to soil particles in similar way to soil organic compounds (De Jonge et al., 2001; Borggaard and Gimsing, 2008). Glyphosate is strongly adsorbed by soil minerals, compared with other herbicides (Mamy and Barriuso, 2005).

In soil, with pH range between 4 and 8, glyphosate is found as a mono or divalent anion, with high affinity to trivalent cations such as Al^{3+}, Fe^{3+} and divalent cations in alkaline pH, such as Ca^{2+}. Additionally, the phosphonic acid fraction of the glyphosate molecule promotes the occlusion of this herbicide to clays and hydroxides (Sprankle et al., 1975b), giving it an adsorption behavior similar to the adsorption of HPO_4^{2-}, whereby the herbicide can compete with this molecule for the same occlusion sites in the soil matrix (De Jonge et al., 2001). Therefore, applications of HPO_4^{2-}, associated with soil fertilization, can exclude the glyphosate from soil occlusion sites, hence increasing its bioavailability and mobility in the soil (De Jonge et al., 2001; Gimsing and Borggaard, 2001).

Commercial formulations of glyphosate contain other compounds listed as inert ingredients that fulfill the functions of surfactants, carrier, antifreeze, antifoam, dye and suction and whose chemical composition is not mentioned. However, these ingredients may also affect the adsorption processes of the herbicide in the soil. Additionally, the adsorption processes also depend on soil pH and mineral characteristics. Mobility of glyphosate in soil and how glyphosate is affected by phosphate additions have been widely studied (Sprankle et al., 1975a; Sprankle et al., 1975b; De Jonge et al., 2001; Gimsing et al., 2004a; Munira et al., 2016), but there are few reports focusing on how phosphate availability and occlusion in soils are affected by glyphosate, and by commercial formulations of the herbicide. Understanding these processes are key to generate strategies for the combined use of herbicides and fertilizers, while trying to preserve or favor biological activity in soils.

Glyphosate and glyphosate-based herbicides may also affect the soil microbial community. Glyphosate can affect soil microorganisms by inhibiting the pathway of shikimic acid, which is carried not only in plants, but also in some bacteria and fungi (Bentley and Haslam, 1990; Ratcliff et al., 2006). Some surfactants used in glyphosate herbicide formulations have also been reported as harmful to microorganisms, for example, the surfactant polyoxyethylene amine (POEA) has shown to be genotoxic for *Escherichia coli* (Nobels et al., 2011). It is known that microbes are important drivers of biogeochemical cycles (Falkowski et al., 2008), and the effects of glyphosate and its commercial formulations could lead to alterations in nutrient cycling. Some studies have shown how glyphosate affects the carbon cycling, increasing the number of substrates that microbial community can use as a carbon source when high doses of glyphosate are applied (Ratcliff et al., 2006) and increasing the activity of the B-glucosidase enzyme (Panettieri et al., 2013). Effects on the nitrogen cycle also have been reported, for example, Stratton (1990) published those high doses of commercial glyphosate formulations can stimulate the nitrification process in soils with neutral pH. Also, Nguyen et al. (2018) showed increases in organic N degrading enzymes, such as N-acety- glucosaminidase and aminopeptidase after 27 days of soil incubations amended with a glyphosate commercial formulation. Regarding phosphorus cycling functions mediated by microbes, Płatkowski and Telesiński (2016) reported that alkaline phosphomonoesterases and phosphodiesterases activities decreased when pure glyphosate and its commercial formulations were added to soil. The differences found between studies show that the effect of glyphosate on microbial activity and their metabolic processes that alter biogeochemical cycles can differ between soils (Dennis et al., 2018).

Naturally, sites with extreme soil pH facilitate analyzing possible P occlusion effects from glyphosate sorption processes. Because the soil in Cuatro Cienegas Basin (CCB) have alkaline pH (values between 8 and 9.5; Tapia-Torres et al., 2015) and high presence of Ca^{2+} (soil concentration between 0.67 mg Ca g^{-1} in agricultural soils to 0.9 mg Ca g^{-1} in abandoned agricultural sites; non-published data) that favors P occlusion, they provide an in-situ laboratory to understand the glyphosate sorption processes. Additionally, Perroni et al. (2014) reported that more than 80% of the soil P concentration is in occluded forms. Therefore, the soils of CCB are ideal for analyzing competition between inorganic phosphates and other organophosphorus compounds, for sorption sites in alkaline soils.

CCB is also characterized by its low P concentration in soil, but high proportion of organic P (Tapia-Torres and García-Oliva, 2013), a condition that can trigger microbial production of enzymes to cleave different organophosphorus molecules, such as phosphonates; an alternative source of available P (Tapia-Torres et al., 2016). Phosphorus starvation can induce genes for different enzymes such as the C—P lyase complex and phosphonatase (Sviridov et al., 2012), both important to glyphosate degradation. It has been reported that CCB bacteria obtained from non-managed soils can obtain phosphorus from different phosphonates such as 2-aminoethyl phosphonate (2-AEP), phosphonoacetaldehyde (2-PA); Tapia-Torres et al., 2016) and pure glyphosate (Morón-Cruz, 2014), besides, some specific bacteria from CCB, like *Bacillus* sp. M3-13 have been studied and it has been reported to contain *Phn* genes, coding for importers of phosphonates, permeases and C—P lyases (Alcaraz et al., 2010). However, the glyphosate degradation capacity of bacteria is unknown for microorganisms belonging to agricultural soil of CCB, where glyphosate is used.

Here we investigated the effect of glyphosate and the commercial formulation Faena on soil nutrient dynamics and microbial activity. Specifically, we aimed to elucidate possible effects of glyphosate and the commercial herbicide on soil phosphorus sorption in soils under nopal cultivation with constant addition of the herbicides (NP) and soils without previous additions of glyphosate (AP). We also analyzed the effect of glyphosate and the commercial herbicide on soil biogeochemistry, addressing nutrient availability, organic nutrients pools, microbial nutrient immobilization, enzyme activity, and potential C mineralization in soil incubation assays. Additionally, this work is also aimed to understand the effect of glyphosate and the commercial herbicide on the biodiversity of soil microorganisms and the ability of bacteria to use glyphosate and its commercial formulation as a sole P source.

We tested the main hypotheses that glyphosate and its commercial formulation would decrease the soil P occlusion capacity because glyphosate competes with inorganic P for soil sorption sites. Additionally, we expected that glyphosate and the commercial herbicide would decrease soil microbial activity when microorganisms are not adapted to the herbicide as in the abandoned plots, therefore, affecting the transformation of soil nutrients. We also hypothesize that due to the oligotrophic origin and capacity of CCB bacteria to use phosphonates, a wide range of bacterial isolates from an agricultural field would be able to use pure glyphosate as the sole source of P, but commercial formulations of glyphosate can be harmful to these isolates.

2. Materials and methods

2.1. Study site

The study site was in farming plots within the Cuatro Cienegas Basin (CCB), located in the Chihuahuan desert, Mexico (27°1′ N 102°6′ W). The climate is hot and arid, with an average yearly temperature of 21.9 °C, and the average annual precipitation is 211 mm (Montiel-González et al., 2018). The dominant parent material in the west and east sides of the valley are gypsum (Calcium sulphate) and limestone (calcium carbonates), respectively, both from the Cretaceous period (Lehmann et al., 1999). Therefore, according to the WRB classification (IUSS Working Group WRB, 2007), the dominant soil groups are Gypsisols and Calcisols in the west and east side, respectively. Additionally, the native main vegetation types are grassland, dominated by *Sporobolus airoides* and *Allenrolfea occidentalis*; and desert shrubs, dominated by *Jatropha dioica* and *Larrea tridentata* (Perroni et al., 2014; Tapia-Torres et al., 2015).

Despite of the low annual precipitation, agriculture is an important activity in this valley, with alfalfa (*Medicago sativa* L.) as the principal crop (Hernández-Becerra et al., 2016; Tapia-Torres et al., 2018). Because alfalfa is a crop with high water requirements, new crop alternatives have been implemented, such as fodder nopal (*Opuntia* sp.), because of its lower water demand. At the time the soil samples were taken, the farmer had been growing nopal for five consecutive years, but the field had been previously used for alfalfa production. The nopal crop was grown under greenhouse conditions. The crop replacement not only implied a change in the irrigation practices, but also in the chemical supplies used for nopal production. Previously, the herbicides Select (cyclohexadiene), Cedrus (cyclohexadiene) and Pivot (imazethapyr) were used for alfalfa crops, but for nopal production, farmers started to use herbicides with glyphosate as the active agent as Faena® herbicide (Tapia-Torres et al., 2018), so the soil of the nopal crops had been exposed to glyphosate for 5 years, but bare soil with previous alfalfa use was not exposed to this herbicide.

2.2. Field sampling

A first soil sampling was carried out in September 2014 in the east side of CCB under two management conditions: nopal crop plot (NP) within a greenhouse and five years abandoned alfalfa crop plot (AP) outside the nopal greenhouse, covered by grasses. For the NP, soil samples were taken in 10 transects. A sample was taken each 5 m of each transect, obtaining 10 subsamples that were mixed homogeneously for obtaining a composite soil sample per transect, providing a total of 10 composite samples. We also took a soil sample outside the greenhouse in the AP, making parallel transect to the greenhouse transects, and a subsample was taken each 5 m, obtaining one composite sample.

A second soil sampling was carried out in June 2015 in the same plots. In this second sampling, three composite samples were taken from outside the greenhouse (the AP), and 5 composite samples were taken from the NP. Soil samples were taken from the top 15 cm of mineral soil with a soil core sampler, placed in black plastic bags and stored at 4 °C until laboratory analyses.

2.3. Phosphorus sorption experiment

A phosphorus sorption experiment was performed. The experimental design was triple factorial that had as factors the plot, the fertilizer concentration and the herbicide treatment. The plot factor contained two levels (NP and AP); Fertilizer concentration had three levels of different P concentrations (1.09 mg P ml^{-1}, 2 mg P ml^{-1}, and 4 mg P ml^{-1}), and the herbicide treatment factor had three levels (a control without herbicide, glyphosate 96% pure, commercial herbicide Faena®). The factor combinations resulted in 18 treatments (2x3x3) each with six replicates giving a total of 108 experimental units. Soil from the first sampling was used. For the AP sample, the composite sample of the transect was divided into 6 samples. For the NP soil, the 10 composite samples were mixed between them to create 6 samples; each sample was a mixture of two samples randomly selected.

The procedure for measuring the non-biological P fixation reported by Perroni et al. (2014) on the basis of (Lajtha and Jarrell, 1999) was carried out. The glyphosate concentration used was based on the recommended concentration of 4 L of herbicide ha^{-1}. The different P concentrations (working solutions) were obtained from a stock solution of 17.56 g KH_2PO_4 l^{-1}, dissolved on KCl 0.01 M and with two drops of chloroform for inhibiting microbial growth. Glyphosate and the commercial herbicide Faena® were added to separate working solutions with the three P concentrations. Then, 30 ml of a working solution with a known concentration of P were added to aliquots of fresh soil (3 g). Tubes were shaken for 24 h. After shaking, samples were centrifuged at 3500 rpm for 30 min, then they were filtered through Whatman No. 42 and measured as HPO_4^{2-} in a Bran-Luebbe III autoanalyzer (Bran-Luebbe AA3, Norderstedt, Germany). To avoid the effect of herbicide treatments on Pi solubilization, the Pi data of all fertilization treatment was corrected with the Pi concentration in samples of each herbicide treatments without P fertilization, which was determined using the same method of fertilized treatments. The occluded P was calculated by the following equation (Perroni et al., 2014):

$$Xs = F - (c - s) \quad (1)$$

Where Xs refers to the occluded P, c is the total available P and s is the available P without fertilization. F is obtained with the Eq. (2):

$$F = [Pfert]*v/m*\text{dry soil weight}, \quad (2)$$

where Pfert refers to the concentration of P used to fertilize the sample, v is the volume used in the test which is 30 ml and m are the fresh soil (g) used, which were 3 g.

2.4. Incubation experiment

To test the effect of glyphosate and commercial herbicide Faena® on the soil microbial community and nutrient dynamics, soil incubations were performed with soil samples from the NP and the AP of the second sampling. Samples from the AP were mixed between them and then 5 subsamples were taken. Each site was analyzed separately, so the experimental design had only one factor. This factor contained three levels: 1) a control without herbicide, 2) a treatment with technical grade glyphosate (*n*-phosphonomethyl glycine with 96% purity) and 3) a treatment with the commercial herbicide Faena ®, with glyphosate as the active ingredient. Each treatment per site had five replicates. The experiment consisted of adding 100 g of soil to previously sterilized PVC tubes with the extreme ends closed by a fine mesh. Glyphosate and the commercial herbicide treatments were added to their corresponding treatments. The glyphosate concentration used was calculated using the maximum rate recommended for the herbicide Faena ® corresponding to 1.452 kg of active ingredient ha^{-1} (where the active ingredient is the glyphosate acid), a soil density of 1.16 g cm^{-3} and a glyphosate mobility in soil of 1 cm deep according to Ratcliff et al. (2006); obtaining a glyphosate concentration of 12.517 μg g^{-1} soil. Using this calculation, 1.251 mg of glyphosate (equivalent to 7.399 μmol of glyphosate) were added to 100 g of soil, dissolved in 400 μl of water for each sample. Deionized water was added to soil at field capacity in all samples.

After the treatment preparations, samples were incubated in previously sterilized glass flasks for 28 days at 28 °C, measuring CO_2 produced by C-mineralization, which is an indicator of microbial activity. After the incubation period, biogeochemical and enzymatic analyses were performed (total C, N and P; microbial biomass C, N and P, available HPO_4^{2-}, NH_4^+ and NO_3^- and enzymatic activities as B-gluosidase(BG), *N*-acetyl glucosaminidase (NAG), phosphomonoesterase (Phm), phosphodiesterase (Phd), phosphonatase (Phn) and laccase (LAC) with the methods described below.

2.4.1. Carbon mineralization measurement

PVC tubes with soil were placed inside 1 L glass flasks with a vial containing 10 ml NaOH serving as a C-CO_2 trap. Different NaOH concentrations were used depending on the sample; NaOH was used initially with a concentration of 0.2 N. At the first lecture of C mineralization, NaOH traps of samples NP1, NP2 and NP3 showed saturation. This can be noticed when low volume of HCl is necessary to change the pH of the solution. Due to this, NaOH normality was adjusted for this samples to 0.4 N for NP1 and NP3 and to 0.6 N to NP2. For further lectures, HCl concentration used to titrate was also adjusted to the correspondent normality. C-mineralization was assessed every two days titrating the NaOH vial with HCl at the corresponding normality of the NaOH for each sample and using $BaCl_2$.

Samples were incubated at 28 °C for 27 days, and this procedure were performed every 2 days.

2.4.2. Biogeochemical analyses

Soil pH was measured in deionized water (1:10 w:v) with a digital pH meter (Thermo Scientific Orion 3star Plus). For soil moisture determination, a subsample was oven-dried at 80 °C to constant weight, in order to allow nutrient concentrations and enzymatic activities to be corrected using dry soil mass.

Total nutrients were determined using oven-dried soil. Total carbon (TC) and inorganic carbon (IC) were determined by coulometric detection (Huffman, 1977) in a total carbon analyzer (UIC model CM5012, Chicago, USA). Total organic carbon (TOC) was calculated subtracting IC from TC. For total N (TN) and total P (TP) determination, acid digestion at 360 °C was performed using H_2SO_4, H_2O_2, K_2SO_4 and $CuSO_4$. N was determined by the macro Kjeldahl method (Bremmer, 1996), while P was determined by the molybdate colorimetric method after ascorbic acid reduction (Murphy and Riley, 1962). N and P were determined colorimetrically in a Bran-Luebbe Auto analyzer 3 (Norderstedt, Germany).

Available forms of N and P, dissolved and microbial forms of C, N, and P, were extracted from field moist soil samples. Available forms of inorganic nitrogen (NH_4^+ and NO_3^-) were extracted from soil using 10 g of fresh samples with 2 M KCl, followed by filtration through a Whatman No. 1 paper filter (Robertson et al., 1999), and determined colorimetrically by the phenol-hypochlorite method in a Bran-Luebbe Auto analyzer 3 (Norderstedt, Germany). Available phosphate (HPO_4^{2-}) was extracted from soil using 0.5 M $NaHCO_3$ (pH 8.5 adjusted with NaOH 5M), followed by filtration through a Whatman No. 42 paper filter, according to Hedley sequential P fractionation (Tiessen and Moir, 1993) and determined colorimetrically by the molybdate-ascorbic acid method (Murphy and Riley, 1962) in a Bran-Luebbe Auto analyzer 3 (Norderstedt, Germany).

Dissolved nutrients were extracted from soil using deionized water (1:4 w:v), shaking for 45 min and then, the samples were filtered through a Millipore 0.45 μm filter (Jones and Willett, 2006). The filtrate was used to determine dissolved ammonium (D-NH_4^+) and dissolved inorganic phosphate (D-HPO_4^{2-}). To determine total dissolved nitrogen (TDN), acid digestion was made, using the macro-Kjeldahl method. Total dissolved P (TDP) was also acid digested and determined by colorimetry. Dissolved N and P forms were determined using a Bran-Luebbe Auto analyzer 3 (Norderstedt, Germany). Total dissolved carbon (TDC) was measured with a Carbon Auto Analyzer (TOC CM 5012) using the module for liquids (UIC-COULOMETRICS). Dissolved inorganic carbon (DIC) was determined in an acidification module CM5130. Dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) was calculated by the subtraction of the inorganic dissolved forms from the total dissolved forms.

Microbial C (Cmic) and microbial P (Pmic) concentrations were determined by the chloroform fumigation-extraction method (Vance et al., 1987). For Cmic, aliquots of 20 g of fumigated and non-fumigated samples were incubated for 24 h at 27 °C and constant moisture. Cmic were extracted from fumigated and no-fumigated samples with 80 ml of 0.5 M K_2SO_4 (Brookes et al., 1985). Samples were filtered using Millipore filters of 0.45 μm. C concentration was measured from each extract as total carbon (TCmic) and inorganic carbon (ICmic) by the method described before (dissolved carbon). The organic C concentration was calculated by the subtraction of ICmic from TCmic, and the organic C was used for the Cmic calculations. Cmic was the difference between the extracted organic carbon in fumigated samples and the organic carbon from no-fumigated samples, and the result was divided by a conversion factor, k_{EC} (extractable part of microbial biomass C), of 0.45 (Joergensen, 1996).

Microbial P was extracted using $NaHCO_3$ 0.5 M at pH 8.5 after the chloroform fumigation-extraction technique described above was performed (Cole et al., 1978), using 5 g of soil for each fumigated and no-fumigated sample. Samples were shaken for 16 h, then centrifugated at 6000 rpm 25 min (Thermo Scientific SL 16), filtered through Whatman No. 42 paper filters and then digested using 11 N H_2SO_4 and a 50% w/v solution of ammonium persulfate. After digestion, samples were neutralized with 5 M, 1 M, and 0.5 M NaOH. Microbial P was calculated as for Cmic and converted using a K_p value (the extractable part of microbial biomass P after fumigation) of 0.4 (Lajtha and Jarrell, 1999). Microbial P was determined colorimetrically by the molybdate-ascorbic acid method using an Evolution 201 Thermo Scientific Inc. spectrophotometer, at a wavelength of 880 nm (Murphy and Riley, 1962). Finally, Cmic and Pmic values were normalized on a dry soil basis.

2.4.3. Enzymatic analyses

The activity of six enzymes associated to C, N and P acquisition was determined. These enzymes were phosphomonoesterase (Phm), phosphodiesterase (Phd), Beta-glucosidase (BG), *N*-acetyl glucosaminidase (NAG), laccase and phosphonatase (Phn). The first four enzymes were determined from a spectrophotometric determination of p-nitrophenol (pNP) using pNP-substrates according to (Tabatabai and Bremner, 1969; Verchot and Borelli, 2005) . Laccase was measured using an ABTS substrate (Johannes and Majcherczyk, 2000; Chavez-Vergara et al., 2014) and phosphonatase was determined by the colorimetric quantification of the inorganic P released by the enzyme (Murphy and Riley, 1962) using (2-Aminoethyl) phosphonic acid (2-AEP) as substrate (Tapia-Torres et al., 2016).

For this analysis, 2 g of fresh soil and 30 ml of modified universal buffer (MUB) at pH 8 were used for the enzyme extraction. Three replicates and one control (sample without substrate) were prepared per sample. Three substrate controls (substrate without sample) were also included per assay, and all were incubated at 30 °C. The tubes were centrifugated after the incubation period and then 750 μl of supernatant were diluted in 2 ml of deionized water and 75 μl of NaOH 1 N were added to stop the enzymatic reaction, having a final volume of 2.825 ml. For enzymes with substrates linked to pNP, the absorbance of pNP was measured at 410 nm on an Evolution 201 spectrophotometer (Thermo Scientific, Inc.), enzyme activities (EA) were expressed as micromoles of pNP formed per gram of soil dry weight per hour (μmol pNP [g SDW]-1 h-1). The laccase activity was determined after extraction with MUB and oxidation of ABTS. The assay was carried out as described above, except that the supernatant resulting from centrifugation was measured directly (without the addition of NaOH or dilution in deionized water) in an Evolution 201 spectrophotometer (Thermo Scientific Inc., USA) at a wavelength of 460 nm. Control samples were prepared as described above and results are expressed in units of μmol-pNP [g SDW]$^{-1}$ h^{-1} for Phm, Phd, NAG and BG and in units of μmol of Tyrosine [g SDW]$^{-1}$ h^{-1} for the laccase enzyme (Chavez-Vergara et al., 2014). Phn was determined by the molybdate-ascorbic acid method (Murphy and Riley, 1962) quantifying colorimetrically the inorganic P released by the enzyme, using 2-Aminoethyl phosphonic acid (2-AEP) as substrate, according to (Tapia-Torres et al., 2016). The absorbance of the inorganic P released was measured at 882 nm. Phn activity was expressed as micromoles of phosphate released per gram of soil dry weight per hour (μmol PO_4^{3-} [g SDW]$^{-1}$ h^{-1}).

Specific enzyme activities (SEA) were calculated to determine how much enzyme was synthetized per concentration of nutrient immobilized in microbial biomass. The SEA was calculated according to Waldrop et al., 2000.

$$\text{SEA} = \text{Enzymatic activity}/\text{Nutrient in microbial biomass} \quad (3)$$

Where enzymatic activity was in units of μmol pNP gSDW^{-1} h^{-1}; and nutrient in microbial biomass could be C, N or P, and their units where mgC g SDW^{-1}, mgN g SDW^{-1} and mgP g SDW^{-1} respectively. All enzyme activities were divided with microbial C. Phd, Phm and Phn activities were divided also between microbial P to determine P immobilization efficiency.

2.5. Homeostasis and threshold element ratio

For the soil incubation results, a soil microbial homeostasis analysis

was made, performing simple linear regressions between Log_e(DOC:DOP) vs Log_e (Cmic:Pmic), and testing if the slope was no different from 0, which indicates strong microbial community homeostasis (Elser and Sterner, 2002).

We followed Sinsabaugh et al. (2009) to calculate the Threshold Element Ratio for C:P using the following modified equation:

$$TER_{C:P} = ((BG/Phm + Phd)B_{C:P})/\rho_o \quad (4)$$

Where $TER_{C:P}$ is the threshold ratio (dimensionless), BG/Phm+Phd is the ecoenzymatic activity ratio for B-1,4-glucosidase and the addition of phosphomonoesterase and phosphodiesterase activities, $B_{C:P}$ is the C:P ratio for microbial biomass (Cmic:Pmic) and ρ_o is the dimensionless normalization constant. This normalization constant is the intercept calculated with a Type II regression using SMATR (SMATR, R Core Team, 2020) for Log_e(BG) vs Log_e(Phm). Data were log_e-transformed prior to regression analysis to conform to the conventions of stoichiometric analyses and to normalize variance (Elser and Sterner, 2002; Sinsabaugh and Follstad Shah, 2012). To see a detailed analysis of the derivation of the TER equations see Sinsabaugh et al., 2009. Because of the importance of the phosphodiesterase enzyme in CCB, TER calculations where performed using the addition of the activities of Phd and Phm, in Eq. (3) and in the corresponding SMATR analysis.

2.6. Fatty acids profile

A fatty acid profile analysis was performed for the soil samples obtained from the incubation experiment, to assess changes in soil microbial community. For this analysis, an aliquot of 3 g of soil from each sample was stocked at -20 °C; then, samples were lyophilized and pulverized.

For the fatty acid extractions, 1 g of lyophilized soil was used for each replicate, and a saponification was performed using NaOH, methanol and water. Later, a methanol methylation was performed to increase fatty acids volatility for the gas chromatography. A liquid-liquid extraction process was performed with a 1:1 v/v hexane:terbutilic ether methyl solution; after that, an alkaline wash was made to eliminate free fatty acids and residuals agents, with a NaOH solution.

Fatty acids were identified with Agilent 7890B gas chromatograph, using the Sherlock software. Quantification of the individual fatty acids was done with an internal standard 19:0 in a volume of 100 μl per sample in a concentration of 0.025 μg μl^{-1}. The molecular weight of the standard is 312 g mol^{-1}, so each sample contained 8.013 nmol of standard. This standard was used to calculate the concentration of the fatty acids obtained with the chromatography analysis.

The Simpson's index and equitability calculations were made with the results from fatty acids analysis, using each fatty acid obtained as a specie, using the formula of Simpson's diversity index (D) and equitability (E) from Begon et al. (2006).

2.7. Isolation of glyphosate degrading bacteria

To identify the capacity of soil bacteria to use glyphosate as a sole P source, microorganisms from the soil incubations were cultured and isolated, growing them in media with glyphosate and the commercial herbicide as sole source of P.

The extraction of bacteria from the soil was based on the procedure carried out by Tapia-Torres et al. (2016). To obtain microorganisms from the soil, 6.6 g of soil from the incubations were weighed and 30 ml of previously sterilized Modified Universal Buffer (MUB) were added. The samples were shaken for 60 min (Tapia-Torres et al., 2016) and 33 μl of the extract, and serial dilutions 1:10 and 1:100 of the extract, were placed in Petri dishes containing marine medium (MM), a complex medium that includes peptone, yeast extract and dibasic sodium phosphate (Cerritos et al., 2008) spreading the extract with glass beads. Petri dishes were incubated at 28 °C for 3 days.

To evaluate the use of glyphosate herbicides as P source, a defined medium was used (DM) whether without P added or containing different P sources. The base DM was prepared according to Tapia-Torres et al. (2016) and contained Tris-base, 6.057 g adjusted to pH 8; NH_4NO_3, 0.26 g; $MgSO_4$, 0.48 g; disodium citrate, 1.99 g; $ZnCl_2$, 0.000136 g; NaCl 5 g; FeCl3, 0.27 g; KCl, 0.1 g; MnCl2, 0.2 g; CaCl, 0.4 g; glucose, 9 g and an amino acid mixture, 0.93 g; heat-labile substrates (vitamin B complex) were filter sterilized and added aseptically after autoclaving (Tapia-Torres et al., 2016).

The DM was added to Petri dishes divided into numbered squares and 20 colonies per sample were selected from MM to be cultured in DM, inoculating one colony per square, obtaining pure cultures (most of the chosen colonies were taken from the 1: 100 dilution), having a total of 600 isolates. These bacteria were incubated at 28 °C for 7 days because their growth was slower. The objective of this step was to carry out a cleaning pass so that the microorganisms used up their phosphorus reserves.

DM was prepared using different P sources for bacteria including: i) Potassium phosphate 2 mM; ii) Glyphosate 96% pure, iii) Commercial herbicide containing glyphosate. For all P sources the concentration was calculated to be 2 mM of P. Isolates from DM were plated in DM with all P sources and DM without P as a negative control. Colonies were placed so that colony in Square No. 1 from one sample was the same bacterial colony in all DM plates with each P source for the specific sample. Plates were incubated at 28 °C for 7 days. After this, bacteria were re-inoculated in the same media they grew, to test that effectively, these bacteria can use the P sources examined, and the same growth conditions were used. After 7 days, isolates on each media were counted and a data base with each treatment from incubation (commercial herbicide, glyphosate and control), phosphorus source (without P, KH_2PO_4, glyphosate and commercial herbicide), and a binary indicator for growth (1 if the bacteria grew, 0 if growth did not occur) was made.

2.8. Statistical analyses

For the non-biological P fixation, a two-way ANOVA was performed with P occlusion as response variable (X_s in the Eq. (1)), and using as factors the treatment with three levels (control, glyphosate and commercial herbicide) and the fertilizer concentration with three levels (1.09 mg P ml^{-1}, 2 mg P ml^{-1} and 4 mg P ml^{-1}). This analysis was performed for each site separately. When significant differences were obtained a Tukey's HSD test was performed. All statistical analyzes were made with the software STATISTICA (StatSoft, 2000). and the R statistical program (R Core Team, 2020). For the C mineralization results, one-way ANOVA was performed, using as response variable the accumulated C mineralization (the addition of all the C mineralization measurements per treatment, per site), and the treatments as factors (control, glyphosate and commercial herbicide). When significant differences were obtained, a Tukey HSD test was performed. Using the same factors, one-way ANOVAs were performed for each biogeochemical variable and enzyme activity (EA) measured for the incubation experiment; Tukey HSD Test was performed when significant differences were found. LSD Test was performed when ANOVA shown significant differences, but Tukey's HSD test did not, which was the case of the SEA of phosphodiesterase. A *t*-test between the two plots (AP and NP) was performed to compare biogeochemical variables after the incubation experiments.

A multiple linear regression was performed, using the data from the incubation experiment to explain the factors affecting C mineralization, which was the independent variable of this analysis. As dependent variables, the relationships BG:Cmic (SEA BG), NAG:Cmic (SEA NAG), Phd:Cmic (SEA Phd), Phm:Cmic (SEA Phm), DOC:DON, DOC:DOP, DON:DOP and Cmic:Pmic were used.

For bacterial growth data, all isolates that grew without P in the second pass, were subtracted and accounted as bacteria without growth. A logistic regression was performed with the results of bacterial growth,

where the independent variable was bacterial growth represented as 1 when bacterial growth occurred and 0 when bacteria did not grow. The dependent variables where the herbicide treatment for soil incubation (control, glyphosate treatment and commercial herbicide treatment), the P source added to the culture media (KH_2PO_4, glyphosate or commercial herbicide) and the interaction of the two variables (Treatment × P source). These analyses were performed using STATISTICA software. A Pearson's correlation analyses were made between Simpson's Index, Equitability, C mineralization and growth of bacteria in the three P sources (KH_2PO_4, glyphosate and CH). Separate correlations were made for each treatment inside the AP data, while one correlation was made using the data obtained for the NP, without separation between treatments.

A redundancy analysis (RDA) was made to relate bacterial growth with biogeochemical characterization. The number of isolates that grew in each sample with each P source (KH_2PO_4, glyphosate, and commercial herbicide) was used as the response variable, and DOC, DON, DOP, NH_4^+, NO_3^-, HPO_4^{2-}, DOC: DON, DOC:DOP and DON:DOP were used as independent variables.

3. Results

3.1. Phosphorus occlusion

In the NP soil, the P occlusion was lower in the treatment with the commercial herbicide (27.6 ± 2.9 mg P g^{-1}) than in the control (29.3 ± 3.4 mg P g^{-1}), while treatment with glyphosate had similar values than the other two treatments (29.5 ± 3.4 mg P g^{-1}; $p = 0.027$). Additionally, the P occlusion was higher with the increase of fertilizer concentration ($p < 0.0001$), independently of the herbicide treatment. However, in the AP soil, the P occlusion was not significantly different between treatments (values ranged between 20.1 and 21.0 mg P g^{-1}), while for the factor fertilization P occlusion increased with the fertilization level ($p < 0.0001$) (Fig. 1a and b).

3.2. Soil incubation experiment

3.2.1. Carbon mineralization

In the AP soil, the soil microbial community had higher C mineralization in the untreated soil than in soil treated with glyphosate or the commercial herbicide (3.14 ± 0.317 mg CO_2-C, 1.41 ± 0.107 mg CO_2-C and 1.54 ± 0.10 mg CO_2-C, respectively; $p < 0.0001$), while the microbial community in NP soil did not show any differences between treatments (5.44 ± 0.46 mg CO_2-C for the control, 5.11 ± 0.46 mg CO_2-C for glyphosate treatment and 5.51 ± 0.56 mg CO_2-C for the commercial herbicide treatment). The accumulation of CO_2-C evolved in the incubation experiment is shown in Fig. S1.

3.2.2. Biogeochemical analysis

The NP soil had higher nutrient concentrations (TN, TP, DON, NO_3^- and HPO_4^{2-}) and activity of three enzymes (Phd, Phm and Lac) than that of AP soil, but soil pH was higher in AP soil than in NP soil (Table 1). Also, the Cmic:Pmic ratio was higher in NP soil suggesting that microorganisms had low P content in their biomass (Table 1). However, no differences were found between herbicide treatments in the NP soil with respect to biogeochemical values (Table S2); but in the AP soil, soil applied with the commercial herbicide (6.2 ± 3.4 μg P g^{-1}) had lower microbial *P* values than the soil with glyphosate and soil from untreated samples (19 ± 5.5 and 18.7 ± 3.4 μg P g^{-1}, respectively; $p = 0.014$; Table S3).

3.2.3. Enzymatic analysis

No differences were found between treatments in NP soil and only laccase activity had significant effects in AP soil ($p = 0.01$), where the control soil and soil with commercial herbicide had the highest and the lowest values (0.094 ± 0.022 and 0.019 ± 0.006 μmol of tyrosine [g SDW]$^{-1}$ h^{-1} for the control soil and soil with the commercial herbicide), respectively ($p = 0.01$; Table S4).

In AP soil, the SEA PhD/Cmic and SEA PhN/Cmic were higher in the soil with the commercial herbicide than in the control soil (Fig. 2b and c). However, the SEA BG/Cmic had no different among treatments (Fig. 2a). In contrast in NP soil SEA BG/Cmic was higher with the commercial herbicide than the control treatment (Fig. 2d), but the SEA

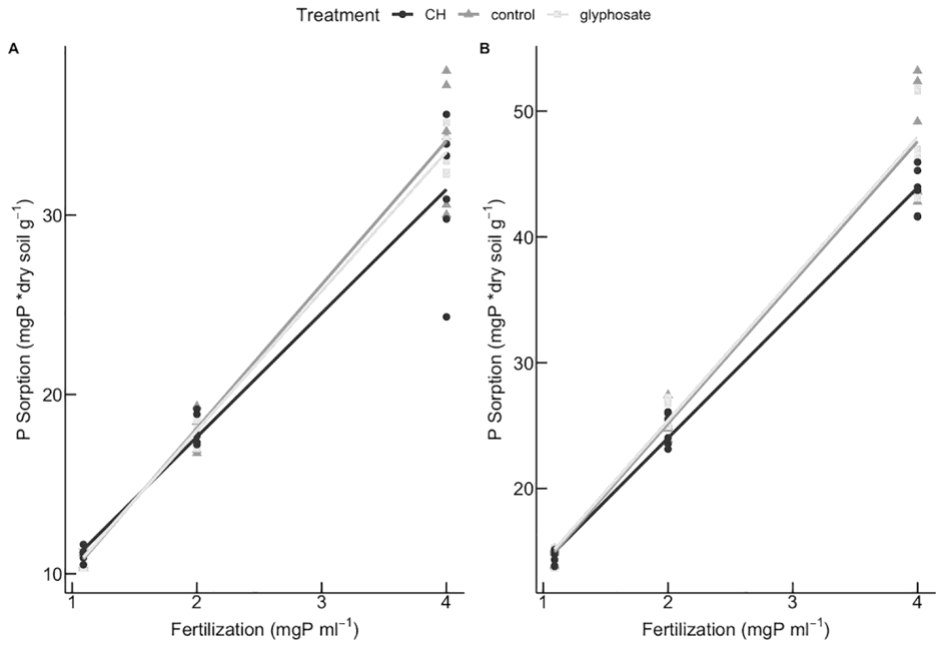

Fig. 1. Soil phosphorus sorption capacity (Y axis) for the three phosphorus fertilization concentrations added (X axis; 1.09 mgP ml^{-1}, 2 mgP ml^{-1} and 4 mgP ml_{-1}) in the A) abandoned plot (AP) and the B) Nopal plot (NP). Each line represents the applied herbicide treatments (control, glyphosate and commercial herbicide CH; $n = 6$).

Table 1
Means and standard error of biogeochemical variables in each site obtained after the incubation experiment; *p* values showing significant differences ($p < 0.05$) are showing with an asterisk (*).The letters AP are for samples from abandoned plot, while NP is for samples from nopal plot.

Variable	AP		NP		p
pH	8.31	(±0.018)	8.15	(±0.04)	0.001*
TOC (mg $gSDW^{-1}$)	26.7	(±0.97)	74.3	(±6.74)	5.13E-06*
TN (mg $gSDW^{-1}$)	36.3	(±1.42)	106	(±10.4)	1.01E-05*
TP(mg $gSDW^{-1}$)	33.4	(±0.93)	107	(±7.29)	7.06E-08*
DOC (μg $gSDW^{-1}$)	146	(±28.5)	94	(±21)	0.15
DON (μg $gSDW^{-1}$)	6.119	(±0.86)	11.3	(±1.02)	0.0006*
DOP (μg $gSDW^{-1}$)	4.664	(±1.06)	4.8	(±1.40)	0.93
Cmic (μg $gSDW^{-1}$)	403	(±113.73)	593	(±83.74)	0.19
Pmic (μg $gSDW^{-1}$)	13.4	(±2.86)	8.9	(±2.50)	0.25
NH_4^+ (μg $gSDW^{-1}$)	2.3	(±0.68)	1.87	(±0.56)	0.62
NO_3^- (μg $gSDW^{-1}$)	22.7	(±1.57)	138	(±34.21)	0.005*
HPO_4^{2-} (μg $gSDW^{-1}$)	2.2	(±0.37)	26.8	(±3.94)	2.10E-05*
BG (μmol PNP [g SDW]$^{-1}$ h^{-1})	0.035	(±0.01)	0.069	(±0.02)	0.097
NAG (μmol PNP [g SDW]$^{-1}$ h^{-1})	0.059	(±0.04)	0.047	(±0.01)	0.77
Phd (μmol PNP [g SDW]$^{-1}$ h^{-1})	0.101	(±0.01)	0.231	(±0.02)	4.81E-05*
Phm (μmol PNP [g SDW]$^{-1}$ h^{-1})	0.052	(±0.01)	0.124	(±0.01)	6.95E-07*
Phn (μmol Pi [g SDW]$^{-1}$ h^{-1})	0.033	(±0.003)	0.039	(±0.01)	0.35
Lac (μmol tyrosine [g SDW]$^{-1}$ h^{-1})	0.051	(±0.012)	0.150	(±0.021)	0.0004*
DOC:DON	188	(±164)	8.28	(±1.9)	0.29
DOC:DOP	45.6	(±18)	32.0	(±9.4)	0.54
DON:DOP	2.27	(±0.8)	5.59	(±2.02)	0.16
CMIC:PMIC	47.0	(±12.956)	270	(±80)	0.015*

[TOC = Total Organic Carbon, TN = Total Nitrogen, TP = Total Phosphorus, Cmic = C in microbial biomass, Nmic = N in microbial biomass, Pmic = P in microbial biomass, NH_4^+ = Available ammonium, NO_3^- = Available nitrate, PO_4^{3-} = Available phosphate, DON = Dissolved Organic Nitrogen, DOP = Dissolved Organic Phosphorus, DOC = Dissolved Organic Carbon, * = significant p values].

PhD/Cmic and SEA PhN/Cmic was not significantly different between treatments (Fig. 2e and f).

SEA and dissolved organic nutrient stoichiometry where important factors determining differences in C mineralization in AP soil (adjusted $R^2 = 0.7$ and $p = 0.089$). This analysis show that the CO_2-C is explained by the specific enzyme activity of Phm:Cmic, Phd:Cmic, the dissolved organic nutrient ratios DOC:DON, DOC:DOP and DON:DOP, and the microbial ratio Cmic:Pmic (Table 2). However, the model was not significant for NP soil ($p = 0.13$ and adjusted $R^2 = 0.54$).

3.3. Homeostasis and threshold element ratio

When samples were tested for homeostasis, the linear regression analysis did not show any slopes different from 0, which indicates that the soil microbial community in both AP soil and NP soil with and without herbicide applications showed a homeostatic behavior (Fig. S1).

When $TER_{C:P}$ was analyzed to see differences between treatments, no differences were found. However, when the $TER_{C:P}$ analysis was compared with the ratio of DOC:DOP to test if microbial community was limited by nutrients (P in this case) or energy (organic carbon), we observed significant results in the samples associated to NP soil. Fig. 3 shows the Log_e $TER_{C:P}$ for both sites (AP and NP); values of Log_e $TER_{C:P}$ were higher than the Log_e DOC:DOP in the NP soil for the three treatments, while the Log_e $TER_{C:P}$ did not differ from the Log_e DOC:DOP in the AP soil. These results suggest that the microbial community was limited by C in both sites.

3.4. Fatty acid profiles

The amount of fatty acids was higher in NP soil than in AP soil, indicating more total microbial biomass (1.18 nmol and 0.36 nmol of fatty acids per gram, respectively; Tables S4 and S5). However, no significant differences were shown when fatty acids were compared among the three incubation treatments in both sites.

Fatty acids were clustered in four microbial groups: i) Gram positive bacteria, ii) Gram negative bacteria, iii) Actinobacteria and iv) Saprotrophic Fungi. Soil microbial community resulted significantly higher in Gram + bacteria and Gram - bacteria, followed by Actinobacteria and fungi in both sites ($p < 0.00001$for AP and NP; Fig. S2). Microbial community structure determined using fatty acids did not show any effect in fatty acid concentration for each microbial group when glyphosate and commercial herbicide were applied. Despite this, the relative abundances of fatty acids were different when comparing the control to the glyphosate and commercial herbicide treatments in AP soils (Fig. 4a). In the control, the group of fatty acids Summed feature 3 (General biomarker) was the most abundant, but in treatments with herbicides the relative abundance of these fatty acids decreased and the saturated fatty acid 16:0 increased and became the most abundant. The group of fatty acids Summed Feature 5, which is a fungal fatty acid became less abundant in the soil with commercial herbicide. The fatty acid 16:0-2OH, a Gram-negative bacterial biomarker, was the second most abundant in the control, and decreased in abundance in both glyphosate and commercial herbicide treatments. In contrast, the relative abundances of fatty acids were not affected by the treatments in the NP site (Fig. 4b).

Simpson's index and equitability values were calculated for both soil (Fig. 5). NP soil showed a greater Simpson's index than the AP soil (8.35 ± 0.27 and 6.9 ± 1.68 for the NP and the AP, respectively; $p < 0.01$), suggesting more diversity of fatty acids in the first site, however the comparisons between treatments did not show any significant difference within both sites. Similarly, the equitability values were higher in AP soil than in NP soil (0.53 ± 0.03 and 0.4 ± 0.01, respectively; $p = 0.004$), suggesting that the microbial community in NP soil is dominated by fewer groups than the AP.

3.5. Isolation of glyphosate degrading bacteria

We accounted a total of 511 isolates that grew using KH_2PO_4 as P source in the defined media (DM). Among these isolates, 252 were obtained from AP soil, and 259 from the NP soil. A total of 442 isolates (86.5%) grew using glyphosate as sole P source of which 209 isolates came from the AP soil and 233 from NP soil. Microbial growth using the commercial herbicide as sole P source was less successful with only 152 (30%) of the isolates able to use this. Among these isolates 57 came from AP soil and 95 from NP soil.

The logistic regression made for each soil, demonstrated the factors that affected bacterial growth. For AP soil the factors with significant results were the *P* source ($p < 0.00001$) and the treatment of the soil incubation ($p = 0.000019$). The results showed that bacterial growth was higher when bacteria came from herbicide treatments and lower when isolates came from control soils (Fig. 6B). Concerning the P source added to DM, we accounted more isolates growing when KH_2PO_4 was used, followed by glyphosate as P source, and less isolates grew when the commercial herbicide was used (Fig. 6A).

For NP soil, the P source was the only significant factor ($p < 0.0001$). Bacterial growth was greater when KH_2PO_4 or glyphosate were used in DM as P source, but fewer bacteria were able to grow when the commercial herbicide was used as P source (Fig. 7).

The redundancy analysis (RDA) tested for the number of bacterial isolates in each P source, showed that for the AP soil, organic and inorganic nutrient concentrations in incubation soils (DOC, DOP, DON, NH_4^+, NO_3^- and HPO_4^{2-}) and nutrient ratios (DOC:DON, DOC:DOP and DON:DOP) significantly affected bacterial growth (RDA model

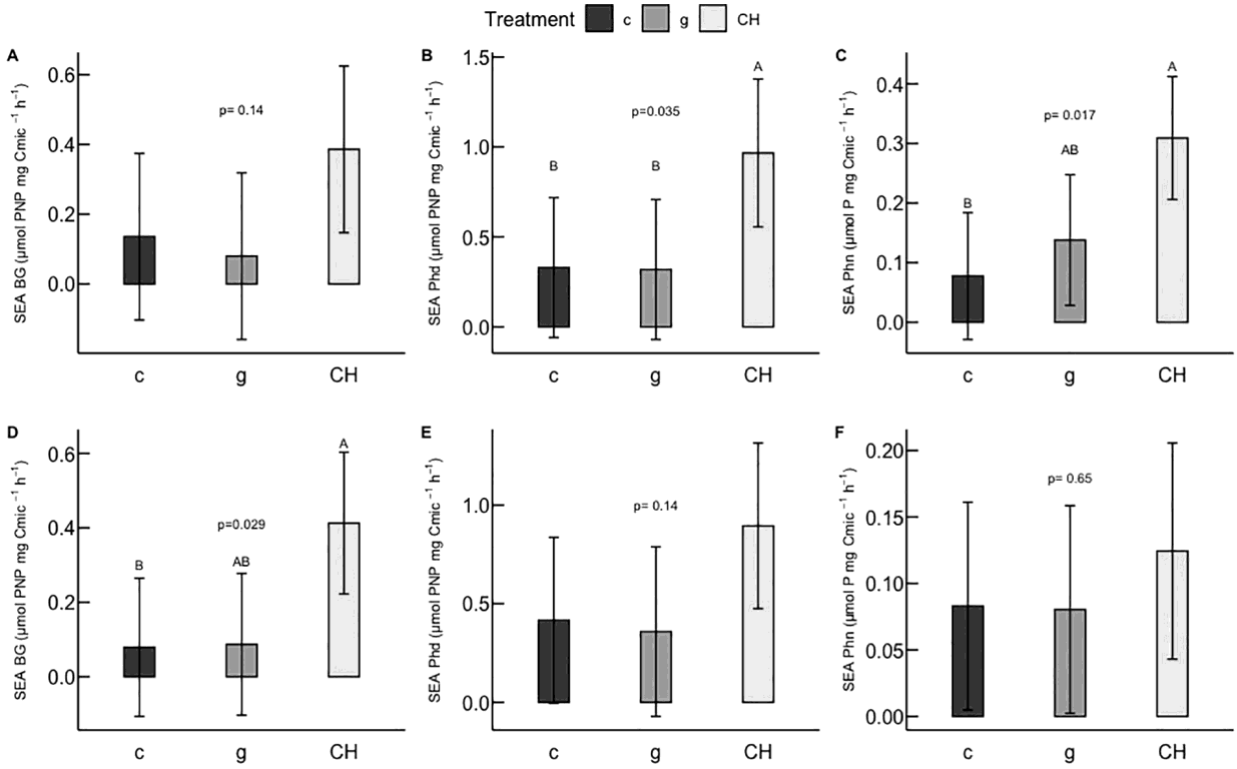

Fig. 2. Specific enzyme activity (SEA) from the Abandoned Plot (AP; figs. A, B and C) and the Nopal Plot (NP; figs. D, E, F) calculated dividing the enzymatic activity between Cmic, for the enzymes B-glucosidase (A and D), Phosphodiesterase (B and E) and Phosphonatase (C and F). Letters showing significant differences when $p < 0.05$. (c = control, CH = commercial herbicide, g = glyphosate) and vertical lines denote 0.95 confidence intervals. Means and ANOVA were calculated with $n = 5$.

Table 2
Variables used in the multiple linear regression analysis and *p*-value of each variable in the AP site. **b*** is the regression coefficient of each variable of this model.

	b*	Std. Error of **b***	p-Value
Intercept			0.107
SEA BG	0.61	0.3	0.14
SEA NAG	−0.24	0.2	0.282
SEA Phd	0.81	0.3	0.0495*
SEA Phm	−1.4	0.4	0.0325*
DOC:DON	2.3	0.75	0.0383*
DOC:DOP	−2.2	0.6	0.0215*
Cmic:Pmic	−2.23	0.6	0.0241*
DON:DOP	2.4	0.6	0.0168*

significant $p = 0.034$; Fig. 8a) and components RDA1 and RDA2 explained 77.6% of the variance, while these variables did not affect bacterial growth in NP soil ($p = 0.38$; Fig. 8b).

In the AP soil, the number of bacteria isolates growing in with glyphosate had only a marginal significant negative correlation with the Simpson's Index (-0.88; $p = 0.05$) in the samples coming from the soil incubation with glyphosate. Also, the number of bacteria growing with KH_2PO_4 had only a significantly negative correlation with that of bacteria growing with the commercial herbicide as P source (-0.95; $p = 0.01$) in the samples coming from the soil incubation with the commercial herbicide. In contrast, the number of isolates growing with KH_2PO_4 correlated positively with the number of bacteria growing with glyphosate in the NP soil (0.55; $p = 0.03$).

4. Discussion

Here we show that glyphosate alter soil nutrient dynamics as well as soil microbial community structure and activity providing useful information when evaluating non-target effects and environmental impacts of this pesticide commonly applied to most agroecosystems worldwide. Overall, our results confirmed the main hypotheses, which will be discussed in the corresponding sections below.

4.1. Phosphorus sorption is affected by herbicide formulations

Our results suggest that glyphosate herbicides affect P sorption differently in agricultural soils (NP soil) compared to non-managed soils (AP soil) with less capacity to adsorb P. A gradient of phosphorus sorption was obtained for the NP soil in the following way, commercial herbicide < glyphosate < control. The observed decrease of adsorbed P when the commercial formulation of glyphosate was applied in the samples with the highest level of fertilization can be attributed to the adjuvants contained in the herbicide formulation. Adjuvants are defined as ingredients in the prescription of the herbicide that facilitate or modify the action of the main ingredient (Foy, 1989; Krogh et al., 2003). In general, adjuvants are added to increase the effectiveness (bioavailability) of the pesticide, increasing the solubility or compatibility of the active ingredients (Krogh et al., 2003) and comprise a large group of substances among which surfactants are widely used (Foy and Pritchard, 1996). In addition to surfactants, glyphosate-based-herbicides contain several minor components that include anti-foaming and coloring agents, biocides, and inorganic ions to adjust the pH (Bradberry et al., 2004). In glyphosate-based-herbicides, various surfactants belonging to the ethoxylated alkylamine group are often used (Krogh et al., 2003). Ethoxylated alkylamines (ANEOs) can bind to soil particles in different ways. ANEOs have hydrophobic alkyl chains that can be adsorbed to the organic matter by hydrophobic bonds. These compounds also have hydrophilic ethoxy chains, which contain oxygens linked by ether bonds (RO-R ‘) bound by hydrogen bonds to polar clay minerals on the soil (Krogh et al., 2003). Also, in the ANEOs, the hydrophilic and hydrophobic portions are connected by an N atom. This N can be protonated depending on the dissociation constant (pKa) value of the molecule and the surrounding pH; agricultural soils of CCB tend to have pH values

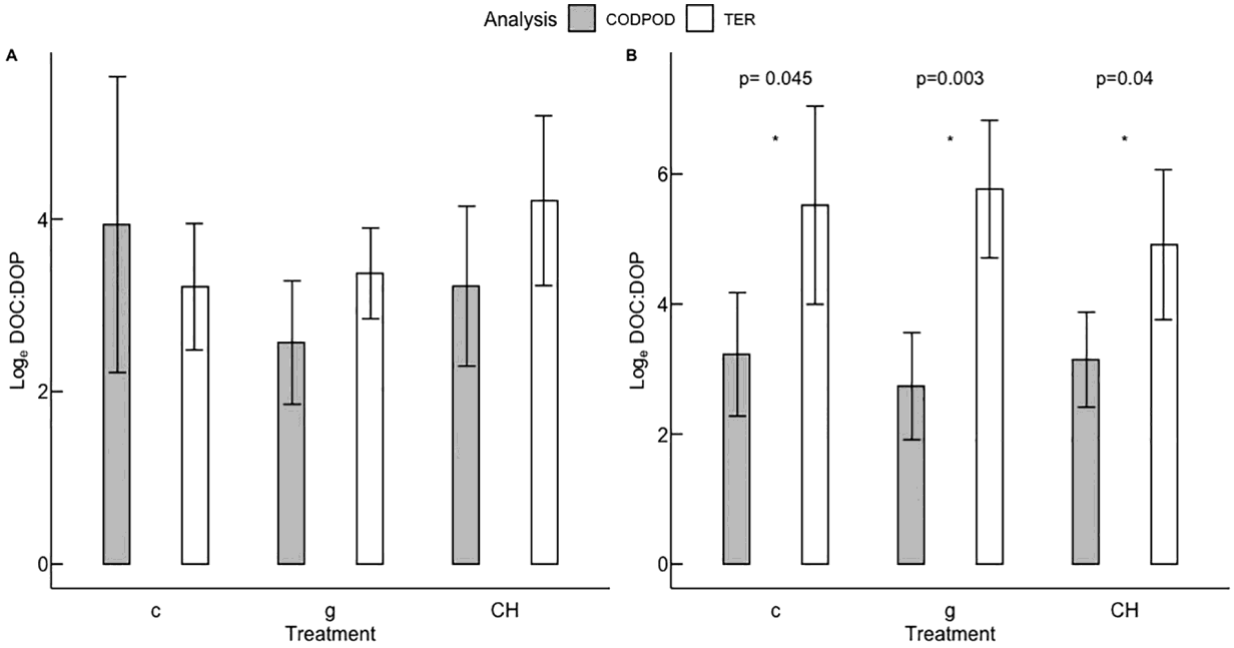

Fig. 3. Values on Y axis represents Log$_e$ Means of Threshold Element Ratio (TER$_{C:P}$; black bars) and log$_e$ COD:POD ratios (gray bars) compared using *t*-test for each treatment for A) Abandoned Plot (AP) and B) Nopal Plot (NP). Significant comparisons are shown with an (*) and *p* values are shown for significant differences. Vertical lines denote 0.95 confidence intervals. Treatments are represented as c = control, g = glyphosate and CH = commercial herbicide. Log$_e$ COD:POD and Log$_e$ are dimensionless values.

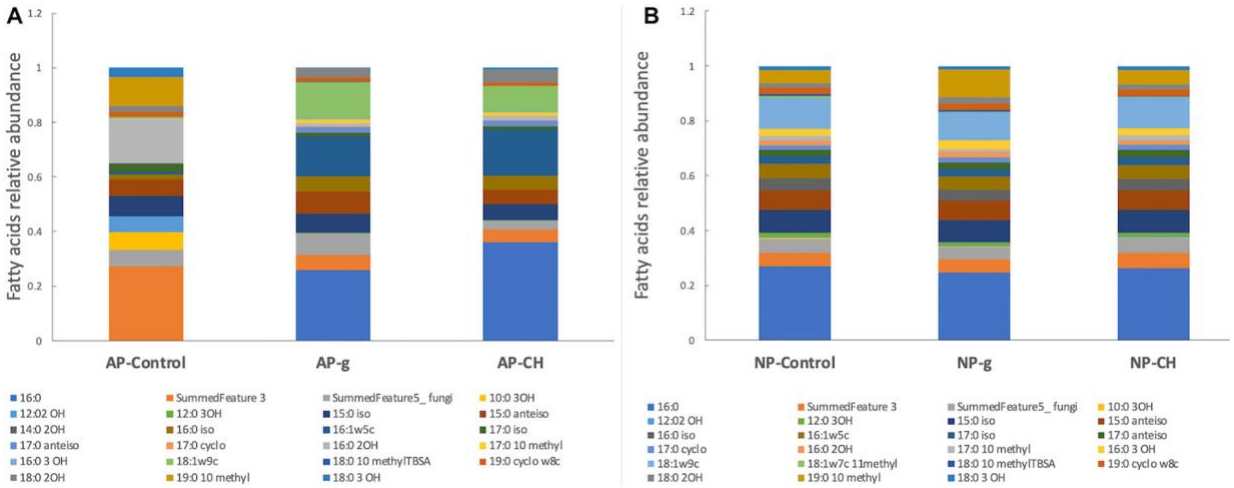

Fig. 4. Relative abundance of fatty acids in the treatments control, g (glyphosate) and CH (commercial herbicide) for the samples of the A) abandoned plot and B) nopal plot.

around 8, so ANEOs are deprotonated; therefore, these molecules can form complex bonds with minerals, making a strong bind between ANEOs and soil minerals (ICI, 1987; Krogh et al., 2003).

Within the ANEOs, one of the most used surfactants in glyphosate herbicide formulations is polyoxyethylene tallow amine (POEA). This non-ionic surfactant consists of a mixture of polyethoxylated long-chain alkylamines (Tush et al., 2013), synthesized from fatty acids derived from animals (Williams et al., 2000). Studies about the mobility of this compound in soil are limited. However, it has been found that the POEA is strongly adsorbed to the soil (Tush et al., 2018), and in addition, cations in the soil such as Na and Ca increase the soil adsorption of POEA (Tush and Meyer, 2016), both cations are abundant in CCB's soils (Perroni et al., 2014). The adsorption of surfactants in soil particles can affect the mobility of other chemicals, such as pesticides (Krogh et al., 2003; Rodríguez-Cruz et al., 2006). Tush et al. (2013) suggested that POEA could change the sorption and desorption of glyphosate in soil similarly to other pesticides and surfactants, but there is a lack of studies about this. It has been determined that the addition of inorganic P displaces pure glyphosate from sorption sites (Gimsing and Borggaard, 2001; Padilla and Selim, 2019) and that the application of commercial formulation of glyphosate does not affect phosphate sorption (Munira et al., 2018), but few studies have shown that at certain conditions, such as high glyphosate application rates, the herbicide can displace inorganic P from sorption sites (Barret & McBride, 2005). If the POEA could increase glyphosate occlusion in soil, as occurs with the increased adsorption of other pesticides with the addition of surfactants (Rodríguez-Cruz et al., 2006), it means that POEA could promote greater competitiveness for the soil sorption sites by glyphosate, affecting P sorption rates as occurred in the NP samples of the present study.

More studies about how surfactants like POEA can influence P and

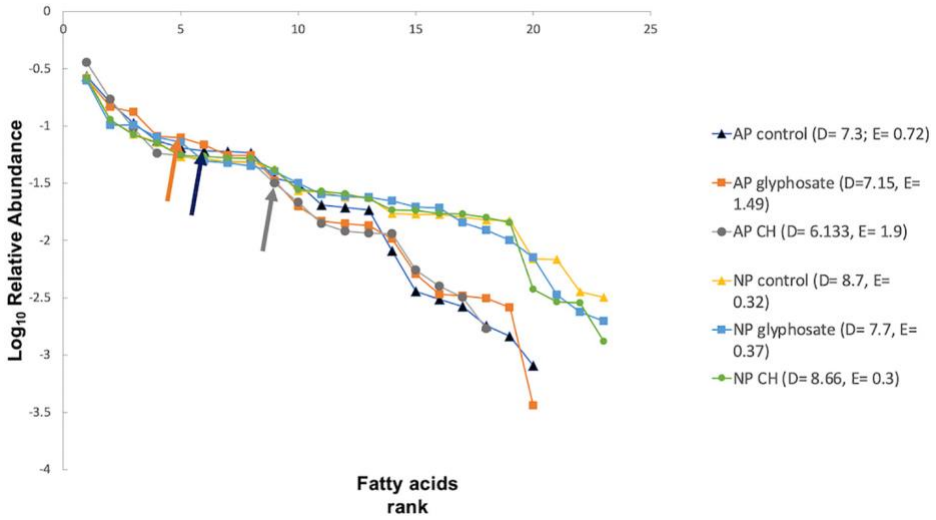

Fig. 5. Rank-Abundance graphic using fatty acids profile results. In this graph, the relative abundance of fatty acids was converted to log values. In the legend of this graphic, Simpson's index values (D) and equitability values (E) are shown for each treatment. Arrows point to the fatty acid Summed feature 5, corresponding to fungal groups, in the three treatments of AP.

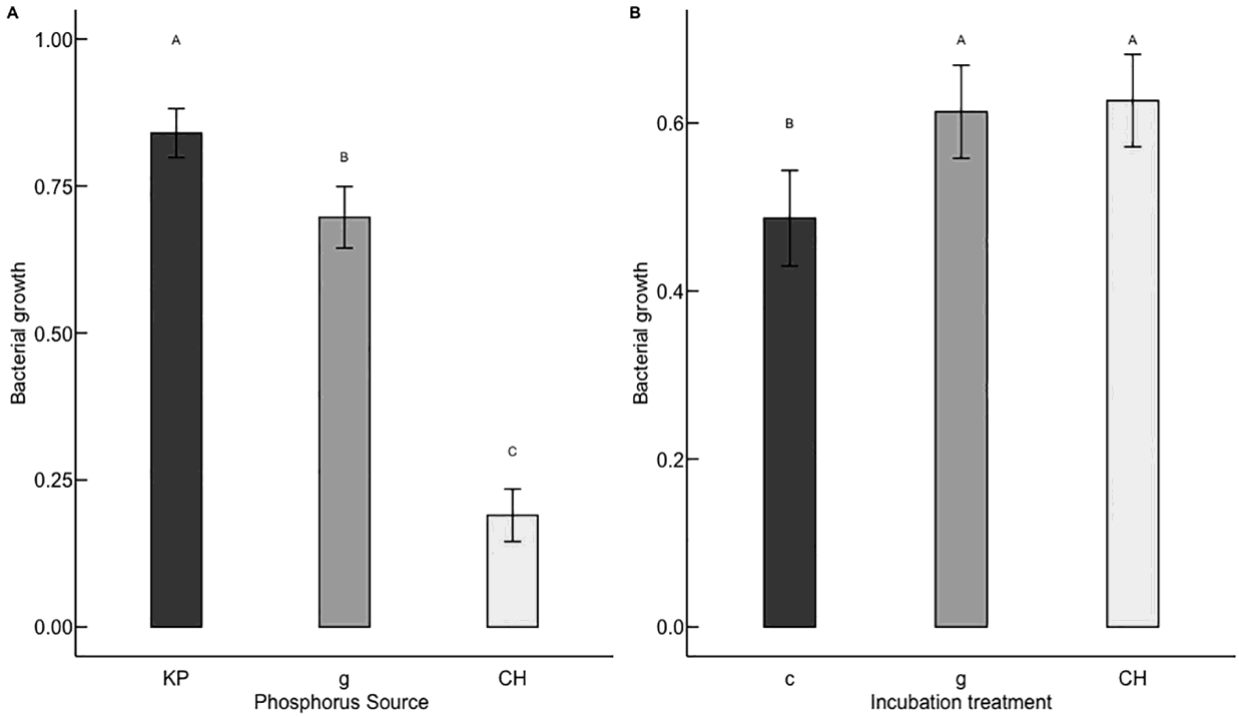

Fig. 6. Mean of bacterial growth for logistic regression with confidence interval lines for the AP site regarding to A) Phosphorus source of the DM (KP = KH_2PO_4, g = Glyphosate, CH = Commercial herbicide) and B) Treatment of the soil incubation (c = control treatment, g = glyphosate, CH = Commercial herbicide). Vertical lines denote 0.95 confidence intervals.

glyphosate mobility are required, nevertheless, in this study, we show that in soil with agricultural use and glyphosate history (NP soil), inorganic phosphate is less adsorbed in soil particles when the herbicide is used, probably due to the ingredients in its formulation.

4.2. *Effect of glyphosate on biogeochemical variables*

Glyphosate and the commercial herbicide had strong effects on microbial activity and soil nutrient dynamics in AP soil. We suggest that this can be related to the reduction of soil sorption capacity with commercial herbicide in the NP soil, but most likely can also be explained with the continuously fertilization of this soil in this site, thus increasing the soil P bioavailability.

Glyphosate and the commercial herbicide decreased microbial soil carbon mineralization in AP soil obtained from a field without glyphosate use. This suggest that when soils have no previous history of glyphosate use, application of glyphosate negatively affect soil microbial activity. Some authors have reported an increase of C mineralization after glyphosate applications (Araújo et al., 2003; Haney et al., 2000; Lane et al., 2012), however, a meta-analysis about glyphosate effects on microbial respiration, suggest that glyphosate concentrations below 200 mg kg^{-1} and a soil pH higher than 7.5 resulted in negative effects on soil respiration (Nguyen et al., 2016). Our glyphosate concentration of 12.5 mg kg^{-1} is smaller than this value and the pH of both plots was

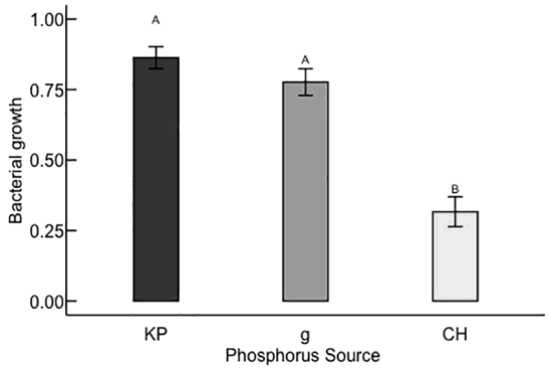

Fig. 7. Means for bacterial growth for logistic regression with error bars for the phosphorus source (KP = KH_2PO_4, g = Glyphosate, CH = Commercial herbicide) in the NP site. Vertical lines denote 0.95 confidence intervals.

higher than 7.5 (Table 1).

Additionally, multiple regression analysis (Table 2) showed that in the AP soil, C mineralization was explained by the specific enzymatic activity (SEA of Phm and Phd), available nutrient organic ratios (DOC:DON, DOC:DOP, DON:DOP) and C:P ratio within microbial biomass. In particular, the C mineralization in soil from this site had negative regression coefficients of three variables associated with soil P dynamic (SEA Phm, DOC:DOP and Cmic:Pmic; Table 2). These results suggest that application with commercial herbicide to AP soil affect microbial P acquisition. For example, soil with the commercial herbicide had lower Pmic (Table S4) than the other two treatments, but higher SEA of both Phn and Phd (Fig. 2B and C). Several authors have reported that the microbial community must produce soil extracellular enzymes for P mineralization from organic molecules when soil P availability is low (Sinsabaugh et al., 2009; Sinsabaugh and Follstad Shah, 2012). When the SEA is higher, it indicates P limitation, because microorganisms must invest their carbon resources (their energy reservoirs) on enzyme production for organic P mineralization (Raiesi and Beheshti, 2014), which is consistent with the lower Pmic concentration found in the samples treated with commercial herbicide in the AP soil (Table S4).

The higher SEA of phosphonatase (Phn) in the AP soil can be an indicator of enzyme production for glyphosate degradation stimulated by the commercial herbicide, as a result of microbial adaptation (De Andréa et al., 2003). Glyphosate can be metabolized through the CP-lyase enzyme, breaking the C—P bond in the molecule and producing sarcosine, or it can be oxidated through glyphosate oxidoreductase with the production of aminomethyl phosphonic acid (AMPA), which is later hydrolyzed by a C—P lyase (Singh et al., 2020), or by a phosphonatase, yielding formaldehyde and inorganic phosphorus (Sviridov et al., 2012; Singh et al., 2020).

In contrast, the enzymatic activity of Laccase was negatively affected by the commercial herbicide in AP soil (Table S6). Laccases depolymerize lignin compounds and they are mainly produced as extracellular enzymes by fungi (Gianfreda et al., 1999; Dwivedi et al., 2011). Although the abundance of total fungal fatty acid biomarkers was not affected by glyphosate, lower relative abundance of fungal biomarker fatty acids was observed in soil with the commercial herbicide (Fig. 4; Log of relative abundance in control = -1.45; in glyphosate = -1.49; in CH = -1.8). It has been reported that glyphosate formulations can be toxic to some culturable fungal species (Busse et al., 2001; Morjan et al., 2002; Tanney and Hutchison, 2010), however this is not often shown in the whole soil fungal community composition (Busse et al., 2001). No studies of glyphosate affecting laccase activities were found; on the contrary, fungal laccases have been tested for glyphosate oxidation, with positive results when other compounds like ABTS, Tween 80 and $MnSO_4$ are present (Pizzul et al., 2009).

These results suggest that the commercial herbicide soil addition constraints the release of glucose from recalcitrant molecules, as lignin, reducing the available organic C for microbial activity as indicated by the $TER_{C:P}$ values (Fig. 3). Similarly, in NP soil, C mineralization was not affected by glyphosate or the commercial herbicide applications, but SEA BG increased in the commercial herbicide treatment, suggesting that the microbial community must invest to obtain glucose. This result is consistent with the $TER_{C:P}$ values, which suggest also energy

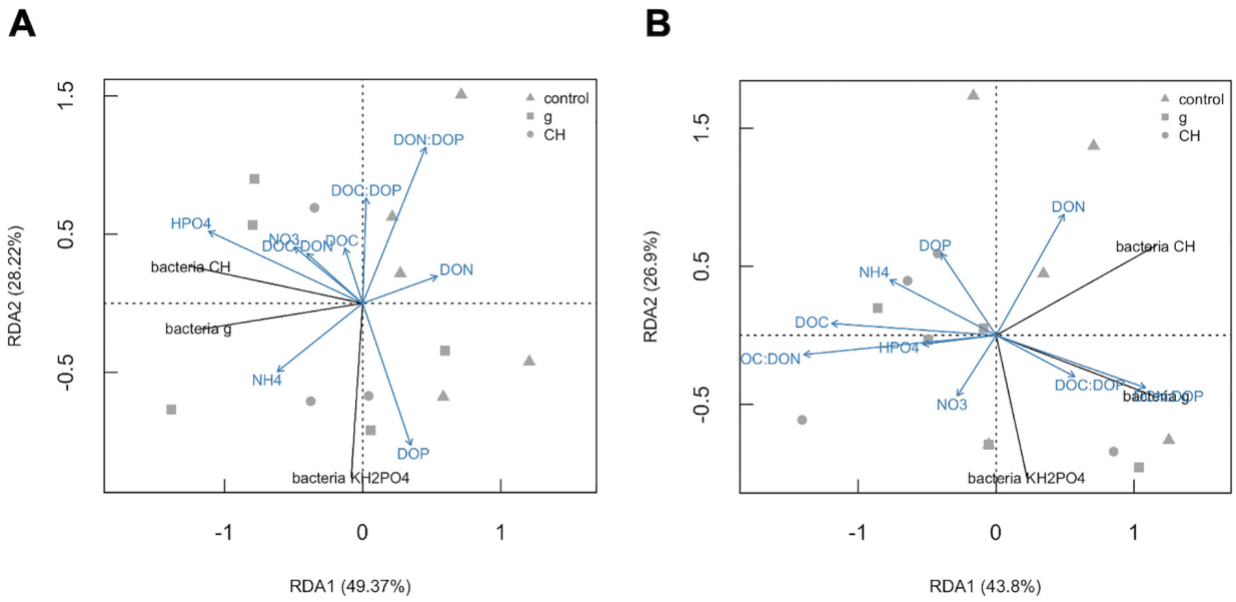

Fig. 8. Results for RDA analysis in the a) AP and in the b)NP, showing RDA1 and RDA2 axis with the proportion of variance explained by each axis between parentheses. Each sample from each treatment is represented by the figures circle for control, triangle for glyphosate and square for commercial herbicide. In the figure, the variables in black represent the response variables, and they refer to number of bacterial growth in KH_2PO_4 defined media (bacteria KH2PO4), number of bacterial growth in glyphosate DM (bacteria Gly), number of bacterial growth in commercial herbicide (bacteria CH). Variables in blue represent independent variables where HPO4 is available phosphorus (HPO_4^{2-}), NH4 represents available ammonium, NO3 is available nitrate, DOC, DON and DOP represents dissolved organic carbon, nitrogen and phosphorus respectively and DOC:DON, DOC:DOP and DON:DOP dissolved organic nutrient ratios. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

limitation for this soil. We can conclude that in soils without previous glyphosate application as the AP site, the mechanism for microbial P acquisition is disturbed when the commercial herbicide was added; while agricultural soils with a previous history of glyphosate use, the main constrains for microbial community is the limitation of soil available C molecules.

4.3. Microbial community diversity and glyphosate degrading bacteria

Our results suggest that glyphosate effects on soil nutrient dynamics are tightly linked with the microbial community composition within the soil from both sites. It has been determined that constant application of glyphosate can select microbial populations capable of using it as a nutrient source (De Andréa et al., 2003; Lancaster et al., 2010), so microorganisms from the NP soil, after 5 years of applications may be adapted to the herbicide and did not show differences regarding their metabolic activity as mentioned in Section 4.2.

For example, the microbial community structure in the NP soil did not show major rank-abundance changes among treatments regarding to biomarker fatty acids (Fig. 5). Additionally, the NP soil showed a higher diversity index than the AP soil (Fig. 5.), suggesting a more resistant microbial community, probably with higher functional redundancy, which is consistent with the results that soil application with the commercial herbicide and glyphosate had no effect on soil nutrient transformations and microbial activity, discussed in Section 4.2. In contrast, the microbial community was affected by the herbicide when added to the AP soil. For example, the commercial herbicide treatment reduced the dominance of fungi and two Gram negative bacteria groups (the fatty acid 16:0 2-OH and 10:0 3 OH), while another Gram negative bacteria fatty acid (16:1 ω5c) increased in relative abundance after application of glyphosate and the commercial herbicide (Fig. 4). Moreover, the actinobacteria group (fatty acid 19:0 10 methyl) was not found in soil with glyphosate and the commercial herbicide applications. These results suggest that the herbicide applications induce changes in microbial functional groups in soil without glyphosate use (AP soil), but these treatments did not affect the structure of microbial functional groups in soils with a previous history of glyphosate use (NP soil), because the constant application of glyphosate favors certain microbial populations (De Andréa et al., 2003; Lancaster et al., 2010; Rainio et al., 2021) and may eliminate gradually glyphosate-sensitive species (Zabaloy et al., 2012).

Assay with bacteria isolated from CCB soil confirmed the adverse effect of commercial herbicide on soil bacteria in both AP and NP soil, whereas glyphosate had no significant effect on bacterial growth. Instead, most bacteria had the ability to use glyphosate as a sole P source. Tapia-Torres et al. (2016) reported that some of the CCB bacteria have the enzymatic tools to breakdown phosphonate compounds, as well as they also reported phosphonatase activity in CCB soils. Glyphosate utilization as P source is an indicator of the presence of C—P lyase enzymes (Pipke and Amrhein, 1988; Sviridov et al., 2015) or phosphonatases (Sviridov et al., 2012, 2015) of these bacteria. However, the 60% of growth reduction in media with the commercial herbicide in comparison to glyphosate suggest the presence of harmful ingredients in this formulation for soil bacteria. Tsui and Chu (2003) reported that the median lethal dose of POEA (10.2 mgL-1) is lower than the dose for glyphosate (LD50 = 17.5 mg l^{-1}) for the bacteria *Vibrio fischeri,* being a more toxic substance. However, this bacterium was the least sensitive to POEA toxicity compared with other organisms. More recent studies (Sihtmäe et al., 2013) had shown that both glyphosate and glyphosate with POEA formulations are able to inhibit growth of some soil indigenous Gram positive bacteria, and that herbicide formulations with POEA are more toxic for soil bacterial strains than for water strains. Other studies have determined that in *E. coli*, the surfactant POEA can cause cellular membrane damage, general cellular injuries, oxidative stress and DNA damage (Nobels et al., 2011) leading to the induction of certain DNA markers (RecA, UmuDC, and SfiA) that are part of the bacterial SOS response, a repair mechanism in bacteria that is activated so that bacteria can survive sudden increases in DNA damage (Michel, 2005), which coincide with the genotoxicity of the surfactant. These results suggest that several bacteria have the capacity to breakdown the glyphosate molecule in our study soil, but this capacity was strongly reduced when the commercial herbicide was used. However, the bacteria that could use the commercial herbicide as P source, are of biotechnological importance, since experiments could be carried out with these to be used for bioremediation of sites affected by glyphosate herbicides.

Lack of molecular taxonomic identification of the isolated cultivable bacteria growing on glyphosate and the commercial herbicide is a limitation of the present study, which should be further addressed in future work. This would provide information about possible differential effects of the commercial herbicide on bacterial species as well as identification of bacteria able to degrade and grow on glyphosate-based herbicides.

5. Conclusions

Our results show that glyphosate and one of its commercial herbicide alter some aspects of soil nutrient cycling, especially related to C and P soil dynamics and the performance of microorganisms participating in biogeochemical cycles measured as soil respiration and enzyme activities involved in organic P decomposition. The observed collateral non-target effects were strongest with the commercial herbicide, especially in soil without previous herbicide exposure indicating some degree of microbial adaptation to the herbicide. Indeed, soil bacteria mainly from soil exposed to glyphosate were able to grow in-vitro on glyphosate as sole P source, which could be further explored for bioremediation of soils polluted with glyphosate-based herbicides.

We suggest that further research should be done about the adsorption of commercial formulations of glyphosate with soil particles, its drivers and conditions, and how glyphosate molecules interact with the rest of the formula, as this can alter its adsorption and consequently, phosphorus mobility. Also, further research is needed on the effects of glyphosate on microbial stoichiometry and enzyme stoichiometry; because previous research about how glyphosate and its commercial formulations affect microorganisms, has primarily been focused on C mineralization, microbial growth and microbial community composition changes. Novel biochemical pathways of glyphosate degradation are also of major interest, specifically, how different enzymes, like phosphonatases, can be used on the breakdown of glyphosate, and their biotechnological potential to bioremediation.

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 data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.104256.

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CONCLUSIONES

El trabajo realizado en la presente tesis permitió resaltar la importancia de las comunidades microbianas del suelo en procesos que regulan la disponibilidad de nutrientes, como es la descomposición de la materia orgánica, un proceso importante para mantener la fertilidad de suelos agrícolas. A pesar de que la mayoría de las enzimas encargadas de procesos de despolimerización (B-glucosidasa, N-acetil glucosaminidasa, fosfodiesterasa) y mineralización (Fosfomonoesterasa) están ampliamente distribuidas entre distintos phyla microbianos, es difícil determinar su distribución a nivel especie, y menos del 5% de los OTUs bacterianos son compartidos entre sitios separados. Además, la revisión bibliográfica mostró que existen pocos estudios sobre la enzima fosfodiesterasa, su actividad y distribución entre los microorganismos del suelo, a pesar de que los diésteres de fosfato son una molécula importante de P en la materia orgánica. Aunque los microorganismos cuenten con los genes para producir las enzimas necesarias para descomponer la materia orgánica, la estequiometría entre su biomasa y los recursos disponibles es un regulador crítico para la producción de estas. Por esto mismo, en los capítulos 2 y 3, se consideró que los análisis estequiométricos brindan información importante, pero son poco utilizados en estudios realizados en suelos agrícolas.

En el presente trabajo, además se pudo determinar las diferencias entre fertilizaciones orgánicas e inorgánicas con P, y dentro de las fertilizaciones orgánicas, las diferencias entre diferentes moléculas. Se comprobó que moléculas lábiles con fósforo aumentan la respiración microbiana (mineralización potencial de C), pero no cualquier molécula orgánica funciona de la misma manera, pues el ácido fítico o fitato, tiene efectos similares a las fertilizaciones inorgánicas. Los microorganismos del suelo agrícola del VCC, donde se realizó el muestreo del capítulo 2, mostraban una fuerte limitación por C y por P, a pesar de ser suelos fertilizados y de que los suelos naturales del VCC suelen tener concentraciones muy bajas de C orgánico y fósforo disponible. Sin embargo, se observó que, al fertilizar con cualquier compuesto fosfatado, los microorganismos respondieron a la limitación inmovilizando fósforo en su biomasa, siendo mayor este proceso en suelos con tratamientos

de RNA y AMP. No obstante, al revisar las relaciones estequiométricas de biomasa microbiana, se observó que en todos los tratamientos distintos del control, se inmovilizó la misma proporción de P con respecto al C microbiano. Además, se observó que las fertilizaciones orgánicas desencadenaron un "efecto priming", el cual se presenta cuando al fertilizar con compuestos orgánicos sencillos, les da a los microorganismos la energía para degradar materia orgánica más recalcitrante. Este efecto influyó en la disminución del carbono orgánico disuelto y, al no continuar con más aplicaciones de moléculas lábiles con C, la biomasa microbiana finalmente disminuyó. El C orgánico es el alimento de microorganismos heterótrofos, y al no obtenerlo fácilmente, propició el aumento de microorganismos autótrofos que eran capaces de realizar procesos de nitrificación. El nitrato (NO_3^-) es una forma de N disponible para plantas y microorganismos, pero en comparación con el amonio (NH_4^+) es una molécula más susceptible para perderse por lixiviación o por desnitrificación, por lo que es importante estudiar los factores que desencadenan el aumento de la nitrificación y cómo se puede reducir.

La adición de otro tipo de compuestos organofosforados, el herbicida glifosato, tanto en forma pura como en formulación comercial, desencadenó efectos negativos en los microorganismos del suelo principalmente en un suelo abandonado donde el glifosato no había sido aplicado previamente, en comparación con un suelo agrícola con nopal con un historial de 5 años de uso del herbicida. Existen otros trabajos que evalúan el efecto del glifosato en microorganismos de suelo donde se utiliza el herbicida, los cuales han demostrado que la mineralización potencial de C llevada a cabo por microorganismos del suelo se mantiene constante o incluso aumenta; sin embargo, muy pocos trabajos evalúan el efecto del herbicida sobre suelos donde no ha sido aplicado con anterioridad. El artículo presentado en el capítulo 3 de la presente tesis, demuestra la importancia de estos trabajos debido a que se obtuvieron resultados contrastantes entre el comportamiento de la comunidad microbiana en la parcela de nopal con previo uso de glifosato (5 años) y la del sitio abandonado sin historial de uso del herbicida. En la presente tesis también se determinó que los efectos son mayores al utilizar la formulación comercial del herbicida, probablemente por otros ingredientes como los surfactantes utilizados, que actualmente se conoce que tienen un grado mayor de toxicidad que el glifosato. Estos efectos se observaron principalmente en la

mineralización potencial de C en el suelo, la cual disminuyó notablemente, y en los aislados bacterianos, pues al cultivar microorganismos en medio de cultivo con glifosato o herbicida comercial como única fuente de fósforo, se observó que la gran mayoría de microorganismos crecieron, lo que significa que la mayoría de las bacterias cultivadas podía producir las enzimas necesarias para romper el enlace directo C-P del glifosato. Sin embargo, el número de aislados obtenido en medio de cultivo con herbicida comercial como fuente de P, disminuyó notablemente, pues mientras que el 85% de los microorganismos cultivados podía crecer utilizando el glifosato puro, sólo el 30% logró crecer en medio con formulaciones comerciales. En este trabajo también se estudió como el glifosato y su formulación, afectan a la adsorción de P, ya que debido al grupo fosfonato en la molécula de glifosato, este compuesto compite con los iones ortofosfato que se encuentran en el suelo por los sitios de adsorción, lo que es importante para suelos fertilizados con P. Los efectos obtenidos se dieron también con la formulación comercial, que, en fertilizaciones altas de fósforo inorgánico, disminuye la adsorción de P, esto probablemente relacionado con la interacción de los surfactantes en el suelo. Es necesario realizar más investigaciones sobre cómo los componentes de las formulaciones comerciales de herbicida interactúan con las partículas y minerales del suelo, así como con nutrientes como el fósforo.

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