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ECOLOGÍA

## Vulnerabilidad de la dinámica de nutrientes a escenarios de cambio climático global en un ecosistema desértico de México

# TESIS

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PRESENTA:


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Por medio de la presente me permito informar a usted, que el Subcomité de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, en su sesión ordinaria del día **26 de febrero de 2018**, aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** a la alumna **MONTIEL GONZÁLEZ CRISTINA,** con número de cuenta **99214050**, con la tesis titulada, **"Vulnerabilidad de la dinámica de nutrientes a escenarios de cambio climático global en un ecosistema desértico de México",** realizada bajo la dirección del **DR.FELIPE FRANCISCO GARCIA OLIVA:**

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Sin otro particular, quedo de usted.

**A T E N T A M E N T E**
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# Niqitoa

Niqitoa ni Nesaualkoyotl:
¿Kuix ok neli nemoua in tlaltikpak?
An nochipa tlaltikpak:
san achika ya nikan.
Tel ka chalchiuitl no xamani,
no teokuitlatl in tlapani,
no ketsali posteki.
An nochipa tlaltikpak:
san achika ye nikan.

# Yo lo Pregunto

Yo Nezahualcóyotl lo pregunto:
¿Acaso de veras se vive con raíz en la tierra?
Nada es para siempre en la tierra:
Sólo un poco aquí.
Aunque sea de jade se quiebra,
Aunque sea de oro se rompe,
Aunque sea plumaje de quetzal se desgarra.
Nada es para siempre en la tierra:
Sólo un poco aquí.

Nezahualcóyotl, el Rey poeta

(Texcoco, 1402-1472)

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# RESUMEN

Uno de los ecosistemas considerados como más amenazados por el Cambio Climático Global (CCG) son las zonas áridas, estas comprenden aproximadamente el 41% de la superficie del planeta. La gravedad de esta amenaza se empeora porque varias hectáreas dentro de las zonas áridas son utilizadas como tierras de cultivo. Las prácticas de adición de fertilizante y riego excesivos han llevado a que los suelos lleguen a ser improductivos con altos rangos de salinidad y a que estos suelos sean abandonados produciendo así aumento de la desertificación.

Los modelos de CCG predicen cambios de los regímenes de precipitación y temperatura para el 2100, estos cambios incluyen incremento en el número y la severidad de eventos de lluvia, sequia, ondas de calor e inviernos severos. Uno de los principales retos para los tomadores de decisiones en cualquier ecosistema es tomar decisiones adecuadas para la adaptación ante el CCG. Así mismo, tomar decisiones con base únicamente en los escenarios proyectados a escalas globales podría representar un error ya que cada ecosistema presenta una dinámica climática propia que depende de factores regionales e incluso locales. Por ello, es necesario utilizar otras herramientas analíticas diferentes a los escenarios globales, que permitan entender la magnitud y la dirección del CCG actual a diferentes niveles. Dentro de estas herramientas se encuentran los análisis de tendencias climáticas a escala regional y local.

En zonas áridas, en donde la evapotranspiración potencial anual excede la precipitación (pp), tanto la temperatura, como la humedad del suelo son factores ambientales que controlan la productividad primaria neta. En condiciones naturales las zonas áridas están constantemente expuestas a pulsos de humedad y de disponibilidad de recursos. Lo anterior ha favorecido que las comunidades del suelo desarrollen estrategias para utilizar los recursos cuando están disponibles y para hacer frente a limitación de recursos. En el suelo, uno de los principales procesos que son afectados cuando se alteran las variables climáticas es la transformación de la materia orgánica (MO) que se lleva a cabo por los microorganismos. Así mismo, se ha observado que la alteración de los factores ambientales influye

directamente en las tasas de descomposición de la materia orgánica y la disponibilidad de nutrientes por parte de los microorganismos del suelo.

El objetivo general de la presente tesis fue "Estudiar la vulnerabilidad de la dinámica de nutrientes (C, N y P) bajo escenarios de cambio climático en suelo de un desierto oligotrófico en el valle Cuatro Ciénegas, Coahuila". Para ello, primero se identificó si existe huella de cambio climático en este sitio, posteriormente se estudió cómo responden las comunidades microbianas del suelo ante la variabilidad de la lluvia en condiciones naturales y por último, se describió cuáles son los procesos que determinan que las comunidades sean eficientes en la obtención de recursos ante un pulso de nutrientes orgánicos.

Los resultados sugieren que en el valle de Cuatro Ciénegas, durante los últimos 36 años las temperaturas media y mínima aumentaron aproximadamente 2°C a lo largo del año, así mismo en verano la oscilación térmica se está acercando aproximadamente 2°C. La precipitación mostró un aumento de equitabilidad a lo largo de año, presentando más lluvia en invierno y menos lluvia en verano. Los datos de eventos extremos sugieren que los inviernos se volverán cada vez más fríos y los veranos se volverán más cálidos con una alta variabilidad en la disponibilidad de agua de un año a otro, aumentando el estrés ambiental para los organismos que habitan el sitio de estudio.

Este estudio muestra que las comunidades microbianas son más resilientes que vulnerables a la variación en la precipitación. Especialmente la comunidad microbiana del suelo con menores recursos (comunidad de sotol) se ha adaptado a la disminución de P en años secos disminuyendo su metabolismo durante este periodo y presentando una sobre regulación enzimática (PME y PDE) para obtener nutrientes cuando aumenta la precipitación, además de que es muy eficiente en inmovilizar P cuando hay pulsos de nutrientes orgánicos especialmente ricos en C y N. Se propone que bajo los escenarios de cambio climático global para ecosistemas desérticos que predicen una reducción de la precipitación anual y una mayor intensidad y frecuencia de lluvias torrenciales y eventos de sequía, las comunidades microbianas del suelo dentro de ambos sitios podrían ser vulnerables a la sequía debido a la combinación de co-limitación C -P y a la reasignación de energía y nutrientes hacia estrategias de aclimatación fisiológica para sobrevivir

# ABSTRACT

Currently, one of the ecosystems considered to be most vulnerable by Global Climate Change (GCC) are the arid zones, which comprise approximately 41% of the planet's surface. The seriousness of this risk is worsened because large parts of the arid zones are used for crop cultivation. The practices of fertilizer addition and excessive irrigation have led to the soil becoming unproductive due to high salinity ranges and subsequently abandoned thus producing increased desertification.

The GCC models predict changes in precipitation and temperature regimes by 2100. These changes include an increase in the number and severity of rainfall, drought, heat waves, and severe winters. One of the main problems that we face today in any ecosystem is to make appropriate decisions for adapting to the CCG. Likewise, making decisions based only on the projected scenarios at global scales could represent an error since each ecosystem has its own climate dynamics that depend on regional and even local factors. Therefore, it is necessary to use other analytical tools different from the global scenarios, which allow us to understand the magnitude and direction of the current GCC at different levels. One of these tools are the analysis of climatic trends at regional and local scales.

In arid zones, where annual potential evapotranspiration exceeds precipitation (pp), both temperature and soil moisture are environmental factors that control net primary productivity. Under natural conditions, arid zones are constantly exposed to pulses of humidity and resources availability. The constant resources availability has favored soil communities to develop strategies to use resources when they are available and to cope with resource limitations. In the soil, one of the main processes that are affected when the climatic variables are altered is the transformation of the organic matter (OM) that is carried out by the microorganisms. Likewise, it has been observed that the alteration of environmental factors directly influences the rates of decomposition, mineralization of organic matter and the availability of nutrients by soil microorganisms.

The aim of the present thesis was "Study the vulnerability of nutrient dynamics (C, N and P) under scenarios of climate change in soil of an oligotrophic desert in the Cuatro Ciénegas Valley, Coahuila". To meet the objective, we first identified if there is a GCC

footprint in this site, later we identified how the soil microbial communities respond to the variability of rainfall in natural conditions and finally we identified the processes that determine that communities are efficient in the obtaining resources with an input of organic nutrients.

Our results suggest that in the Cuatro Ciénegas Valley the last 36 years the average and minimum temperatures increased approximately 2 °C throughout the year, likewise in summer the thermal oscillation is approaching approximately 2 °C. The rainfall showed increased equitability throughout the year, with more rain in winter and less rain in summer. Our results from extreme events suggest that the winters will become increasingly cold and the summers will become warmer with a high variability in water availability from one year to the next, increasing the environmental stress for the organisms that inhabit our study site.

We observed that microbial communities are more resilient than vulnerable to variation in precipitation. Especially the soil microbial community with fewer resources (sotol community) has adapted to the decrease of P in dry years by decreasing its metabolism during this period and presenting an enzymatic up-regulation (PME and PDE) to obtain nutrients when precipitation increases and it is very efficient in immobilizing nutrients when there are pulses of organic nutrients particularly rich in C and N. We propose that under the scenarios of CCG for desert ecosystems that predict a reduction of annual precipitation and a greater intensity and frequency of torrential rains and events of drought, microbial soil communities within both sites could be vulnerable to drought due to the combination of C-co-limiting and the reassignment of energy and nutrients towards physiological acclimatization strategies to survive

# CAPÍTULO I

# INTRODUCCIÓN GENERAL

## 1.1 Introducción

Aproximadamente el 41% de la superficie del planeta está ocupada por son zonas secas (e.i. desiertos, zonas áridas y semiáridas), en las cuales habitan aproximadamente el 38% de la población mundial (Maestre et al. 2015). Se ha proyectado que para el 2100, estas zonas secas aumenten del 11 al 23% del territorio global (Huang et al. 2016). Aproximadamente, el 70% de las zonas secas productivas están actualmente amenazadas por diversas formas de desertificación, producto de las actividades humanas y del cambio climático global (Huang et al. 2016).

### Ecosistemas desérticos frente al Cambio Climático Global (CCG)

El cambio climático global (CCG) ha sido identificado como un cambio en el valor promedio de las variables climáticas (como precipitación y temperatura), este es un cambio que persiste durante largos periodos de tiempo, generalmente decenios o periodos más largos (IPCC 2013; WMO 2017). El CCG puede deberse a procesos naturales internos, a forzamientos externos o a cambios persistentes en la composición de la atmósfera asociados a actividades antropogénicas (IPCC 2013). Por otro lado, se ha llamado evento climático extremo (ECE) a la ocurrencia del valor de una variable climática (como precipitación y temperatura) en los extremos o umbrales de la distribución normal de valores observados de las variables climáticas (en el 10 y 90 percentil de probabilidad) (Gutschick y BassiriRad 2003; IPCC 2012). Estos eventos son completamente estocásticos y pueden alterar la estructura y el funcionamiento de los ecosistemas. Los ECE incluyen principalmente: ondas de frio y calor, heladas, sequías, lluvias torrenciales y ciclones (Gutschick y BassiriRad 2003; IPCC 2012; Jentsch y Beierkuhnlein 2008). El CCG ha ocasionado un desplazamiento del valor promedio y un aumento en la varianza de los valores de las variables climáticas (temperatura y precipitación) lo cual ha propiciado el aumento de la frecuencia e intensidad de los ECE en todos los ecosistemas (IPCC 2012). Por ello se pronostica que de no disminuir las emisiones antropogénicas que aceleran la velocidad del CCG, este último, podría propiciar ECE cada vez más frecuentes y severos (Figura 1).

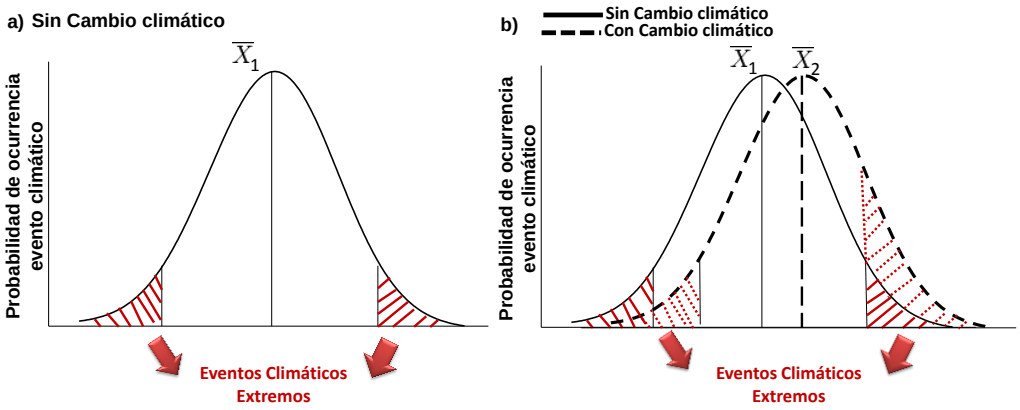


**Figura1**. Comportamiento de la distribución de probabilidad de ocurrencia de variables climáticas (Temperatura y precipitación) y ubicación de los Eventos Climáticos Extremos: a) sin cambio climático, b) con cambio climático. Versión de Montiel-González C. modificada de IPCC (2012)

En los ecosistemas desérticos, la precipitación es escasa y errática y la evaporación es tan alta que la proporción de la precipitación media anual / evapotranspiración potencial es inferior a 0.65 (FAO 2007). Así mismo, en los ecosistemas desérticos la precipitación y la temperatura son altamente variables entre años y ésta variabilidad ha incrementado recientemente debido al CCG (Bell et al. 2014; IPCC 2013; Singh et al. 2014). La vulnerabilidad de un ecosistema ante el CCG, se define como la susceptibilidad del ecosistema ante eventos "anormales" de precipitación y temperatura producidos por el CCG (D'Odorico et al. 2013). Un ecosistema desértico será más o menos vulnerable dependiendo de: 1) el carácter, la magnitud y la rapidez de una perturbación a la cual está expuesto; y 2) su sensibilidad, su resistencia (a los cambios causados por una perturbación) y su resiliencia (la capacidad para que el sistema vuelva a un nivel previo a la perturbación) (IPCC 2014; Orwin y Wardle 2004). Los ecosistemas desérticos son considerados extremadamente vulnerables a las variaciones en temperatura y precipitación, producidas por el CCG y ECE. Se considera vulnerable a los ecosistemas desérticos ya que en su mayoría plantas, animales y organismos que lo habitan en general, viven cerca de sus límites fisiológicos en términos de requerimientos de agua y temperatura (Archer y Predick 2008; Lioubimtseva y Henebry 2009). Los modelos de CCG predicen cambios en los regímenes de precipitación y temperatura para el 2100, estos cambios incluyen incremento de ECE como: aumento en el número y la severidad de eventos de lluvia, sequía, ondas de calor e inviernos severos (IPCC 2013; IPCC 2014).

Actualmente existen pocos trabajos que analizan la dirección y los efectos del CCG particularmente en las regiones áridas y semiáridas, especialmente en los ecosistemas del desierto de América Latina (IPCC 2013). Los escenarios de cambio climático se construyen comúnmente a partir de los Modelos Climáticos Globales de la Atmósfera y Océano acoplados (AOGCM por sus siglas en inglés) (IPCC 2013). Sin embargo, estos modelos no incluyen la dinámica climática local, lo cual aumenta la incertidumbre de los escenarios proyectados a esta escala y las propuestas de adaptación ante el CCG basadas en esas proyecciones. Por ello, es necesario utilizar otras herramientas analíticas permitan entender la magnitud y la dirección del CCG actual a diferentes escalas espaciales. Dentro de estas herramientas se encuentran los análisis de tendencias climáticas a escala regional y local (Tabari y Hosseinzadeh Talaee 2011), entre estos análisis de tendencias que se incluyen análisis de tendencias de series temporales (Bautista et al. 2013; Jain et al. 2013) y los análisis de la frecuencia de ECE (Easterling et al. 2000). Un aporte de la presente tesis es el análisis de las tendencias climáticas a escala local para identificar si está presente la huella de cambio climático global para un desierto oligotrófico de México.

**Comunidades microbianas del suelo frente al CCG**

El suelo es el almacén más grande de C, N y P orgánico de los ecosistemas terrestres, ya que puede contener hasta dos veces más C que la atmósfera y tres veces más C, N y P que el existente en la vegetación (Schlesinger 2000; Singh et al. 2010). Así mismo, las comunidades microbianas del suelo juegan un papel central en la estructura y funcionamiento de los ecosistemas desérticos, ya que estos organismos representan un importante almacén de C, N y P en los suelos (Singh et al. 2010). Diversos estudios han mostrado que las comunidades microbianas de estos ecosistemas son altamente eficientes, principalmente en el uso de C, lo cual favorece la conservación, transformación y disponibilidad de los nutrientes (Steinweg et al. 2008). Así mismo, se ha sugerido que la cantidad de N y P contenido en la biomasa microbiana es comparable al N y P contenido en la biomasa de las plantas en los ecosistemas desérticos (Coleman y Whitman 2005).

En los ecosistemas desérticos, en donde la evapotranspiración potencial anual (PET) excede la precipitación (pp), tanto la temperatura, como la humedad del suelo son factores ambientales que controlan la productividad primaria neta (PPN) (Plante y Conant 2014;

Williams 2014). En el suelo, uno de los principales procesos que son afectados cuando se alteran las variables climáticas es la transformación microbiana de la materia orgánica (MO) (Plante y Conant 2014; Tiemann y Billings 2011). Se ha observado que la variabilidad climática afecta la composición de la comunidad microbiana del suelo (Maestre et al. 2015), las tasas de descomposición y la mineralización de la MO, así como en la disponibilidad de nutrientes y las relaciones estequiométricas de la MO y de los microorganismos del suelo (Schimel y Schaeffer 2012; Yang et al. 2013; Yoo et al. 2006).

Las distintas OTUS microbianos del suelo pueden vivir en determinados rangos óptimos de temperatura y humedad, por esta razón, un cambio en la temperatura y en la humedad del suelo puede afectar la estructura y composición de las comunidades microbianas, lo cual a su vez podría ocasionar cambios en la dinámica de la transformación de los nutrientes del suelo (Singh et al. 2010; Singh et al. 2014). Los microorganismos del suelo también pueden contar con capacidad de adaptación o aclimatación a los cambios ambientales (Evans y Wallenstein 2014; Gutknecht et al. 2012). La resistencia que los microorganismos presenten ante el cambio de temperatura y humedad dependen en gran medida de su historia de vida (Evans y Wallenstein 2012; Pritchard 2011). En los ecosistemas desérticos, los microorganismos del suelo han desarrollado estrategias de aclimatación que les permite tolerar un estrés específico a la variabilidad climática. Algunos ejemplos de estas estrategias de aclimatación ante la variabilidad climática son (Figura 2): entrar en latencia, formar esporas (quistes o esclerosios), construir una capa mucilaginosa rica en polisacáridos para prevenir desecación, algunos microorganismos se protegen de la presión osmótica (potencial mátrico y osmótico negativo) y de la lisis celular por medio de adquisición de osmolitos generalmente ricos en N, como aminoácidos (prolina, glutamina, etc.), síntesis de proteínas chaperonas para estabilizar otras proteínas, etc. (Schimel et al. 2007; Tiemann y Billings 2011). Sin embargo, todos estos mecanismos de tolerancia suelen ser costosos metabólicamente, ya que requieren de la reasignación de energía (C) y nutrientes (principalmente N y P (Hamdi et al. 2013; Schimel et al. 2007). Los microorganismos del suelo a menudo responden a los cambios ambientales por medio de "compromisos fisiológicos", lo cual afecta su capacidad metabólica (Classen et al. 2015). Dentro de las consecuencias de la reasignación de recursos están (Figura 2): una producción limitada de maquinaria para la adquisición de nutrientes, es decir, disminución de la producción de

enzimas para la descomposición de la MO (Burns et al. 2013; Steinweg et al. 2008), así como un cambio en la estructura y composición de las comunidades microbianas y una reducción del crecimiento de quienes constituyen a la comunidad microbiana (Bouskill et al. 2013; Evans y Wallenstein 2012; Maestre et al. 2015).

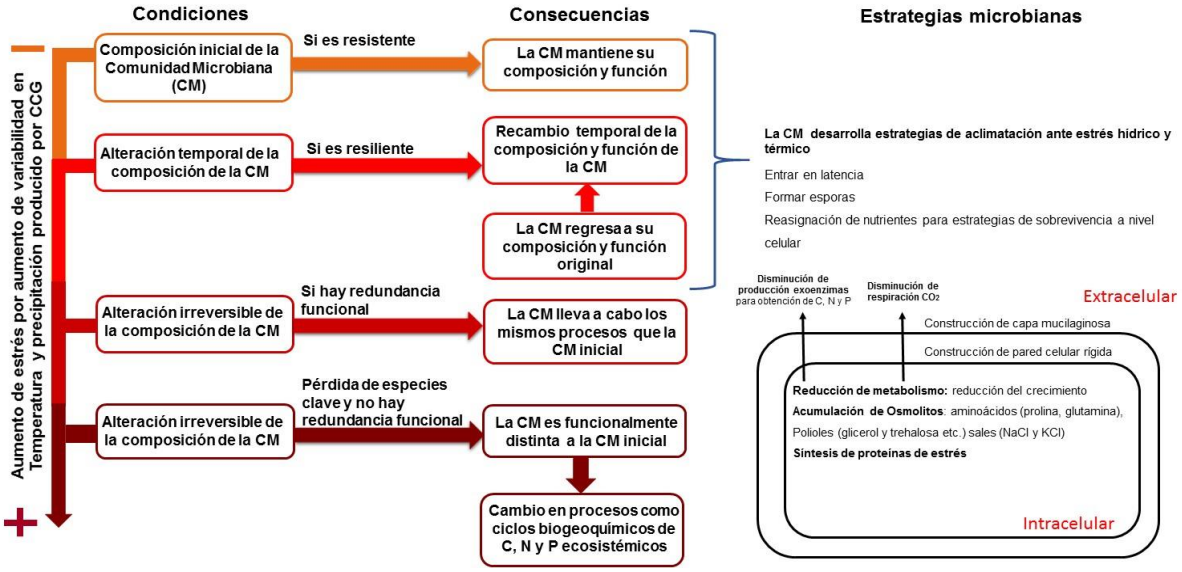


**Figura 2.** Efecto de la variabilidad climática ocasionada por el cambio climático global (CCG) sobre la estructura y función de las comunidades microbianas (CM) del suelo y algunas estrategias de las CM para aclimatarse ante esta variabilidad. Versión de Montiel-González C. modificado de Allison y Martiny (2008) y de Schimel et al. ( 2007).

Bajo los escenarios de CCG para los ecosistemas desérticos que plantean un incremento en la variabilidad climática se espera que el estrés constante al que se expongan las comunidades produzca un fuerte inversión de energía y nutrientes y que promuevan estrategias de aclimatación que impliquen redireccionar los recursos. Los ajustes fisiológicos que realizan los comunidades para afrontar esta variabilidad climática ha sido poco documentado para ecosistemas desérticos, especialmente en América Latina. Un aporte de la presente tesis fue proponer la evaluación de los ajustes fisiológicos que las comunidades microbianas de suelo de un desierto oligotrófico de México realizan ante la variabilidad de la precipitación a lo largo de 4 años.

**Procesos biogeoquímicos que favorecen el uso eficiente de nutrientes en ecosistemas limitados por nutrientes orgánicos.**

Las comunidades microbianas del suelo responden a determinados requerimientos nutricionales ya sea en condiciones "normales" o de estrés, o bien mediante la modificación en sus tasas de inmovilización y mineralización de nutrientes. En los ecosistemas desérticos esta modificación puede responder a la alta variabilidad climática que a su vez puede generar variabilidad temporal de la energía y de los nutrientes disponibles para los organismos del suelo y para las plantas.

En ecosistemas limitados por nutrientes inorgánicos disponibles, para que se mantenga el crecimiento de las comunidades microbianas del suelo, el proceso más importante es la descomposición de la materia orgánica del suelo (MOS) el cual va desde la fragmentación y la despolimerización hasta la mineralización del C, N y P (Bell et al. 2014; Madigan et al. 2015). A través de la descomposición de la MOS ocurren flujos de nutrientes como son la mineralización e inmovilización, los cuales son regulados por los microrganismos a través de relaciones sinérgicas de diversas actividades exoenzimáticas (Elser et al. 2000; Heuck et al. 2015; Sinsabaugh et al. 2009). A sí mismo, se ha propuesto que los microrganismos del suelo mineralizan e inmovilizan el C y el N en respuesta a sus requerimientos nutricionales principalmente de C y N y que el flujo de P dependen exclusivamente de la demanda biótica por P (Bünemann et al. 2012; McGill y Cole 1981; Mooshammer 2012).

Además de la composición de la comunidad microbiana, en ecosistemas limitados por agua, las tasas de mineralización e inmovilización de N por los microorganismos del suelo, son reguladas principalmente por tres variables: 1) la relación C/N en los sustratos orgánicos utilizados por los microorganismos, 2) el uso eficiente de N por los microorganismos y 3) el uso eficiente de C por los microorganismos (Austin et al. 2004). Sin embargo, las tasas de mineralización e inmovilización microbianas de P son reguladas por: 1) la relación C/P del sustrato orgánico y 2) el uso eficiente del P.

Un concepto que nos permite entender como los microorganismos del suelo inmovilizan los nutrientes es el "uso eficiente de nutrientes" ya sea de C, N o de P (CUE, NUE y PUE, respectivamente por sus siglas en inglés). El uso eficiente de C, N y P por los microorganismos del suelo (Figura 3) es la cantidad de biomasa microbiana producida

(nutriente inmovilizado) por unidad de nutriente consumido (nutriente potencialmente disponible) y la excreción metabólica del nutriente no utilizado (nutriente mineralizado) (Bridgham et al. 1995; Manzoni et al. 2012; Sinsabaugh et al. 2009). El uso eficiente de nutrientes por parte de los microorganismos del suelo está en función de su capacidad expresar o alterar su expresión enzimática y de modificar la composición elemental de la biomasa microbiana (por medio de adaptaciones fisiológicas o por medio de la selección de la población), para disminuir las diferencias entre la composición de los recursos y los requerimientos nutricionales permitiendo maximizar las tasas de crecimiento microbiano (Sinsabaugh et al. 2016).

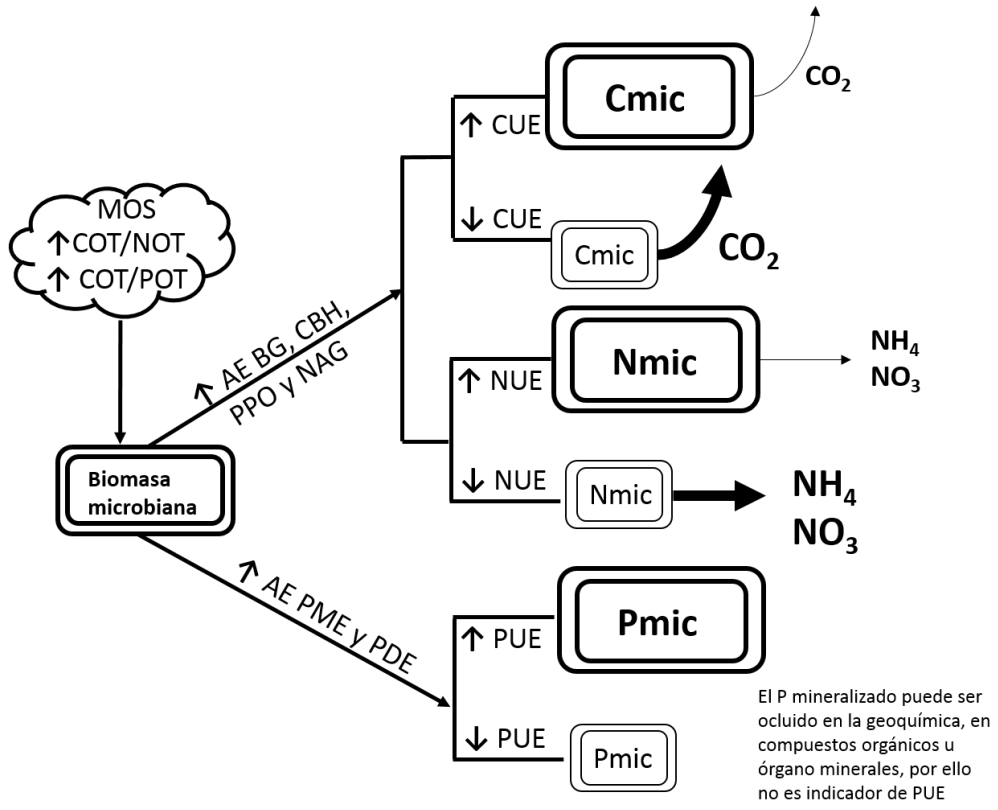


**Figura 3.** Diagrama de las posibles respuestas de una comunidad microbiana del suelo cuando la MOS está limitada por nutrientes (N y P), se produce alta actividad enzimática para compensar la limitación. En el caso del C y N, su uso eficiente del también podría afectar la mineralización. Imagen de Montiel-González C.

Diversos estudios han cuantificado el uso eficiente de C y N (Manzoni et al. 2012; Mooshammer 2012), pero sólo en un estudio se reporta el uso eficiente del P (Sinsabaugh et al. 2016). Sin embargo, se desconoce cuáles son los procesos que favorecen el uso eficiente de los nutrientes en ecosistemas que están limitados por nutrientes orgánicos.

En esta tesis se propone que la combinación de una serie de indicadores como son: 1) la actividad enzimática específica (SEA) de las enzimas involucradas en los procesos de la descomposición de la MOS, 2) las tasas potenciales de transformación de los nutrientes orgánicos, principalmente de C, N y P 3) las tasas potenciales de mineralización e inmovilización, y 4) la relación umbral de limitación de un nutriente ($TER_{C:N}$ Y $TER_{C:P}$), podrían indicarnos cuáles son los procesos que favorecen el uso eficiente de nutrientes por parte de las comunidades microbianas del suelo. Por ello, un aporte de la presente tesis fue estudiar, de manera experimental y potencial (por medio de incubaciones en laboratorio), cómo las comunidades microbianas provenientes de un desierto oligotrófico responden a el ingreso de nutrientes orgánicos disueltos lábiles. Así mismo, se buscó identificar si estas comunidades microbianas tienen la capacidad de utilizar de manera eficiente el C, N y P en respuesta a los nutrientes limitantes y a la estequiometría de sus recursos y conocer cuáles son los procesos que podrían favorecer que las comunidades utilicen los nutrientes eficientemente.

**1.2 Descripción de área de estudio**

El valle de Cuatro Ciénegas se localiza entre las coordenadas 26°45'00" y 27°00'00" de latitud norte, y 101°48'49" y 102°17'53" de longitud oeste, a una altitud promedio de 740 msnm; ubicado en la región conocida como Altiplano septentrional (Espinosa et al. 2005). Presenta una extensión aproximada de 150,000 $km^2$; de esta superficie, aproximadamente el 40% está ocupado por las sierras: La Madera y La Menchaca al norte, La Purísima y San Vicente al oeste, San Marcos y Pinos al sur y La Fragua al sureste. El 60% restante son terrenos planos (Velasco-Molina 1991). Estas barreras geográficas han favorecido que las condiciones climáticas se diferencien del resto del desierto Chihuahuense (Archer y Predick 2008). Su clima seco a semicálido, con estacionalidad de la precipitación, aunque presenta pocas lluvias en verano y ocasionalmente algunas en invierno. La temperatura media anual es de 21.4°C y la precipitación promedio anual es de 246.2 mm (http://smn.cna.gob.mx/). La primera temporada, de noviembre a abril, es fría y seca, con temperaturas mínimas y máximas de 4 y 31°C, respectivamente, y 51 mm de precipitación. La segunda temporada, de mayo a

octubre, es caliente (con temperaturas mínimas y máximas de 15 y 35°C, respectivamente) y alrededor del 60% (155 mm) de la precipitación anual total se concentra en esta temporada.

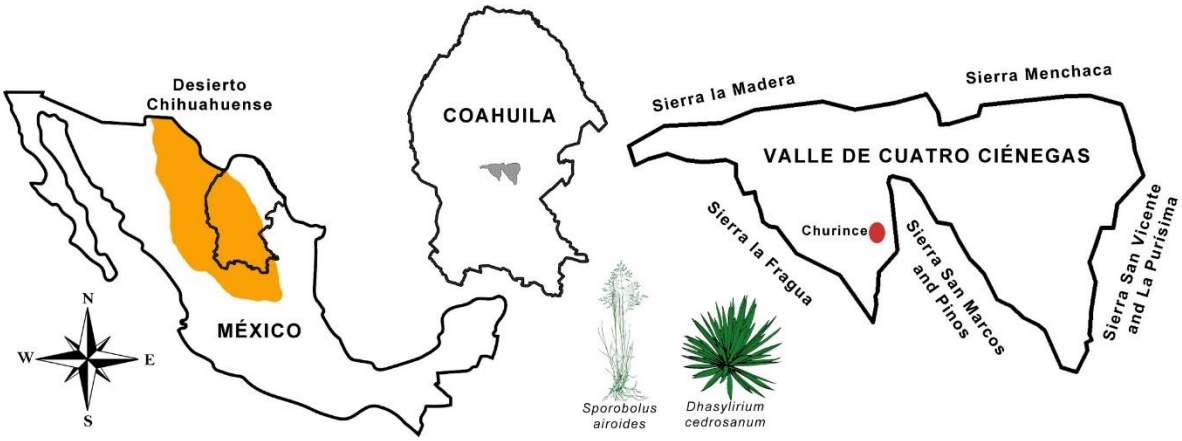


**Figura 4.** Mapa de la ubicación del valle de Cuatro Ciénegas de Carranza Coahuila, México. El pentágono indica la ubicación del sitio de muestreo (Churince). Se observan dos de las especies dominantes del sitio de muestreo. Imagen de Montiel-González C.

El yeso de la era jurásica es el material parental dominante en el lado occidental del valle, mientras que las calizas de la era jurásica dominan el lado oriental (McKee et al. 1990). Según la clasificación de la WRB (2007), los suelos predominantes son *Gypsisol* y *Calcisol* en los lados occidental y oriental de la cuenca, respectivamente. A pesar de la poca precipitación, existen manantiales, pozas y ríos alimentados por corrientes subterráneas (Souza et al. 2004). De acuerdo con la clasificación de las Provincias florísticas de México reportada por Rzedowski (2006), Cuatro Ciénegas se encuentra dentro del reino florístico Neotropical, la región Xerofitica Mexicana y la provincia del Altiplanicie.

Los principales tipos de vegetación son: 1) pastizal (G) dominado por *Sporobolus airoides* (Torr.) Torr. y *Allenrolfea occidentalis* (S. Watson) Kuntze; 2) Matorral micrófilo dominados por *Jatropha dioica* Cerv., *Larrea tridentata* (DC) Cov. y *Fouqueria* sp. Kunth (Perroni et al. 2014); y 3) matorral rosetófilo (RS) dominados por *Dhasylirium cedrosanum* Trel., y *Yucca treculeana* Carriére (González 2012).

## 1.3 Preguntas generales

1. ¿El desierto de Cuatro Ciénegas Coahuila presenta evidencia de cambio climático?
2. ¿Cómo responden las comunidades microbianas del suelo de un desierto oligotrófico ante la variabilidad climática actual y la limitación de nutrientes orgánicos?

## 1.4 Objetivo general

Estudiar la vulnerabilidad de la dinámica de nutrientes (C, N y P) bajo escenarios de cambio climático en suelo de un desierto oligotrófico en el valle Cuatro Ciénegas, Coahuila, México.

## 1.5 Objetivos particulares

a) Evaluar el efecto del GCC en el valle de Cuatro Ciénegas, Coahuila durante los últimos 70 años (1941 a 2013). Concretamente, se buscó: 1) identificar tendencias en el comportamiento de las variables climáticas (temperatura y precipitación); 2) evaluar la naturaleza y dirección de los cambios en la frecuencia de los Eventos Climáticos Extremos; y 3) detectar los cambios en la variabilidad interanual de la precipitación a lo largo de todos los meses durante los últimos 70 años.

b) Examinar los efectos de la variación de la lluvia sobre los ajustes fisiológicos que realizan las comunidades microbianas del suelo: 1) para la obtención de energía y nutrientes y 2) que determinan la vulnerabilidad de las comunidades microbianas en dos sistemas suelo-vegetación, cada uno con diferente entrada de materia orgánica en el desierto oligotrófico: del valle de Cuatro Ciénegas, Coahuila.

c) Evaluar: 1) La respuesta de las comunidades microbianas del suelo de un desierto oligotrófico a la entrada de diferentes compuestos de MOS lábil rica en C, N y P, 2) La capacidad fisiológica de las comunidades microbianas al uso eficiente de los nutrientes, y 3) Los procesos que determinan que las comunidades microbianas utilicen eficientemente los nutrientes en este ecosistema oligotrófico.

### 1.6 Estructura de la tesis

Para cumplir con los objetivos en esta tesis se desarrollaron tres capítulos que abordan cada uno de los objetivos particulares (Capítulos II, III y IV), además de presentar una introducción general en el **Capítulo I** y las conclusiones generales en el **Capítulo V**.

**Capítulo II. The Global Climate Change footprint at Cuatro Ciénegas Basin, Mexico: the increasing frequency of extreme climatic events.**

En este capítulo se presenta un estudio que tuvo como objetivo identificar la existencia de una posible huella de cambio climático en el valle de Cuatro Ciénegas Coahuila (CCC). Así mismo, se planteó identificar sobre qué variables climáticas se refleja el cambio de clima y cuál es la dirección de estos cambios. Para responder a estas preguntas se analizaron los registros disponibles para los principales parámetros climáticos (temperatura y precipitación) de la estación meteorológica 5044 localizada dentro del valle de CCC. En este estudio se presenta un análisis de tendencias climáticas mensuales de: temperatura (mínima, máxima y promedio), oscilación térmica y precipitación recabados a los largo de 70 años. De acuerdo a la Organización Meteorológica Mundial, un cambio en el clima se identifica en periodos prologados de por lo menos 30 años. Por ello, en éste capítulo se identificó el tipo de clima de hace 70 años y de hace 30 años con base en la clasificación de Köppen modificada para México por Enriqueta García (1981). Adicionalmente se analizó el cambio en los eventos climáticos extremos (ECE) y de probabilidad de lluvia. Se propone que la identificación de estos cambios a escala local como en el caso del valle de CCC representa una herramienta para el entendimiento, la predicción, y la prevención más precisa de los efectos presentes y futuros del CCG sobre los componentes y el funcionamiento de los procesos de un ecosistema.

**Capítulo III. Rainfall variability increases the vulnerability of the soil microbial community in an oligotrophic desert ecosystem**

Este capítulo presenta un estudio que tuvo como objetivo general identificar cuáles son los ajustes metabólicos que hacen las comunidades microbianas del suelo de un ecosistema oligotrófico para sobrevivir ante la variabilidad anual de la precipitación; y si esos ajustes

podrían favorecer que las comunidades sean vulnerables ante la variación anual de la precipitación. Para responder al objetivo se realizaron muestreos de suelo en el valle de Cuatro Ciénegas Coahuila (CCC) en los años 2011, 2012, 2013 y 2014, en los cuales varió la precipitación anual. El año más húmedo fue 2011 (348 mm y 25°C), el año 2012 fue particularmente caluroso y seco (89mm y 28°C) y los años siguientes fueron húmedos (217 mm y 230 mm para el 2013 y 2014, respectivamente) con menores temperaturas (24.9 y 24.8 $^{\circ}$C para 2013 y 2014, respectivamente). El muestreo fue realizado en el sitio Churince, el cual se localiza en la parte este del valle de CCC, en este sitio el suelo está caracterizado como Yermosol. Así mismo, el muestro se realizó bajo dos coberturas vegetales: pastizal dominado por *Sporobolus airoides* (Torr.) Torr. y matorral rosetófilo dominado *Dhasylirium cedrosanum* Trel. Durante el muestreo se colectaron muestras de suelo y vegetación (parte aérea y radical). Solo para el año más húmedo (2011) se realizó la determinación taxonómica de la comunidad bacteriana del suelo. Para los cuatro años se realizaron análisis biogeoquímicos (humead, pH, C, N y P totales y microbianos). Para los años 2012 a 2014 se analizaron los nutrientes disueltos y disponibles, así como la actividad enzimática de β-1, 4-glucosidasa (BG), celobiohydrolasa (CBH), β-1, 4-N-acetylglucosaminidasa (NAG), polyfenol oxidasa (PPO), fosfomonoesterasa (PME) y fofodiesterasa (PDE). Así mismo, de los años 2012 a 2014 se realizaron análisis de C, N y P de la vegetación. Con estos datos se realizó: 1) regresión entre la concentración y los cocientes de C, N y P en biomasa microbiana con la precipitación anual 2) la actividad enzimática específica (SEA) por año y vegetación; y 3) la estequiometría ecoenzimática, homeostasis y la relación umbral de los elementos ($TER_{C:N}$ y $TER_{C.P}$). Con este estudio se propone que la vulnerabilidad de una comunidad microbiana a la variación de la precipitación anual en un desierto oligotrófico como CCC puede ser determinada con: 1) La co-limitación por energía:nutriente, la cual fue determinada con la comparación del $TER_{C:N}$ y $TER_{C.P}$ entre años y vegetaciones. 2) la reasignación de recursos, la cual fue determinada con los SEA para la adquisición de C, N y P comparadas entre años y vegetaciones y 3) La resiliencia funcional de la comunidades microbianas, la cual fue determinada con las concentraciones y relaciones de los nutrientes disueltos, disponibles y nutrientes en biomasa microbiana.

**Capítulo IV: The organic nutrient limitation determines the intensity of the processes involved in the efficient use of C, N and P by soil microbial communities in an oligotrophic arid ecosystem in México.**

El objetivo principal de este capítulo fue contestar la siguiente pregunta: ¿Cuáles son los procesos que determinan que las comunidades microbianas utilicen eficientemente los nutrientes en este ecosistema oligotrófico? Para responder esta preguntase realizó un muestreo de suelo en el valle de Cuatro Ciénegas Coahuila (CCC) en Septiembre del 2014 bajo dos coberturas vegetales que difieren en la cantidad y calidad de la MO que producen: pastizal dominado por *Sporobolus airoides* (Torr.) Torr. y matorral rosetófilo dominado *Dhasylirium cedrosanum* Trel. Se eligieron 5 puntos de muestreo en cada vegetación cubriendo un área aproximada de 1 $km^{2.}$ Inmediato a la colecta el suelo fue incubado por un periodo de 4 días bajo condiciones controladas de temperatura, humedad y luz en una cámara de crecimiento para la aclimatación de las comunidades a la incubación. Posteriormente, a cada suelo se le adicionaron nutrientes orgánicos lábiles (menores a1kDa) en solución (necesario para llevar al cada suelo a su capacidad de campo), para un total de 5 tratamientos: 1) control, adición de agua desionizada; 2) C, adición de ribosa; 3) CN, adición de adenosina; 4) CP, adición de ribosa 5-fosfato y 5) CNP, adición de adenosin difosfato. La concentración simulo una entrada equivalente al doble la concentración de COD bajo condiciones "normales". Los suelos fueron incubados durante un periodo de 21 días con una trampa química para $CO_2$ la cual fue cambiada cada 3 días. En cada cambio de trampa de $CO_{2,}$ también se controló y repuso la pérdida de agua por evaporación. Se cuantificó pre y post incubación: 1) Los nutrientes potencial mente solubles (COD, NOD, POD, $NH_4$, $NO_3$, Pi); 2) lo nutrientes potencialmente disponibles ($NH_4$-KCl $NO_3$-KCl y PI-$NaHCO_3$); 3) la actividad enzimática (BG, CBH, PPO, NAG, PME y PDE) y 4) los nutrientes en biomasa microbiana (Cmic, Nmic, Pmic). Con los resultados se determinaron las tasas potenciales de transformación de nutrientes orgánicos, las tasas potenciales de mineralización e inmovilización, la actividad enzimática específica y la relación umbral del elemento (TER por sus siglas en ingles). Con este estudio proponemos que la adición de nutrientes orgánicos promovió la disminución de co-limitación por nutrientes y cambios en las magnitudes de procesos de inmovilización y mineralización de N y P. En los procesos de mineralización,

inmovilización implicados en la transformación del C las comunidades microbianas del suelo respondieron de manera similar a los tratamientos especialmente a l adición de C orgánico, así mismo, se perdió el efecto de la cubierta vegetal (origen de la comunidad). Los resultados sugieren la comunidad microbiana de sotol es más eficiente en obtener N y P que la comunidad microbiana de pastizal, lo anterior se debe a que la comunidad microbiana de sotol inmoviliza más nutrientes por enzima producida.

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# CAPÍTULO II

## THE GLOBAL CLIMATE CHANGE FOOTPRINT AT CUATRO CIÉNEGAS BASIN, MEXICO: THE INCREASING FREQUENCY OF EXTREME CLIMATIC EVENTS

*Manuscrito enviado a Journal Arid Land*

# The Global Climate Change footprint in a Mexican desert ecosystem: the increasing frequency of extreme climatic events

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**Abstract:** While the accuracy of scenarios of Global Climate Change has been improved, the lack of climatic data from several regions of the world means that some predictions remain misleading. For this reason, local climate studies are critical for the calibration of global climate scenarios. Our objective was to evaluate the climate trends within the Cuatro Ciénegas Basin (CCB). Specifically, we aimed to: 1) identify potential trends in the behavior of temperature and precipitation; 2) assess the nature and direction of changes in the frequency of extreme climate events (ECE); and 3) detect changes in inter-annual rainfall variability. To achieve these aims, we analyzed a 70-year database of climatic variables from the CCB weather station. Data were subjected to trend analyses using two different software packages (EViews and Clic-MD); ECE frequency was evaluated by Chi-square analysis and rainfall probability by the gamma distribution function. Minimum temperature ($T_{min}$) increased in almost every month, while mean temperature ($T_{mean}$) increased only in the summer months. Lower $T_{min}$ frequency increased in the winter months, while the frequency of upper event extremes increased during the summer months, as did the extreme events of maximum temperature ($T_{max}$). Winters have therefore become colder while summers have become warmer, increasing the frequency of heat waves over the last 36 years. However, monthly rainfall patterns presented high variability that obscured any trend in the frequency of extreme rainfall events. Over the last 36 years, frequencies of events of both intense rainfall associated with tropical cyclones and intense drought associated with the ENSO were higher than before.

**Keywords:** Climate trends, Chihuahuan desert, extreme climate events, precipitation, temperature.

# 1. Introduction

Global Climate Change (GCC) affects both the components and functioning of ecosystems (Rustad, 2008; IPCC, 2013). According to the IPCC, the globally averaged combined land and ocean surface temperature data as calculated by a linear trend, shows a warming of 0.85°C, over the period from 1880 to 2012, but global changes in precipitation show no clear trend (IPCC, 2013). Similarly, heat waves have increased in intensity since the mid-20$^{th}$ century in the majority of world regions, but the temporal pattern of torrential rains and drought remains unclear at global level (IPCC, 2013).This lack of a clear pattern in the trends of rainfall events is explained by the absence of long-term rainfall data for several regions of the planet and also because precipitation patterns depend mainly on regional phenomena, which are not reflected in global models (Easterling et al., 2000; Archer and Predick, 2008; Grimes and Pardo-Igúzquiza, 2010). Rainfall variability complicates the accurate assessment of contemporary rainfall distribution trends and the potential impacts of GCC (Batisani and Yarnal, 2010). This lack of data is particularly critical in arid and semiarid regions, especially in Latin American desert ecosystems (IPCC, 2013).

In desert ecosystems, the precipitation is both scarce and erratic and evaporation is so high that the ratio of average annual precipitation to potential evapotranspiration is below 0.65 (FAO, 2007), which produces a water deficit stress for plants and animals. The quantity of rainfall and environmental temperature are both important factors in determining the amount of water available for primary productivity and for the biological activity of organisms (Holmgren et al., 2006; Williams, 2014). Inter-annual climatic variability in these ecosystems determines the occurrence, duration and intensity of flood and drought conditions (Jun et al., 2012; D'Odorico et al., 2013). However, variability in annual levels of precipitation could be increased by GCC over the 21$^{st}$century (Jain and Kumar, 2012).This variability is a consequence for increasing frequency and intensity of extreme climate events (ECE) (D'Odorico and Bhattachan, 2012; IPCC, 2012). An ECE is defined as the occurrence of a value of climate variable with very low probably of occurrence (IPCC, 2012). These events are completely stochastic and can alter, sometimes irreversibly, the structure and functioning of ecosystems (Jentsch and Beierkuhnlein, 2008). Some predicted scenarios for the inter-annual variability in desert precipitation include: decreased variability in Africa (Namib desert) and Australia (Tanami, Simpson and Stzelecki deserts), and increased

variability in India (Thar desert), as well as an increase then decrease in the USA (Mojave desert) and Botswana (Kalahari desert) (Archer and Predick, 2008; D'Odorico and Bhattachan, 2012).

Desert ecosystem vulnerability is defined as its susceptibility to disturbances such as those produced by GCC or ECE (D'Odorico et al., 2013; IPCC, 2013). In this ecosystem, vulnerability is governed by: 1) the character, magnitude and rate of a disturbance to which an ecosystem is exposed, and 2) the sensitivity and adaptability of the ecosystem to that disturbance (IPCC, 2012). Desert ecosystems are considered extremely vulnerable to GCC, particularly ECE, because the plants and animals in these ecosystems live near to their physiological limits in terms of water and temperature requirements, and can therefore be very sensitive to even moderate changes in climate (Archer and Predick, 2008; Lioubimtseva and Henebry, 2009). For this reason, it has been proposed that failure to mitigate the GCC will lead to an increased frequency and severity of ECE in the future, which could have negative and irreversible impacts on the functioning of desert ecosystems (Jentsch and Beierkuhnlein, 2008; Reichstein et al., 2013).

The scenarios for the Sonoran and the Chihuahuan deserts in Mexico include decreased annual rainfall and, an increased number and intensity of individual precipitation events, accompanied by rising mean annual temperatures (Archer and Predick, 2008; Loarie et al., 2009; Bell et al., 2014). In addition, the ECE projections imply lower frost frequencies and higher frequencies of heat waves, droughts, storms and floods (IPCC, 2013).The Chihuahuan desert has been classified as one of Earth's most biologically outstanding habitats by the World Wildlife Fund (Archer and Predick, 2008). The Cuatro Ciénegas Basin (CCB), which is the study site of the present research, is located within in the Chihuahuan desert and is considered the most important wetland of Mexico because of its high levels of endemism and biodiversity (Souza et al., 2011). However, the alfalfa fields that represent the main agricultural crop within the CCB demand great quantities of water and the practice of irrigation is mainly done by flood irrigation, which promotes soil degradation and biodiversity loss (Hernández-Becerra, 2014).

Climate change scenarios are commonly constructed based on Atmosphere Ocean Global Climate Models (AOGCMs) (IPCC, 2013); however, these models do not take local climate dynamics into account, and their use therefore increases the uncertainty of projected

scenarios at this scale. The integration of other analytical tools is therefore required for climatic trends at regional and local scales (Tabari and Hosseinzadeh Talaee, 2011; Jun et al., 2012). Among these tools, trend analyses of time series (Jain and Kumar, 2012; Bautista et al., 2013) and analyses of ECE frequency can be of particular value (Easterling et al., 2000).

The objective of the present study was therefore to evaluate the effect of GCC in the CCB over the last 70 years (1941 to 2013). Specifically, we aimed to: 1) identify trends in the behavior of climatic variables (temperature and precipitation); 2) assess the nature and direction of changes in the frequency of ECE; and 3) detect changes in inter-annual variability of rainfall throughout the year over the last 70 years. We hypothesize increases in atmospheric temperature, frequency of ECE and rainfall variability. To test these hypotheses, we analyzed a 70-year database of climate variables from the CCB weather station.

# 2. Materials and methods

## 2.1. Study site

The study was carried out in the Cuatro Ciénegas Basin (CCB; 26°45'- 27°00' N and 101°48'- 102°17' W) in central-northern Mexico, which is part of the Chihuahuan Desert. The CCB has an area of 150,000 $km^2$ and the study area had an elevation of 740 m.a.s.l. and it is completely surrounded by mountains. These geographical barriers are favored that the climate dynamic differentiated from the rest of the Chihuahuan Desert (Archer and Predick, 2008). The climate is seasonally arid with two contrasting seasons and an average annual temperature of 21.2°C with 252.5 mm of annual rainfall (SMN-Conagua, 2018). The first season, from November to April, is cold and dry with minimum and maximum temperatures of 4.8 and 29.8°C, respectively, and 65.9 mm of precipitation. The second season, from May to October, is hot (with minimum and maximum temperatures of 15.7 and 33.4°C, respectively) and around 60% (186.6 mm) of the total annual rainfall is concentrated within this season (SMN-Conagua, 2018). Jurassic-era gypsum is the dominant parent material at the western side of the basin, while Jurassic-era limestones dominate the eastern side (McKee et al., 1990). According to the World Reference Base for soil resources (WRB, 2015) the predominant soils are *Gypsisol* and *Calcisol* at the western and eastern sides of the basin, respectively. The main vegetation types are: 1) grassland (G), dominated by *Sporobolus*

*airoides* (Torr.) Torr., and *Allenrolfea occidentalis* (S. Watson) Kuntze; 2) microphyll scrub, dominated by *Jatropha dioica* Cerv., *Larrea tridentata* (DC) Cov., and *Fouqueria sp* Kunth (Perroni et al., 2014); and 3) rosetophylous scrub (RS) dominated by *Dhasylirium cedrosanum* Trel., and *Yucca treculeana* Carriére (González, 2012).

### 2.2. Climate data set

For the present study, a data set covering a 70-year period (1941 to 2013) was analyzed. The data came from two weather stations within CCB (Cuatro Ciénegas station of the Mexican National Water Commission (http://smn.cna.gob.mx/) and the "Rancho Pozas Azules" station of INIFAP (http://clima.inifap.gob.mx/redinifap/). Daily and monthly data were used to evaluate the following parameters: precipitation (pp), mean temperature ($T_{mean}$), maximum temperature ($T_{max}$) and minimum temperature ($T_{min}$). The pp, $T_{max}$ and $T_{min}$ databases were subjected to quality control in order to identify possible errors (i.e., pp $\geq$ 0 or $T_{min} > T_{max}$; (Moberg and Jones, 2005). The monthly thermal oscillation was calculated from the difference between $T_{max}$ and $T_{min}$ (Tabari and Hosseinzadeh Talaee, 2011).

### 2.3. Trend analysis: temperature and precipitation

EViews version 7.0 (Quantitative Micro Software) for parametric statistics (López-Diáz et al., 2013) and Clic-MD version 1 for nonparametric statistics (Bautista et al., 2013) were used for analyzing temporal trends in temperature and precipitation. For the EViews model, the following assumptions were tested before performing the trend analysis: normality, functional form, no autocorrelation, correct specification, structural permanence, multicollinearity and no homoscedasticity. To test if the slope of the relationship between the climate variable and time differed from zero, EViews adjusted this relationship to a least squares linear regression model (Jain and Kumar, 2012):

$$y = C\, a_j x_j + e \quad \text{for } j = 1...n \qquad (1)$$

Where "y" is the climate variable (temperature or precipitation), $x_j$ the time, $C$ a constant coefficient, $a_j$ the regression parameter (slope), and $e$ the residual error. An increase or decrease of the trend for the climate variable under analysis was indicated by a positive or negative "a" value, respectively. Slope intensity was analyzed by Pearson correlation analysis (López-Diáz et al., 2013).

The Clic-MD software (Bautista et al., 2014) included two statistical analyses: 1) a Spearman simple linear correlation was used to evaluate changes in climate variable intensities, and 2) a non-parametric Mann-Kendall test (MK-T) was used for analysis of the temporal trends of climatic variables (Jain and Kumar, 2012). The MK-T tests the null hypothesis of no temporal trend where the slope is equal to zero; (Tabari and Hosseinzadeh Talaee, 2011). The null hypothesis is rejected when $Z > 1.96$. Positive or negative values of Z indicate increments or decrements of the climate variable in the time series, respectively (Jun et al., 2012; Bautista et al., 2013).

### 2.4. Analyses of Extreme Climate Events (ECE).

The ECEs were identified as those values below the $10^{th}$ (lower extremes) or above the $90^{th}$ (upper extremes) percentile distribution values in any climate parameter (Ben-Gai et al., 1999; Easterling et al., 2000; IPCC, 2012). For this analysis, we used daily temperature data and monthly precipitation data. In order to analyze whether the frequency of temperature ($T_{max}$ and $T_{min}$) and precipitation ECEs had changed over the last 36 years, the70-year CCB dataset was divided into two time periods: a) from 1941 to 1976 and b) from 1977 to 2013. A chi-square test was used to identify changes in the frequency of lower and upper extreme events of $T_{max}$, $T_{min}$ and for upper extreme events of precipitation between the two time periods. To identify monthly changes between the two time periods, we applied a residual analysis to the lower and upper extreme events of $T_{max}$, $T_{min}$ and precipitation. Where the residual value was >1.96 or <-1.96, the change in frequency was considered to be significant (Everitt, 1992; Ben-Gai et al., 1999)

### 2.5. Analysis of Rainfall probability

The analysis of rainfall probability utilized the precipitation data of the two time periods defined above. To calculate the monthly probability distribution between the two periods, we used the density function of the Gamma distribution with Clic-MD (Liang et al., 2012; Bautista et al., 2013).

The Gamma function is the probability model used in the analysis of historical monthly precipitation data. Adjustment of the Gamma function to monthly precipitation records is based on the calculation of the parameters that shape the function.

First, an auxiliary variable A is calculated:

$$A = In\bar{X} - \left(\frac{1}{N}\right)(\sum InX) \quad (2)$$

Where *ln X* = natural logarithm of the average of the data; *N* = amount of data: $\sum InX$ = sum of the natural logarithms of the data. With this variable A, it is then possible to calculate the two variables that will shape the probability function adjusted to the values for each month, α = alpha and β = beta:

$$\alpha = \frac{1+\sqrt{1+\frac{3}{4}A}}{4A} \; ; \; \beta = \frac{\overline{X}}{\alpha} \quad (3)$$

Once the values of the parameters alpha and beta are obtained, the gamma probability function can be calculated.

$$f(X) = \frac{x^{\alpha-1}\exp(-\frac{x}{\beta})}{\beta^{\alpha}\tau(\alpha)} \quad (4)$$

Thus, the area under the curve of this function, calculated with its corresponding integral, represents the probability of finding avalue less than or equal to the limit value to the right used as a reference. For precipitation analysis, we need to know the probability of finding a higher or equal precipitation to each value, so it is necessary to calculate the complement of those previously obtained by simply subtracting 1 from each value.

We also calculated the following parameters: the amount of rain in a typical rainy month (r), the rainfall concentration or the number of rainy months (P) and the equitability, which is a relative measurement of rainfall concentration (E). We calculated these parameters using the equations proposed by Ezcurra and Rodrigues (1986) for the total period (1941 to 2013) and the two defined periods, where "x" is the amount of precipitation in one month per year and "n" is the number of months (12):

$$P = \frac{(\sum X)^2}{\sum x^2} \quad (5)$$

$$r = \frac{\sum x^2}{\sum x} \quad (6)$$

$$E = \frac{P}{n} \quad (7)$$

### 2.6. Climate type classification

For classification of climate type, the analysis of data records for a period of at least 30 years is required (WMO, 2018). To detect changes in the climate type within the last 36 years, the 70-year CCB database was divided into two periods: a) from 1941 to 1976 and b) from 1977

to 2013. For each period, climate type was characterized using the Köppen classification method modified for Mexico by García (1981).

# 3. Results

## 3.1. Trends analysis: temperature, thermal oscillation and precipitation

$T_{min}$ (Table 1) and $T_{mean}$ (Table 2) presented a significant increase of approximately 2 °C from 1941 to 2013 in January to March, and in May. $T_{min}$ also increased 2 °C in the summer months (from June to August) and approximately 1 °C in winter months (November to December; Table 1). Additionally, the $T_{mean}$ increased of approximately 1°C in June and 2°C in November. We found no significant trend in the $T_{max}$ from 1941 to 2013 with either of the software packages used. For thermal oscillation, we observed a negative trend by a decrease of *ca.* 2 °C in March, July, August and September (Table 3).

For precipitation, we observed an increasing trend in July only. However, it is likely that the lack of observable trends in the other months was due to the wide variability of the data (Table 4).

**Table 1.** Trend analysis of monthly minimum temperatures from 1941 to 2013 in the CCB. The correlation coefficient is shown. Asterisk denotes statistical significance (P<0.05 and Z <1.96).

| Month | Eviews | | Clic-MD | | Trend |
|---|---|---|---|---|---|
| | R | P | R | Z | |
| January | 0.03 | 0.0001***** | 0.43 | 3.83***** | ↑ 2°C |
| February | 0.02 | 0.0089***** | 0.3 | 3.04***** | ↑ 2°C |
| March | 0.03 | 0.0005***** | 0.4 | 3.43***** | ↑ 2°C |
| April | 0.01 | 0.09 | 0.24 | 1.79 | NS |
| May | 0.03 | 0.0001***** | 0.46 | 4.00***** | ↑ 2°C |
| June | 0.02 | 0.0000***** | 0.46 | 3.86***** | ↑ 2°C |
| July | 0.02 | 0.0046***** | 0.32 | 2.59***** | ↑ 2°C |
| August | 0.02 | 0.0002***** | 0.48 | 4.09***** | ↑ 2°C |
| September | 0.02 | 0.0303***** | 0.3 | 2.00***** | ↑ 2°C |
| October | 0.02 | 0.0949 | 0.23 | 1.9 | NS |
| November | 0.03 | 0.0029***** | 0.38 | 3.06***** | ↑ 1°C |
| December | 0.02 | 0.0558 | 0.22 | 1.96***** | ↑ 1°C |

**Table 2**. Trend analysis of monthly means temperatures from 1941 to 2013 in the CCB. The correlation coefficient is shown. Asterisk denotes statistical significance (P<0.05 and Z <1.96).

| Month | Eviews | | Clic-MD | | Trend |
|---|---|---|---|---|---|
| | R | P | R | Z | |
| January | 0.028 | 0.0088* | 0.43 | 2.36* | ↑ 2°C |
| February | 0.011 | 0.081 | 0.3 | 2.01* | ↑ 2°C |
| March | 0.026 | 0.007* | 0.39 | 2.43* | ↑ 2°C |
| April | 0.003 | 0.826 | 0.23 | 1.69 | NS |
| May | 0.024 | 0.0008* | 0.46 | 3.21* | ↑ 2°C |
| June | 0.015 | 0.0185* | 0.46 | 1.8 | ↑ 1°C |
| July | -0.0003 | 0.964 | 0.32 | -0.08 | NS |
| August | 0.005 | 0.4731 | 0.48 | 0.69 | NS |
| September | 0.008 | 0.3042 | 0.3 | 0.41 | NS |
| October | 0.015 | 0.1119 | 0.23 | 0.87 | NS |
| November | 0.023 | 0.0115* | 0.38 | 2.35* | ↑ 2°C |
| December | 0.014 | 0.1381 | 0.22 | 1.12 | |

**Table 3.** Trend analysis of monthly thermal oscillation from 1941 to 2013 in the CCB. The correlation coefficient is shown. Asterisk denotes statistical significance (P<0.05 and Z <1.96).

| Month | Eviews | | Clic-MD | | Trend |
|---|---|---|---|---|---|
| | R | P | R | Z | |
| January | -0.01 | 0.53 | -0.113 | -1.74 | NS |
| February | -0.03 | 0.09 | 0.007 | -0.89 | NS |
| March | -0.02 | 0.22 | -0.01 | -1.98* | ↓ 2°C |
| April | -0.02 | 0.11 | 0.06 | -1.14 | NS |
| May | -0.01 | 0.34 | 0.028 | -0.95 | NS |
| June | -0.02 | 0.06 | -0.094 | -1.32 | NS |
| July | -0.04 | 0.0003 * | -0.281 | -2.79* | ↓ 2°C |
| August | -0.04 | 0.0003 * | -0.305 | -2.66* | ↓ 2°C |
| September | -0.03 | 0.012 * | -0.174 | -2.23* | ↓ 2°C |
| October | -0.01 | 0.46 | 0.018 | -1.06 | NS |
| November | -0.01 | 0.50 | -0.017 | -1.7 | NS |
| December | 0.00 | 0.88 | 0.032 | -0.61 | NS |

**Table 4.** Trend analysis of monthly precipitation from 1941 to 2013 in the CCB. The correlation coefficient is shown. Asterisk denotes statistical significance (P<0.05 and Z <1.96).

| Month | Eviews | | Clic-MD | | Trend |
|---|---|---|---|---|---|
| | R | P | R | Z | |
| January | 0.00 | 0.96 | 0.00 | 0.07 | NS |
| February | -0.02 | 0.78 | -0.03 | -0.32 | NS |
| March | 0.02 | 0.70 | 0.04 | 0.93 | NS |
| April | -0.04 | 0.58 | -0.06 | -0.71 | NS |
| May | 0.15 | 0.32 | 0.11 | 0.68 | NS |
| June | 0.13 | 0.42 | 0.09 | 0.73 | NS |
| July | 0.37 | 0.04 * | 0.20 | 2.25 * | ↑ |
| August | 0.12 | 0.51 | 0.07 | 1.01 | NS |
| September | 0.03 | 0.89 | 0.01 | -0.06 | NS |
| October | -0.03 | 0.81 | -0.02 | 0.01 | NS |
| November | -0.02 | 0.85 | -0.02 | 0.3 | NS |
| December | 0.05 | 0.57 | 0.06 | -0.14 | NS |

### 3.2. Analyses of Extreme Climate Events (ECE).

The Chi-square analysis was significant ($p<0.005$) in all the cases of temperature, but was not significant for monthly precipitation (Table 5). For the $T_{min}$ of January, February and May, we observed an increase in the frequency of lower temperature (months increasingly colder) from the first (1941-1976) to the second time period (1977-2013). Likewise in April, June, July, November and December, we observed a decrease in the lower extremes of $T_{min}$ (months increasingly less cold) from the first to the second period (Figure 1a).

For the upper extremes of $T_{min}$ in February, July, August and November, we observed an increase of frequencies (increasingly warmer $T_{min}$) from the first to the second period. For the months of March, May, June, September and December, the frequencies of upper extremes (colder $T_{min}$) of $T_{min}$ decreased from the first to the second period (Figure 1b).

In January, April, June and September, we observed a decrease of the frequencies of the lower extremes (increasingly warmer months) of $T_{max}$ from the first to the second period. In the months of July, August, October, November and December, we observed an increase in the frequencies of lower extremes (less warm months) of $T_{max}$ from the first to the second period (Figure 1c).

In the upper extremes of $T_{max}$, we observed in January, February, June and July an increase of frequencies (increasingly warmer months) from the first to the second period. In March, April and September, we observed a decrease of frequencies of upper extreme events (less warm months) of $T_{max}$ from the first to the second period (Figure 1d).

We did not find significant differences in the frequencies of extreme monthly precipitation between the first and second periods (Table 5).

**Table 5.** Chi-square of frequency of Extreme Climate Events (ECE), in terms of temperature, for two periods in the CCB: 1)1941-1976 and 2)1977-2013, $Chi^2$ value 11.34.

| | **$Chi^2$ Value** | **df.** | **P** |
|---|---|---|---|
| **T min lower extreme** | 102.56 | 12 | <0.005 |
| **T min upper extreme** | 134.81 | 12 | <0.005 |
| **T max lower extreme** | 158.83 | 12 | <0.005 |
| **T max upper extreme** | 212.61 | 12 | <0.005 |
| **Precipitation upper extreme** | 2.97 | 12 | NS |

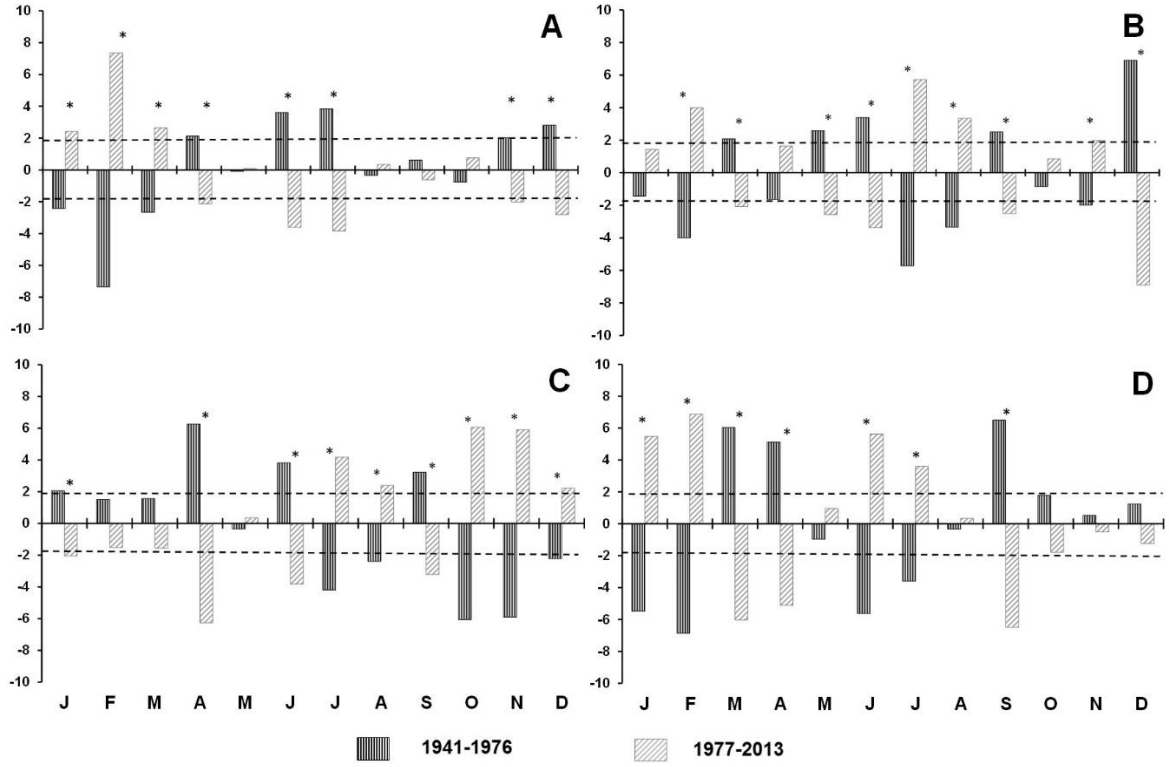


**Figure 1.** Analysis of residuals from January to December of the frequencies of Extreme Climate Events (ECE) over two periods: 1) 1941-1976 and 2) 1977-2013 in the CCB. A) Tmin lower extremes, B) Tmin upper extremes, C) Tmax lower extremes, and D) Tmax upper extremes. The asterisk (*) indicates the values above dashed lines that are significantly different to the expected value ($p<0.05$).

### 3.3. Analysis of Rainfall probability

We identified a trend of increasing rainfall in the month of June using the Mann Kendall test (Table 4); this situation was also reflected in the analysis of the probability of precipitation (Figure 2). The probability curves of the rainy months were convex rather than the concave curve typical of the more humid sites.

The calculated "r", "P" and "E" values, respectively, for the complete period (1941-2013) were 54 mm, 277 and 0.32 mm; for the first period, these values (1941-1976) were 51, 137 and 0.32 mm and for the second period (1977-2013), they were 57, 142 and 0.32 mm.

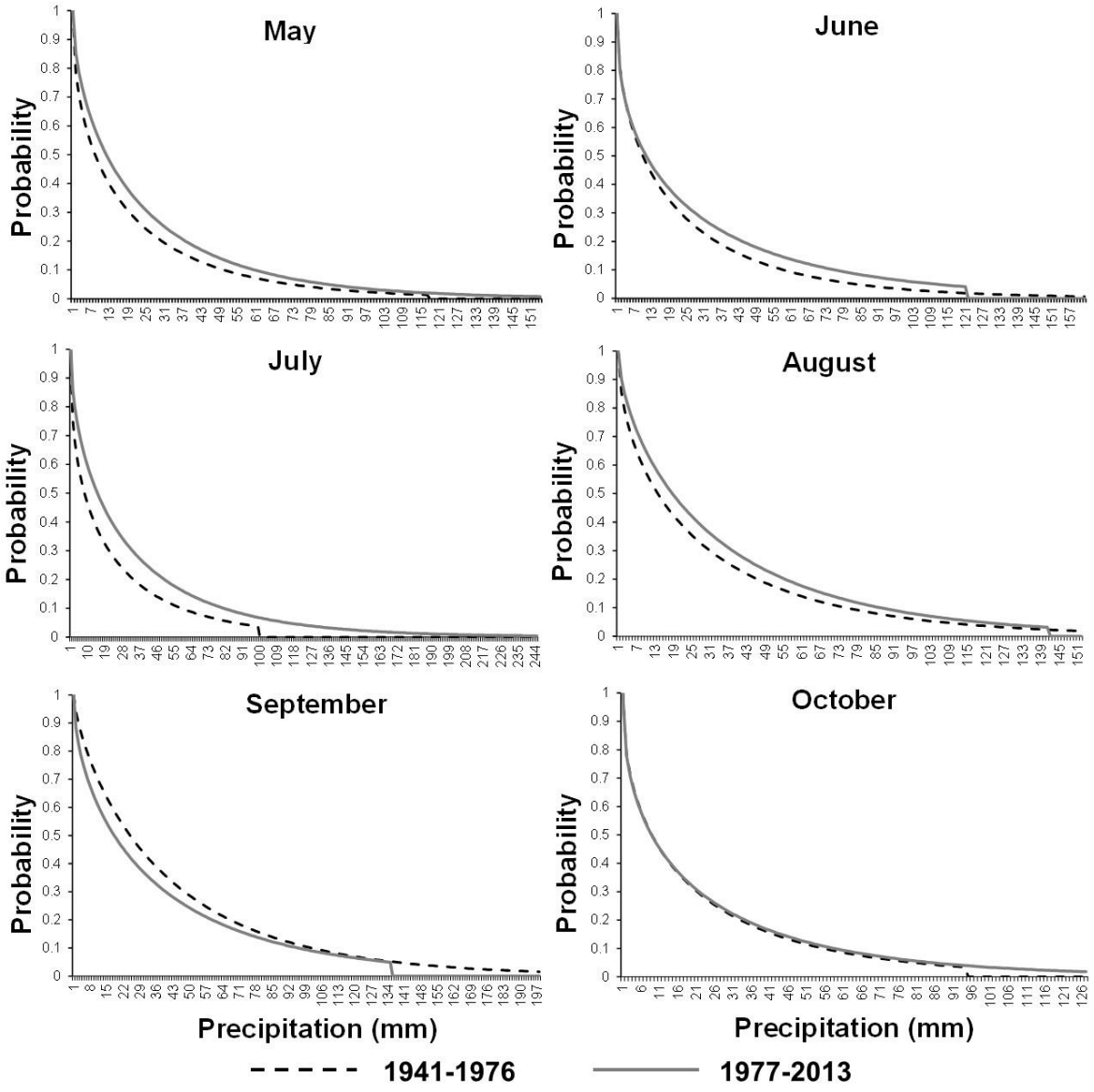


**Figure 2.** Gamma distribution density function of monthly rainfall probability for the months where changes in climate type were detected. Dotted and solid lines indicate the periods1941-1976 and 1977-2013, respectively.

### 3.4. Climate type classification for two periods

For the two defined time periods, we found a change of climate type. For the first period from 1941 to 1976, the climate classification according to Köppen was BWhw(x´)(e´). This climate is defined as very dry, semi-warm, and had an annual average temperature of 21.4 °C, the temperature of the coldest month (January) was 12.3 °C, while that of the hottest

month (July) was 28.4 °C. Rains were markedly seasonal (summer), the percentage of rains that fell in winter was 10.7%. Thermal oscillation was extreme (Figure 3a).

In the second period, from 1977 to 2013, the climate classification was BWhwx´(w)(e´). This climate is defined as very dry, semi-warm, and presented an annual average temperature of 21.9 °C. The temperature of the coldest month (January) was 12.9 ° C, while that of the hottest month (July) was 28.8 ° C. Summer rains concentrated around 90% of the annual precipitation and the percentage of winter rain was 9.3%. Thermal oscillation was very extreme (Figure 3b).

The change in the climate type classification from first to the second period was therefore mainly caused by a decrease in the temperature of the coldest month (January), an increase in the temperature of the warmest month (July), higher annual thermal oscillation and a reduction in the percentage of winter rain.

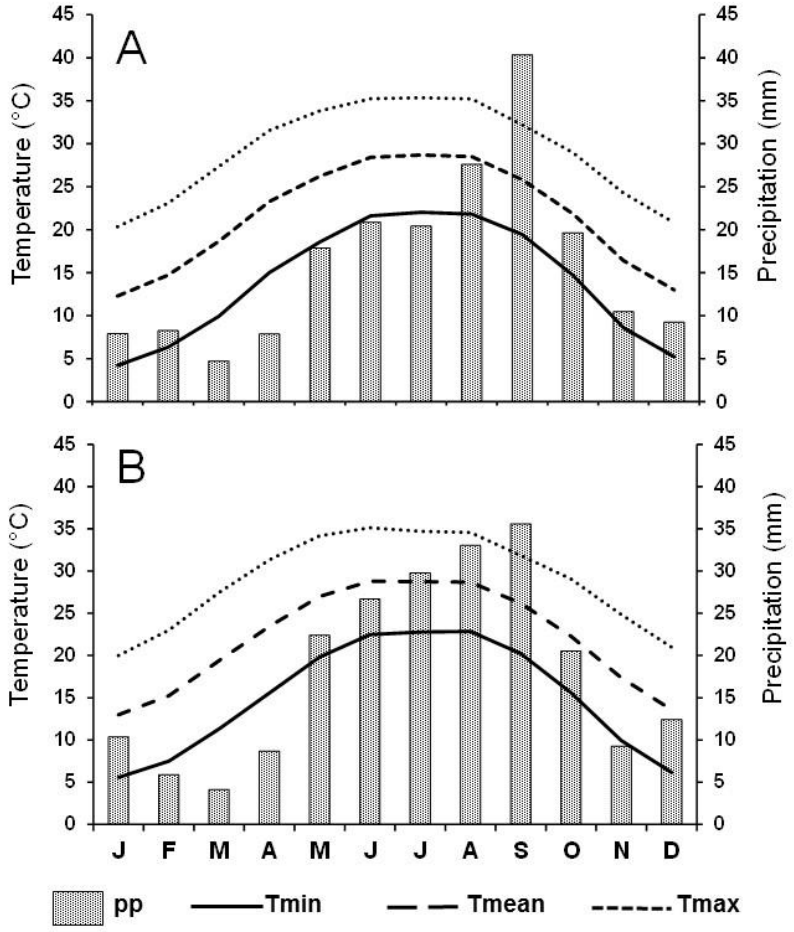


**Figure 3.** Monthly minimum, average and maximum values for temperature and precipitation recorded over two periods: a) From 1941 to 1976 and b) From 1977 to 2013.

## 4. Discussion

### 4.1. Temperature

Our working hypotheses were increases in atmospheric temperature and the frequency of temperature ECEs, as proposed for the Chihuahuan desert by Loarie et al. (2009) and by the IPCC scenarios (2012). For our study site, $T_{min}$ increased in almost all the months but $T_{mean}$ increased only in the summer months. Moreover, the frequency of lower $T_{min}$ increased in the winter months, while the frequency of upper event extremes increased during the summer months, as well as the extreme events of $T_{max}$. This means that the winters were colder and the summer months were warmer with higher $T_{min}$ and $T_{max}$ extreme events, increasing the frequency of heat waves over the last 36 years.

Positive trends of $T_{mean}$ in the summer months have also been found in other arid ecosystems, such as the Sahara desert in Libya (Mamtimin et al., 2011) and Almeria in Spain (del Río et al., 2011); while other studies have reported $T_{mean}$ increments throughout the year in other desert sites such as Jerusalem, Tripoli (Hasanean, 2001) and Iran (Tabari and Hosseinzadeh Talaee, 2011; Tabari et al., 2011).

As expected, we detected a positive trend in $T_{min}$, implying that $T_{min}$ has become warmer in recent years for most months of the year. This was found in other arid ecosystems at Iran (Ben-Gai et al., 1999; Tabari et al., 2011), Jordan (Hamdi et al., 2009) and North Carolina at the USA (Boyles and Raman, 2003), and it was also observed in other regions of the world, such as Italy (Brunetti et al., 2000) and Turkey (Türkes et al., 1996). It was also observed at global level by Easterling et al. (1997) and Vose et al. (2005). Several studies in other ecosystems (Hurrell, 1995; Easterling et al., 1997; Brunetti et al., 2000; Boyles and Raman, 2003; Vose et al., 2005) have attributed the summer increase of the Tmin in specific years to abnormalities in the ENSO combined with an increase of the positive phase of NAO and is probable that in our study site the increase in $T_{min}$ may be related with this phenomena. However, there was an increased frequency of lower extreme events for $T_{min}$ during the winter months, a finding also reported for Israel (Ben-Gai et al., 1999), Utah in the USA (Santos, 2011) and Tlaxcala in Mexico (López-Diáz et al., 2013). This result indicates that winters with colder nocturnal events in the CCB have been more frequent during the last 36 years. The increase in Tmin of winter months was explained by the negative phase of NAO during the winter months in other ecosystems (Hurrell, 1995; Easterling et al., 1997; Brunetti

et al., 2000; Boyles and Raman, 2003; Vose et al., 2005). Thus we propose that this phenomenon could also cause colder winters in specific years within in our study site.

In contrast, the frequency of the upper extremes for $T_{min}$ and $T_{max}$ during the summer increased over the last 36 years, promoting higher summer nocturnal and diurnal temperatures. Other studies in North America (Hasanean, 2001; López-Diáz et al., 2013; Peterson et al., 2013) observed that in the summer months the increase in extreme temperature was produced by El Niño events in combination with the positive phase of the Pacific Decadal Oscillation (PDO). In turn we proposed that these phenomena could also generate the changes in the upper extremes for $T_{min}$ and $T_{max}$ during the summer in our study site. These results have also been reported in other studies; for example, Alexander et al. (2006) observed a marked increase of warm nocturnal temperatures at global level throughout the year, while several authors have found a higher frequency of upper extreme temperature events of $T_{max}$ during the summer months (Ben-Gai et al., 1999; Santos, 2011; López-Diáz et al., 2013).

### 4.2. Precipitation

In the case of precipitation, our working hypothesis had been an increase in rainfall variability but we found that the precipitation did not show any temporal trend, due to the large variability of monthly rainfall. This variability obscured any trend in the frequency of extreme rainfall events. In desert ecosystems, rainfall events are scarce and very erratic, producing a skewed temporal distribution of the data (Ezcurra and Rodrigues, 1986), as was the case in our study site. It is possible that this behavior differed from the expected trend in the CCG and ECE scenarios for the Chihuahuan Desert, explained mainly by the effect of geographical barriers on climatic dynamics within CBB. This lack of a trend in precipitation throughout the year was also observed in other arid ecosystems in Israel (Modarres and Silva, 2007), Jordan (Hamdi et al., 2009) and India (Jain et al., 2013).

Jain and Kumar (2012) expected that the inter-annual variability of annual precipitation could be increased by GCC during the 21$^{st}$century, mainly as a result of the increasing frequency and intensity of ECE (D'Odorico and Bhattachan, 2012; IPCC, 2012). However, while we did not observe a significant change in the precipitation frequency of rainfall ECE, the records of annual precipitation for the second period show a higher

incidence of years with annual rainfall higher than 300 mm distributed throughout the year (9 years: 1978, 1981, 1984, 1986, 1991, 1992, 1997, 2003 and 2010), in contrast to the first period where the rainfall was concentrated in the tropical cyclone seasons (5 years: 1949, 1958, 1963, 1971 and 1976).The calculated rainfall value for a typical rainy month for CCB (1941-2013) was 54 mm, and it increases from 51 mm to 57 mm from the first to the second analyzed period. The rainfall probability was also increased in the second period. These results suggested that intensive extreme rainfall events had increased, but were obscured by rainfall variability. We also observed a higher incidence of years with rainfall below 100 mm in the second period (6 years: 1983, 1988, 1994, 1995, 2011 and 2012) compared to the first period (4 years: 1942, 1952, 1956 and 1959).The increased frequency of heavy precipitation events has been attributed to years with strong cyclones from the Gulf of Mexico, produced by a combination of La Niña and either the NAO or the PDO (Brunetti et al., 2000; Boyles and Raman, 2003; Vose et al., 2013), while drought events have been associated in other ecosystems with warm subtropical anticyclones attributed to the coincidence of El Niño with either the NAO or the PDO (Brunetti et al., 2000; Boyles and Raman, 2003; Peterson et al., 2013). Unfortunately, the incidences of intensive ENSO or NAO abnormalities have increased in recent decades (IPCC, 2013), promoting rainfall variability.

In arid and semi-arid regions such as the CCB, changes in temperature and precipitation will affect environmental water balances, increasing the water stress experienced by organisms and leading to a significant reduction in ecosystem productivity. Unfortunately, our results suggest that winters will become colder and summers will become warmer with a high variability in the availability of water in CCB, increasing the environmental stress for organisms. For this reason, is very important to fully understand how climate is changing in order to design appropriate management strategies for adapting to such climate variability in the near future. Moreover, local climate studies, such as the present study, are critical for the calibration and development of global scenarios under GCC (Tabari and Hosseinzadeh, Talaee 2011).

## 5. Conclusions

We observed a global climate change footprint in the desert of Cuatro Ciénegas Basin Mexico. At CCB, $T_{min}$ increased in almost all the months of the study period, but $T_{mean}$

increased only in the summer months. The frequency of lower $T_{min}$ increased for the winter months, while the frequency of upper event extremes increased during the summer months, as did the extreme events of $T_{max}$. This implies that the winters have become colder and the summer months warmer, increasing the frequency of heat waves over the last 36 years. Monthly rainfall showed high variability, which obscured any potential trend in the frequency of extreme rainfall events; nevertheless, over the last 36 years, frequencies of events of both intensive rainfall associated with tropical cyclones and intense drought probably associated with ENSO were higher than before. As a consequence, the organisms within CCB are expected to face higher levels of environmental stress.

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# CAPÍTULO III

## THE RESPONSE OF SOIL MICROBIAL COMMUNITIES TO VARIATION IN ANNUAL PRECIPITATION DEPENDS ON SOIL NUTRITIONAL STATUS IN AN OLIGOTROPHIC DESERT

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# The response of soil microbial communities to variation in annual precipitation depends on soil nutritional status in an oligotrophic desert

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## ABSTRACT

**Background**. Soil microbial communities (SMC) play a central role in the structure and function of desert ecosystems. However, the high variability of annual precipitation could results in the alteration of SMC and related biological processes depending on soil water potential. The nature of the physiological adjustments made by SMC in order to obtain energy and nutrients remains unclear under different soil resource availabilities in desert ecosystems. In order to examine this dynamic, the present study examined the effects of variation in annual precipitation on physiological adjustments by the SMC across two vegetation-soil systems of different soil organic matter input in an oligotrophic desert ecosystem.

**Methods**. We collected soil samples in the Cuatro Ciénegas Basin (Mexico) under two vegetation covers: rosetophylous scrub (RS) and grassland (G), that differ in terms of quantity and quality of organic matter. Collections were conducted during the years 2011, 2012, 2013 and 2014, over which a noticeable variation in the annual precipitation occurred. The ecoenzymatic activity involved in the decomposition of organic matter, and the concentration of dissolved, available and microbial biomass nutrients, were determined and compared between sites and years.

**Results**. In 2011, we observed differences in bacterial taxonomic composition between the two vegetation covers. The lowest values of dissolved, available and microbial nutrients in both cover types were found in 2012. The G soil showed higher values of dissolved and available nutrients in the wet years. Significant positive correlations were detected between precipitation and the ratios Cmic:Nmic and Cmic:Pmic in the RS soil and Cmic:Pmic and Nmic:Pmic in the G soil. The slopes of the regression with Cmic and Nmic were higher in the G soil and lower in the RS soil. Moreover, the SMC under each vegetation cover were co-limited by different nutrients and responded to the sum of water stress and nutrient limitation.

**Discussion**. Soil community within both sites (RS and G) may be vulnerable to drought. However, the community of the site with lower resources (RS) is well adapted to acquire P resources by ecoenzyme upregulation during years with adequate precipitation, suggesting that this community is resilient after drought occurs. Under

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the Global Climate Change scenarios for desert ecosystems that predict reduced annual precipitation and an increased intensity and frequency of torrential rains and drought events, the soil microbial communities of both sites could be vulnerable to drought through C and P co-limitation and reallocation of resources to physiological acclimatization strategies in order to survive.

**Subjects** Biodiversity, Ecology, Ecosystem Science, Microbiology, Soil Science
**Keywords** Nutrients, Enzymes, Chihuahuan desert, Microbial physiological adjustments, Stoichiometry ratio, Threshold elemental ratio

## INTRODUCTION

In desert ecosystems, precipitation is highly variable among years and this variability has increased in recent years due to the effect of Global Climate Change (GCC) (*Bell et al., 2014*; *IPCC, 2013*). The scenarios derived from GCC models for desert ecosystems predict reduced annual precipitation, as well as increases in the annual precipitation variability by the end of the 21st century, including an increase in the frequency and intensity of both torrential rain and drought events (*Holmgren et al., 2006*; *IPCC, 2013*). The high variability of annual precipitation projected for desert ecosystems could alter biological processes dependent on soil water potential, as is the case with the processes related to soil organic matter (SOM) decomposition (*D'Odorico & Bhattachan, 2012*; *Fay et al., 2008*; *Thomey et al., 2011*). For example, enzymatic activity stimulated by rainfall in desert ecosystems may result in most of the total annual mineralization that occurs in desert soils (*Manzoni, Schimel & Porporato, 2012*). However when soil water potential decreases, the metabolic activity of most soil microbial species is reduced, and thus a decline in nutrient mineralization can occur. Additionally, soil drying reduces enzymatic activity and microbial mobility, which reduces substrate supply for the decomposers (*Henry, 2013*; *Manzoni, Schimel & Porporato, 2012*). Likewise, studies in a semiarid region in New Mexico (*Cregger et al., 2012*) and in the Chihuahuan Desert (*Bell et al., 2009*; *Bell et al., 2014*) showed that the high precipitation variability significantly altered the structure of the soil microbial community, mainly due to a change in the fungal/bacterial ratio and consequently altered microbial community functional dynamics.

Microbial communities play a central role in the structure and functioning of desert ecosystems since they represent an important pool of soil C, N, and P. Indeed, it has been suggested that the amount of N and P contained within the soil microorganism biomass is comparable to the N and P content within the plant biomass in desert ecosystems (*Coleman & Whitman, 2005*). Moreover, microbial communities can help accelerate the transformation of molecules containing C, N, and P by producing soil extracellular enzymes (ecoenzymes) (*Sinsabaugh & Follstad Shah, 2012*; *Sinsabaugh, Hill & Shah, 2009*) that lead to the fragmentation, depolymerization and mineralization of organic matter (*Singh et al., 2014*). Microorganisms can only assimilate soluble organic compounds of a molecular weight lower than 1 kDa and must therefore break down, or depolymerize, most of the organic matter molecules (where between 72 and 87% of the DOC in grassland soils is

larger than 1 kDa) in order to access the nutrients and energy contained within the organic molecules (*Cregger et al., 2012*; *Farrell et al., 2014*; *Jones et al., 2012*). The microorganisms produce hydrolytic or oxidative ecoenzymes that degrade organic matter, producing assimilable dissolved organic nutrients that are rapidly immobilized within their biomass (*Conant et al., 2011*; *Sinsabaugh & Follstad Shah, 2012*). Additionally, in desert ecosystems, the natural distribution of different vegetation types can produce spatial heterogeneity in the quantity and quality of organic matter (*Austin et al., 2004*; *Housman et al., 2007*). In these ecosystems, the depolymerization process will therefore require the production of different ecoenzymes, since the organic matter under each vegetation type contains a particular combination of structurally simple and complex molecules that promote differences in the soil nutrient dynamics mediated by the microbial community (*Conant et al., 2011*). However, the complete organic matter decomposition process requires a chain of enzymatic reactions where each ecoenzyme acts on a different substrate and is produced by different microbial groups (*Ekschmitt et al., 2005*). Additionally, the soil microbial communities can exhibit functional redundancy in the ecoenzyme production (*Allison & Martiny, 2008*).

Soil microorganisms have developed mechanisms of physiological acclimatization to cope with precipitation variability (*Schimel & Schaeffer, 2012*). These mechanisms generate physiological costs for the microbial community that derive from the need for high investments of energy (C) and nutrients (N and P) in order to survive (*Classen et al., 2015*; *Schimel, Balser & Wallenstein, 2007*; *Schimel & Schaeffer, 2012*). This high demand for energy (C) and nutrients (N and P) can be offset by reallocation of these resources, generating a trade-off in which the microbial community invests C, N and P in either growth or survival (*Evans & Wallenstein, 2012*; *Schimel, Balser & Wallenstein, 2007*). Some consequences of such resource redirection are: (1) a limited production of ecoenzymes for nutrient acquisition (i.e., for SOM decomposition) (*Burns et al., 2013*; *Henry, 2013*; *Steinweg et al., 2013*) and (2) reduced growth of the microbial community (i.e., decreased protein synthesis) (*Schimel, Balser & Wallenstein, 2007*). Resource reallocation increases the vulnerability of some microbial groups that produce a change in the structure and function of the soil microbial community also affecting the energy flow (C) and nutrient dynamics of N and P at the ecosystem level (*Esch, Lipson & Cleland, 2017*; *Evans & Wallenstein, 2012*; *Thibault & Brown, 2008*). This variability strongly affects microbial community development in resource-limited environments, because the adaptation rates of microbial species are constrained by the resource cost of physiological adjustment (*Wallenstein & Hall, 2012*). *Wallenstein & Hall (2012)* proposed that sites limited by nutrients are more vulnerable to annual rainfall variability, because the microbial community must invest energy in nutrient acquisition, and consequently reducing its capacity for adaptation required by fluctuation in water availability. Sites with low resource availability could be therefore more vulnerable to annual precipitation variability.

The Chihuahuan desert has been classified as one of the most biologically outstanding habitats globally by the World Wildlife Fund (*Archer & Predick, 2008*). The Cuatro Ciénegas Basin (CCB), which is the study site of the present investigation, is part of the Chihuahuan desert and is considered the most important wetland of Mexico for its high levels of

endemism and biodiversity (*Souza et al., 2011*). Moreover, the CCB has been listed as an ultra-oligotrophic site due to low P concentrations in the water and soil, which can constitute a strong potential for P limitation of microbial growth (*Elser et al., 2005*; *Tapia-Torres et al., 2015a*). A study in the CCB desert reported that, in the same soil type with different vegetation cover (grassland and desert scrub) differences in OM content promotes variation in DOC concentration, which represents the main energy source for soil microorganisms (*Tapia-Torres et al., 2015b*). The higher DOC concentration under grassland soil compared to desert scrub soil favored a higher microbial N immobilization and a higher C availability, therefore significantly reducing soil N losses (*Tapia-Torres et al., 2015b*). Another study in the CCB that compared two sites with different soil moisture content showed that the site with the highest moisture content and concentration of DOC also exhibited higher $NH_4^+$, microbial C and N concentrations, and also presented higher diversity, richness and evenness of soil bacterial community compared to the dry site (*López-Lozano et al., 2012*). Both studies suggest that differences in DOC concentration (energy availability) and microbial community composition promoted different nutrient dynamics. In the sites with organic matter providing lower DOC concentrations, the microbial communities may be co-limited by energy and nutrients and yet they must invest more energy in order to obtain the most limiting nutrients. An indicator that helps us understand how resources are reallocated by the microbial community to cope with the nutrient limitation is the combination of: (1) the stoichiometry ratios of C:N:P in the soil and microbial biomass (*Cleveland & Liptzin, 2007*) and (2) the Threshold Elemental Ratio (TER) (*Sinsabaugh & Follstad Shah, 2012*; *Tapia-Torres et al., 2015a*), which defines the element ratio at which growth is affected by nutrient limitation (represented by N and P, at high C:N or C:P) and by energy limitation (represented by C, at low C:N or C:P) (*Frost et al., 2006*; *Sterner & Elser, 2002*). The combination of stoichiometry ratios and TER indicate how resources are reallocated towards enzyme activity depending on the availability of energy (C) and nutrients (N and P) in the soil. This microbial co-limitation between energy and nutrient acquisition was also found in CCB by comparing the $TER_{C:N}$and $TER_{C:P}$ from two sites with the same vegetation cover (grassland), but different soil moisture and DOC availability values (*Tapia-Torres et al., 2015a*). The microbial communities were co-limited by C and N in the site with higher water and C availability (Churince) and were co-limited by C and P in the site with lower water and C availability (Pozas Azules). In addition, these authors argue that this limitation favors an elevated allocation of N-acquisition enzymes relative to energy/C enzymes in Churince, while for Pozas Azules, an elevated investment in ecoenzymes of P acquisition is found (*Tapia-Torres et al., 2015a*). These results support the notion that soil microbial communities can adjust their metabolism by allocating more resources (i.e., energy and production of ecoenzymes) to the accumulation of scarcer nutrients, and fewer resources to the acquisition of abundant nutrients. The ratios of C:N:P in microbial biomass are therefore constrained relative to nutrient (*Cleveland & Liptzin, 2007*) and energy availability. These studies suggest that both vegetation and soil moisture content may determine differences in: (1) soil nutrient dynamics, (2) the diversity of the soil microbial community and (3) the C:N:P ratios of the microbial biomass in this ecosystem.

To date, the physiological adjustments made by the soil microbial communities under different soil resource availability in order to obtain energy and nutrients in desert ecosystems with high precipitation variability remain unclear. To elucidate this dynamic, the present study examined the effects of rainfall variation on the physiological adjustments made in order to obtain energy and nutrients by the soil microbial community from two vegetation-soil systems with different soil organic matter inputs in an oligotrophic desert ecosystem. Our hypothesis is that, in a site with high soil resources availability, the soil microbial communities invest less energy in the acquisition of nutrients (i.e., ecoenzymatic production), favoring nutrient accumulations within the biomass (i.e., immobilization). Our predictions are: (1) in a site that presents low soil nutrient availability (rosetophylous scrub—RS), the soil microbial community will invest more energy in the production of ecoenzymes in order to depolymerize and mineralize, thus favoring nutrient availability; while in a site with high soil nutrient availability (grassland—G), the soil microbial community will invest more energy in biomass growth; and (2) in the site with greater soil resources availability (G), the microbial community will be less vulnerable to changes in precipitation. To test the hypothesis, we collected soil samples in the CCB from sites under two vegetation covers (RS and G) that differ in terms of the quantity and quality of the organic matter present. Collections were conducted during years: 2011 (February), 2012, 2013 and 2014 (September), over which a noticeable variation in annual precipitation took place. The ecoenzyme activity involved in the decomposition of organic matter, as well as the concentration of dissolved, available and microbial biomass nutrient, were determined and compared between sites and years. With the ecoenzymatic and biogeochemistry data we calculated the $TER_{C:nutrient}$, SEA, the nutrient ratios and performed regressions between the precipitation and the concentrations and ratios of C, N and P in microbial biomass.

## MATERIAL AND METHODS

### Study site

The study was carried out in the Cuatro Ciénegas Basin (CCB; 26°45′-27°00′N and 101°48′-102°17′W) in central northern Mexico, within the Chihuahuan Desert. The CCB has an area of 150,000 $km^2$, with an elevation of 740 m.a.s.l. The climate is arid with an average annual temperature of 21 °C and 252 mm of annual rainfall, which is concentrated during the summer months (http://smn.cna.gob.mx/). However in the last 30 years the annual precipitation showed a high variability among years. In this study the annual precipitation was estimated as the amount of rain accumulated 9-months before the sampling month. The precipitation data were obtained from meteorological station 5044 "Cuatro Cienegas" located at 26°59′0″N and 101°04′0″W (http://smn.cna.gob.mx/). Annual precipitation and the average temperature of the sampling months varied strongly during the four studied years: the year 2011 was the wettest year (348 mm and 25 °C), 2012 was particularly dry and hot (89 mm and 28 °C) and was followed by two wet years (217 mm and 230 mm for 2013 and 2014, respectively) with lower temperatures (24.9 and 24.8 °C for 2013 and 2014, respectively).

Jurassic-era gypsum is the dominant parent material on the western side of the basin (*McKee, Jones & Long, 1990*). According to the WRB classification (2007), the predominant

soil on the western side of the basin is *Gypsisol*. The main vegetation types are: (1) grassland (G), dominated by *Sporobolus airoides* (Torr.) Torr. and *Allenrolfea occidentalis* (S. Watson) Kuntze; (2) microphyll scrub, dominated by *Jatropha dioica* Cerv., *Larrea tridentate* (DC) Cov. and *Fouqueria sp* Kunth (*Perroni, García-Oliva & Souza, 2014*); and (3) rosetophylous scrub (RS) dominated by *Dhasylirium cedrosanum* Trel., and *Yucca treculeana* Carriére (*González, 2012*).

## Sampling

Mean air temperature for the sampling month (September) and annual rainfall data in each studied year were obtained from the meteorological station "Rancho Pozas Azules" INIFAP. Soil collection was carried out in Churince on the west side of the CCB, where *Gypsisol* is the predominant soil type (*Perroni et al., 2014*). The samples were taken from two vegetation cover types, rosetophylous scrub (RS) and grassland (G), during February (2011) and September (rainy of 2012, 2013 and 2014). For each vegetation cover, we sampled seven sites located at a distance of 140 m apart, along a one km north-to-south transect. At each sampling site, a 4 × 4 m plot was demarcated and five soil samples were taken from the first 15 cm of soil depth within the plot, and mixed to produce one compound sample per site. A total of seven composite samples were therefore obtained from each vegetation cover in each sampling year. The soil samples were stored in black plastic bags at 4 °C until subsequent laboratory analysis.

## Moisture and pH

Soil pH was measured in deionized water (soil/solution, 1:2 w:v) with a digital pH meter (Corning$^{TM}$). A subsample of 100 g was oven-dried at 75 °C to constant weight for soil moisture determination using the gravimetric method.

## Biogeochemical analyses

### *Nutrient analysis*

All Carbon (C) forms analyzed were determined with a Total Carbon Analyzer (UIC Mod. CM5012; Chicago, USA), while nitrogen (N) and phosphorus (P) concentrations were determined by colorimetric analyses, using a Bran Luebbe Auto Analyzer III (Norderstedt, Germany). Microbial P and enzymatic activity were determined by colorimetric analyses using a spectrophotometer Evolution 201 (Thermo Scientific Inc.).

### *Total nutrients*

Prior to analysis of total nutrient forms, soil samples were dried and milled with a pestle and agate mortar. Total C (TC) and inorganic C (IC) were determined by combustion and coulometric detection (*Huffman, 1977*). Organic total C (OTC) was calculated as the difference between TC and IC. For total N (TN) and total P (TP) determination, the samples were digested in a mixture of concentrated $H_2SO_4$, $H_2O_2$ (30%) and $K_2SO_4$ plus $CuSO_4$, the latter acting as a catalyst at 360 °C. Nitrogen was determined by the macro Kjeldahl method (*Bremmer, 1996*), while P was determined by the molybdate colorimetric method, following ascorbic acid reduction (*Murphy & Riley, 1962*).

### Dissolved and available nutrients and those within the microbial biomass

The dissolved, available and microbial nutrient forms were extracted from fresh field soil samples. Dissolved nutrients were extracted from 20 g of soil with deionized water after shaking for 45 min and then filtering through a Whatman No. 42 and a 0.45 μm nitrocellulose membrane (*Jones & Willett, 2006*). The filtrate was used to determine the total dissolved C (TDC), as measured with an Auto Analyzer of carbon (TOC CM 5012) module for liquids (UIC-COULOMETRICS). Inorganic dissolved C (IDC) was determined in an acidification module CM5130. One aliquot of the filtrate was used to determine ammonium ($DNH_4^+$) and dissolved inorganic P (DIP) in a deionized water extract. Total dissolved N and P (TDN and TDP, respectively) were digested in a mixture of concentrated $H_2SO_4$, $H_2O_2$ (30%) at 250 °C. Nitrogen was determined by the macro Kjeldahl method (*Bremmer, 1996*), while P was determined by the molybdate colorimetric method, following ascorbic acid reduction (*Murphy & Riley, 1962*). Dissolved organic C, N and P (DOC, DON and DOP respectively) values were calculated as the difference between the total dissolved forms and the inorganic dissolved forms.

Available inorganic nitrogen forms ($NH_4^+$ and $NO_3^-$) were extracted from 10 g of soil with 2M KCl, followed by filtration through a Whatman No. 1 paper filter, and determined colorimetrically by the phenol-hypochlorite method (*Technicon, 1977*). Available inorganic phosphorous (Pi) was extracted with 0.5 M $NaHCO_3$, pH 8.5 (*Tiessen & Moir, 2008*) and determined colorimetrically using the molybdate-ascorbic acid method (*Murphy & Riley, 1962*).

Carbon (Cmic) and N (Nmic) concentrations within the microbial biomass were determined from 20 g of soil by the chloroform fumigation extraction method (*Vance, Brookes & Jenkinson, 1987*). Fumigated and non-fumigated samples were incubated for 24 h at 25 °C and constant relative humidity. Cmic and Nmic were extracted from fumigated and non-fumigated samples with 0.5 $MK_2SO_4$, filtered through a 0.45 μm nitrocellulose membrane (*Brookes, Powlson & Jenkinson, 1984*). Carbon concentration was measured from each extract, as the total (TC) and inorganic (IC) carbon contents, using the method described before. The difference between TC and IC was used for Cmic calculation. To determine the Nmic concentration one aliquot of the filtrate extracted was acid digested and determined as TN by Macro-Kjeldahl method (*Brookes, Powlson & Jenkinson, 1984*). Phosphorus within microbial biomass (Pmic) was extracted from 5 g of soil by the chloroform fumigation extraction and incubation method (*Vance, Brookes & Jenkinson, 1987*). Pmic was extracted using $NaCO_3$ 0.5M, pH 8.5 and digested in a mixture of $H_2SO_4$ 11N and $(NH_4)_2S_2O_8$ at 50%, with the latter acting as a catalyst at 120 °C (*Lajtha et al., 1999*). Pmic was determined colorimetrically by the molybdate-ascorbic acid method (*Murphy & Riley, 1962*). The values of Cmic, Nmic and Pmic were calculated as the difference between fumigated and non-fumigated samples using correction factors of $K_{EC}$ 0.45 (*Joergensen, 1996*), $K_{EN}$ 0.54 (*Joergensen & Mueller, 1996*) and $K_P$ 0.4 (*Lajtha et al., 1999*) for Cmic, Nmic and Pmic, respectively. Finally, the values of Cmic, Nmic and Pmic were corrected to a dry soil basis.

## Molecular analysis

Bacterial composition analysis was performed on the samples from the wettest year (2011). We extracted DNA from each soil sample using the methodology described in *López-Lozano et al. (2013)* and sent it to J. Craig Venter Institute (JCVI) in order to construct a 16S library using 454 ROCHE tag, 50,000 reads per site of 500 bp and primers 341F-926R. Sequences were trimmed and chimeras eliminated using JCVI protocols. Taxa were assigned using Blast via JCVI pipeline, these methods are detailed by *Tanenbaum et al. (2010)*.

## Ecoenzyme activity analyses

The activities of six ecoenzymes (extracellular enzymes) involved in the cleavage of organic molecules with C, N and P were measured: $\beta$-1,4-glucosidase (BG), cellobiohydrolase (CBH), $\beta$-1,4-N-acetylglucosaminidase (NAG), polyphenol oxidase (PPO), phosphomonoesterase (PME) and phosphodiesterase (PDE), using assay techniques reported by *Tabatabai & Bremner (1969)*, *Eivazi & Tabatabai (1977)*, *Eivazi & Tabatabai (1988)*, *Verchot & Borelli (2005)* and *Johannes & Majcherczyk (2000)*.

For all ecoenzymes, we used 2 g of fresh soil and 30 ml of modified universal buffer (MUB) at pH 9 for ecoenzyme extraction. Three replicates and two control samples (soil extract with no substrate, and pure MUB with substrate) were included per assay. All ecoenzyme assays were incubated at 40 °C: the BG and CBH for 2 h, NAG for 3 h, PPO for 2.5 h, PME and PDE 1.25 h. Following the incubation period, the tubes were centrifuged at 10,000 rpm for 2 min and 750 µl of supernatant was recovered.

For all ecoenzymes with substrates containing p-nitrophenol (pNP), we diluted the supernatant in 2 ml of deionized water with 75 µl of NaOH and measured the absorbance of pNP liberated at 410 nm on an Evolution 201 spectrophotometer (Thermo Scientific Inc.). For the PPO, we used 2,2′-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) as a substrate. The resulting supernatant was measured directly at 410 nm. Ecoenzyme activities were expressed as nanomoles of pNP per gram of dry soil per hour (nmol pNP [g SDE]$^{-1}$ h$^{-1}$) for substrates containing p-nitrophenol (pNP) and $O_2$ formed per gram of dry soil per hour (nmol$O_2$ [g SDE]$^{-1}$ h$^{-1}$) for the PPO, respectively. Specific enzymatic activity was calculated using Eqs. (1)–(3) (*Chavez-Vergara et al., 2014*; *Waldrop, Balser & Firestone, 2000*):

$$\text{SEA } \mu\text{mol}/(\text{mg}C_{\text{mic}}\text{h}) = A/(\text{C}_{\text{mic}} \times 0.001) \quad (1)$$

$$\text{SEA } \mu\text{mol}/(\text{mg}N_{\text{mic}}\text{h}) = B/(\text{N}_{\text{mic}} \times 0.001) \quad (2)$$

$$\text{SEA } \mu\text{mol}/(\text{mgP}_{\text{mic}}\text{h}) = C/(\text{P}_{\text{mic}} \times 0.001) \quad (3)$$

where $A$ is the enzymatic activity of BG or CBH or PPO, $B$ is the enzymatic activity of NAG and $C$ is the enzymatic activity of PME or PDE.

## Data analysis

### *Biogeochemistry and ecoenzymatic analysis*

Soil biogeochemistry and ecoenzymatic data were subjected to a repeated measures analysis of variance (RMANOVA) (*Von Ende, 2001*). Vegetation cover types (RS and G) were considered as a between-subject factor and year (2012, 2013 and 2014), and their interaction,

were considered as within-subject factors. When RMANOVA indicated significant factor effects, mean comparisons were performed with Tukey's multiple comparisons test (*Von Ende, 2001*). Ecoenzyme activities were normalized to units per μg of available organic carbon (OC) using the DOC data corresponding to each sample (*Tapia-Torres et al., 2015a*). Data were $\log_e$-transformed prior to regression analysis in order to conform to the conventions of stoichiometric analyses and to normalize variance (*Sinsabaugh & Follstad Shah, 2012*; *Sterner & Elser, 2002*). After that, relationships between ecoenzyme activities were calculated with a type II regression, using SMATR (*R Development Core Team, 2007*).

To detect the relationship between precipitation and nutrients immobilized by microbial biomass, we applied two simple regression analyses using the annual accumulated precipitation prior to the sampling date with: (1) nutrient concentration within the microbial biomass (Cmic, Nmic and Pmic) and (2) the microbial biomass nutrient ratios (Cmic:Nmic, Cmic:Pmic and Nmic:Pmic). The data used in the regression analyses corresponded to the years 2011, 2012, 2013 and 2014.

### *Stoichiometric analyses and threshold elemental ratio*

We calculated the degree of soil community-level microbial C:N and C:P homeostasis by calculating the slope of $\log_e$ C:$N_R$ (resources) versus $\log_e$ C:$N_B$ (microbial biomass) or the slope of $\log_e$ C:$P_R$ versus $\log_e$ C:$P_B$ scatterplot (*Sterner & Elser, 2002*). Moreover, we followed *Sinsabaugh, Hill & Shah (2009)* in order to calculate the TER for C:N and C:P to relate the measured ecoenzyme activity with Ecological Stoichiometry Theory (EST) and the Metabolic Theory of Ecology (MTE), using Eqs. (4) and (5):

$$TER_{C:N} = ((BG/NAG)B_{C:N})/n_0 \quad (4)$$

$$TER_{C:P} = ((BG/PME)B_{C:P})/p_0 \quad (5)$$

where $TER_{C:N}$ and $TER_{C:P}$ are the threshold ratios (dimensionless), BG/NAG is the ecoenzymatic activity ratio for $\beta$-1,4-glucosidase and $\beta$-1,4-N-acetylglucosaminidase, BG/PME is the ecoenzymatic ratio for $\beta$-1,4-glucosidase and phosphomonoesterase, $B_{C:N}$ and $B_{C:P}$ are the C:N or C:P ratios of the microbial biomass (respectively) and $n_0$ and $p_0$ are the dimensionless normalization constants for N and $P$, respectively. These normalization constants $p_0$ and $n_0$ are the intercepts in the SMA regressions for $\log_e$ (BG) *vs.* $\log_e$ (NAG) and $\log_e$ (BG) *vs.* $\log_e$ (PME) respectively (*Tapia-Torres et al., 2015a*). For a more detailed analysis of the derivation of the equations, see *Sinsabaugh, Hill & Shah (2009)*.

## RESULTS

### Soil moisture, and pH

Regardless of vegetation cover, soil moisture was higher in 2013 and 2014 than in 2012; while the G soil had higher soil moisture than the RS soil, regardless of year (Tables 1 and 2). In the driest year (2012), soil pH was higher than in the wetter years (2013 and 2014), with an exception in the G soil in 2014 (Tables 1 and 2). Soil pH correlated with annual precipitation in both sites ($R^2 = -0.85$ and $R^2 = -0.61$ for RS and G, respectively), as well as soil moisture correlated with annual precipitation ($R^2 = 0.76$ and $R^2 = 0.88$ for RS and G, respectively).

**Table 1 Means and (standard errors) of soil nutrients and ratios in the rosetophylous scrub (RS) and grassland (G) soils over three consecutive years (2012, 2013 and 2014) in the Cuatro Ciénegas Basin, Coahuila, Mexico.** Different uppercase letters (A and B) indicate significantly different means ($P < 0.05$) between vegetation cover types (rosetophylous scrub and grassland) within the same sampling year (2012, 2013 and 2014); whereas different lowercase letters (a, b and c) indicate significantly different means ($P < 0.05$) among sampling dates within the same site.

| | Year | | | | | |
|---|---|---|---|---|---|---|
| | 2012 | | 2013 | | 2014 | |
| | RS | G | RS | G | RS | G |
| Moisture (%) | 12.7 (1.1)$^{Bc}$ | 24.6 (2.5)$^{Ab}$ | 24.6 (3)$^{Bab}$ | 43.5 (1.3)$^{Aa}$ | 16.4 (1.0)$^{Bb}$ | 37.1 (7.1)$^{Aa}$ |
| pH | 8.5 (0.06)$^{Aa}$ | 8.3 (0.04)$^{Ba}$ | 8.1 (0.03)$^{Ab}$ | 8.1 (0.02)$^{Ab}$ | 8.1 (0.02)$^{Ab}$ | 8.1 (0.1)$^{Aab}$ |
| Dissolved organic nutrient concentration | | | | | | |
| DOC ($\mu g\ g^{-1}$) | 9 (1)$^{Ab}$ | 19 (4)$^{Ac}$ | 23 (4)$^{Ba}$ | 52 (1)$^{Ab}$ | 28 (2)$^{Ba}$ | 67 (4)$^{Aa}$ |
| DON ($\mu g\ g^{-1}$) | 4.1 (0.5)$^{Bb}$ | 7.0 (0.6)$^{Ab}$ | 5.5 (0.5)$^{Bab}$ | 10.8 (0.8)$^{Aa}$ | 6.9 (0.3)$^{Ba}$ | 7.8 (0.4)$^{Aab}$ |
| DOP ($\mu g\ g^{-1}$) | 1.2 (0.2)$^{Ab}$ | 0.3 (0.3)$^{Ab}$ | 2.8 (0.2)$^{Ba}$ | 5.1 (0.2)$^{Aa}$ | 2.8 (0.2)$^{Ba}$ | 5.3 (0.5)$^{Aa}$ |
| DOC:DON | 2.3 (0.6) | 3.1 (0.6) | 4.2 (0.6) | 4.9 (0.6) | 4.0 (0.6) | 6.8 (0.6) |
| DOC:DOP | 7.9 (3.6) | 15.3 (3.6) | 8.2 (1.3) | 18.8 (1.3) | 10.3 (1.2) | 13.0 (1.1) |
| DON:DOP | 3.6 (0.4) | 6.8 (3.3) | 2.0 (0.1) | 2.1 (0.1) | 2.6 (0.4) | 1.5 (0.1) |
| Available nutrient concentration | | | | | | |
| $NH_4^+$ ($\mu g\ g^{-1}$) | 2.8 (0.2)$^{Ba}$ | 6.3 (0.5)$^{Ac}$ | 3.6 (0.2)$^{Ba}$ | 11.8 (1.1)$^{Aa}$ | 2.7 (0.4)$^{Ba}$ | 8. 9 (0.2)$^{Ab}$ |
| $NO_3^-$ ($\mu g\ g^{-1}$) | 10.4 (1.4)$^{Aa}$ | 6.7 (1.4)$^{Ba}$ | 1.7 (0.3)$^{Ab}$ | 3.2 (0.4)$^{Aab}$ | 1.7 (0.1)$^{Ab}$ | 1.0 (0.1)$^{Ab}$ |
| Pi ($\mu g\ g^{-1}$) | 1.9 (0.2) | 2.5 (0.2) | 2.9 (0.4) | 4.5 (0.4) | 3.9 (0.6) | 5.3 (0.6) |

**Notes.**
DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; $NH_4^+$, available ammonium; $NO_3^-$, available nitrate; Pi, Available inorganic phosphorus.

## Dissolved organic nutrients and available nutrients

For the two vegetation covers, the lowest values of DOC, DON and DOP were found in 2012 (Table 1). In this year, the RS and the G soils had similar DOC and DOP concentrations, while the G soil had a higher DON concentration than the scrub soil; moreover, the G soil had higher dissolved organic nutrient concentrations than in the RS soil in both 2013 and 2014 (Tables 1 and 2). Consequently, the DOC:DON ratio was lower in 2012 than in the other two years (2013 and 2014) and the RS soil had lower values than the G soils (3.5 and 4.9, respectively); the RS soil also had lower DOC:DOP ratios than the G soil (9 and 16, respectively).

The year trends of available $NH_4^+$ concentration differed between the two vegetation cover types. Available $NH_4^+$ concentration was similar over the three years in the RS soil, while G soil samples from 2012 and 2013 had the lowest and the highest $NH_4^+$ concentrations, respectively (Tables 1 and 2). However, the G soil had higher values than the RS soil in the three studied years. In contrast, the $NO_3^-$ concentration was higher in the samples collected in 2012 than those of the other two years, while the RS soil had higher $NO_3^-$ concentration than the G soil only in the 2012 samples (Tables 1 and 2). The 2012 samples had lower available P concentration than in those collected in the other two years, and the G samples had 40% higher available P concentration than the RS samples, regardless of the sampling year (4.1 and 2.9 $\mu g\ P\ g^{-1}$, respectively).

**Table 2** F-ratios and significant levels of the repeated-measures ANOVA for soil variables quantified in the rosetophylous scrub and grassland soils over three consecutive years (2012, 2013 and 2014) in Cuatro Ciénegas Basin, Coahuila Mexico.

| Parameters | Source of variation | | |
|---|---|---|---|
| | Between subjects | Within subjects | |
| | Vegetation cover | Year | Vegetation cover X Year |
| Moisture | 90.7 (<0.0001) | 49.1 (<0.0001) | 2.7 (0.08) |
| pH | 7.3 (0.02) | 28.0 (<0.0001) | 5.4 (0.01) |
| Dissolved nutrients | | | |
| DOC | 102.1 (<0.0001) | 79.2 (<0.0001) | 14.5 (<0.0001) |
| DON | 38.5 (<0.0001) | 25.1 (<0.0001) | 3.8 (0.03) |
| DOP | 14.1 (0.002) | 55.0 (<0.0001) | 13.2 (0.0001) |
| DOC:DON | 6.4 (0.02) | 11.6 (0.0002) | 2.0 (0.1) |
| DOC:DOP | 9.1 (0.01) | 0.5 (0.6) | 1.8 (0.2) |
| DON:DOP | 1.8 (0.2) | 3.0 (0.07) | 1.2 (0.3) |
| Available nutrients | | | |
| $NH_4^+$ | 236.8 (<0.0001) | 19.0 (<0.000) | 10.5 (0.0005) |
| $NO_3^-$ | 1.8 (0.1) | 47 (<0.0001) | 5.4 (0.01) |
| Pi | 14.2 (0.003) | 12.9 (0.002) | 1.1 (0.3) |

**Notes.**
DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; $NH_4^+$, available ammonium; $NO_3^-$, available nitrate; Pi, Available inorganic phosphorus.

## Microbial nutrients and ecoenzymatic activities

The highest and the lowest values of Cmic and Nmic were found in 2014 and 2012, respectively, and Cmic values of the G soil samples were 39% higher than in the RS soil samples, regardless of sampling year (254 and 184 $\mu g\ C\ g^{-1}$, respectively). This was also the case with the Nmic and Pmic concentrations, with an exception in the 2012 samples (Tables 3 and 4). In contrast, Pmic concentrations presented no differences among years within the RS samples, while the 2012 samples had lower Pmic values than was the case in the other two years, within the G samples (Tables 3 and 4). The 2014 samples had lower Cmic:Nmic than the other two years regardless of vegetation cover type (2012 and 2013), while the lowest and the highest Cmic:Pmic and Nmic:Pmic ratios were found in 2012 and 2014, respectively (Tables 3 and 4). The RS soil samples had higher Cmic:Pmic and Nmic:Pmic ratios than in the G soil samples, with an exception in the 2012 samples (Tables 3 and 4).

Significant positive correlations were observed between precipitation and immobilized nutrients within the microbial biomass (Cmic, Nmic, Pmic), in both soils. Moreover, significant positive correlations were detected between precipitation and the Cmic:Nmic and Cmic:Pmic ratios in the RS soil and the Cmic:Pmic and Nmic:Pmic ratios in the G soil. The slopes of the regression with Cmic and Nmic were higher in the G soil and lower in the RS soil (Figs. 1, 2 and Table S1).

The specificenzymatic activity of BG under both vegetation cover types was lower in the wet (2014) than in the dry year (2012; Fig. 3A, Table 4), while that of CBH in the dry year was lower than in both wet years (2013 and 2014), in both vegetation covers (Fig. 3B).

**Table 3** **Means and (standard errors) of microbial biomass nutrients, and microbial nutrient ratios in the rosetophylous scrub (RS) and the grassland (G) soils over three consecutive years (2012, 2013 and 2014) in the Cuatro Ciénegas Basin, Coahuila, Mexico.** Different uppercase letter (A and B) indicate that means differ significantly ($P < 0.05$) between vegetation cover types (RS and G) within the same sampling year (2012, 2013 and 2014); whereas different lowercase letters (a, b and c) indicate significantly different means ($P < 0.05$) among sampling dates within the same site.

| | Year | | | | | |
|---|---|---|---|---|---|---|
| | 2012 | | 2013 | | 2014 | |
| | RS | G | RS | G | RS | G |
| Nutrients concentration within microbial biomass | | | | | | |
| Cmic ($\mu g\ g^{-1}$) | 68 (12) | 93 (12) | 191 (20) | 289 (20) | 287 (16) | 379 (1) |
| Nmic ($\mu g\ g^{-1}$) | 4.2 (0.6)$^{Ab}$ | 6.4 (0.6)$^{Ac}$ | 10.0 (1.0)$^{Bb}$ | 22.0 (2.8)$^{Ab}$ | 42.2 (1.7)$^{Ba}$ | 59.8 (1.9)$^{Aa}$ |
| Pmic ($\mu g\ g^{-1}$) | 2.3 (0.6)$^{Aa}$ | 2.5 (1.3)$^{Ab}$ | 2.4 (0.1)$^{Ba}$ | 6.4 (0.6)$^{Aa}$ | 2.2 (0.02)$^{Ba}$ | 6.1 (0.4)$^{Aa}$ |
| Cmic:Nmic | 20 (4) | 15 (4) | 20 (2) | 14 (2) | 7 (0.3) | 6 (0.3) |
| Cmic:Pmic | 17 (5)$^{Ac}$ | 9 (5)$^{Ac}$ | 79 (6)$^{Ab}$ | 48 (6)$^{Bb}$ | 127 (0.2)$^{Aa}$ | 63 (4)$^{Ba}$ |
| Nmic:Pmic | 0.9 (0.3)$^{Ac}$ | 0.4 (0.2)$^{Ac}$ | 4.2 (0.4)$^{Ab}$ | 3.6 (0.6)$^{Ab}$ | 18.7 (0.8)$^{Aa}$ | 10.1 (0.7)$^{Ba}$ |

**Notes.**
Cmic, microbial carbon; Nmic, microbial nitrogen; Pmic, microbial phosphorus.

**Table 4** **F-ratios and significant levels of the repeated measures ANOVA for microbial nutrient concentration, microbial nutrient ratios and specific enzymatic activity quantified in the rosetophylous scrub (RS) and the grassland (G) soils over three consecutive years (2012, 2013 and 2014) in Cuatro Ciénegas Basin, Coahuila Mexico.**

| Parameters | Source of variation | | |
|---|---|---|---|
| | Between subject | Within subjects | |
| | Vegetation cover | Year | Vegetation cover X Year |
| Dissolved nutrients | | | |
| Cmic | 62.1 (<0.0001) | 93.3 (<0.0001) | 2.3 (0.11) |
| Nmic | 48.7 (<0.0001) | 484 (<0.0001) | 12.9 (0.0001) |
| Pmic | 24.6 (0.0003) | 5.8 (0.008) | 5.7 (0.009) |
| Cmic:Nmic | 4.0 (0.07) | 12.3 (0.0002) | 0.7 (0.5) |
| Cmic:Pmic | 107 (<0.0001) | 92 (<0.0001) | 11 (0.0005) |
| Nmic:Pmic | 42 (<0.0001) | 316 (<0.0001) | 34 (<0.0001) |
| Specific enzymatic activity | | | |
| BG | 1.2 (0.28) | 22.8 (<0.0001) | 1.1 (0.33) |
| CBH | 3 (0.1) | 9.9 (<0.0001) | 0.2 (0.7) |
| NAG | 8.1 (0.01) | 52 (<0.0001) | 10.8 (<0.0001) |
| PPO | 8.8 (0.011) | 34 (<0.0001) | 4 (0.03) |
| PME | 137 (<0.0001) | 444 (<0.0001) | 80 (<0.0001) |
| PDE | 67 (<0.0001) | 232 (<0.0001) | 19 (<0.0001) |

**Notes.**
Cmic, microbial carbon; Nmic, microbial nitrogen; Pmic, microbial phosphorus; BG, β-1,4-glucosidase; CBH, cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; PPO, polyphenol oxidase; PME, phosphomonoesterase; PDE, phosphodiesterase.

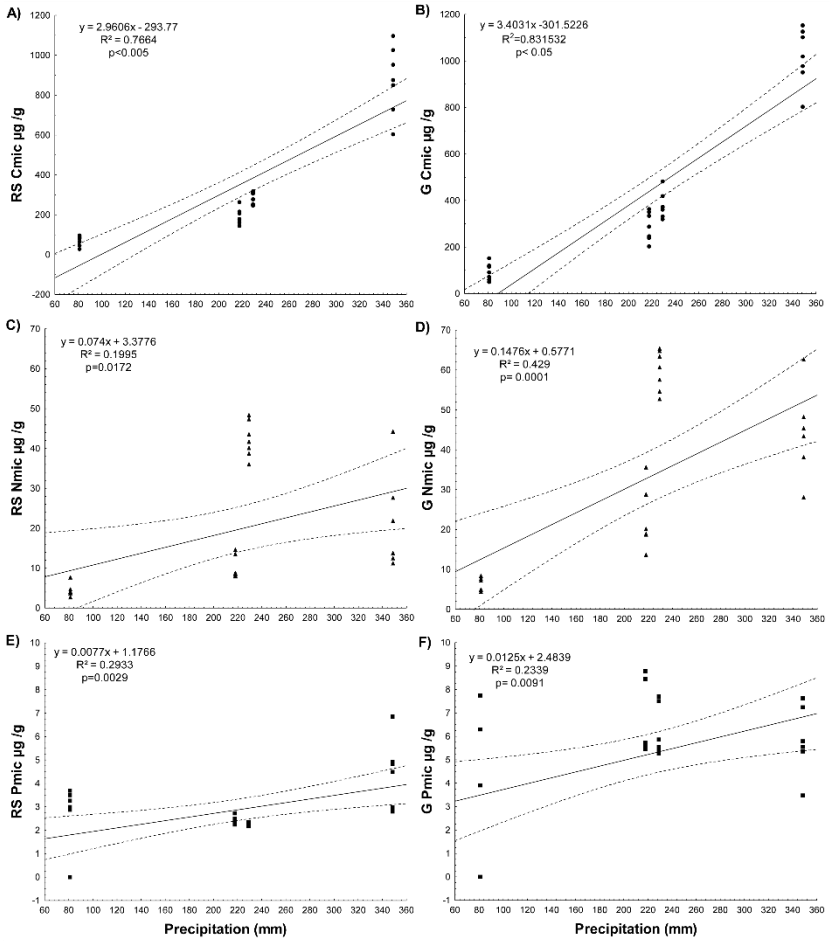


**Figure 1 Simple linear regressions between annual accumulated precipitation before the sampling date for four years and nutrients immobilized by microbial biomass for RS soil and G soil.** The dotted line represents the standard deviation at 0.95.

Full-size DOI: 10.7717/peerj.4007/fig-1

The specific enzymatic activity of the PPO in the scrub soil did not differ among years, while the dry year (2012) had lower values than the wet years (2013 and 2014) in the G soil (Fig. 3C and Table 4). Furthermore, the G soil had higher specific PPO enzimatic activity than the RS soil in the wet year (2014). In contrast, the wet year (2014) had the lowest NAG specific enzymatic activity under both vegetation cover types, and the RS soil had lower values only in the dry year (2012; Fig. 3D). The specific enzymatic activity of

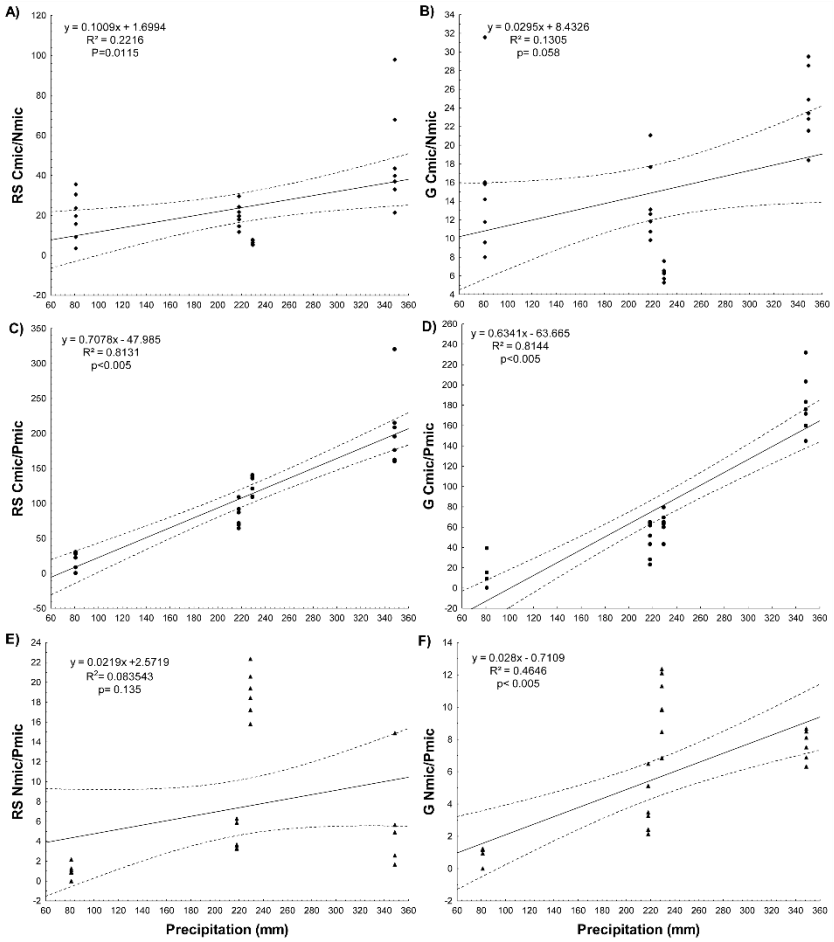


**Figure 2** **Simple linear regressions between the annual accumulated precipitation before the sampling date for four years and ratios of nutrients immobilized by microbial biomass for RS soil and G soil.**
Full-size DOI: 10.7717/peerj.4007/fig-2

PME and PDE was similar, and the lowest values of specific enzymatic activity were in the driest year (2012). In the two wet years, the RS soil presented higher specific activities than the G soil (Figs. 3E and 3F).

## Soil bacterial composition

Even at 97% similarity, a very high diversity was found, encompassing all the known phyla of bacteria but a very low diversity and abundance of Archaea. A total of 46,898 sequences were obtained for the RS soil and 9,979 for the G soil, comprising 24 phyla. We

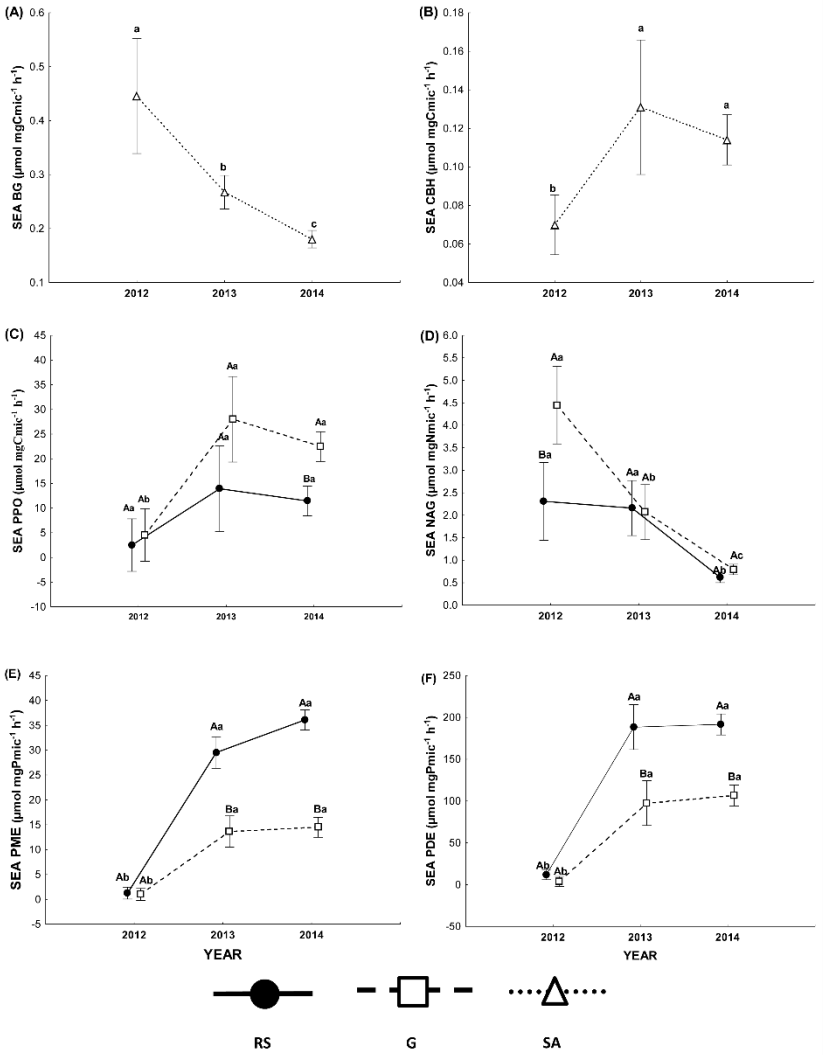


**Figure 3** **Specific enzymatic activity.** (A) $\beta$-1,4-glucosidase (BG), (B) cellobiohydrolase (CBH), (C) polyphenol oxidase (PPO), (D) $\beta$-1,4-N-acetylglucosaminidase (NAG), (E) phosphomonoesterase (PME) and (F) phosphodiesterase (PDE) in the rosetophylous scrub (RS) and grassland (G) soils over three consecutive years (2012, 2013 and 2014) in the Cuatro Ciénegas Basin, Coahuila, Mexico. Different uppercase letters (A and B) indicate significantly different means ($P < 0.05$) between vegetation cover types (RS and G) within the same sampling year (2012, 2013 and 2014); whereas different lowercase letters (a, b and c) vertically indicate significantly different means ($P < 0.05$) among sampling dates within the same site.
Full-size DOI: 10.7717/peerj.4007/fig-3

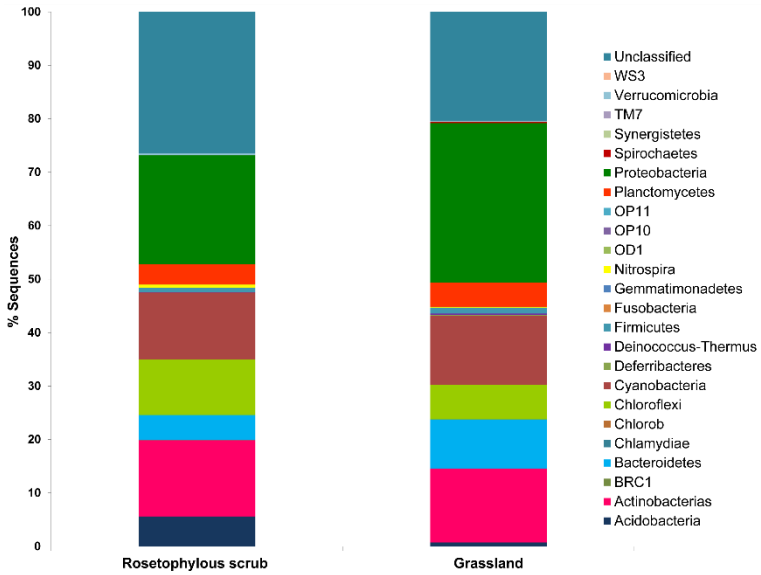


**Figure 4** **Taxonomic distribution of sequences obtained from Pyrosequencing of 16S rRNA tags of rosetophylous scrub and grassland soils during a wet year (2010).**
Full-size DOI: 10.7717/peerj.4007/fig-4

observed a high number of unclassified bacteria; 26% for the RS soil and 20% for the G soil (Fig. 4). In the two vegetation cover types, the Proteobacteria was the most abundant bacterial phylum, accounting for 20% in the RS soil and 30% in the G soil. Similarly, Actinobacteria was the second most dominant phylum in the RS soil and in the G soil, with an abundance of 14% in both soils. Interestingly, the Cyanobacteria was the third most dominant phylum, with 13% of abundance both soils, suggesting the importance of the desert crust in both sites. Other important phyla observed were: Chloroflexi (10%), Bacteroidetes (5%), Plantomycetes (4%), Firmicutes (4%), Nitrospira (1% in the RS and 0.5% in the G soils) and Acidobacteria (6% in RS and 0.8% in G; Fig. 4).

## Ecoenzymatic stoichiometry, homeostasis and threshold elemental ratios

In all of the model II regressions analyzed, there were no differences found in slopes between soils of the two vegetation cover types within sampling years (Figs. S1 and S2). To test the strength of stoichiometric homeostasis, we analyzed for associations between microbial biomass elemental ratios and those in the soil resources (*Tapia-Torres et al., 2015a*). In both soil vegetation cover types, the relationships between log C:$N_R$ and log C:$N_B$, and between log C:$P_R$ and log C:$P_B$ did not differ from zero ($p > 0.05$), regardless of year (Figs. S1 and S2); indicating strong community-level elemental homeostasis in the soil of both sites.

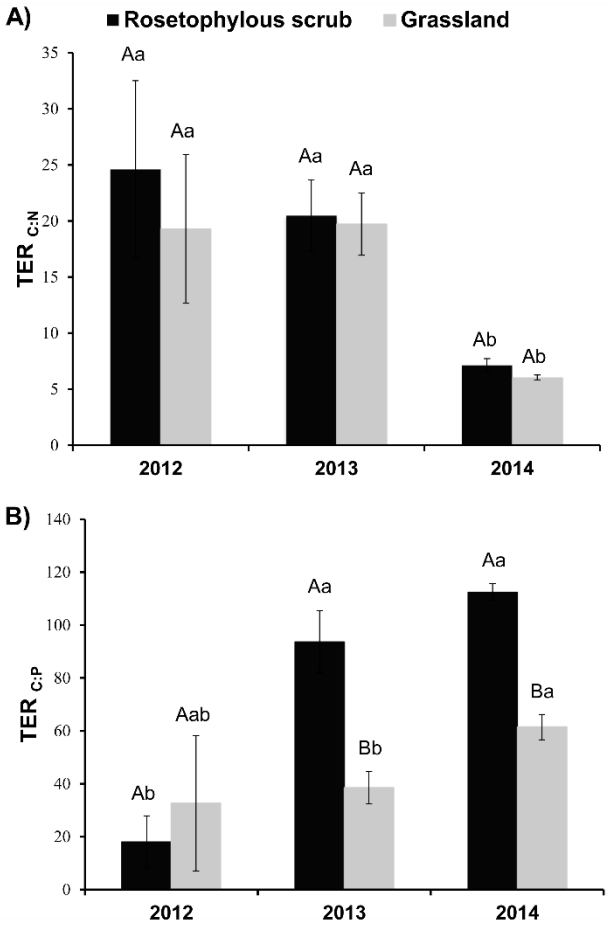


**Figure 5** **Threshold Elemental Ratio C:N and C:P (A and B, respectively) of the soil microbial community over three consecutive years (2012, 2013 and 2014).**

Full-size DOI: 10.7717/peerj.4007/fig-5

Moreover, we used the parameters generated from the type II regressions using enzymatic data and microbial C:N:P stoichiometric values to estimate $TER_{C:N}$ and $TER_{C:P}$ values. The lowest $TER_{C:N}$ values were observed in 2014 (wet year), but no differences were observed between 2012 and 2013, or even between vegetation cover types (RS and G) among study years (Fig. 5). The opposite was found for $TER_{C:P}$, where we obtained the lowest value in the dry year (2012), but only in the RS soil. For the dry year (2012), no differences were observed between vegetation cover types, while we observed lower $TER_{C:P}$ values in the G soil than in the RS soil for the wet years (2013 and 2014; Fig. 5).

## DISCUSSION

### Reallocation of resources by the soil microbial community

Our first prediction, that the soil microbial community invests more energy in the production of ecoenzymes to acquire nutrients in sites of low resource availability, such as the RS soil, was confirmed. We observed that the RS soil showed a lower concentration of available P than the G soil in the three years studied and, consequently, the RS soil microbial community invested more energy in the acquisition of P (increased enzymatic activity of phosphomonoesterase and phosphodiesterase) than the G soil microbial community only during the two wet years. In contrast, the G soil had higher Cmic, Nmic and Pmic concentrations and lower enzymatic activity of phosphomonoesterase and phosphodiesterase than the RS soil during both wet years, which also supports our prediction (Fig. 1 and Table 3). These results suggest that the microbial community in the RS soil, with lower resource availability, must reduce growth as a result of: (1) the physiological cost associated with a low reallocation to P-rich ribosomal RNA, as suggested by the growth rate hypothesis (GRH) (*Sinsabaugh & Follstad Shah, 2012*; *Sterner & Elser, 2002*; *Zechmeister-Boltenstern et al., 2015*) and (2) the required investment of energy towards the acquisition of P in order to produce ecoenzymes (*Evans & Wallenstein, 2012*; *Schimel, Balser & Wallenstein, 2007*; *Wallenstein & Hall, 2012*). The microbial C:N:P ratio was greater in the RS soil (127:19:1) than in the G soil (63:10:1), suggesting that the microbial community in the former site is more P-constrained (*Cleveland & Liptzin, 2007*). The studied soils are characterized by low P availability and a high capacity for P occlusion within inorganic molecules, mainly by Ca-bound (*Perroni et al., 2014*). Therefore, the main source of available P is mineralization of organic P mediated by phosphatase activity (*Waring, Weintraub & Sinsabaugh, 2014*). Among organic P molecules, phosphodiester forms are the preferred substrate in P-limited ecosystems (*Karl, 2014*; *Tapia-Torres et al., 2016*), although phosphomonoester forms may also be an important source of available P in most soils (*Turner, Mahieu & Condron, 2003*). In our study sites, phosphodiesterase activity was almost ten times higher than that of phosphomonoesterase, mainly in the scrub soil, suggesting mineralization of phosphodiesters as the main source of soil available P. Several bacteria isolates from CCB soils prefer to grow in DNA as a P source, associated with phosphodiesterase activity (*Tapia-Torres et al., 2016*). We suggest that the main P source in sites with low nutrient availability, such as the RS soil, is recycling of the organic molecules that are the product of cellular lysis.

However, the G soil had higher enzymatic activity of polyphenol oxidase in the wet year 2014 than was the case in the RS soil. This result is consistent with other studies (*Sinsabaugh, 2010*; *Sinsabaugh & Follstad Shah, 2011*), which have reported that polyphenol oxidase activity does not present the same behavior as the $\beta$-1,4-glucosidase and other hydrolases that degrade labile C. Microbial community size begins to be limited by the availability of labile C, which produces a change in the microbial community composition towards microbial guilds with lower growth rates (low concentration of Cmic), but with the capacity to produce polyphenol oxidase to break down structurally complex molecules and obtain C (*Moorhead & Sinsabaugh, 2006*). This situation is comparable to the conditions of the G

soil in the wet year 2014, where the microbial community was required to cleave lignin in order to maintain its growth rate.

Furthermore, the differences in soil nutrient dynamics between both sites can be strongly affected by soil microbial composition. While analyses of soil bacterial composition were only determined for 2011, this year presented the highest soil water availability and also showed higher concentrations of Cmic, Nmic, and Pmic than in the other studied years. Several studies (*Nemergut et al., 2010*; *Philippot et al., 2009*) have reported that heterotrophic decomposition depends on the relative abundance of specific taxa because different species process organic matter at different rates, even under similar soil conditions. The G soil had a higher proportion of Proteobacterias, Actinobacterias and Bacteroidetes than the RS soil, and some species of these taxa have the capacity to produce $\beta$-glucosidase (BG) (*Moreno et al., 2013*), cellobiohydrolase (CBH), poliphenoloxidase (PPO) (*Kirk & Farrell, 1987*), glucanases and glycosidases (*Xie et al., 2007*), which act to cleave C molecules.

In contrast, the scrub soil had higher proportion of Acidobaterias and Firmicutes, including species with the capacity for producing enzymes for P mineralization (*Koch et al., 2008*; *Tan et al., 2013*). Moreover, the acidobacterias of the RS soil could contribute to the release of unavailable P through organic acid release (*Tan et al., 2013*) and, together with Firmicutes, can mineralize P via the production of phosphatases, as has been observed in isolates of acidobacterias from substrates with low C concentrations (*Koch et al., 2008*; *Tan et al., 2013*). Chloroflexi was present in a higher proportion in the RS than in the G soil, but both soils had a similar proportion of Cyanobacteria suggesting that the amount of microbial desert crust is similar in both sites. Both phyla are facultative autotrophic bacteria (*Smith, 1983*) and therefore have the capacity to fix atmospheric C and to produce ecoenzymes for depolymerization and mineralization of C (*Berg et al., 2010*; *Mitsui et al., 1986*; *Smith, 1983*). The Cyanobacteria also have the capacity to fix atmospheric N. Fixation in the microbial biomass of C and N by these taxa could represent an important input of both nutrients to the soil (*Mitsui et al., 1986*; *Smith, 1983*). *Wallenstein & Hall (2012)* proposed that sites limited by nutrients are more vulnerable to rainfall variability, because the microbial community must invest energy in nutrient acquisition, thus reducing its capacity for adaptation required by fluctuation in water availability. We proposed that sites with low resources availability, such as the RS soil, could be thus more vulnerable to annual precipitation variability.

### Resilience in the face of precipitation changes

Our second prediction, that the microbial community will be more vulnerable to variability in precipitation in the site with lower soil resources (RS), was not confirmed because the soil community was resilient to soil P coinstrains by ecoenzyme upregulation during times of adequate moisture. In both vegetation cover types, nutrient availability increased with increased precipitation. The correlation between precipitation and the Cmic, Nmic and Pmic, indicate that a higher amount of rainfall favored the microbial immobilization of these nutrients under both vegetation cover types. Nevertheless, compared to the RS soil, the G soil showed steeper slopes in regressions between the precipitation and the concentrations of Nmic and ratios of Cmic:Pmic and Nmic:Pmic (Table S1), suggesting

that the microbial community of the grassland soil has the ability to immobilize more N within its microbial biomass and more rapidly than the microbial community of the RS soil. Positive correlations between Cmic and rainfall have been reported for an oak forest (*Baldrian et al., 2010*) and a semiarid grassland (*Zhou et al., 2013*), but a correlation between precipitation with Nmic and Pmic concentrations has not hitherto been reported for natural ecosystems.

Furthermore, in the soil community homeostasis analyses, the relationships between log $C:N_R$ and log $C:N_B$, and between log $C:P_R$ and log $C:P_B$ in the G and the RS soils had slopes that did not differ significantly from zero (Figs. S1 and S2), suggesting that the soil microbial communities adjust physiologically (*Sinsabaugh & Follstad Shah, 2012*) to processing low N and P resources in order to cope with the nutrient limitation, particularly in dry years. Our data also suggest that these physiological adjustments occurred differently in the soil microbial communities of the two vegetation covers and was related to both precipitation quantity and nutrient availability.

Our results show how values of TERC:N and $TER_{C:P}$ may shift with respect to variation in annual rainfall and different vegetation cover. The estimated $TER_{C:N}$ was lower in the wet year for both sites, indicating greater sensitivity to N limitation due to the rapid growth of the microbial community produced by the water availability. For $TER_{C:P}$, we observed site-specific differences. The $TER_{C:P}$ was higher in the RS soil than in the G soil for 2013 and 2014, indicating a greater sensitivity of the microbial community to P limitation in the G soil. However, in order to determine the nutritional limitations of the microbial community, we also compared the estimated TER values and the C:N or C:P ratios of the organic matter. If the C:N or C:P ratio of the organic matter being consumed is greater than the TER for that element, this would suggest nutrient limitation (*Sterner & Elser, 2002*). We observed P limitation in both soils, regardless of year ($C:P > TER_{C:P}$; $p < 0.05$) and N limitation in the G soil in the wet year ($C:N = 11.3$ and $TER_{C:N} = 6$; $p = 0.002$). Our results for the dry year (2012) showed that the ecoenzymatic activities associated with C and P acquisition were lowest in the RS and G soils. Values for TERC:N and $TER_{C:P}$ were similar between the RS and G soils, suggesting that both sites may be vulnerable to drought. However, with the increase of the annual precipitation (years 2013 and 2014), the G soil microbial community requires more P and N to meet its metabolic demands and it makes metabolic adjustments in order to maintain its growth which makes it more susceptible or sensitive to resource limitation. Similarly, increased ecoenzyme activities associated with P acquisition and elevated $TER_{C:P}$ values when the water is not limiting (2013 and 2014) suggest that the RS soil microbial community is well adapted to acquire P resources via ecoenzyme upregulation post drought.

We suggested that, under the scenario proposed by Global Climate Change models for desert ecosystems that predict reduced annual precipitation and increased rainfall variability, the microbial community from both sites could be vulnerable to drought events, but the RS soil microbial communities can make adjustments in order to obtain nutrients in wet years, suggesting that this community is resilient post drought.

## CONCLUSION

Soil communities of both sites (RS and G) may be vulnerable to drought. However, the community at the site with lower resources (RS) may have evolved adaptations, such as rapid ecoenzymatic upregulation, under chronic P limitation. This adaptation confers greater resilience within the community to respond to precipitation events post drought. Under the Global Climate Change scenarios for desert ecosystems that predict reduced annual precipitation and an increased intensity and frequency of torrential rains and drought events, soil microbial communities within both sites could be vulnerable to drought through the combination of C and P co-limitation and reallocation of energy and nutrient resources to physiological acclimatization strategies in order to survive.

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## ADDITIONAL INFORMATION AND DECLARATIONS

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### Competing Interests

Valeria Souza is an Academic Editor for PeerJ.

### Author Contributions

- Cristina Montiel-González conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables.
- Yunuen Tapia-Torres analyzed the data, wrote the paper.

- Valeria Souza contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Felipe García-Oliva conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper.

### Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.4007#supplemental-information.

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# CAPÍTULO IV

## THE ORGANIC NUTRIENT LIMITATION DETERMINES THE INTENSITY OF THE PROCESSES INVOLVED IN THE EFFICIENT USE OF C, N AND P BY SOIL MICROBIAL COMMUNITIES IN AN OLIGOTROPHIC ARID ECOSYSTEM IN MÉXICO

# The organic nutrient limitation determines the intensity of the processes involved in the efficient use of C, N and P by soil microbial communities in an oligotrophic arid ecosystem in México.

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**Abstract**

In water-limited ecosystems the soil microbial communities (SMC) fulfill their specific nutritional requirements by the modification of immobilization and mineralization rates to cope with changes in the resources availability. In these ecosystems to sustain the microbial biomass production, the macronutrients C, N and P are primarily obtained by the heterotrophic soil microorganisms from the decomposition of biopolymers contained in the soil organic matter (SOM). During SOM decomposition, C, N and P fluxes in the soil, such as mineralization or immobilization, are regulated by the soil microorganisms through the synergistic interaction of several exo-enzymatic activities. However the efficient use of nutrients could vary depending on the physiological capacity of the communities to produce enzymes and to immobilize the mineralized resources. In the present study we propose that the combination of the specific enzymatic activity (SEA) with the potential rates of transformation of organic nutrients, and potentials of mineralization and immobilization could provide us with clues to identify the processes that favor the efficient use of nutrients in SMC. We aimed to answer: 1) How the SMC of an oligotrophic desert respond to an input of the labile SOM?, 2) Do the SMC of this soil have the physiological capacity to use the nutrients efficiently?, and 3) What are the processes that determine that The SMC efficiently use nutrients in this oligotrophic ecosystem?. To answer our questions, we collected soil samples in September 2014, in the CCB under two vegetation covers (rosetophylous scrub or RS and grassland or G) that differ in the quantity and quality of their organic matter. The soils were incubated under five treatments (control, ribose, adenosine, ribose 5-phosphate and adenosine diphosphate) in controlled conditions. The ecoenzymatic activity involved in the decomposition of organic matter, as well as the concentration of dissolved, available and microbial biomass nutrient, were determined in pre and post incubated samples and compared between sites and treatments. With the ecoenzymatic and biogeochemical data we calculated the potential nutrient transformation rates (mineralization and immobilization), the SEA, the $TER_{C:N}$ and the $TER_{C:P}$. We found a decrease of the $TER_{C:N}$ and the $TER_{C:P}$, which suggests that in both SMCs the co-limitation for resources decreased as a result of the input of labile C-N. We also found that after the input of labile organic C and N the site effect was lost in the transformation of organic C and P (ΔCOD, ΔPOD), the mineralization of C (CO2-C) and the net immobilization of N and P (ΔNmic and ΔPmic). This suggests that both communities

are strongly limited by C which represents a nutritional element that constrain limits the processes for obtaining N, P and also limits the growth of the community. We also found that the RS SMC were the most co-limited by P and, after the input of labile organic CN, had high C, N and P immobilization with low ecoenzymatic activities which suggest that this soil microbial community could be efficient to obtain P. However, the G SMC was the most co-limited by N but was also inefficient at obtaining this resource.

**Keywords.** Ecoenzyme activity, stoichiometric theory, nutrient use efficiency, nutrient Co-limited

## 1. Introduction

Soils are the most metabolically diverse habitat on Earth (Voroney & Heck 2015). Specifically, soil microbial communities consists of a diverse community of organisms occupying vastly divergent phylogenetic lineages and showing significant variation in form, physiology, metabolism (Cleveland & Liptzin 2007; Schimel & Schaeffer 2012), and in their ability to mobilize macronutrients as carbon (C), nitrogen (N) and phosphorus (P).

The C, N and P constitute approximately 50%, 13% and 2.5%, respectively of the dry weight of a microbial cell from soil (Madigan et al. 2015). Moreover soil microorganisms contain from 70% to 85% water and the remaining dry matter consists of 50% protein, 10 to 20% cell wall material, 10% lipids (mainly cell membrane material), 10% to 20% RNA, and 3% to 4% DNA (Coyne 1999), but these values vary among microbial groups and species.

To sustain the microbial biomass production, the macronutrients C, N and P are primarily obtained by the heterotrophic soil microorganisms from the decomposition of biopolymers contained in the soil organic matter (SOM) (Bell et al. 2014; Madigan et al. 2015). Throughout SOM decomposition, C, N and P fluxes in the soil, such as mineralization or immobilization, are regulated by the soil microorganisms through the synergistic interaction of several exo-enzymatic activities (Elser et al. 2000; Heuck et al. 2015; Sinsabaugh et al. 2009).

A conceptual model that differentiates the factors that regulate the mineralization of C, N and P in the soils suggest that the exo-enzymes involved in N mineralization may be less sensitive to variations in the demand for N, than the enzymes that mineralize P (McGill & Cole 1981). While inorganic P can be released directly by enzymatic activity, the N in organic matter is closely linked to the C atoms in various configurations and the inorganic N ($NH_4$) can only be released by means of multiple steps involving the participation of a series of enzymes that selectively eliminate particle types with C-N skeletons (Aerts & Chapin 1999). Therefore, the biotic demand for P determines the production of phosphatase and mineralization of P (Bünemann et al. 2012), while the demand for C or energy may be determining the N mineralization (Mooshammer et al. 2014). This may result in an inverse relationship between availability of P and mineralization of P, but, whether there is a weak relationship or no relationship between the availability of N and the N mineralization (Olander & Vitousek 2000).

In water-limited ecosystems, the rates of N mineralization and immobilization by soil microbial communities are regulated primarily by three variables: 1) the C/N ratio in organic substrates used by microorganism, 2) Efficient use of N in the microbial community and 3) efficient growth (GE) or C use efficiency by the microbial community (Austin et al. 2004). However, the mineralization and immobilization of P will be regulated by 1) the C/P ratio in organic substrates used by microorganisms (resources available for the enzymes elaboration) and 2) P use efficiency by the microbial community. A study in a grassland soil with very low inorganic P availability, showed that the input of organic phosphorus induced high phosphatase activity and extremely rapid microbial P immobilization (Bünemann et al. 2012). Also, a study in forest soils poor in available P found no relationships between microbial P mineralized and microbial C and N mineralized (Achat et al. 2010).

As not all soil microorganisms require of macronutrients at the same time and in the same amounts (Madigan et al. 2015), mineralization or immobilization rates respond mainly to the soil microbial community nutritional requirements (Elser et al. 2000; Fierer et al. 2007; Heuck et al. 2015). Two indicators that help us identify the soil microbial community nutritional requirements are the "ecoenzyme activity" (EA) and the "threshold element ratio" (TER), both derived from the integration of the ecological stoichiometric theory (EST) and the metabolic theory of ecology (MTE) (Allen & Gillooly 2009; Sinsabaugh et al. 2009).

The EA includes the activity of enzymes occurring outside the membranes of living cells, released by microorganisms or by cell lysis that catalyze the degradation of specific organic substrates to terminal monomers (Sinsabaugh et al. 2009; Sinsabaugh et al. 2008). The EA derives from both EST and MTE because the enzyme expression of the soil microbial community is a product of the cellular metabolism specifically regulated by its nutritional requirements and by the environmental nutrient availability which can have repercussions in the nutrient availability at the ecosystem level (Allen & Gillooly 2009; Sinsabaugh et al. 2009; Sinsabaugh et al. 2008).

The TER indicates the transition point for a consumer of an ecological system from being controlled by the energy flow or C (where the N or P will be in excess relative to the soil microbial nutritional requirements) to being controlled by the limiting nutrient flow as N or P (even with high C availability) (Mooshammer 2012; Zechmeister-Boltenstern et al. 2015). The TER derives from both MTE and EST because it integrates the biomass elemental

composition, the energetics of growth and respiration (Allen & Gillooly 2009). When the C:N or C:P ratios from the SOM consumed is greater than the $TER_{C:N}$ or $TER_{C:P}$ respectively. This would suggest a nutrient limitation by N or P (Sterner & Elser 2002). A study that compared the $TER_{C:nutrient}$ and the SOM stoichiometry under two different vegetation covers (grassland G and rosetophylous scrub RS) in an oligotrophic desert showed that with no water limitation, there was a P limitation in both soils (C:P = 248.9 with $TER_{C:P}$ = 112.6 and C:P = 175.2 with $TER_{C:P}$ = 61.4 for RS and G respectively) and a N limitation in the G soil (C:N = 11.3 and $TER_{C:N}$ = 6) (Montiel-González et al. 2017).

An indicator of the amount of ecoenzymes synthesized by the soil microbial community that efficiently produce immobilization of a nutrient is the "specific ecoenzymatic activity" or SEA. The SEA is the expression of enzyme activities per unit of nutrient in microbial biomass (EA/MB). A high SEA value indicates that the soil microbial community has a high enzymatic synthesis and produces low nutrient immobilization, while a low SEA value indicates that the soil microbial community invests little in ecoenzymatic synthesis and produces a high nutrient immobilization. (Chavez-Vergara et al. 2014; Raiesi & Beheshti 2014). Two studies found that in different ecosystems the SEA of C and N was the highest in soil with lower amount of organic matter (Chavez-Vergara et al. 2014; Raiesi & Beheshti 2014), thus indicating under low OM availability the soil microbial communities immobilize few nutrients with high production of enzymes.

Additionally, a concept that allows us to understand how soil microorganisms mobilize nutrients (mineralization and immobilization) is the microbial "nutrient use efficiency" mainly of C, N or P (Mooshammer 2012; Sinsabaugh et al. 2016). For the soil microorganisms, the nutrient use efficiency (either of C, N, P) is the amount of microbial biomass produced (growth) per unit of nutrient used minus the metabolic excretion (mineralization) of unused nutrient (Bridgham et al. 1995; Manzoni et al. 2012; Mooshammer 2012; Sinsabaugh et al. 2009; Veneklaas et al. 2012; Zechmeister-Boltenstern et al. 2015). The microbial "nutrient use efficiency" is a function of the capacity of soil microbial communities, obtained through physiological adaptation and population selection, to alter enzyme expression and biomass composition to diminish differences between environmental resources and growth requirements, with the goal of maximizing the growth rate (Sinsabaugh et al. 2016).

The C use efficiency has been determined using different approaches: 1) the decrease in substrate concentration and the measuring of cumulative respiration, 2) the increase in microbial biomass and the measuring of cumulative respiration, 3) the substrate uptake rate and the measuring of respiration, 4) microbial growth rate and the measuring of respiration; and 5) decomposition models (Manzoni et al. 2012). The N use efficiency is calculated as the partitioning of organic N taken up, between N incorporation into microbial biomass and N recycling to the environment as inorganic N (mainly as ammonium; N mineralization) (Mooshammer et al. 2014). Also, there is experimental evidence that the C and N use efficiency of soil microbial communities depends on substrate quality (Manzoni et al. 2012).

The EST suggests that at low substrate C:N ratios (N-sufficient conditions), strictly homeostatic microorganisms have low N use efficiency but high C use efficiency; in contrast, at high substrate C:N ratios (N-deficiency) they are expected to lower their C use efficiency while increasing their N use efficiency (Mooshammer 2012; Sterner & Elser 2002). A study in two tundra sites showed that: 1) the microbial communities can efficiently capture N in N-limited ecosystems, and 2) the resultant stoichiometric imbalance between resources and microbial decomposers, produces a significant stoichiometric constraint on the physiology of microorganisms, and consequently lead to a reduction in microbial N use efficiency at low resource C:N (N-sufficient substrate) (Mooshammer 2012). Likewise, it has been observed that in ecosystems with high fluctuations in nutrient input, the capacity of a cell to respond quickly to environmental resource pulses is linked to rRNA gene copy number, i.e., the capacity to quickly produce new ribosomes (Sinsabaugh et al. 2016). The growth rate hypothesis suggests that the cellular P content controls growth rates, therefore, the microbial growth increases with cellular P content because most cellular P is in the form of ribosomal RNA (Sterner & Elser 2002; Zechmeister-Boltenstern et al. 2015).

In a previous study in an oligotrophic desert, we observed that when water availability is not limiting (rainy season), the main constraining factor for the growth of soil microbial communities are the organic nutrients. Also, in the same study we observed that the microbial communities coming from the same type of soil but with different vegetation cover are highly homeostatic and are strongly co-limited in a site by C-N (glassland) and in another site by C-P (rosetophylous scrub), and that the ability of the microbial community to cope with this co-limitation depends mainly on metabolic adjustments to growth, enzymatic

activity production and the initial amount of available organic nutrients (Montiel-González et al. 2017).

In the present study we propose that the combination of the SEA with the potential rates of transformation of organic nutrients, and potentials of mineralization and immobilization could provide us with clues as of which are the processes that favor the efficient use of nutrients in soil microbial communities. We aimed to answer the following questions: 1) How the microbial communities of an oligotrophic desert respond to an input of the labile SOM?, 2) Do the microbial communities of this soil have the physiological capacity to use the nutrients efficiently?, and 3) What are the processes that determine that microbial communities efficiently use nutrients in this oligotrophic ecosystem?

Our hypotheses were that in response to the limitation of a single nutrient or the co-limitation of nutrients, a pulse of labile organic nutrients (twice the normal): 1) could cause that the microbial communities with higher nutritional requirements will respond in greater magnitude in processes of mineralization and immobilization of nutrients than the community with greater nutritional requirements, which would imply that this community has a physiological limit to make use of labile organic resources; and 2) Microbial communities will increase the efficient use of the most limiting nutrient (or nutrients) by increasing potential rates of organic nutrient transformation, increased SEA, and decreased mineralization.

To test the hypotheses, we collected soil samples in September 2014, in the CCB under two vegetation covers (RS and G) that differ in the quantity and quality of their organic matter. The soils were incubated under five treatments (control, ribose, adenosine, ribose 5-phosphate and adenosine diphosphate) in controlled conditions. The ecoenzymatic activity involved in the decomposition of organic matter, as well as the concentration of dissolved, available and microbial biomass nutrient, were determined in pre and post incubated samples and compared between sites and treatments. With the ecoenzymatic and biogeochemical data we calculated the potential nutrient transformation rates (mineralization and immobilization), the SEA, the $TER_{C:N}$ and the $TER_{C:P}$.

## 2. Methods

### 2.1. Study site

The study was carried out in the Cuatro Ciénegas Basin (CCB; 26°45'- 27°00' N and 101°48'- 102°17' W) in central northern México, within the Chihuahuan Desert. The CCB has an area of 150,000 $km^2$, with an elevation of 740 m.a.s.l. The climate is seasonally arid with an average annual temperature of 21°C and an annual rainfall of 252 mm, which is concentrated during the summer months (http://smn.cna.gob.mx/). Jurassic-era gypsum is the dominant parent material in the western side of the basin (McKee et al. 1990). According to the WRB classification (WRB 2015), the predominant soil is Gypsisol in the western side of the basin. The main vegetation types are: 1) grassland (G) dominated by *Sporobolus airoides* (Torr.) Torr. and *Allenrolfea occidentalis* (S. Watson) Kuntze; 2) microphyll scrub, dominated by *Jatropha dioica* Cerv., *Larrea tridentata* (DC) Cov. and *Fouqueria* sp Kunth (Perroni et al. 2014); and 3) rosetophylous scrub (RS) dominated by *Dhasylirium cedrosanum* Trel., and *Yucca treculeana* Carriére (González 2012).

### 2.2. **Sampling**

Soil collection was carried out in Churince on the west side of the CCB from two vegetation covers: grassland (G) and rosetophylous scrub (RS) during the rainy season (September 2014). For each vegetation cover we sampled 5 sites separated from each other by 140 m, along a one kilometer north-to-south transect. At each sampling site we established a 4×4 m plot, a soil sample was taken at each plot corner with a soil-core sampler (5 cm diameter × 15 cm depth), and combined to form a representative composite sample per site. Thus, a total of 5 composite samples were obtained from each vegetation cover. The soil samples were stored in black plastic bags at 4°C before the incubation experiment and laboratory analyses.

### 2.3. Laboratory incubation

#### 2.3.1. Water Holding Capacity (WHC) determination

Soil bulk density of the G and the RS soil samples were 0.85 ± 0.04 and 0.81 ± 0.03 g $cm^{-3}$, respectively. To compare the two sites at the same water content during the experiment, we estimated the water holding capacity (WHC) for each site. Therefore, we measured WHC for each site as follows: a 50 g sub-sample from each soil was sieved to remove coarse plant

debris (>4 mm) and placed in an oven at 60° C for 48 h to determine the gravimetric soil moisture. Subsequently, the dried soil samples were used to determine WHC as follows: 5 g of dry soil were placed in a Whatman No.1 filter paper on a funnel and saturated with deionized water. The funnels with soil were covered to prevent evaporative loss and allowed to drain overnight. WHC is determined by subtracting the dry weight form the wet weight (Fierer & Schimel 2002; Tiemann & Billings 2011). Additionally, the soil water percentage of these samples was also determined with a Soil Moisture and Temperature Sensor Kit (SM300, Delta-T Devices). The WHC was 45 and 25 % for the G and the RS soils, respectively.

### 2.3.2. Soil preparation before incubation

From each soil sample, we weighted 5 sub-samples of 200 g that were sieved (>4 mm) to remove large organic particles and coarse plant debris. The soil samples were placed in a PVC core of 5 cm diameter by 10 cm depth fitted on the bottom with filter paper Whatman No.1 and each core was placed in a 1L jar. Before starting the incubation, the soil samples were pre-incubated for three days in a Plant Growth Chamber (ICP, Lumistell) at 25° C (average temperature of sampling month) at its respective WHC moisture in dark conditions, to allow microbial activity to resume.

### 2.3.3. Experimental design

The experimental design was a full factorial design with soil from two vegetation covers (G and RS) and five treatments of organic nutrients fertilization, where each treatment had 5 replicates. The organic nutrients fertilization treatments were: 1) a control without organic fertilization (W), 2) ribose (MW 0.15 kDa) to test organic C limitation (Cf), 3) Adenosine (MW 0.27 kDa) to test organic N limitation, this molecule has a 2:1 C:N ratio (CNf), 4) Ribose 5-phosphate (MW 0.23 kDa) to test organic P limitation, this molecule has a 5:1 C:P ratio (CPf), and 5) Adenosine diphosphate or ADP (MW 0.42 kDa) to test organic N-P co-limitation, this molecule has the following ratios 2:1, 5:1 and 2.5 for C:N, C:P and N:P ratios, respectively (CNPf).

The concentration at which we fertilized with each substrate was chosen to ensure a higher input of DOC than would normally be present in a wet year (2014), simulating a year with

high organic nutrient availability through OM input. To ensure a higher input we determined the DOC concentration (see dissolved nutrients; the DOC concentrations were 28 μg C $g^{-1}$ for the RS soil and 70 μg C $g^{-1}$ for the G soil) and we duplicated this concentration with the fertilization. In average, the DOC concentrations used for the fertilizations were: 56 μg C $g^{-1}$ and 140 μg C $g^{-1}$ for the RS soil and the G soil, respectively. The substrates were dissolved in the amount of deionized water necessary to reach the WHC of each soil. A single fertilization was applied at the beginning of incubation for all treatments.

Each soil core was placed in a 1 L jar with a vial containing 10 ml of 0.5 N NaOH solution to trap the emitted $CO_2$. Soil cores were incubated for 20 days at 25°C in the dark. The soil cores were kept at their WHC by weighting and adding deionized water to replace the loss by evaporation every three days, at the same time alkali traps were substituted. We used five jar-blanks, containing the $CO_2$ trap and the PVC core but no soil. The jar-blanks were used to make subsequent corrections in all treatments. The $CO_2$ was determined by adding 2 ml of $BaCl_2$ 1.5 N and a few drops of phenolphthalein indicator to the alkali traps, which were titrated to neutral pH with HCl 0.5 N (Stotzky 1965).

### 2.3. Soil Analysis

At the end of the incubation period, soil samples were collected and stored at 4° C in black bags until the corresponding analyzes. The data of pre incubation samples was published in a previous study (Montiel et al. 2017) and they were used for the calculation of nutrients transformations.

#### 2.4.1. Biogeochemical analyses

The biogeochemical analyses applied to post Incubations soil samples were: 1) pH, 2) Total C, N and P (TOC, TN, TP), 3) dissolved C, N and P (DOC, DON, DOP, $DNH_4^+$and $DNO_3$), 4) available N and P ($NH_4^+$, $NO_3^-$, Pi), and 5) C, N and P within the microbial biomass (Cmic, Nmic and Pmic) with the same methods used in the samples pre-I (Montiel-González et al. 2017)

**Nutrients Analysis**

All Carbon (C) forms analyzed were determined with a Total Carbon Analyzer (UIC Mod. CM5012; Chicago, E.U.A), while nitrogen (N) and phosphorus (P) forms were determined

by colorimetrical analyses using a Bran Luebbe Auto Analyzer III (Norderstedt, Germany). However, available and microbial P concentrations were determined by colorimetric analyses using a spectrophotometer Evolution 201 (Thermo Scientific Inc.).

***Dissolved, available and nutrients within microbial biomass***

The dissolved, available and microbial nutrient forms were extracted from fresh pos-I soil samples. Dissolved nutrients were extracted from 20g of soil with deionized water after shaking for 45 min and then filtering through a Whatman No. 42 and a 0.45 μm nitrocellulose membrane (Jones & Willett 2006). The filtrate was used to determine the total dissolved C (TDC), measured with an Auto Analyzer of carbon (TOC CM 5012) module for liquids (UIC-COULOMETRICS). Inorganic dissolved C (IDC) was determined in an acidification module CM5130. One aliquot of the filtrate was used to determine ammonium ($DNH_4^+$) and dissolved inorganic P (DIP) in deionized water extract. Total dissolved N and P (TDN and TDP respectively) were digested in a mixture of concentrated $H_2SO_4$, $H_2O_2$ (30%) at 250 °C. N was determined by the macro Kjeldahl method (Bremmer 1996), while P was determined by the molybdate colorimetric method following ascorbic acid reduction (Murphy & Riley 1962). The dissolved organic C, N and P (DOC, DON and DOP respectively) were calculated as the difference between total dissolved forms and inorganic dissolved forms.

Available inorganic nitrogen forms ($NH_4^+$ and $NO_3^-$) were extracted from 10 g of post-I soil with 2M KCl, followed by filtration through a Whatman No. 1 paper filter, and determined colorimetrically by the phenol-hypochlorite method (Technicon 1977). Available inorganic phosphorous (Pi) was extracted with 0.5 M $NaHCO_3$, pH 8.5 (Tiessen & Moir 2008) and was determined colorimetrically by the molybdate-ascorbic acid method (Murphy & Riley 1962). Carbon (Cmic) and N (Nmic) concentrations within microbial biomass were determined from 20 g of post-I soil by the chloroform fumigation extraction method (Vance et al. 1987). Fumigated and non-fumigated samples were incubated for 24 h at 25°C and constant moisture. Cmic and Nmic were extracted from fumigated and non-fumigated samples with 0.5 $MK_2SO_4$, filtered through a 0.45 μm nitrocellulose membrane (Brookes et al. 1984). C concentration was measured from each extract as total (TC) and inorganic (IC) by the method described before. The difference between TC and IC was used for Cmic calculation. To determine the Nmic concentration one aliquot of the filtrate extracted was acid digested and

determined as TN by Macro-Kjeldahl method (Brookes et al. 1984). Phosphorus within microbial biomass (Pmic) was extracted from 5 g of soil by the chloroform fumigation extraction and incubation method (Vance et al. 1987). Pmic was extracted using $NaCO_3$ 0.5M, pH 8.5 and digested in a mixture of $H_2SO_4$ 11N and ($(NH_4)_2S_2O_8$ at 50%), the latter as a catalyst at 120 °C (Lajtha et al. 1999). Pmic was determined colorimetrically by the molybdate-ascorbic acid method (Murphy & Riley 1962). The Cmic, Nmic and Pmic were calculated as the difference between fumigated and non-fumigated samples using correction factors of $K_{EC}$ 0.45 (Joergensen 1996), $K_{EN}$ 0.54 (Joergensen & Mueller 1996) and $K_P$ 0.4 (Lajtha et al. 1999) for Cmic, Nmic and Pmic, respectively. Finally, the values of Cmic, Nmic and Pmic were corrected to dry soil basis.

### 2.4.2. Post-I Ecoenzyme Activity Analyses

The activities of six ecoenzymes (extracellular enzymes) involved in the cleavage of organic molecules with C, N and P were measured: β-1, 4-glucosidase (BG), cellobiohydrolase (CBH), β-1, 4-N-acetylglucosaminidase (NAG), polyphenol oxidase (PPO), phosphomonoesterase (PME) and phosphodiesterase (PDE) with assay techniques reported by Tabatabai & Bremner (1969), Eivazi & Tabatabai (1977), Eivazi & Tabatabai (1988),Verchot & Borelli (2005) and Johannes & Majcherczyk (2000).

For all ecoenzymes, we used 2 g of fresh soil and 30 ml of modified universal buffer (MUB) at pH 9 for ecoenzyme extraction. Three replicates and two control samples (soil extract with no substrate, and pure MUB with substrate) were included per assay. All ecoenzyme assays were incubated at 40°C: the BG and CBH for 2 h, NAG for 3 h, PPO for 2.5 h, PME and PDE 1.25 h. Following the incubation period, the tubes were centrifuged at 10,000 rpm for 2 minutes and 750µl of supernatant was recovered.

For all ecoenzymes with substrates containing p-nitrophenol (pNP), we diluted the supernatant in 2 ml of deionized water with 75 µl of NaOH and measured the absorbance of pNP liberated at 410 nm on an Evolution 201 spectrophotometer (Thermo Scientific Inc.). For the PPO, we used 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) as a substrate. The resulting supernatant was measured directly at 410 nm. Ecoenzyme activities were expressed as nanomoles of pNP per gram of dry soil per hour

(nmol pNP [g SDE]$^{-1}$ h$^{-1}$) for substrates containing p-nitrophenol (pNP) and $O_2$ formed per gram of dry soil per hour (nmol$O_2$ [g SDE]$^{-1}$ h$^{-1}$) for the PPO, respectively.

To infer the amount of enzyme efficiently used per nutrient immobilized in microbial biomass we calculated the Specific enzymatic activity (SEA) using equations (1) to (3) (Chavez-Vergara et al. 2014; Waldrop et al. 2000):

$$\text{SEA } \mu\text{mol}/(\text{mgC}_{\text{mic}}\ \text{h}) = \text{A}/(\text{C}_{\text{mic}} \text{ x } 0.001) \quad (1)$$

$$\text{SEA } \mu\text{mol}/(\text{mgN}_{\text{mic}}\ \text{h}) = \text{B}/(\text{N}_{\text{mic}} \text{ x } 0.001) \quad (2)$$

$$\text{SEA } \mu\text{mol}/(\text{mgP}_{\text{mic}}\ \text{h}) = \text{C}/(\text{P}_{\text{mic}} \text{ x } 0.001) \quad (3)$$

Where A is the enzymatic activity of BG or CBH or PPO, B is the enzymatic activity of NAG and C is the enzymatic activity of PME or PDE.

## 2.5. Organic nutrients transformation, nutrients mineralization, nutrients immobilization within microbial biomass by soil microorganisms

The calculation of potential DOC transformation and potential microbial C immobilization were calculated with the following equations:

$$\Delta\ \text{DOC}\ (\mu\text{C g}^{-1}) = \text{DOC post incubation} - \text{DOC pre incubation} \quad (4)$$

$$\Delta\ \text{C}_{\text{mic}}(\mu\text{C}_{\text{mic}}\ \text{g}^{-1}) = \text{C}_{\text{mic}}\text{post incubation} - \text{C}_{\text{mic}}\text{pre incubation} \quad (5)$$

The potential soil C-mineralization was calculated as the $CO_2$-C accumulated during the incubation period (equation 4):

$$\text{CO}_2 - \text{C}(\mu\text{C g}^{-1}) = \sum \text{CO}_2 - \text{C} \quad (6)$$

Similarly, the potential DON transformation, potential N microbial immobilization and potential N mineralization were estimated using the following equations:

$$\Delta\ \text{DON}(\mu\text{gN g}^{-1}) = \text{DON post incubation} - \text{DON pre incubation} \quad (7)$$

$$\Delta\text{N}_{\text{mic}}\ (\mu\text{N}_{\text{mic}}\ \text{g}^{-1}) = \text{N}_{\text{mic}}\ \text{post incubation} - \text{N}_{\text{mic}}\ \text{pre incubation} \quad (8)$$

$$\text{Net Amonification(NA; } \mu\text{gNH}_4\ \text{g}^{-1}) = \text{NH}_4\ \text{posincubation} - \text{NH}_4\ \text{pre incubation} \quad (9)$$

$$\text{Net Nitrificación (NN, } \mu\text{gNO}_3\ \text{g}^{-1}) = \text{NO}_3\ \text{post incubation} - \text{NO}_3\text{pre incubation} \quad (10)$$

$$\text{Net Mineralization (NM, } \mu\text{gN g}^{-1}) = \text{Net amonification} + \text{Net nitrification} \quad (11)$$

Finally, the potential POD transformation, potential P microbial immobilization and the potential transformation of availability of inorganic P were calculated with the following equations:

$$\Delta\ \text{DOP}(\mu\text{gP g}^{-1}) = \text{DOP post incubation} - \text{DOP pre incubation} \quad (12)$$

$$\Delta\ P_{mic}(\mu P_{mic}\ g^{-1}) = P_{mic}\ \text{post incubation} - P_{mic}\ \text{pre incubation} \quad (13)$$

### 2.6. Threshold elemental ratio

The threshold elemental ratios were calculated using: 1) Ecoenzymatic activity ratios (EEA) post-incubation( BG, NAG, PME and PDE) and 2) The elemental ratio of the substrate consumed (TOC, NT and PT)

The TER was calculated with the equations proposed by Sinsabaugh et al. (2016) (equations 14 to 19)

$$TER_{C:N} = (L_{C:N})(EEA_{C:N}) \quad (14)$$

$$TER_{C:P} = (L_{C:P})(EEA_{C:P}) \quad (15)$$

$$L_{C:N} = TOC/NT \quad (16)$$

$$L_{C:P} = TOC/PT \quad (17)$$

$$EEA_{C:N} = BG/NAG \quad (18)$$

$$EEA_{C:P} = BG/PME\ \text{or}\ PDE \quad (19)$$

### 2.7. Data analysis

Soil biogeochemistry and ecoenzymatic data were subjected to factorial analyses of variance (F-ANOVA; Von Ende 2001). The vegetation cover factor had two levels (RS and G) and the fertilization treatments had five levels (W, Cf, CNf, CPf and CNPf). When F-ANOVA indicated significant factor effects, mean comparisons were performed with Tukey's multiple comparisons test (Von Ende 2001).

## 3. Results

*3.1. Dissolved organic nutrients and available nutrients in the Post-I samples*

The DOC, DOP and Pi concentrations were higher in the G soil (95.1 ± 6.7, 5.5 ± 0.4 and 8.1 ± 0.4 µg $g^{-1}$, respectively) than in the RS soil (57.8 ± 5.3, 3.3 ± 0.4 and 4.7 ± 0.4 µg $g^{-1}$, respectively).The CNf and W treatments had the highest and the lowest DOC concentrations, respectively (Fig 1A), while the CNf treatment had higher DOP concentration than in the Cf and W treatments (Fig 1B). The CNf and CNPf had the highest values of $NO_3$ concentration (Fig. 1C), while the Pi concentration was higher in the CPf treatment than in the W treatment (Figure 1D).

The DON and $NH_4^+$ concentrations, and DOC:DON ratio were affected by the interaction between vegetation cover and fertilization treatments (Table 2). The CNf treatment had higher DON concentration than the other treatments within the G soil, but the DON concentration had no differences among fertilization treatments within the RS soil (Table 1). Therefore, the DOC:DON ratio was different among treatments only within the G soil, the CNPf and CNf treatments had the highest and the lowest values respectively (Table 1). In contrast, the $NH_4$ concentration had the highest value in the CNf treatment in both soils, but the lowest value was in the CPf treatment within the G soil samples, while the Cf and CPf treatments had the lowest values within the RS soil samples (Table 1).

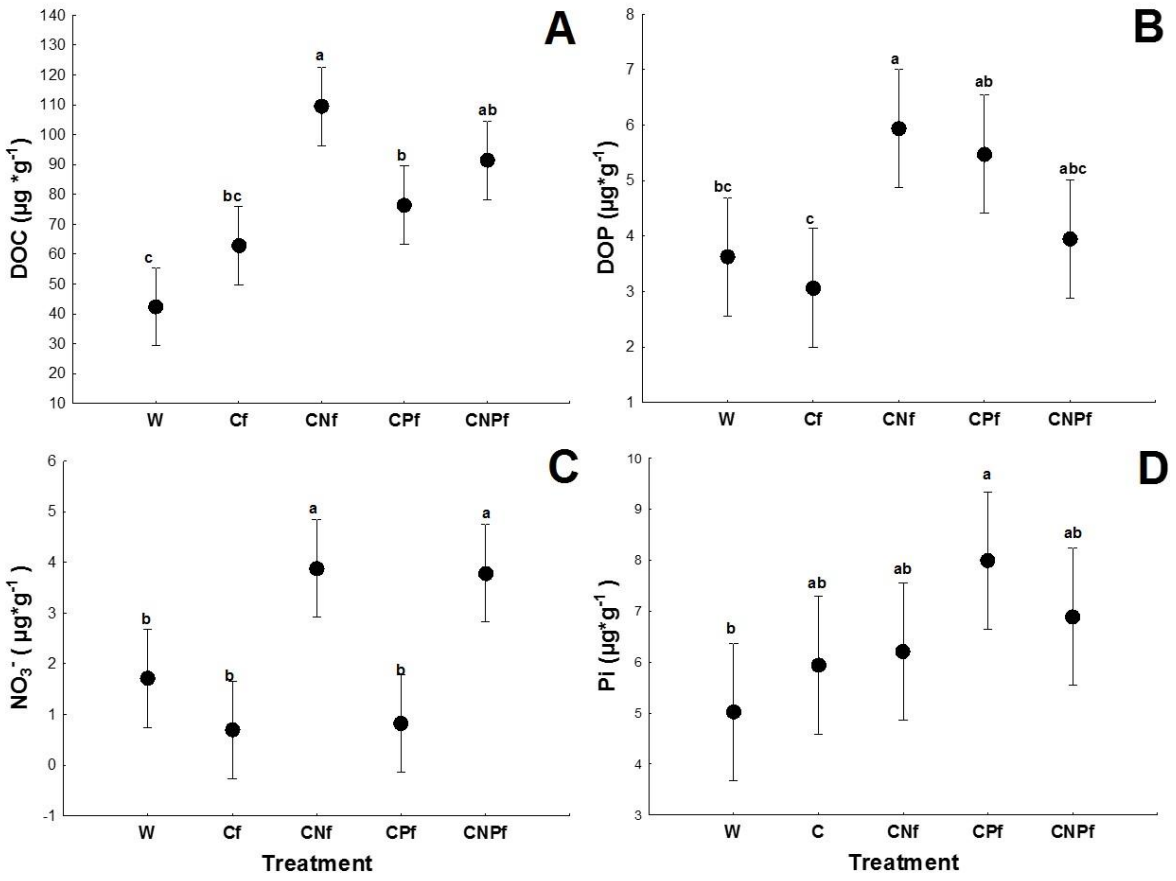


**Figure 1.** Means and standard errors of soil nutrient concentrations under five treatments (W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization) in rosetophylous scrub soil (RS) and grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different lower-case letters indicate significant differences between treatments ($P < 0.05$).

**Table 1.** Means and standard errors of concentrations and ratios of soil nutrients post-I under five treatments in the rosetophylous scrub soil (RS) and the grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different uppercase letters indicate significant differences ($P < 0.05$) between vegetation covers (RS and G) within the same treatment. Different lower-case letters indicate significant differences ($P < 0.05$) between treatments (W, Cf, CNf, CPf and CNPf) within the same vegetation cover. Values without letters did not show statistically significant differences in the interaction between vegetation and fertilization treatment.

| | Fertilization treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RS | | | | | G | | | | |
| | W | Cf | CNf | CPf | CNPf | W | Cf | CNf | CPf | CNPf |
| | Dissolved organic nutrient concentration | | | | | | | | | |
| DOC (μg $g^{-1}$) | 20(4.8) | 44(7.8) | 87(5.1) | 62(2.1) | 76(1.2) | 87(8.5) | 106(12) | 159(13) | 114(8.9) | 129(15) |
| DON (μg $g^{-1}$) | 5.2(0.4)$^{Ba}$ | 4.8(0.2)$^{Ba}$ | 6.1(0.7)$^{Ba}$ | 5(0.3)$^{Ba}$ | 5.3(1.0)$^{Ba}$ | 8(0.5)$^{Ab}$ | 9.1(0.6)$^{Ab}$ | 15.8(1)$^{Aa}$ | 10(1)$^{Ab}$ | 10.2(1.5)$^{Ab}$ |
| DOP (μg $g^{-1}$) | 3.1(0.9) | 2.3(0.4) | 4.2(0.6) | 4(0.9) | 2.9(0.8) | 4.1(0.3) | 3.8(0.6) | 7.7(0.8) | 7(0.6) | 5(0.7) |
| DOC:DON | 3.8(0.9)$^{Ba}$ | 9.3(1.8)$^{Aa}$ | 15.2(2.3)$^{Aa}$ | 12.4(0.6)$^{Aa}$ | 16.8(3.1)$^{Aa}$ | 11(1.3)$^{Ab}$ | 11.9(1.7)$^{Aab}$ | 10.4(1.4)$^{Bc}$ | 11.6(0.6)$^{Ab}$ | 13.6(1.9)$^{Aa}$ |
| DOC:DOP | 11.4(4) | 24.7(7.1) | 22.9(3.6) | 21.1(5.5) | 31.7(6.3) | 22(3.4) | 30.7(5.4) | 20.9(1.1) | 17.6(3.3) | 52.8(4.7) |
| DON:DOP | 2.8(1) | 2.7(0.2) | 1.6(0.4) | 1.7(0.5) | 2.1(0.5) | 2(0.7) | 2.6(0.3) | 2.2(0.3) | 1.5(0.3) | 2.2(0.4) |
| | Available nutrient concentration | | | | | | | | | |
| $NH_4^+$ (μg $g^{-1}$) | 2(0.5)$^{Bbc}$ | 1.5(0.2)$^{Bc}$ | 7.1(2.8)$^{Ba}$ | 1.2(0.2)$^{Ac}$ | 3.8(2.5)$^{Bb}$ | 4.6(0.5)$^{Ac}$ | 4(0.4)$^{Ac}$ | 31.2(3.1)$^{Aa}$ | 2.5(0.4)$^{Ad}$ | 14.3(5.2)$^{Ab}$ |
| $NO_3^-$ (μg $g^{-1}$) | 1.6(0.3) | 0.6(0.1) | 4.4(0.7) | 0.7(0.2) | 4.5(1.4) | 1.8(0.3) | 0.8(0.2) | 3.3(0.7) | 1.0(0.3) | 3.0(0.6) |
| Pi (μg $g^{-1}$) | 3.6(1.3) | 4.5(0.9) | 4.1(0.8) | 5.7(0.7) | 5.7(0.7) | 6.4(0.8) | 7.4(0.6) | 8.4(0.9) | 10.3(1.2) | 8.1(0.6) |

DOC: dissolved organic carbon; DON: dissolved organic nitrogen; DOP: dissolved organic phosphorus; $NH_4^+$: available ammonium; $NO_3$: available nitrate; Pi: Available inorganic phosphorus. Treatments: W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

**Table 2.** F-ratios and significant levels of the factorial ANOVA for concentrations and ratios of soil nutrients post-I under five fertilization treatments in the Rosetophylous scrub soil (RS) and the Grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico.

| Parameters | Source of variation | | |
|---|---|---|---|
| | Vegetation cover | Fertilization treatments | Interaction |
| | Dissolved nutrients | | |
| DOC | 41.3(<0.0001) | 15.8(<0.0001) | 0.32 (0.86) |
| DON | 92.6 (<0.0001) | 7.9 (<0.0001) | 4.4 (0.005) |
| DOP | 22.3 (<0.0001) | 5.5(<0.0001) | 1.0 (0.4) |
| DOC:DON | 0.03(0.85) | 4.7(0.003) | 3.3(0.02) |
| DOC:DOP | 0.19(0.6) | 2.3(0.08) | 0.8(0.5) |
| DON:DOP | 0.04(0.8) | 1.18(0.4) | 0.4(0.8) |
| | Available nutrients | | |
| $NH_4^+$ | 27.5(<0.0001) | 17.1 (<0.0001) | 7.5 (0.0001) |
| $NO_3^-$ | 0.88(0.3) | 10.7(<0.0001) | 0.71(0.6) |
| Pi | 32.2 (<0.0001) | 2.7 (0.04) | 0.6 (0.7) |

DOC: dissolved organic carbon; DON: dissolved organic nitrogen; DOP: dissolved organic phosphorus; $NH_4^+$: available ammonium; $NO_3^-$: available nitrate; Pi: Available inorganic phosphorus.

### *3.2 Microbial nutrients, specific enzymatic activity and ecoenzymatic ratios*

Cmic, Nmic and Pmic showed vegetation cover effect and fertilization effect (Table 4). Regardless of the fertilization treatment, the Cmic, Nmic, and Pmic concentrations were higher in the G soils (492 ± 34, 76 ± 3 and 12 ± 0.8 µg $g^{-1}$) than in the RS soils (359 ± 15, 66 ± 3, 7 ± 0.4 µg $g^{-1}$). In the same way and regardless of the vegetation, the Cmic and Nmic concentrations were higher in the CNf treatment than in the W, Cf and CPf treatments (Figure 2A and 2B). The Pmic concentration was lower in the W treatment than the other four fertilization treatments (Figure 2C). The interaction effect between vegetation cover and fertilization treatments was significant for Cmic:Pmic ratio (Table 4), because the W treatment had the highest ratio values only in the RS soil samples (Table 3). In contrast, the Cmic:Nmic ratios were not affected for any factor analyzed (Table 4).

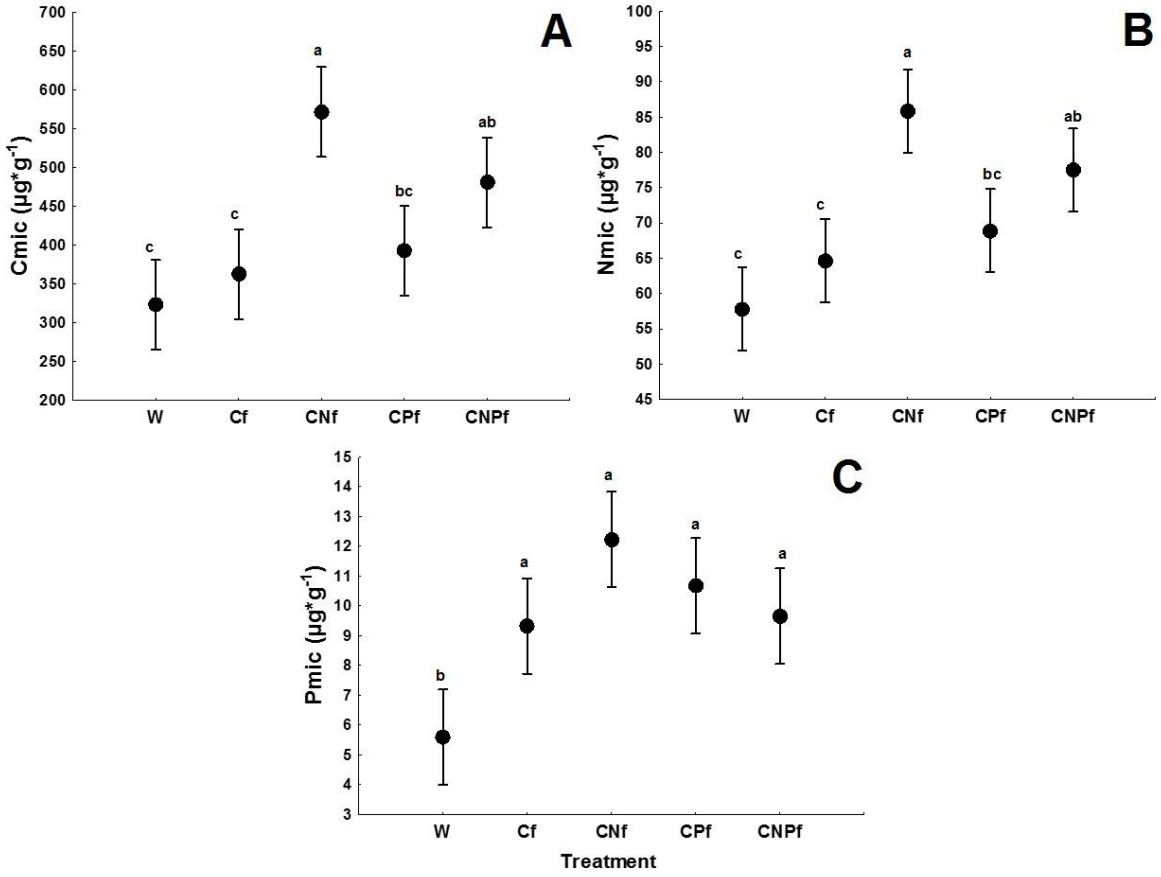


**Figure 2**. Means and standard errors of microbial biomass nutrient concentrations under five treatments in rosetophylous scrub soil (RS) and grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different lower-case letters indicate significant differences between treatments ($P < 0.05$). Treatments: W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

The specific enzymatic activity (SEA) of the BG, CBH, NAG and PME were higher in the G soil samples than in the RS soil samples (Figure 3). In contrast, the SEA of the NAG, PME and PDE were affected by the fertilization treatments (Table 4), where the W treatment had the lowest values (Figures 4A, 4B and 4C, respectively). The BG:NAG ratio was higher in the G soils ($2 \pm 0.08$) than in the RS soils ($1.5 \pm 0.09$), as well as, this ratio was lower in the CNPf treatment than in the W treatment (Figure 4D). In contrast, the BG:PDE, NAG:PME and NAG:PDE ratios were affected by the interaction between vegetation cover and fertilization treatments (Table 4). In the RS soil samples, the CNPf had the lowest BG:PDE ratio, while the Cf treatments had the lowest values of NAG:PDE ratio. In contrast in the G soils, the lowest values of BG:PDE ratios were in Cf and CNf treatments, while the

NAG:PDE ratio had the lowest values in the Cf treatment, and CNf and CNPf has the highest values of NAG:PME ratio.

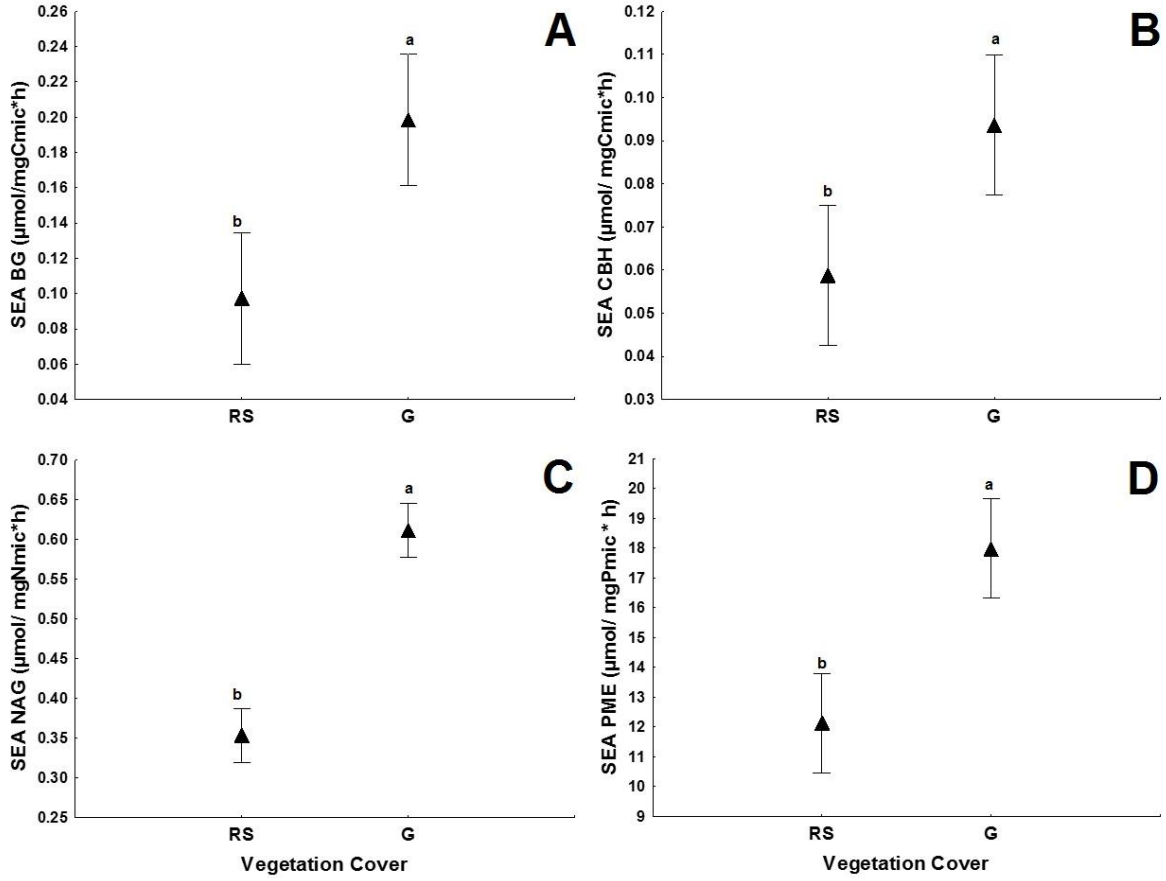


**Figure 3.** Means and standard errors of specific enzymatic activity (SEA) by vegetation cover (RS: rosetophylous scrub soil and G: grassland soil) within the Cuatro Ciénegas Basin, Coahuila, Mexico. (A) β-1,4-glucosidase (BG), (B) cellobiohydrolase (CBH), (C) β-1,4-N-acetylglucosaminidase (NAG) and (D) phosphomonoesterase (PME). Different lower-case letters indicate significant differences between treatments ($P < 0.05$).

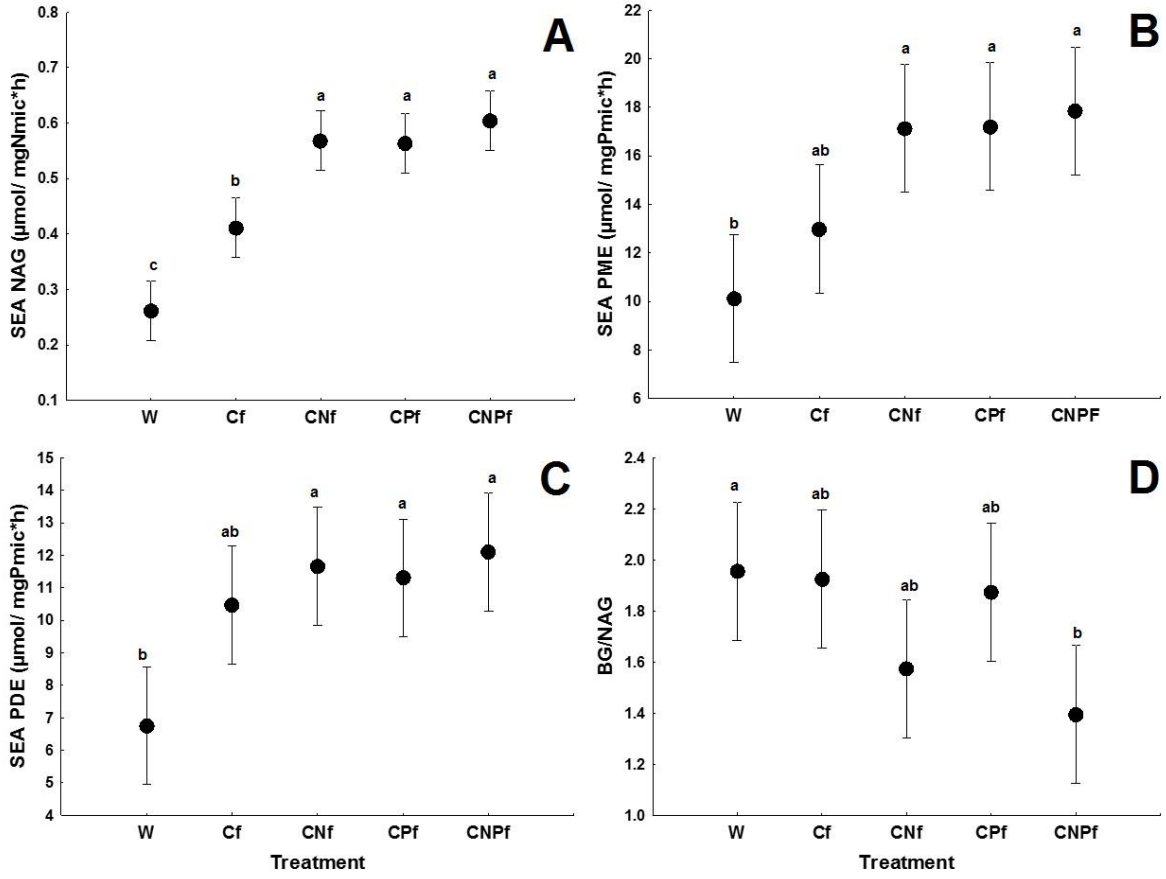


**Figure 4.** Means and standard errors of specific enzymatic activity (SEA) under five fertilization treatments in rosetophylous scrub soil (RS) and grassland soil (G) within the Cuatro Ciénegas Basin, Coahuila, Mexico. (A) β-1,4-N-acetylglucosaminidase (NAG), (B) phosphomonoesterase (PME), (C) phosphodiesterase (PDE) and BG/NAG ratio. Different lower-case letters indicate significant differences between treatments ($P < 0.05$). Treatments: W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

**Table 3.** Means and standard errors of microbial biomass nutrients, ratios and ecoenzyme activities under five treatments in the rosetophylous scrub soil (RS) and the grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different uppercase letters indicate significant differences ($P < 0.05$) between vegetation covers (RS and G) under the same treatment. Different lower-case letters indicate significant differences ($P < 0.05$) between fertilization treatments (W, Cf, CNf, CPf and CNPf) under the same vegetation cover. Values without letters did not show statistically significant differences in the interaction between vegetation and treatment.

| | Fertilization treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RS | | | | | G | | | | |
| | W | Cf | CNf | CPf | CNPf | W | Cf | CNf | CPf | CNPf |
| | Nutrients concentration within microbial biomass | | | | | | | | | |
| Cmic (μg $g^{-1}$) | 259 (8) | 311 (33) | 454 (18) | 333 (3) | 381 (26) | 325 (63) | 414 (79) | 690 (18) | 453 (11) | 581 (20) |
| Nmic (μg $g^{-1}$) | 60 (4) | 61 (6) | 79 (4) | 66 (2) | 71 (2) | 62 (2) | 69 (2) | 93 (1) | 72 (1) | 84 (6) |
| Pmic (μg $g^{-1}$) | 4.4 (0.5) | 7.2 (0.5) | 9.5 (0.3) | 7.7 (0.9) | 7.2 (0.3) | 7.6 (0.1) | 11.4 (0.9) | 15.0 (2.2) | 13.6 (1) | 12.1 (1.5) |
| Cmic:Nmic | 4 (0.3) | 5 (0.3) | 6 (0.4) | 5 (0.2) | 5 (0.4) | 5 (1) | 6 (1.3) | 7 (0.2) | 6 (0.2) | 7 (0.4) |
| Cmic:Pmic | 59 (2)$^{Aa}$ | 43 (3)$^{Ab}$ | 48 (2)$^{Ab}$ | 47 (7)$^{Ab}$ | 53 (4)$^{Ab}$ | 43 (8)$^{Ba}$ | 36 (6)$^{Aa}$ | 53 (11)$^{Aa}$ | 34 (2)$^{Aa}$ | 51 (6)$^{Aa}$ |
| | Ecoenzyme activity ratios | | | | | | | | | |
| BG/NAG | 1.9(0.21) | 1.6(0.1) | 1.3(0.04) | 1.6(0.2) | 1.1(0.1) | 2.3(0.2) | 2.3(0.15) | 1.6 (0.05) | 2.1(0.1) | 1.7(0.1) |
| BG/PME | 0.78(0.01) | 0.37(0.05) | 0.35(0.04) | 0.44(0.03) | 0.31(0.04) | 0.46(0.03) | 0.44(0.02) | 0.42(0.01) | 0.40(0.03) | 0.44(0.03) |
| BG/PDE | 0.91 (0.16)$^{Aa}$ | 0.41(0.03)$^{Bc}$ | 0.52(0.02)$^{Bb}$ | 0.55(0.02)$^{Bb}$ | 0.35(0.02)$^{Bd}$ | 0.76(0.06)$^{Ba}$ | 0.59(0.03)$^{Ab}$ | 0.6(0.02)$^{Ab}$ | 0.71(0.06)$^{Aa}$ | 0.82(0.08)$^{Aa}$ |
| NAG/PME | 0.41(0.06)$^{Aa}$ | 0.23(0.02)$^{Ab}$ | 0.26(0.02)$^{Ab}$ | 0.27(0.02)$^{Ab}$ | 0.28(0.02)$^{Ab}$ | 0.2(0.01)$^{Bb}$ | 0.2(0.01)$^{Ab}$ | 0.25(0.01)$^{Aa}$ | 0.19(0.01)$^{Bb}$ | 0.25(0.01)$^{Aa}$ |
| NAG/PDE | 0.48(0.1)$^{Aa}$ | 0.26(0.02)$^{Ac}$ | 0.38(0.01)$^{Ab}$ | 0.35(0.02)$^{Ab}$ | 0.34(0.04)$^{Bb}$ | 0.34(0.01)$^{Bb}$ | 0.27(0.01)$^{Ac}$ | 0.36(0.02)$^{Ab}$ | 0.33(0.01)$^{Ab}$ | 0.48(0.04)$^{Aa}$ |

Cmic: microbial carbon; Nmic: microbial nitrogen; Pmic: microbial phosphorus; BG: β-1,4-glucosidase; CBH: cellobiohydrolase; NAG: β-1,4-N-acetylglucosaminidase; PPO: polifenol oxidase; PME: phosphomonoesterase; PDE: phosphodiesterase. Treatments: W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

**Table 4.** F-ratios and significant levels of the factorial ANOVA for concentrations and ratios of soil nutrients, and enzymatic activity post-I under five fertilization treatments in the Rosetophylous scrub soil (RS) and the Grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico.

| Parameters | Source of variation | | |
|---|---|---|---|
| | Vegetation cover | Fertilization treatments | Interaction |
| Nutrients concentration within microbial biomass | | | |
| Cmic | 27 (<0.0001) | 12 (<0.0001) | 2.51 (0.056) |
| Nmic | 14 (0.0006) | 14 (<0.0001) | 0.4 (0.8) |
| Pmic | 48 (<0.0001) | 9.6 (<0.0001) | 0.3 (0.9) |
| Cmic:Nmic | 3.6 (0.06) | 0.7 (0.56) | 1.4(0.2) |
| Cmic:Pmic | 24 (<0.0001) | 6.6 (0.004) | 2.6 (0.04) |
| Specific enzymatic activity (SEA) | | | |
| BG | 15 (0.0003) | 0.6 (0.6) | 1.4 (0.2) |
| CBH | 9.3 (0.003) | 0.5 (0.7) | 0.8 (0.5) |
| PPO | 3.3 (0.07) | 1.2 (0.3) | 0.8 (0.5) |
| NAG | 117 (<0.0001) | 29 (<0.0001) | 1.1 (0.3) |
| PME | 25 (<0.0001) | 6.6 (0.0003) | 2.4 (0.06) |
| PDE | 3.5 (0.06) | 5.7 (0.0009) | 2.3 (0.06) |
| Ecoenzyme activities ratios | | | |
| BG/NAG | 19 (<0.0001) | 3.4 (0.05) | 0.6 (0.64) |
| BG/PME | 1.3 (0.26) | 0.3 (0.85) | 0.4 (0.85) |
| BG/PDE | 103 (<0.0001) | 3.4 (0.016) | 10 (<0.0001) |
| NAG/PME | 18.1(0.0001) | 3.5(0.01) | 4.4 (0.004) |
| NAG/PDE | 0.075(0.78) | 4(0.007) | 3 (0.03) |

Cmic: microbial carbon; Nmic: microbial nitrogen; Pmic: microbial phosphorus; BG: β-1,4-glucosidase; CBH: cellobiohydrolase; NAG: β-1,4-N-acetylglucosaminidase; PPO: polifenol oxidase; PME: phosphomonoesterase; PDE: phosphodiesterase

### *3.3. Potential mineralization, nutrient transformation, and immobilization by the soil microorganisms*

The potential C mineralization was affected by the vegetation cover and by the fertilization treatments (Table 6). Regardless of the fertilization treatment, the $CO_2$-C value was higher in the G soil than in the RS soil (488 ± 27 and 188 ± 14 µgC $g^{-1}$); while CNf and CNPf showed higher values of $CO_2$-C than the other three treatments despite of the vegetation cover (Figure 5A ). In contrast, the interaction effect of vegetation cover and fertilization treatments was significant for the net amonification (NA) and net nitrogen mineralization (NNM; table 6). The CNf treatment had the highest values of NA and NNM in the G soils, while the NA and NNM values were not different among fertilization treatment in the RS soil (Table 5).

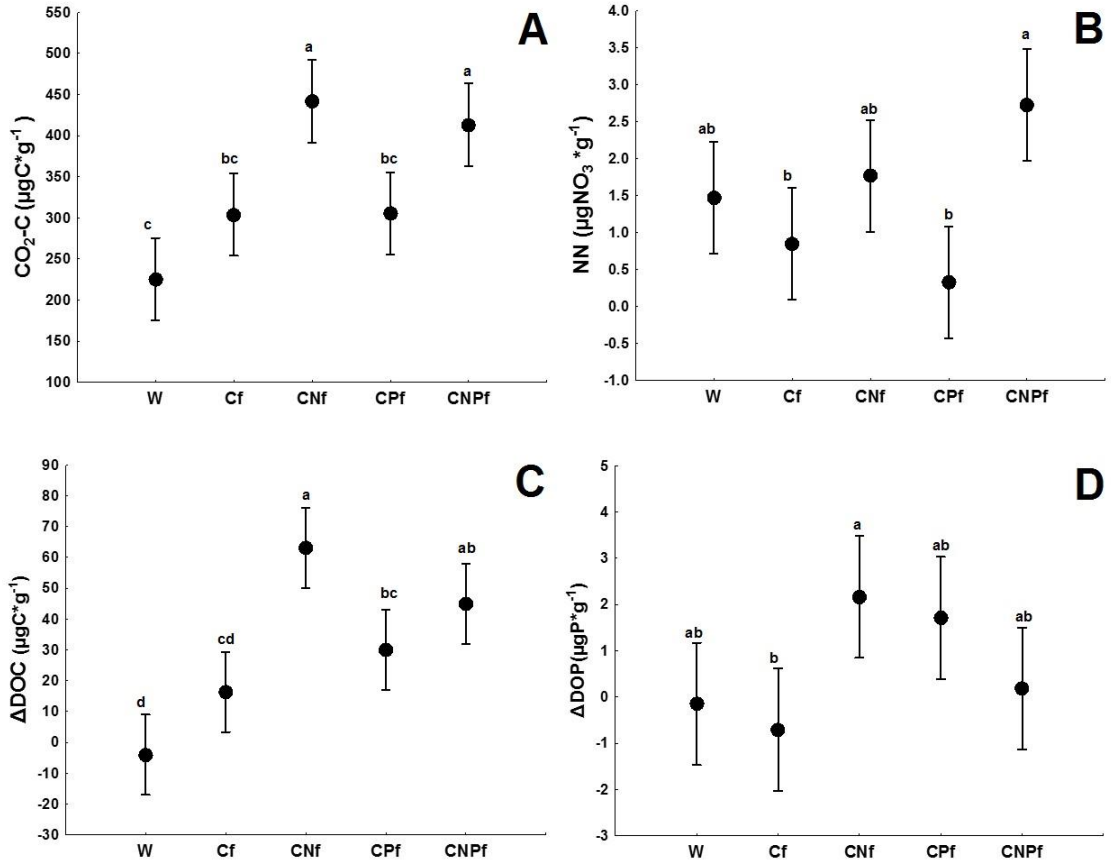


**Figure 5.** Means and standard errors of A) Net carbon mineralization, B) Net nitrification, C) Dissolved organic carbon transformation, D) Dissolved organic phosphorus transformation, under five fertilization treatments: W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization. Different lower-case letters indicate significant differences between treatments ($P < 0.05$).

However, the net nitrification (NN) was only affected by fertilization effect (Table 6): the CNPf treatment had higher values of NN than that in the Cf and CPf treatments (Figure 5B).

The CNf and W treatments had the highest and the lowest values of transformation of dissolved organic carbon (ΔDOC; Figure 5C); while CNf treatment had higher values of transformation of dissolved organic phosphorus (ΔDOP) than that in the Cf treatment (Figure 5D). Conversely, the transformation of dissolved organic nitrogen (ΔDON) was affect by the interaction between vegetation cover and fertilization treatments (Table 6). The CNf treatment had higher ΔDON values than the other four treatments in the G soil, while these values had no difference among fertilization treatment in the RS soil (Table 5).

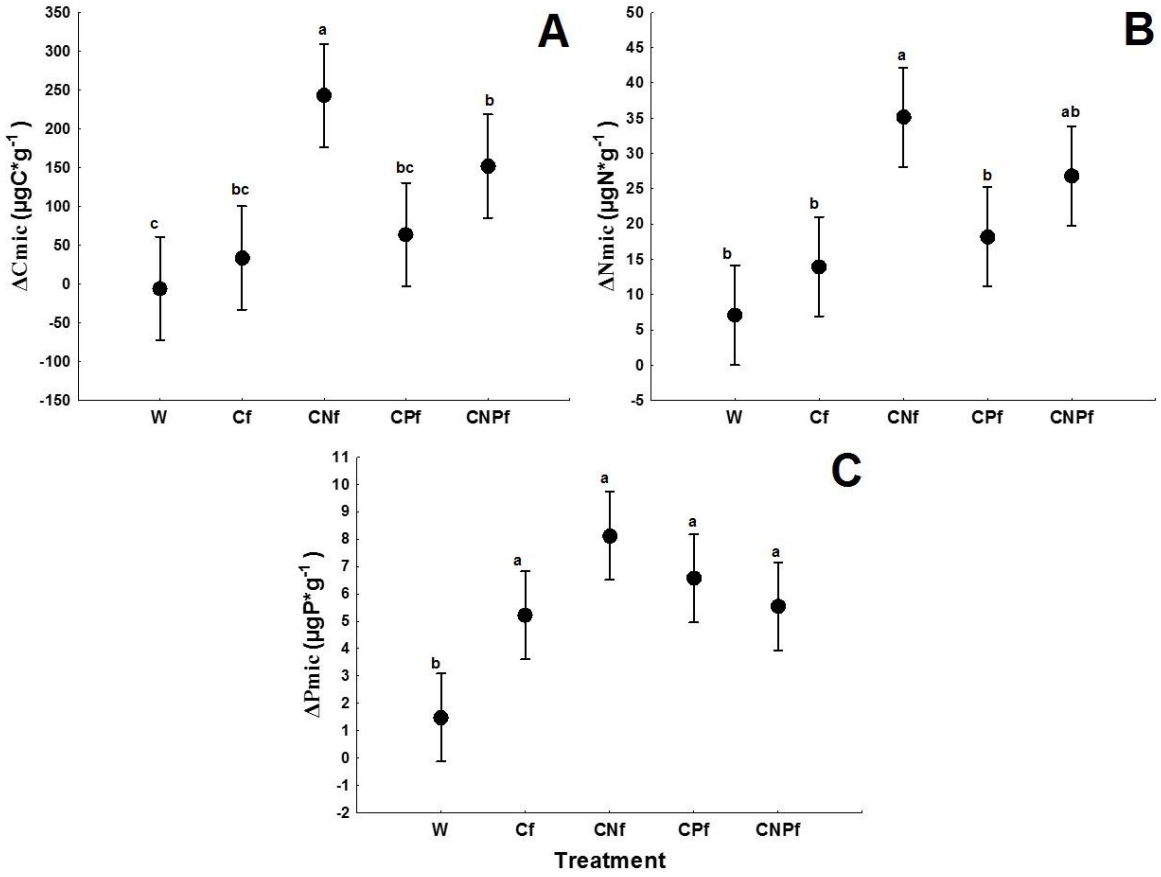


**Figure 6.** Means and standard errors of potential nutrient immobilization within microbial biomass (A: carbon, B: nitrogen and C: phosphorus) under five treatments in rosetophylous scrub soil (RS) and the grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different lower-case letters indicate significant differences between treatments ($P < 0.05$). W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

The CNf treatment had higher potential nutrient immobilization within microbial biomass (ΔCmic, ΔNmic and ΔPmic) than in the W treatment (Fig 6). Additionally, the potential carbon immobilization (ΔCmic) was higher in the G soil than in the RS soil despite of the fertilization treatments (133 ± 35 and 62 ± 16 µg g-1, respectively).

values were similar among fertilization treatments in the G soil (Fig. 7B). Furthermore, the RS soil had higher $TER_{C:P}$ values than in the G soil only in the W treatment (Figure 7B).

### *3.4 Nutrient use efficiency and Threshold elemental ratio*

$TER_{C:N}$ showed both vegetation cover effect and fertilization effect (Table 6 ). Regardless of the fertilization treatment, the $TER_{C:N}$ was higher in the G soil than in the RS soil (20 ± 1 and 15 ± 1, respectively). Additionally, the $TER_{C:N}$ values were lower in the CNf and CNPf treatments than in the W treatment despite of the vegetation cover (Figure 7A). In contrast, the interaction vegetation cover and fertilization affect the $TER_{C:P}$ (Table 6). The W treatment had higher $TER_{C:P}$ values than the other four treatments only in the RS soil, while these

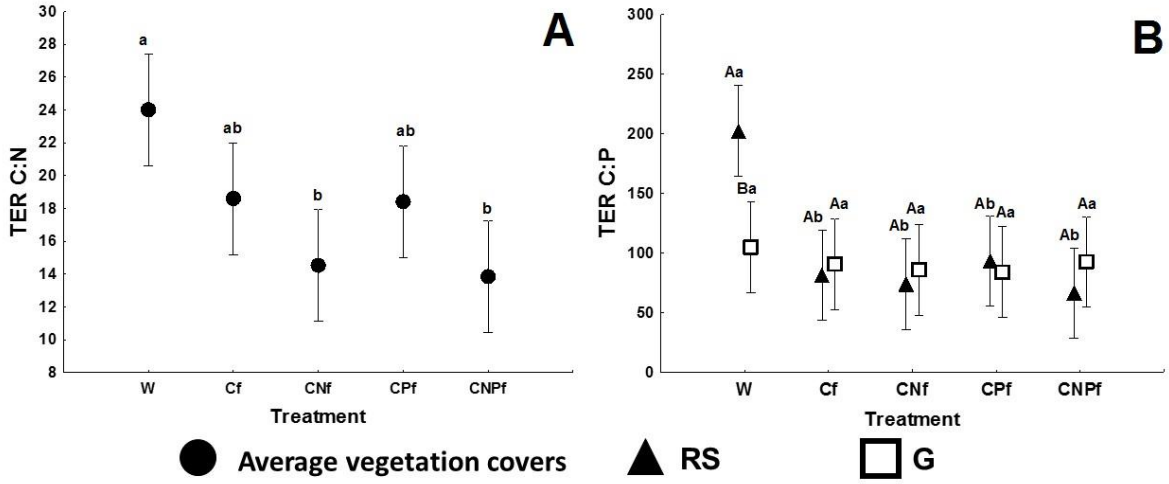


**Figure 7.** Means and standard errors of: Threshold elemental ratio A) TERC:N, B) TERC:P; under five fertilization treatments in rosetophylous scrub soil (RS) and the grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different uppercase letters (A and B) indicate significantly different means ($P < 0.05$) between vegetation cover (RS and G) within the same treatment; whereas different lowercase letters vertically indicate significantly different means ($P < 0.05$) among fertilization treatments within the same vegetation. W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

**Table 5**. Means and standard errors nutrient transformation, mineralization and immobilization by the soil microorganisms under five treatments in the rosetophylous scrub soil (RS) and the grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico. Different uppercase letters indicate significant differences ($P < 0.05$) between vegetation covers (RS and G) under the same treatment. Different lower-case letters indicate significant differences ($P < 0.05$) between treatments (W, Cf, CNf, CPf and CNPf) under the same vegetation cover. Values without letters did not show statistically significant differences in the interaction between vegetation and treatment.

| | Fertilization treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RS | | | | | G | | | | |
| | W | Cf | CNf | CPf | CNPf | W | Cf | CNf | CPf | CNPf |
| Potential dissolved nutrient transformation rates | | | | | | | | | | |
| ΔDOC(μgC $g^{-1}$) | -8.8(4.7) | 15(8.8) | 57.9(6.2) | 32.5(2.7) | 47.2(2.7) | 23(7.9) | 42.2(12.6) | 95 (13.7) | 49.8(9.1) | 65(16.3) |
| ΔDON(μgN $g^{-1}$) | -1.9(0.5)$^{Aa}$ | -2.3(0.4) $^{Aa}$ | -1.0(0.8)$^{Ba}$ | -2.1(0.4)$^{Ba}$ | -1.8(0.8)$^{Ba}$ | 0.2(0.9)$^{Ab}$ | 1.4(0.9)$^{Ab}$ | 8.1(0.8)$^{Aa}$ | 2.3(1)$^{Ab}$ | 2.5(1.6)$^{Ab}$ |
| ΔDOP(μgP g-1) | 0.5(1) | -0.3(0.6) | 1.6(0.8) | 1.4(1) | 0.3(0.9) | -0.8(0.8) | -1.1(0.9) | 2.8(0.9) | 2.1(1.2) | 0.1(0.1) |
| Potential mineralization | | | | | | | | | | |
| $CO_2$-C(μgC $g^{-1}$) | 104(12) | 154(13) | 277(11.2) | 197(11) | 253(14) | 346(27) | 454(54.5) | 607(45) | 460(43) | 573(63) |
| NA(μg$NH_4^+$ $g^{-1}$) | -0.8(0.3)$^{Aa}$ | -0.8(0.4) $^{Aa}$ | 4.8(0.9) $^{Ba}$ | -0.7(0.2) $^{Aa}$ | 0.9(0.3) $^{Aa}$ | -4(0.5)$^{Ab}$ | -4.4(0.5)$^{Ab}$ | 14.7(4.5)$^{Aa}$ | -3.8(0.2)$^{Ab}$ | 3.5(3)$^{Ab}$ |
| NN(μg$NO_3$ $g^{-1}$) | 1.13(0.4) | 0.7(0.2) | 1.6(0.9) | 0.3(0.4) | 2.6(0.6) | 1.8(0.5) | 1(0.5) | 2(0.3) | 0.4(0.5) | 2.8(0.6) |
| NNM (μgN $g^{-1}$) | 0.3 (0.5) $^{Aa}$ | -0.1(0.5) $^{Aa}$ | 6.4(1.2)$^{Ba}$ | -0.4(0.4) $^{Aa}$ | 3.5(0.7) $^{Aa}$ | -2.2(0.5)$^{Ac}$ | -3.4(0.5)$^{Ac}$ | 16.6(4.5)$^{Aa}$ | -3.4(0.5)$^{Ac}$ | 6.3(3.2)$^{Ab}$ |
| Potential microbial transformation rates | | | | | | | | | | |
| ΔCmic(μgC $g^{-1}$) | 23(10) | 13.2(9) | 155.5(30) | 34.4(14) | 82.6(34) | -34.5(10) | 54.5(33) | 330(34) | 92.9(9) | 221.2(24) |
| ΔNmic(μgN $g^{-1}$) | 10.7(7) | 17.4(7) | 35.6(3) | 22.7(3) | 27.9(4) | 3.4(3) | 10.5(4) | 34.7(2.3) | 13.7(3.5) | 25.8(7.7) |
| ΔPmic(μgP $g^{-1}$) | 13(0.6) | 5(0.5) | 7.2(0.4) | 5.4(0.9) | 5(.4) | 1.6(0.7) | 5.4(0.9) | 9(2.1) | 7.7(1.2) | 6.1(1.9) |

Δ Change in: DOC: dissolved organic carbon; DON: dissolved organic nitrogen; DOP: dissolved organic phosphorus. NA: Net Amonification, NN: Net Nitrification and NNM: Net Nitrogen Mineralization. Treatments: W: control, Cf: carbon fertilization, CNf: carbon and organic N fertilization, CPf: carbon and organic phosphorus fertilization, and CNPf: carbon, organic N and organic P fertilization.

**Table 6.** F-ratios and significant levels of the factorial ANOVA of post-I: potential nutrient transformation, mineralization and immobilization by the soil microorganisms under five treatments in the rosetophylous scrub soil (RS) and the grassland soil (G) of the Cuatro Ciénegas Basin, Coahuila, Mexico.

| Parameters | Source of variation | | |
|---|---|---|---|
| | Vegetation cover | Fertilization treatments | Interaction |
| | Potential nutrient transformation rates | | |
| ΔDOC | 0.2 (0.65) | 16.1(<0.0001) | 0.33 (0.86) |
| ΔDON | 68.3(<0.0001) | 7.41(<0.0001) | 4.11 (0.01) |
| ΔDOP | 0.02(0.9) | 3.58(0.01) | 0.64 |
| | Potential mineralization | | |
| $CO_2$-C | 182.4(<0.0001) | 12.7(<0.0001) | 0.5(0.8) |
| NA | 0.20(0.65) | 16.3(<0.0001) | 4.8(0.002) |
| NN | 0.93(0.34) | 6.02(<0.0001) | 0.10(0.9) |
| NNM | 0.53(0.47) | 20(<0.0001) | 4.96(<0.0001) |
| | Potential microbial transformation rates | | |
| ΔCmic | 5.8(0.02) | 9.21(<0.0001) | 1.8(0.12) |
| ΔNmic | 2.9(0.09) | 9.9(<0.0001) | 0.25(0.9) |
| ΔPmic | 2.8(0.09) | 9.56(<0.0001) | 0.28(0.8) |
| | Threshold elemental ratio | | |
| $TER_{C:N}$ | 14 (0.0005) | 5.8 (0.0008) | 0.2 (0.9) |
| $TER_{C:P}$ | 1 (0.31) | 5.7 (0.001) | 3.5 (0.01) |

Δ Change in: DOC: dissolved organic carbon; DON: dissolved organic nitrogen; DOP: dissolved organic phosphorus; DIP: dissolved inorganic phosphorus. NA: Net Ammonification, NN: Net Nitrification and NM: Net Mineralization

## 4. Discussion

Our two hypotheses were partially confirmed, we tested that a pulse of labile organic nutrients (twice the normal) in an oligotrophic soil made the soil microbial communities: 1) respond in greater magnitude with processes of nutrient mobilization when it has higher nutritional requirements, and 2) increase the efficient use of the most limiting nutrient (or nutrients) by increasing the potential rates of organic nutrient transformation, increased SEA, and decreased mineralization

We observed that with the input of labile organic C and N the site effect was lost in the transformation of organic C and P (ΔCOD, ΔPOD), the mineralization of C (CO2-C) and the net immobilization of N and P (ΔNmic and ΔPmic). This suggests that both communities are strongly limited by C and that the C represents a nutritional requirement that limits the processes for obtaining N, P and also limits the growth of the community

We also found that the RS soil microbial communities were the most co-limited by P and, after the input of labile organic CN, had high C, N and P immobilization with low ecoenzymatic activities which suggest that this soil microbial community is efficient to obtain P. However, the G soil microbial communities was the most co-limited by N but was also inefficient at obtaining this resource.

### 4.1 Response of the soil microbial communities to the organic nutrient addition in the use of C and N

In a previous study we observed that the G soil microbial community is more limited by N than the RS soil microbial community (Montiel-González et al. 2017). In the present study, we observed that with an input of labile organic nutrients with CN and CNP, there was a decrease in the co-limitation by C-N. Likewise, we observed that the microbial communities from both vegetation covers did not show a difference in the intensity of their response to the co-limitation ($TER_{CN}$). This could suggest that both microbial communities have the physiological capacity to satisfy their metabolic demands by N and respond to a pulse of labile organic nutrients rich in CN even though historically the G soil microbial community was more limited by N.

The effect of the fertilization was stronger than the origin of the soil microbial communities in the production of organic C (COD and ΔCOD) and the C mineralization ($CO_2$-C) especially in the CNf treatment. This means that although both communities produce enzymes and depolymerize more organic C in the CNf treatment, both communities also lose large amounts of C as a metabolic waste. However, the low SEA BG and SEA CBH values in RS soil suggest that the RS soil microbial community had invest low in ecoenzymatic synthesis of BG and CBH to produce high C immobilization.

The high net nitrogen mineralization (NMM), specially in the CNf treatment of G soil in combination with the higher SEANAG values in G soil than in RS soil and the higher

SEANAG value in the CNf and CNPf treatments, indicate that with an input of organic labile N the G soil microbial community can compensate for the N deficiency by setting out a high enzymatic synthesis of NAG but obtains low nutrient immobilization N.

We had previously observed that the G soil microbial community can immobilize more C and N than the RS soil microbial community (Montiel-González et al. 2017). However, in the present study, the Cmic, Nmic, ΔCmic, ΔNmic values suggest that the effect of the inputs of labile organic forms of CN and CNP are stronger than the origin of the soil microbial communities. We suggest that both soil microbial communities seem to be more sensitive to the input of labile organic CN and respond quickly with high C and N immobilization by the ecoenzymatic upregulation of BG, CBH and NAG. The increase in the Cmic and Nmic produced by C addition was also observed in a soil with low P availability (Heuck et al. 2015).

Besides, the vegetation cover effect on the ecoenzymatic ratios of the BG/NAG suggests that the soil microbial community of G soil produces more BG than NAG compared to the RS soil microbial community, which implies greater investment of resources to obtain C than to obtain N by the G soil microbial community. In general, our values of the BG/NAG ratio above 1.6 (Table 3) were higher than those previously reported of 1.33 (Sinsabaugh et al. 2016) and 1.43 (Sinsabaugh & Follstad Shah 2012), suggesting a very low production of NAG by unit of BG compared to other studied ecosystems. Although we observed no vegetation cover or treatment effect for nutrient microbial rates Cmic:Nmic, we observed that our data represents the lowest reported values (between 6.4± 1 and 7.4± 0.8) compared to other studies with means of: 7.91 ± 0.05 (Sinsabaugh et al. 2016), 8.6 ± 0.3 (Cleveland & Liptzin 2007) and 7.6 (Xu et al. 2013). Our results suggests that the microbial communities in these soils tend to protect the N within its biomass.

Therefore, in a pulse of organic nutrient rich in labile organic CN, the response of both microbial communities was the decrease of the co-limitation (low $TER_{C:N}$) through the increase in enzymatic activity to acquire N which produced an increase in its immobilized C and N. However the RS soil microbial community could be more efficient in obtaining C and N with less resource investment than the G soil microbial community. The G soil microbial community offset the co-limitation by CN with ecoenzymatic upregulation of BG, CBH and

NAG but we think that this community, even with the fertilization, is strongly limited by C-N organic.

### 4.2 Response of the soil microbial communities to the organic nutrient addition in the use of P

In a previous study we observed that the RS soil microbial community presented more co-limitation by P than the G soil microbial community (Montiel-González et al. 2017). In our present study we observed the higher $TER_{C:P}$ value in the W treatment of the RS soil indicating that there is a high co-limitation by C:P when there is no addition of organic nutrients.

We also observed that the input of labile organic C favored this co-limitation by C-P to decrease for RS communities. However, communities of G did not respond to the pulse of organic nutrients with changes in co-limitation by C-P. The above could suggest that when humidity and temperature are not limiting for the growth of G soil microbial communities: 1) A pulse of twice the normal labile organic nutrients do not represent a sufficient input of nutrients to reduce the co-limitation for C-P in G soil microbial communities, or 2) The G soil microbial communities have physiological limitations that allow them to respond quickly to these pulses.

The effect of the fertilization was stronger than the origin of the soil microbial communities in the transformation of organic P (ΔPOD). However, the differences among treatments only were observed in low ΔPOD in Cf treatment and high ΔPOD in CNf treatment. The above implies that with the input of labile organic C the low production of POD could be explain by an increase in the P mineralization.

Both soil microbial communities responded to the addition of C organic with an increase in the net P immobilization (ΔPmic). Also, the high SEA PME and PDE values with an input of organic nutrients rich in CN and CP could indicate that both soil microbial communities present high PME and PDE activities but the P immobilization is low. Additionally, regardless of treatments, the low SEA PME value for the RS soil suggests that the RS soil microbial community is more efficient in immobilizing P by PME production than the G soil microbial community. This supports the proposed hypothesis that RS soil

microbial communities face resource limitation by ecoenzymatic upregulation of PME when there are pulses of available resources (Montiel-González et al. 2017).

Additionally, the lower Cmic:Pmic ratio in the W treatment in the RS soil, suggests that the RS soil microbial community responded to the addition of organic C with an increase in the concentration of Pmic by unit of Cmic inmobilizated. For the nutrient microbial rates Cmic:Pmic, we observed that our values (with the exception of the low values in the Cf and CPf treatment for the G soil) were similar to the means reported in other studies: 42.2 ± 1.9 (Sinsabaugh et al. 2016), 59.5 ± 3.6 (Cleveland & Liptzin 2007) and 42 (Xu et al. 2013).

Although the BG/PME ratio showed no difference, we observed a decrease of the BG/PDE ratio for the G soil under Cf and CNf treatments and for the RS soil under Cf and CNPf treatments, which suggests an increase of PDE activity by unit of BG activity in response to the addition of Cf. Additionally, our BG/PME ratios were between the mean values reported in other studies: 0.18 (Sinsabaugh et al. 2016) and 0.61 (Sinsabaugh & Follstad-Shah 2012).

Together these results suggest that although the C:P co-limitation was higher for the microbial communities in the RS soil than in the G soil, both communities responded primarily to the addition of organic nutrients C with the increase in enzymatic activity to acquire P and an increase in the P immobilization. However, the high limitation by P in these ecosystems caused both soil microbial communities to invest high resources in producing ecoenzymes to obtain P.

## 5. Conclusions

The addition of labile organic nutrients, especially of CNf, promoted an increase in the eco-enzymatic activities (EEA) to obtain C, N and P. The changes of EEA in turn produced changes in the fluxes of microbial immobilization and mineralization of C, N and P; which modified the stoichiometry of the microbial biomass of both vegetation covers.

We observed that the addition of organic nutrients produced a change in the process to mobilize N and P. However, the effect of vegetation cover (origin) was lost and we observed that the communities responded similarly to the treatments. An input of labile organic C favors the investment of resources for the production of ecoenzymes that mineralize P, which

is reflected in the increase of immobilized P. On the other hand, the pulse of labile organic CN favors the increase of N mineralization and immobilization.

However, it seems that the microbial community of RS immobilizes the N and P with less investment in enzymes, which would make it more efficient at obtaining these resources. One possible explanation is that since historically the soil microbial communities of RS have lived under a lower availability of nutrients, these communities have become efficient at obtaining resources after pulses of organic nutrients as a survival strategy.

In contrast, even with an input organic nutrient twice as large as that occurring in a wet year, the growth of G soil microbial communities are still limited by nutrients.

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# CAPÍTULO V
# CONCLUSIONES

Con base en los resultados que derivaron de los estudios realizados en la presente tesis, proponemos de manera general las siguientes conclusiones.

En el **capítulo II** se propuso "evaluar el efecto del GCC en el valle de Cuatro Ciénegas, Coahuila durante los últimos 70 años (1941 a 2013). Concretamente, se buscó: 1) identificar tendencias en el comportamiento de las variables climáticas (temperatura y precipitación); 2) evaluar la naturaleza y dirección de los cambios en la frecuencia de la ECE; y 3) detectar los cambios en la variabilidad interanual de la precipitación a lo largo de todos los meses durante los últimos 70 años".

1. Con base en los análisis de tendencias climáticas de Temperatura en el valle de Cuatro Ciénegas en los últimos 36 años:
   - La temperatura máxima (Tmax) no mostró cambio significativo en el comportamiento de los datos. La Temperatura mínima (Tmin) ha aumentado en casi todos los meses del periodo de estudio. La Temperatura media solamente aumentó en los meses de verano.
   - La oscilación térmica disminuyó en los meses de verano, lo cual implica que la Tmax y la Tmin se han acercado, principalmente en estos meses.
2. En Cuatro Ciénegas en los últimos 30 años observamos que lo Eventos Climáticos Extremos (ECE):
   - En invierno aumentó la frecuencia de Tmin y Tmax: inviernos con oscilación térmica cada vez más extrema y aumento de heladas.
   - En verano disminuyó la frecuencia de Tmin y aumentó la frecuencia de Tmax lo cual produce veranos con reducción de oscilación térmica, es decir cada vez más calurosos y aumento de ondas de calor.
   - Hubo aumento de eventos de sequía y lluvias torrenciales
3. La precipitación no mostró tendencia debido a la alta variabilidad de los datos en las series que se estudiaron. Sin embargo, se identificó un cambio de la distribución de lluvias, observamos que en los últimos 36 años hubo mayor equitabilidad interanual de lluvia, disminuyendo el ingreso de precipitación en los meses húmedos y aumentando la precipitación en los meses secos.

4. Para las series de datos climáticos de Cuatro Ciénegas, se detectó un cambio basado en la clasificación climática propuesta por Köppen y modificada por Enriqueta García para México. El clima paso de BWhw(x´)(e´) hacia BWhwx(x´)(e´) en los últimos 36 años, lo cual se debe principalmente a la modificación del patrón de la distribución de lluvias a lo largo del año, el aumento de la temperatura tanto del mes más frio (enero) como del mes más caluroso (julio.).
5. Desafortunadamente, nuestros resultados sugieren que la tendencia en el valle de Cuatro Ciénegas es que los inviernos se volverán más fríos y los veranos se volverán más cálidos con una alta variabilidad en la disponibilidad de agua, aumentando el estrés ambiental para los organismos que habitan nuestro sitio de estudio. Por esta razón, es muy importante entender completamente cómo está cambiando el clima a fin de diseñar estrategias de gestión apropiadas para adaptarse a dicha variabilidad climática en el futuro cercano.

De acuerdo con la figura 1 propuesta en la introducción general de la presente tesis la temperatura del valle de Cuatro Ciénegas, Coahuila, en los últimos 36 años seguiría el comportamiento que se muestra en la figura 1.

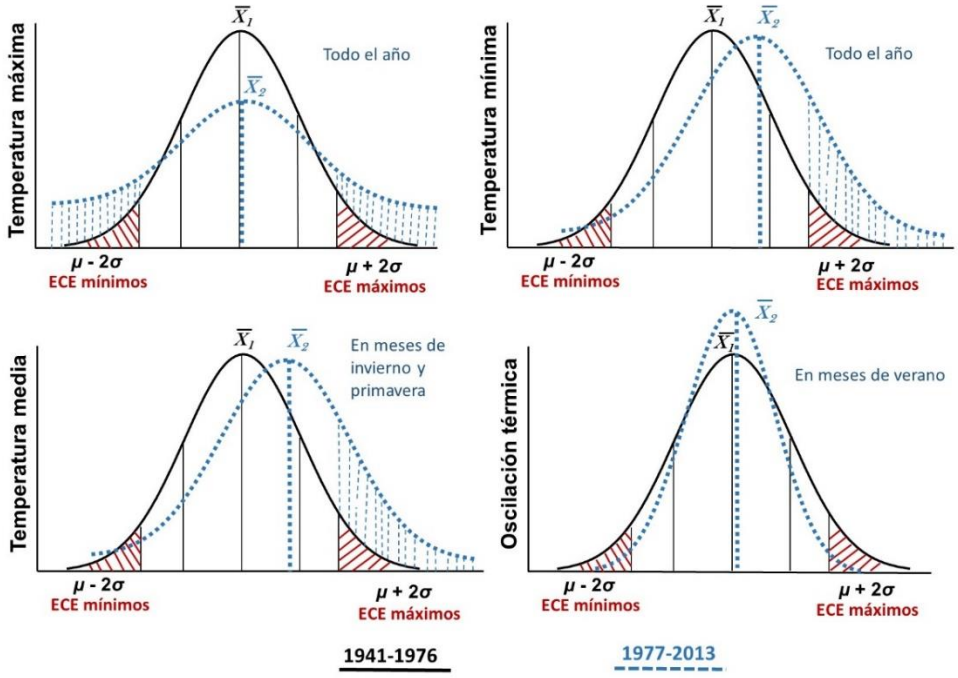


**Figura 1**. Comportamiento de la distribución de probabilidad de ocurrencia de la temperatura y ubicación de los eventos climáticos extremos (ECE) en dos periodos de 30 años para el Valle de Cuatro Ciénegas, Coahuila. Versión de Montiel-González C. modificada a partir de IPCC (2012).

En el **capítulo III** se propuso "Examinar los efectos de la variación de la lluvia sobre los ajustes fisiológicos que realizan las comunidades microbianas del suelo: 1) para obtener energía y nutrientes y 2) que determinan la vulnerabilidad de las comunidades microbianas en dos sistemas suelo-vegetación, cada uno con diferente entrada de materia orgánica en un desierto oligotrófico: el valle de Cuatro Ciénegas, Coahuila". Se concluyó que:

1) En suelos con diferente disponibilidad de recursos el aumento de la precipitación podría favorecer la inmovilización de C, N y P en la biomasa microbiana, ya que estas variables correlacionaron positivamente.
2) Se observó alta limitación por P en ambos sitios independientemente del año. Así mismo, se observó alta limitación por N durante el año húmedo (2013), pero solo en suelos de pastizal.
3) En el año más húmedo (2011) la diferencia de la abundancia relativa de taxas específicos en la comunidad microbiana del suelo podría reflejar la capacidad de obtención de recursos por las comunidades. En el suelo de sotol hay mayor abundancia de acidobacterias y firmicutes los cuales son grupos facultados para obtener P en condiciones limitadas por medio de producción de enzimas y ácidos orgánicos. En el suelo de pastizal existe mayor abundancia de grupos como proteobacterias, actinobacterias y bacteroidetes con la capacidad de obtener el C de diferentes calidades de sustrato por medio de actividad enzimática.
4) En un sitio con baja disponibilidad de recursos (C, N y P) como es el suelo de sotol, las comunidades microbianas invierten más energía en producir enzimas (PME y PDE) para adquirir nutrientes (especialmente P) que en acumular nutrientes dentro de su biomasa (baja inmovilización).
5) Por el contrario en un sitio con alta disponibilidad de recursos como es el pastizal, las comunidades microbianas invierten menos energía en adquirir nutrientes favoreciendo la acumulación de nutrientes dentro de su biomasa.
6) Ambas comunidades microbianas del suelo son altamente homeostáticas; es decir, se ajustan fisiológicamente para procesar bajos recursos de N y P y hacer frente a la limitación de nutrientes, particularmente en años secos.
7) La comunidad microbiana del sitio con mayor disponibilidad de recursos (G) en este ecosistema, aun en años húmedos (2013 y 2014) se ve limitada por N y P para satisfacer

sus demandas metabólicas. Esta comunidad realiza ajustes metabólicos para mantener su crecimiento lo cual la hace más sensible a la limitación por recursos que la comunidad microbiana de sotol.

8) Las comunidades microbianas son más resilientes que vulnerables a la variación en la precipitación. Especialmente la comunidad microbiana del suelo con menores recursos (comunidad de sotol) se ha adaptado a la disminución de P en años secos disminuyendo su metabolismo durante este periodo y presentando una sobre regulación enzimática (de PME y PDE) para obtener nutrientes cuando aumenta la precipitación.
9) Bajo los escenarios de cambio climático global para ecosistemas desérticos que predicen una reducción de precipitación anual y una mayor intensidad y frecuencia de lluvias torrenciales y eventos de sequía, las comunidades microbianas del suelo dentro de ambos sitios podrían ser vulnerables a la sequía debido a la combinación de co-limitación C -P y a la reasignación de energía y nutrientes hacia estrategias de aclimatación fisiológica para sobrevivir

Bajo el esquema propuesto en la introducción general de la presente tesis las comunidades microbianas del suelo de ambos sitios llegan hasta el segundo nivel en donde las comunidades presentan resistencia y resiliencia a la variabilidad anual en la precipitación debido a que realizan estrategias de aclimatación al estrés (Figura 2).

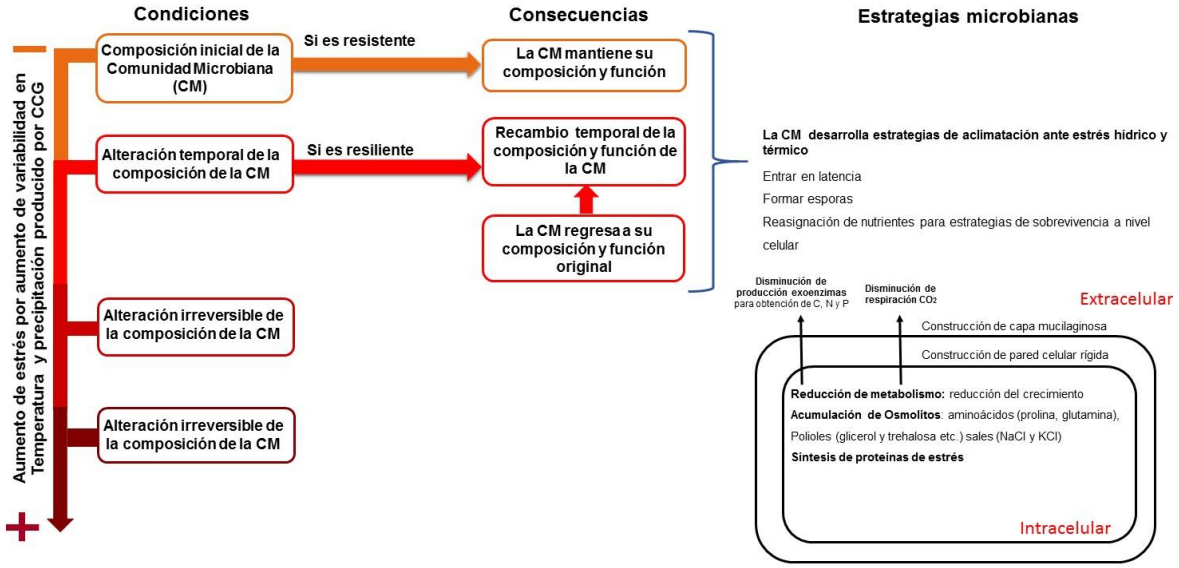


**Figura 2**. Efecto de la variabilidad anual de la precipitación ocasionada CCG sobre la estructura y función de las comunidades microbianas (CM) del suelo y algunas estrategias de las CM para aclimatarse ante esta variabilidad. Versión de Montiel-González C. modificado a partir de Allison y Martiny (2008) y de Schimel et al. (2007).

En el **capítulo IV** se propuso Evaluar: 1) ¿Cómo responde el comunidades microbiana del suelo de un desierto oligotrófico a una entrada del diferentes compuestos con MOS lábil rica en C, N y P?, 2) ¿La comunidad microbiana de este suelo tiene la capacidad fisiológica para usar los nutrientes de manera eficiente?, y 3) ¿Cuáles son los procesos que determinan que las comunidades microbianas utilicen eficientemente nutrientes en este ecosistema oligotrófico?

1) Bajo condiciones de laboratorio la fertilización con nutrientes orgánicos lábiles, especialmente de CN (2:1), favoreció un aumento en las actividades eco-enzimáticas para obtener C, N y P. en suelo de valle de Cuatro Ciénegas.
2) Los cambios producidos en la actividad eco-enzimática derivan en cambio de flujos de inmovilización y mineralización de C, N y P. Como consecuencia de lo anterior, se producen cambios en la estequiometría (C:N y C:P) de la biomasa microbiana del suelo
3) La adición de nutrientes orgánicos produjo un cambio en los procesos para movilizar N y P. Sin embargo, el efecto de la cubierta vegetal (origen de la comunidad) se perdió y observamos que las comunidades respondieron de manera similar a los tratamientos en algunas variables.
4) Una entrada de C orgánico lábil favorece la inversión de recursos para la producción de ecoenzimas que mineralizan P, lo que se refleja en el aumento de P. inmovilizado. Por otro lado, el pulso de CN orgánico lábil favorece el aumento de la mineralización e inmovilización de N.
5) La comunidad microbiana de suelo de sotol inmoviliza el N y P con menor inversión en enzimas, lo que lo haría más eficiente en la obtención de estos recursos. Una posible explicación es que, debido a que históricamente las comunidades microbianas del suelo de sotol han vivido con una menor disponibilidad de nutrientes, estas comunidades se han vuelto muy eficientes en la obtención de recursos después de los pulsos de nutrientes orgánicos como una estrategia de supervivencia. Lo anterior sugiere que probablemente tienen una alta resiliencia ante la variación de recursos.
6) En contraste, incluso con la entrada de nutrientes orgánicos lábiles dos veces mayor a la que se produce en un año lluvioso, el crecimiento de las comunidades

microbianas del suelo todavía está limitado por los nutrientes, ya que estas comunidades muestran alta inversión de recursos para producción enzimática y poca incorporación de nutrientes en su biomasa.

Bajo el esquema propuesto en la introducción general de la presente tesis las comunidades microbianas del suelo de ambos sitios responden de manera diferente al pulso de nutrientes orgánicos. La respuesta principal de las comunidades a la entrada de C y N orgánicos, se reflejó en su capacidad para transformar los nutrientes orgánicos potencialmente disponibles mediante síntesis enzimática (y mineralizar nutrientes) y su capacidad de convertir esos nutrientes en biomasa microbiana de manera eficiente para disminuir la co-limitación por CN y CP.

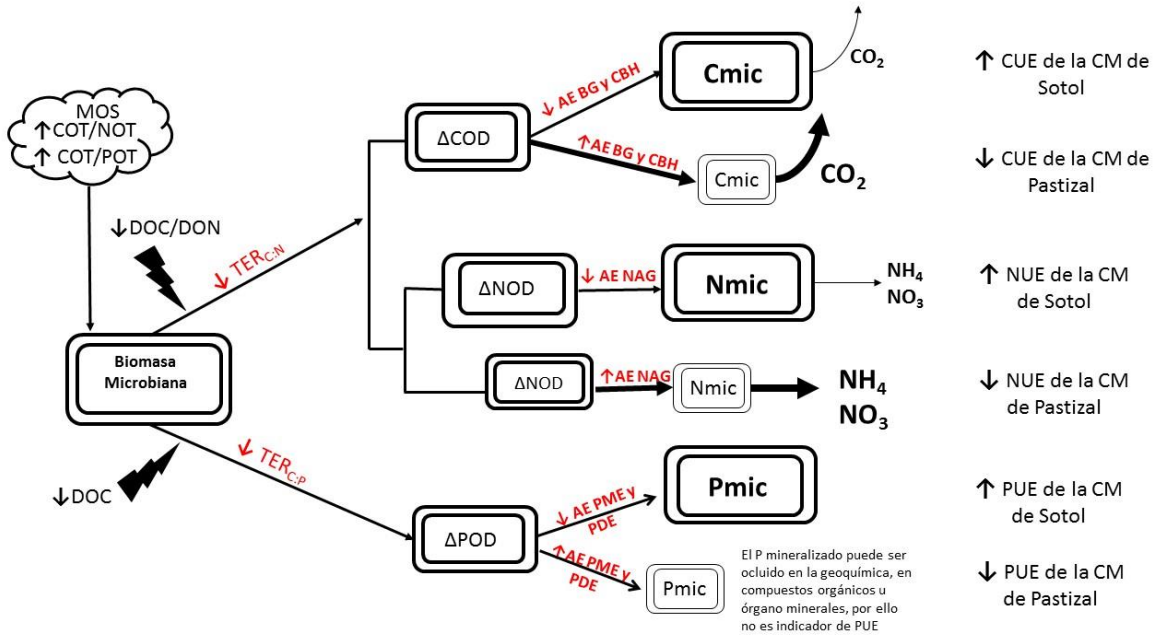


**Figura 3.** Diagrama de posibles respuestas en los procesos que intervienen en el uso de nutrientes cuando se presenta un pulso de nutrientes orgánicos lábiles ricos en CN en una comunidad microbiana de suelo limitado por nutrientes orgánicos en el Valle de Cuatro Ciénegas. Imagen de Montiel-González C.