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**DINÁMICA DEL C Y DEL N EN EL SUELO DE BOSQUES TROPICALES ESTACIONALMENTE
SECOS ANTE LOS ESCENARIOS DE INCREMENTO EN LA DEPOSICIÓN ATMOSFÉRICA**

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MSc. MARYLIN BEJARANO CASTILLO

TUTOR PRINCIPAL DE TESIS: DR: JULIO CAMPO ALVES

INSTITUTO DE ECOLOGÍA

COMITÉ TUTOR: DR. VICTOR PARRA-TABLA

POSGRADO EN CIENCIAS BIOLÓGICAS

DR. JORGE ETCHEVERS BARRA

POSGRADO EN CIENCIAS BIOLÓGICAS

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M. del Coro Arizmendi

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Sí reíste por las buenas noticias

Sí no respiraste durante los retos

Sí te preocupaste en los momentos complicados

Sí anhelaste durante las esperas

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RESUMEN

Durante el último siglo el N reactivo (Nr) se ha incrementado en dos órdenes de magnitud como consecuencia del uso de fertilizantes nitrogenados, la quema de combustibles fósiles y la extensión e intensificación del cultivo de leguminosas. Diversos estudios han comprobado que altas tasas de deposición crónica de N en los bosques templados cambian la estructura, composición y funcionamiento de estos ecosistemas. Los pocos estudios realizados en los BTs limitan nuestra capacidad de entender el efecto del incremento del N en estas mismas variables.

En este proyecto se exploró el efecto del incremento en la disponibilidad de N en los ciclos de carbono (C) y N en BTs. Para ello se realizó el meta-análisis de los datos reportados en la literatura y, por medio de un enfoque experimental, se exploraron los efectos del incremento en la disponibilidad de N, en interacción con el régimen de lluvia, en (1) la dinámica del C y del N en el suelo de BTs estacionalmente secos (BTES) y (2) en la descomposición foliar de dos especies dominantes en estos mismos bosques. Los BTES seleccionados tienen características ambientales y biológicas poco variables, lo cual permitió aislar los efectos de la precipitación media anual en las variables estudiadas. El diseño experimental se basó en la adición de N en BTES ubicados a lo largo de un gradiente de precipitación (536 – 1035 mm de precipitación media anual) en la Península de Yucatán. La adición de N fue realizada siguiendo las tasas esperadas en la deposición de N atmosférico para el año 2030 en bosques tropicales.

Los resultados del meta-análisis demostraron que la adición de N incrementó la productividad primaria neta, la concentración de bioelementos de la hojarasca y del mantillo y las pérdidas de N en los BTs. Estos resultados indican que a pesar de que éstos son ambientes ricos en N, tanto la comunidad de plantas como la de microorganismos del suelo utilizan el N adicionado, soportando las evidencias previas acerca de la existencia de un

gradiente espacial de disponibilidad del N en BTs. En conjunto, estos resultados demuestran que los supuestos basados en el modelo de saturación de N en BTs deben ser reevaluados.

En la primera aproximación experimental se encontró que en el suelo de BTES la adición de N disminuyó las concentraciones de C orgánico en los bosques más secos y húmedos. El carbono en la biomasa microbiana del suelo respondió diferencialmente entre los sitios al incremento de N, mientras que en los suelos del sitio más seco, el incremento de N declinó significativamente las concentraciones microbianas de C y N. En contraste, la adición de N incrementó las concentraciones microbianas de C y de N en los suelos del sitio más húmedo del gradiente. La mineralización de N y las tasas de nitrificación no respondieron ante la adición de N. En el suelo del bosque más seco, la adición de N derivó en el incremento de las pérdidas de C y N debido a las emisiones de CO₂ y N₂O. Sin embargo, el incremento de N no afectó consistentemente las emisiones de CO₂ y N₂O en los suelos del bosque más húmedo.

En el segundo experimento se encontró que la tasa de descomposición fue afectada por la adición de N solo cuando el material originado en el bosque más húmedo y con menor contenido de N fue descompuesto en todos los sitios, o cuando el material proveniente de todos los sitios del gradiente fue descompuesto en el bosque más húmedo. Las concentraciones iniciales de los compuestos solubles de carbono y de lignina fueron los mejores predictores de la masa final remanente para el material de *Gymnopodium*. En un análisis similar, el porcentaje inicial de compuestos de carbono solubles en agua y la concentración de N predijeron la masa final remanente de *Piscidia*.

Las conclusiones de este trabajo son que (1) el incremento futuro de la deposición de N podría contribuir fuertemente a modificar las proyecciones y retroalimentaciones del ciclo global del C por cambios directos en los ciclos del C y del N en BTr., (2) los suelos de los BTES reaccionan al incremento del N dependiendo de la limitación hídrica de cada bosque, lo cual parece ser el mecanismo que controla el ciclo del N en estos suelos y (3) la descomposición foliar en los BTES ante los escenarios de incremento de N atmosférico también podrá variar dependiendo de la limitación hídrica del bosque y de la identidad de la especie.

ABSTRACT

Nitrogen (N) reactive compounds (Nr) have increased by two fold during the last century due to the increment of fertilizer use, biomass burning and rise extent and intensification of leguminous crops. There are evidence demonstrating that chronic N deposition under temperate forest change the structure, composition and function of them. However, the little searching in tropical forests limit our capacity to understand the N chronic deposition effect on these ecosystem variables.

In this work was explored the effect of N-supply availability on carbon (C) and N cycles in tropical forests. First, we achieved a meta-analysis with data reported in per-review references. Then, we explored the effect of experimental N-supply, and its interaction with the mean annual precipitation (MAP), on (1) both C and N soil dynamic in *seasonal dry tropical forest*, and (2) foliar decomposition rate of two dominant species in three study sites. SDTFs selected have similar environmental and biological characteristics that allowed study the MAP effects in our analysis. Experimental desing was based on the N-supply SDTFs along precipitation gradient (536 – 1035 mm MAP) in the Yucatan Peninsula. N-supply treatments were calculated according to expected rate atmospheric N deposition for 2030 in tropical regions.

Meta-analysis results showed that C, N, and P cycles respond to N-supply in ways not previously predicted by the approach based on the N-saturation approach. N-supply changed nutrient concentrations in litterfall, litter, and soil, supporting that both plants and microorganisms use the added N and changing the cycle of C, N, and P. Despite observed changes are based on low number and short-time searching, these provide more support for the NP co-limitation than the N-saturation state in TFs

In the first experimental approach, organic C concentrations in soil from both the driest and the wettest forest sites decreased significantly with elevated N input. However, microbial biomass responded to N enrichment differently across sites. In soils from the driest site, microbial biomass C and N concentrations declined significantly with elevated N input. In contrast, in soils from the wettest site, microbial biomass C and N immobilization increased. Net N-mineralization and nitrification rates did not respond to elevated N input. In soils from the driest site, elevated N input resulted in an increase in C and N losses due to CO₂ and N₂O fluxes. However, elevated N input to soils from the wettest site did not consistently affect CO₂ and N₂O fluxes. In soils from the wettest site, the main response to N enrichment was an increase only in CO₂ fluxes.

In the last experimental approach, N-supply did not have a significant effect on decomposition rates at the drier sites. In contrast, N addition at the wettest site affects litter decomposition. In this site the main response to N-supply was an increase in decomposition of *Gymnopodium*'s litter and a decrease in *Piscidia*'s litter. Overall, we observed that, decomposition was affected by the N-supply only when samples from the N-poor wettest site were decomposed in all sites, or when litter from the all sites were decomposed in the wettest nutrient-poor site. Initial water soluble C and, initial lignin were the best predictors of observed final mass for litter of *Gymnopodium*. In similar analysis, initial percentage of water-soluble C and N concentration predicted observed final mass for *Piscidia* litters.

The main conclusions of this work are that (1) future N-deposition increments could strongly contribute to changing projections and feedback of C cycling worldwide by affecting the C and N cycle in TFs, (2) the reactions of SDTF soils to increases in N deposition will vary, depending on the details of the site's water limitations, which appear as a regulatory mechanism of the N cycle in these soils, and (3) N-supply effects on decomposition processes will vary, depending on the details of the site's N status that results of mean annual precipitation, and the identity of species.

INTRODUCCIÓN GENERAL

Los bosques tropicales (BTs) son ecosistemas importantes a escala global porque son componentes primordiales del ciclo del carbono (C) y del nitrógeno (N). Estos bosques se forman en regiones en las que la precipitación media anual es superior a 500 mm, la biotemperatura es superior a 6° C y la relación de potencial de evapotranspiración es inferior a 4 (Holdridge *et al.*, 1971). En esta combinación climática ocurren tres tipos de biomas: los bosques tropicales y subtropicales secos de hojas amplias, los bosques tropicales y subtropicales húmedos de hojas amplias y, los manglares, que en conjunto ocupan ~ 30 % de la superficie terrestre (Olson *et al.*, 2001). Los BTs existen en una gran heterogeneidad de factores abióticos y bióticos (Townsend *et al.*, 2008). Por ejemplo, éstos se desarrollan en materiales parentales con diferentes orígenes geológicos y relieves, albergando una gran variedad de suelos (Richter & Babbar, 1991). Bióticamente, los BTs conservan ~ 50% de las especies conocidas a nivel mundial (Losos & Leigh, 2004) y se caracterizan por su alta riqueza de plantas (Wilson *et al.*, 2012).

A escala global, los BTs contribuyen con ~ 40% a la productividad primaria neta (Malhi, 2012). La ocurrencia de climas cálidos y húmedos y la radiación solar sostenida durante una buena parte del año, ocasionan que los BTs asignen mayor cantidad del C fijado a la biomasa aérea que a la subterránea (Luyssaert *et al.*, 2007). En cuanto a las emisiones a la atmósfera, se estima que los BTs contribuyeron con ~ 20% del CO₂ de origen antropogénico emitido por deforestación entre el 2000 – 2008 (IPCC 2007), y emiten ~ 25% del CO₂ de la respiración autótrofa (Luyssaert *et al.*, 2007). En conjunto, el balance entre las entradas y las pérdidas de C en los BTs, determina que estos biomas almacenen ~ 55 % del C asociado a los bosques del mundo (Pan *et al.*, 2011).

Globalmente, los BTs también tienen un efecto importante en el ciclo natural del N; participando con ~ 70% del N atmosférico fijado biológicamente (Townsend *et al.*, 2011) y

emitiendo ~ 71% de los óxidos de N hacia la atmósfera por biomasa terrestre (Bai *et al.*, 2012). Un fenómeno que llama la atención en los BTs es que la fijación biológica de N es mantenida a tasas altas a pesar de que este proceso es inhibido por las altas concentraciones de N en el suelo (Hedin *et al.*, 2009). Esta paradoja es explicada en términos de la existencia de una alta heterogeneidad espacial en la disponibilidad de N. De esta manera, en un mismo BT existen ambientes de alta disponibilidad de N, que soportan altas tasas de pérdida del bioelemento y ambientes de baja disponibilidad de N, que favorecen su fijación biológica (Hedin *et al.*, 2009; Menge *et al.*, 2009).

Cambios en el clima y en la deposición atmosférica de N en regiones tropicales

A escala global, los gases con efecto invernadero de origen antropogénico incrementaron ~ 57 % en las últimas tres décadas (IPCC, 2007); específicamente en los BTs durante el mismo periodo, la temperatura superficial incrementó ~ 0.3 ° C y la precipitación media anual descendió ~ 1 % en cada década (Malhi & Phillips, 2004). También a escala global, el N reactivo atmosférico incrementó en dos órdenes de magnitud entre 1809 - 2005; pero tal incremento parece no haber afectado sustancialmente la deposición de N en BTs (1.0 - 7.5 kg N ha año⁻¹) (Galloway *et al.*, 2008). Sin embargo, se estima que esta deposición podría incrementarse a tasas anuales de 32 kg N ha año⁻¹ en el año 2030 (Dentener *et al.*, 2006) y alcanzar 50 kg N ha año⁻¹ en el año 2100 (Lamarque *et al.*, 2005). Estos incrementos serían producidos por la intensificación esperada en el uso de combustibles fósiles, el uso de fertilizantes y en la quema de biomasa en las áreas tropicales (Galloway *et al.*, 2004).

Las evidencias experimentales sugieren que el incremento esperado en la temperatura en regiones con BTs estimularía la actividad de los microorganismos del suelo, la tasa de descomposición (Powers *et al.*, 2009), las tasas de circulación de C y de N en el ecosistema (Malhi & Phillips, 2004; Saxe *et al.*, 2001), y la liberación de CO₂ hacia la atmósfera (Van Der Putten *et al.*, 2009); mientras que disminuiría la productividad primaria neta (Clark *et al.*, 2003). Por otra parte, uno de los efectos indirectos más importantes del incremento

esperado en la temperatura es que el balance hídrico podría ser más negativo en comparación con los valores actuales (Vicente-Serrano *et al.*, 2013); por lo que la disponibilidad de agua será más importante como factor limitante de la actividad microbiana (Davidson & Janssens, 2006). Los estudios en BTs húmedos han demostrado concluyentemente que el ingreso extra de N incrementa la concentración de N total en suelo y las pérdidas de N hacia otros ecosistemas vía lixiviación o emisión de óxidos de N (Lu *et al.*, 2011 a). Sin embargo, aunque la adición de N ha causado cambios en la producción de biomasa aérea (LeBauer & Treseder, 2008), en las tasas netas de mineralización de C y de N, en la tasa neta de nitrificación (Liu & Greaver, 2010; Lu *et al.*, 2011 a), en la biomasa microbiana y en la tasa de respiración del suelo (Liu & Greaver, 2009; Lu *et al.*, 2011 b) de los BTs, la dirección del cambio suele no ser consistente entre los estudios. Finalmente, los efectos de la interacción entre el cambio climático y el incremento de la deposición de N aún permanecen incipientemente estudiados para BTs (Cusack *et al.*, 2012).

Relaciones entre los ciclos del C y del N y el régimen de lluvia en bosques tropicales

Globalmente y a escala ecosistémica, los ciclos del C y del N se encuentran fuertemente acoplados, como consecuencia de que éstos son demandados en proporciones balanceadas por los organismos (Elser *et al.*, 2010). Estudios a lo largo de gradientes de precipitación en BTs, en los que otros factores ambientales y bióticos han permanecido constantes, han permitido determinar el importante papel de la precipitación media anual como variable reguladora del acoplamiento entre ambos bioelementos (Austin, 2002; Schuur, 2003). En los BTs, los rangos de precipitación más estudiados son los húmedos (1400-3500 mm de precipitación media anual) y los hiper-húmedos (>3500 mm de precipitación media anual) (Austin, 2002; Austin & Vitousek, 2000; Santiago & Mulkey, 2005; Santiago *et al.*, 2004, 2005; Schuur & Matson, 2001; Schuur *et al.*, 2001), mientras que el rango seco (500-1400 mm de precipitación media anual) ha recibido menor atención (Austin, 2002; Austin & Vitousek, 2000; Roa-Fuentes *et al.* 2013).

En los BTs, una mayor precipitación media anual estimula la productividad primaria neta, la descomposición de necromasa y las tasas de los ciclos internos de los bioelementos (Powers *et al.*, 2009; Santiago & Mulkey, 2005; Santiago *et al.*, 2004; Schuur & Matson, 2001). Sin embargo, estos procesos alcanzan sus tasas máximas en el rango de 2000-2500 mm de precipitación media anual (Austin, 2002; Powers *et al.*, 2009), mientras que a mayor precipitación anual sus tasas disminuyen (Austin & Vitousek, 2000; Powers *et al.*, 2009; Santiago *et al.*, 2005; Schuur *et al.*, 2001). Los principales factores que explican la reducción de la productividad primaria neta y de las tasa de descomposición y de reciclaje de bionutrientes en los BTs hiper-húmedos son los incrementos en la lixiviación de los bioelementos (Austin, 2002) y el decrecimiento del potencial de óxido-reducción del suelo (Schuur *et al.*, 2001).

Indirectamente, la precipitación media anual tiene un efecto inversamente proporcional en la tasa de descomposición y de mineralización de bioelementos (Powers *et al.*, 2009; Waring, 2012) porque ésta influye determinando los rasgos estructurales y funcionales de las hojas y la calidad de la hojarasca producida por la comunidad vegetal (Pasquini & Santiago, 2012; Santiago *et al.*, 2004). El principal rasgo distintivo a lo largo de un gradiente de precipitación es que las comunidades vegetales de los bosques secos tienen una mayor proporción de especies caducifolias, y con el incremento de la precipitación media anual, los bosques tienden a tener mayor proporción de comunidades perennifolias (Santiago *et al.*, 2004). La producción de hojas de vida corta es una estrategia de las especies de las comunidades vegetales para enfrentar el déficit hídrico y a éstas son asociadas a rasgos que las hacen más susceptibles a la descomposición como altas concentraciones de N y P, y una baja asignación del C fijado a compuestos estructurales foliares (Cunningham *et al.*, 1999; Mass & Burgos, 2011; Wright *et al.*, 2002). En contraposición, la producción de hojas de vida larga es una estrategia de las especies de las comunidades vegetales en ambientes húmedos para enfrentar la baja concentración de bioelementos disponibles biológicamente (Aerts & Chapin III, 1999). A las hojas de vida larga se asocian rasgos que las hacen menos susceptibles a la descomposición que las hojas caducifolias producidas en bosques secos, como la baja

concentración y masa foliar de N (Santiago & Mulkey, 2005; Santiago *et al.*, 2004) y de P (Austin & Vitousek, 2000; Santiago *et al.*, 2004), bajos índices de área foliar (Austin & Vitousek, 2000) y la asignación de una mayor proporción del C fijado a compuestos como la hemicelulosa o la lignina (Austin & Vitousek, 2000). Estos últimos compuestos presentan estructuras moleculares complejas que las hacen degradables por enzimas producidas por microorganismos especializados del suelo (Hatakka, 2001).

Ciclo de C y N en bosques tropicales estacionalmente secos

Los bosques tropicales estacionalmente secos (BTES) abarcan el ~ 42 % de la cobertura potencial de los BTs a escala global (Murphy & Lugo, 1986) y son los biomas tropicales más amenazados por el cambio climático (DeFries *et al.*, 2005). De hecho se estima que en el año 2055 cerca del 80% del área de los BTES podrá ser afectada por el incremento de temperatura y el decrecimiento de precipitación (Miles *et al.*, 2006). Para los BTES no existen estimaciones específicas acerca de la tasa futura de deposición de N atmosférico, sin embargo las evidencias indican que éstos serán susceptibles al incremento de la deposición debido a que en ~ 20% de los BTES del mundo es común como práctica agrícola la combustión de biomasa (Sánchez & Portillo-Quintero, 2011). La combustión de biomasa incrementa la emisión de compuestos de N hacia la atmósfera y su consecuente deposición sobre la superficie terrestre (Crutzen & Andreae, 1990). Adicionalmente, se estima que entre 18 y 80% del bosque húmedo tropical podría perderse o convertirse en BTES (Huntingford *et al.*, 2008), lo que hace prioritaria la investigación y caracterización de los ciclos biogeoquímicos y sus cambios como efecto de las alteraciones en el clima y la deposición de N.

En los BTES la disponibilidad de agua es el principal factor que controla la productividad primaria neta y la dinámica de los bioelementos (Austin *et al.*, 2004; Martínez-Yrizar *et al.*, 1992). Durante la época seca la actividad de los microorganismos y de las plantas disminuye por déficit hídrico (Anaya *et al.*, 2007; Campo *et al.*, 2000, 2001; Solis & Campo, 2004). También, durante esta época las plantas pierden sus hojas para disminuir la transpiración y

las concentraciones de formas minerales de N en el suelo se incrementan, debido a que la menor cantidad de agua en el suelo reduce su transporte y su uso biológico (Saynes *et al.*, 2005; Stark & Firestone, 1995). Durante el inicio de la época de lluvias, los pulsos de disponibilidad de agua afectan fuertemente la actividad de los microorganismos del suelo (Campo *et al.*, 1998; Fierer & Schimel, 2002). Las primeras lluvias causan la lisis de las células microbianas liberando bioelementos que son mineralizados rápidamente, soportando una alta tasa del crecimiento microbiano (Van Gestel *et al.*, 1993) y de la respiración del suelo (Davidson *et al.*, 1991; García-Méndez *et al.*, 1991; Kim *et al.*, 2012). En esta época, la emisión de óxidos de N es estimulada por la acumulación de nitrato en la época seca y la formación de microporos anóxicos en el suelo que favorecen la vía desnitrificadora (Kim *et al.*, 2012). Cuando la demanda microbiana de N es suplida, el N deja de ser inmovilizado y las concentraciones de amonio en suelo sostienen la productividad primaria neta, a la vez que determinan altas tasas de nitrificación (Stark & Firestone, 1995). Estas tasas se relacionan positivamente con la desnitrificación y, en sus pasos intermedios, con la emisión de óxidos de N (Matson & Vitousek, 1987). Finalmente, durante el resto de la época de lluvias, tanto la comunidad microbiana del suelo como la comunidad vegetal son más activas funcionalmente y, en el caso de la comunidad de plantas, se alcanzan las más altas tasas fotosintéticas del año (Gamon *et al.*, 2005).

El desacople entre la época de mayor disponibilidad de bioelementos y la de mayor demanda de bioelementos por parte de las plantas genera que los BTES sean importantes emisores de óxidos de N a escala global (Austin *et al.*, 2004) y que puedan comportarse como bosques limitados por N (Cecon *et al.*, 2004; Jackson *et al.*, 1988; Campo & Vázquez-Yanes, 2004).

Bosques tropicales estacionalmente secos de la Península de Yucatán

En México, los BTES ocupan ~ 10 % del territorio (Challenger & Soberón, 2008). Éstos tienen una gran heterogeneidad climática, topográfica y edáfica (Rzedowski *et al.*, 1979), así como una gran variación en la estructura vegetal, alta riqueza florística (Trejo & Dirzo,

2000) y alto grado de endemismos (~ 60 % de sus especies) (Trejo, 1998). En estos bosques las familias dominantes son las leguminosas, euphorbiáceas, burseráceas, cactáceas, malpigiáceas y anacardiáceas (Rzedowski, 1991).

En México los BTES se distribuyen en las provincias de la Costa del Pacífico, la Depresión del Balsas y en la Península de Yucatán (CONABIO, 1997). Biogeográficamente la Península de Yucatán es la más aislada de estas provincias porque presenta mayor afinidad biológica con los BTES insulares del Mar Caribe (Espinosa *et al.*, 2008) y porque su material parental cárstico (Bautista *et al.*, 2005) le confiere características biogeoquímicas únicas. Por ejemplo, el efecto estabilizador de los carbonatos en la materia orgánica del suelo (Shang & Tiessen, 2003) actúan favoreciendo una alta acumulación de C en el suelo, explicando las más altas concentraciones de C en el suelo del país (99-166 Mg C ha⁻¹) (Balbontín *et al.*, 2009). En estos bosques la tasa de residencia de los bioelementos en los suelos es superior a un año debido a que la tasa de recambio de bioelementos está limitada a la época de lluvia (Campo & Vázquez-Yanes, 2004), limitando a su vez la productividad primaria neta (Campo *et al.*, 2007).

Aunque en la Península de Yucatán existe una marcada estacionalidad climática, a escala regional se presenta un gradiente de mayor precipitación en el norte de la Península, que decrece hacia el sur (Figura 1). Esta variación climática explica la dominancia de Leptosoles en sitios del norte y la asociación de Leptosoles con Cambisoles en los sitios del sur (Bautista-Zuñiga *et al.*, 2004) debido a que los procesos pedogénéticos están principalmente limitados por la duración e intensidad de la época de lluvias. Adicionalmente de norte a sur, las comunidades vegetales presentes son la selva caducifolia espinosa, la selva baja caducifolia y la selva mediana subcaducifolia (Miranda-Hernández, 1963). En la región la familia dominante es leguminosae y las especies dominantes son *Gymnopodium floribundum* Rolfe, *Piscidia piscipula* (L.) Sarg., *Lysiloma latisiliquum* (L.) Benth, y *Bursera simaruba* (L.) Sarg. (Roa-Fuentes *et al.*, 2013).

Los BTES de la Península de Yucatán han sido estudiados en el marco de diversos proyectos que tienen como objetivo su caracterización funcional a lo largo del gradiente de precipitación. Brevemente, los resultados muestran que con el incremento de la precipitación media anual se registra un aumento en la altura y diámetro de los árboles (Roa-Fuentes *et al.*, 2012). Adicionalmente, a lo largo del gradiente de precipitación, los suelos de los sitios más secos tienen menores tasas de mineralización de la materia orgánica y mayores concentraciones de C en la fracción lábil (Cuevas *et al.*, 2013), así como mayores cantidades de mantillo (Roa-Fuentes *et al.*, 2013), concentraciones de C, tasas de respiración basal y concentraciones de N total e inorgánico en comparación con los bosques más húmedos del gradiente (Cuevas *et al.*, 2013; Maldonado, 2013).

Los modelos de estimación de la deposición actual de N calculan una tasa anual de 8 kg de N ha⁻¹ en la Península de Yucatán (Dentener *et al.*, 2006). Sin embargo, es posible que la deposición anual estimada sea mayor debido a que en ésta se ha registrado la mayor tasa anual de incendios de origen forestal o agrícola en Mesoamérica (Yokelson *et al.*, 2009) y, a que la quema de biomasa incrementa la emisión y posterior deposición atmosférica de óxidos y formas reducidas de N (Crutzen & Andreae, 1990; Veldkamp *et al.*, 1992). Adicionalmente, es posible que áreas con producción intensiva de animales o de cultivos en la región puedan ocasionar mayores tasas de deposición local (com. pers. E. B. Allen).

La mayoría de los estudios y las hipótesis planteadas respecto al efecto del incremento de N sobre el funcionamiento de los BTr han sido realizados para los BTr húmedos (Lu *et al.*, 2011). Sin embargo, las evidencias para los BTES que se han recopilado en la Península de Yucatán han documentado que la adición experimental de N puede incrementar la concentración de N foliar en algunas especies del BTES (Campo & Dirzo, 2000), la productividad primaria neta (Campo *et al.*, 2004), la relación C:N en el suelo (Gamboa *et al.*, 2010) y disminuir el tiempo de recambio de los elementos en el suelo (Campo *et al.*, 2004). En conjunto estos cambios suelen ser más intensos en los bosques en regeneración que en los bosques maduros.

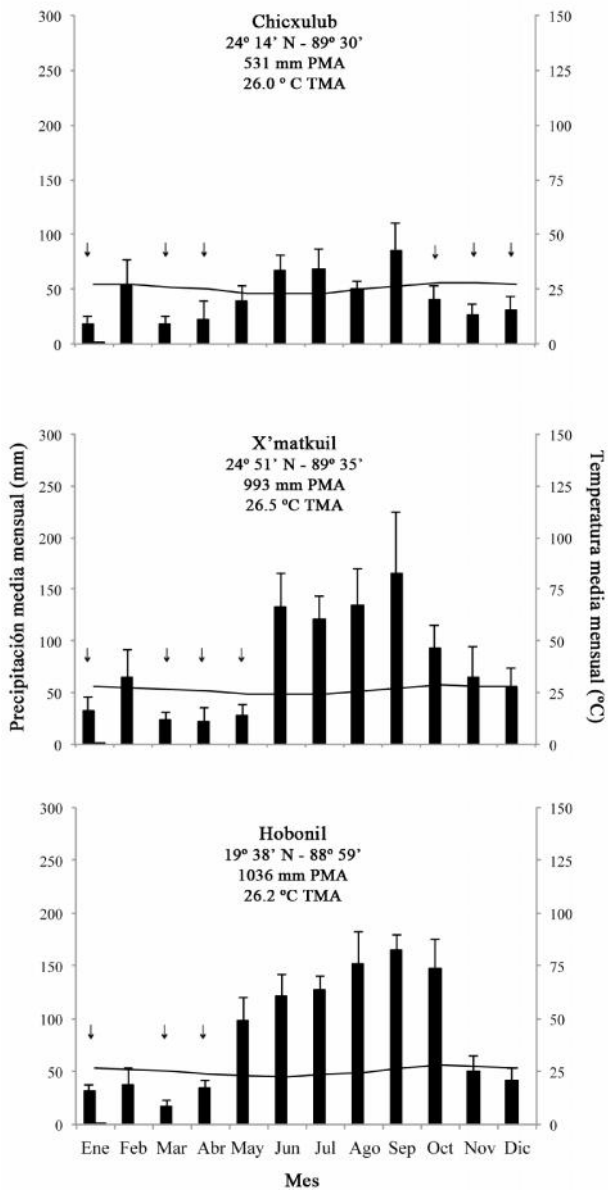


Figura 1. Diagramas ombrotérmicos de tres bosques tropicales estacionalmente secos en la Península de Yucatán. Los datos en barras son medias \pm 1 EE de la precipitación media mensual, y en línea continua son valores medios mensuales de la temperatura durante el período 2001 al 2010. Los meses secos son indicados con flechas. PMA: precipitación media anual, TMA: temperatura media anual. Fuente: CONAGUA comunicación personal.

En este proyecto se exploró el efecto del incremento en la disponibilidad de N en los ciclos de C y N en BTs. Para ello se exploraron los efectos del incremento en la disponibilidad de N, en interacción con el régimen de lluvia, en la dinámica del C y del N en el suelo de BTES. El diseño experimental se basó en la adición de N en BTES ubicados a lo largo de un gradiente de precipitación (536 – 1035 mm precipitación media anual) en la Península de Yucatán. La adición de N fue realizada siguiendo las tasas esperadas en la deposición de N atmosférico para el año 2030 en regiones con BTs (Dentener *et al.*, 2006). Los BTES seleccionados tienen características ambientales y biológicas poco variables, lo cual permitió aislar los efectos de la precipitación media anual en los ciclos del C y del N en estos ecosistemas. Adicionalmente, se realizó el meta-análisis de los cambios en los ciclos de C y N en los BTs que han sido reportados en la literatura.

OBJETIVOS

En este proyecto se exploró el efecto del incremento en la disponibilidad de N en los ciclos de C y N en BTs.

Los objetivos específicos de este estudio fueron:

1. Estimar los patrones de cambio en la dinámica de C y de N como consecuencia del ingreso extra de N en BTs.
2. Analizar los efectos de diferentes tratamientos de adición de N en la dinámica del C y del N en el suelo de BTES de Yucatan ubicados a lo largo de un gradiente de de precipitación.
3. Determinar los efectos de diferentes tratamientos de adición de N en la tasa de descomposición de hojas de *Piscidia piscipula* (L.) Sarg. (Fabaceae) y *Gymnopodium floribundum* Rolfe (Polygonaceae) en BTES de Yucatán ubicados a lo largo de un gradiente de de precipitación.

CAPÍTULO 1.

A meta-analysis on the effects of increased nitrogen availability on macronutrient cycling in tropical forests

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Marylin Bejarano^a and Julio Campo^a

^a*Instituto de Ecología, Universidad Nacional Autónoma de México, México D.F., Mexico*

Resumen

1. El incremento de la deposición atmosférica de nitrógeno (N) puede incrementar hasta por un factor de cuatro en las regiones tropicales para el 2030. Las evidencias sugieren que el incremento en la deposición de N podría afectar los ciclos del carbono (C) y del N. La actual teoría biogeoquímica para bosques tropicales (BTs) se fundamenta en estudios realizados en bosques tropicales maduros, en los que es alcanzada la demanda biológica de N y la adición experimental de N incrementa la transformación y pérdidas del N. De esta manera los BTs son considerados ecosistemas saturados por N. Sin embargo, investigaciones recientes, como la paradoja de N y la existencia de gradientes de limitación por N a la limitación por P, sugieren que los BTs podrían reaccionar a la deposición de N en direcciones no contempladas anteriormente.
2. Nuestro objetivo fue realizar un meta-análisis para explorar las consecuencias de la adición experimental de N en los ciclos del C, N y del fósforo (P) en BTs. El análisis fue realizado considerando tanto las variables aéreas como las subterráneas, así como la importancia de las características experimentales y de los sitios de estudio.
3. Los resultados indican que los ciclos del C, N y del P responden a la adición experimental de N en direcciones no predichas anteriormente por el enfoque de saturación de N en estos ecosistemas. La adición experimental de N cambió la concentración de estos nutrientes en la hojarasca, el mantillo y el suelo, evidenciando que las plantas y los microorganismos usan el N adicionado, cambiando sus ciclos. A pesar de que los resultados reportados en este estudio provienen de estudios cortos (< 5 años) y con pocas réplicas, nuestros resultados soportan más la teoría de N-P co-limitación que la de saturación por N en los BTs.
4. El fuerte efecto de las condiciones experimentales en este estudio indica que para entender el efecto de la deposición atmosférica de N es necesario realizar investigaciones con condiciones experimentales más realistas. Debido a que los BTs son componentes importantes de los ciclos globales de C y de N, el incremento en la deposición de N atmosférico podría cambiar sus proyecciones futuras, retroalimentando el ciclo del C a escala global.

Summary

1. Deposition of atmospheric nitrogen (N) could increase up to a factor of four in tropical regions by 2030. Evidence suggests that this increased N deposition could affect the carbon (C) and phosphorous (P) cycles. Current biogeochemical theory on tropical forests (TFs) usually accounts for mature forests, in which N biological demand is achieved and N-supply increases the soil N transformations and losses; supporting their N-saturation state. However, evidence suggests TFs could react to N deposition in previously understood ways, such as the N-paradox and the existing gradient from P to N limitation in successional and altitudinal TFs.
2. Our objective was to provide a meta-analysis to explore the consequences of N-supply on C, N and P cycling in TFs, analyzing the changes in aboveground and belowground variables. We also explored the importance of experimental and site characteristics.
3. The results show that C, N, and P cycles respond to N-supply in ways not previously predicted by the approach based on the N-saturation approach. N-supply changed nutrient concentrations in litterfall, litter, and soil, supporting that both plants and microorganisms use the added N and changing the cycle of C, N, and P. Despite observed changes are based on low number and short time searching, these provide more support for the NP co-limitation than the N-saturation state in TFs.
4. Experimental conditions effect on variables responses is an indicator of the need for more realistically planned research conditions in order to better understand TFs changes to future N-deposition increments, including the subjacent mechanisms that control them. Since TFs are an important component of the global C and N cycle, future N-deposition increments could strongly contribute to changing projections and feedback of C cycling worldwide.

Key-words Carbon cycling, coupled nutrient cycles, nitrogen deposition, nitrogen paradox, nitrogen saturation, phosphorus cycling.

Introduction

Atmospheric deposition of nitrogen (N) has increased globally by a factor of 3.6 since the pre-industrial era due to fertilizer application, fossil fuel combustion, and legume cultivation (Gruber & Galloway 2008). Evidence from a wide range of terrestrial ecosystems suggests that this increase affects the carbon (C), phosphorous (P), and N cycles (Lu *et al.* 2011, Marklein & Houlton 2012). The impact of N on these cycles is a consequence of their stoichiometric relationship wherein organisms demand these elements in fixed proportions (Sterner & Elser 2002). Recent climate models, which incorporate N-deposition as a part of the CN-coupled models, predict a lower net

C-uptake (37-74%) than values projected by C models alone (Thornton *et al.* 2009; Wania *et al.* 2012), demonstrating the importance of the N cycle in future climate change. For tropical regions, the CN-coupled models have greater uncertainties (Bai, Houlton & Wang 2012) due to the unknown sensitivity of the C-cycle to changes in N-supply (Zaehle & Dalmonech 2011). Tropical forests represent nearly 32% of the world's forests (Olson *et al.* 2001) and play a central role in global C and N cycles (Pan *et al.* 2011; Bai, Houlton & Wang 2012). Therefore, their responses to N-deposition could substantially influence climate change and global nutrient cycles (Vitousek *et al.* 2010).

In meta-analyses of N-deposition effects, there is a remarkably low representation of TFs compared to non-tropical forests (~5% of studies) (e.g. Lu *et al.* 2011a, Lu *et al.* 2011b). There are two main explanations for this. First, TF studies are usually performed under experimental conditions unsuited to criteria used in meta-analyses (e.g. time of N addition > 1 year). Second, TFs have been the subject of biased research due to their biochemical theory was mainly generated from studies of mature lowland moist forests (Cleveland *et al.* 2011), in which N addition did not show an effect on the net primary productivity (NPP) (e.g. Vitousek 1984), supporting the N-saturation model. This model, developed from temperate forests under chronic N-deposition (Aber 1998), posits that there is a threshold after which N-supply achieve the biological demand and becomes lost from the ecosystem (Matson, Lohse & Hall 2002).

However, TFs include ecosystems that differ in their macronutrient limitation according to the age of the soil substrate (Vitousek 1984), elevation (Tanner, Vitousek & Cuevas 1998), and

successional state (Davidson *et al.* 2004). In fact, according to successional and mountain forest distribution, it is estimated that ~30% of TFs are more N-limited than P-limited (FAO 2010; Scatena *et al.* 2011). Thus, TFs could be seen as ecosystems occurring along a gradient of N availability, from N-limited (i.e. early-substrate landscapes, montane elevations, or regenerating forests) to P-limited (i.e. old-substrate landscape, lowland elevations, and mature forests). This TF gradient leans itself to the NP co-limitation state rather than a N-saturation state in the same way described globally (Elser *et al.* 2007).

Studies of the effects of N-supply on TFs differing in their N availabilities have demonstrated that the N retention is determined by the biotic demand, soil retention, and loss pathway (Lohse & Matson 2005). When N-supply is used biologically in N-limited ecosystems, N is immobilized to support new biomass, thereby changing the internal cycling of both C and N (Vitousek *et al.* 1993; Campo & Vázquez-Yanes 2004). Conversely, when N-supply exceeds the biological demand in P-limited ecosystems, a portion of N can be retained by the soil-exchanging cation and anion capacity, while the rest is lost by the emission of N oxides or the leaching of NO_3^- -N (Hall & Matson 2003; Lohse & Matson 2005). In addition, a recent study has demonstrated that N-supply can increase the soil phosphatase concentration, and with this, increase the P biological availability for plants (Marklein & Houlton 2012). However, these effects have not been tested for TFs with differing N availabilities.

Our objective was to synthesize research about the consequences of N enrichment on C, N, and P cycling in TFs by analyzing changes in aboveground and belowground variables (Table 1).

According to the N-limitation gradient in TFs, we hypothesized that N addition would change the C, N, and P ecosystem cycles. We also explored the importance of experimental conditions (N addition rate and length, fertilizer chemical form, and experimental site) and site study characteristics (mean annual precipitation and temperature, and soil C:N ratio) on the responses; each modulator variable (i.e. covariables) with its own hypothesis. For experimental conditions, we hypothesized that high rates and longer durations of N addition would generate the strongest responses in soil N losses, according to N-saturation state of TFs. For fertilizer type, we hypothesized that variables associated with soil microorganism activity would be more sensitive than other variables because fertilizer type directly affects soil conditions (Nason & Myrold 1992), and microorganisms are sensitive to these changes (Myrold 1998). For the study site, we expected that experimental N addition in N-limited TFs would affect both aboveground and belowground variables as a consequence of N use by plant and soil microorganisms. However, additional supplies of N- in P-limited TFs would produce more changes belowground than aboveground variables as an effect of N use by nitrifier and denitrifier bacteria that mediate the N lost. Additionally, experimental N addition could be used to increase the production of phosphatases, accelerating the P cycle; more in P-limited than N-limited TFs (Marklein & Houlton 2012).

Materials and methods

Data collection and classification

Peer-reviewed studies were meta-analyzed using the ISI Web of Science database (<http://apps.isiknowledge.com>) during June 2012. The search includes articles from 1985 to 2012 with *tropical forest* and *subtropical forest* as keywords. The results were refined with the words *nitrogen*, *nitrogen addition*, *nitrogen fertilization*, and *nitrogen deposition*. The resulting papers

were filtered with the following criteria: (1) the study was performed in a tropical or subtropical forest; (2) inorganic N fertilizer was added; (3) it measured at least one of the C, N, or P pools or fluxes; (4) the N application rate, the chemical form, and the experimental duration were reported; and (5) the mean and sample sizes of the selected variables were reported for the control and N-supply treatments. TF studies have been poorly represented in previous meta-analyses (e.g. Lu *et al.* 2011a) because they often do not meet two main criteria: N-supply was conducted for over a year, and the experiment was performed in field conditions. In order to analyze the largest number of studies, these criteria were avoided, but the short-pulse and the ex-situ effects were analyzed as covariables (see *Meta-data analysis* section). Sixty-seven studies met the meta-analysis criteria (Table S1), which were augmented with data from tropical dry forests (M. Bejarano & J. Campo, unpublished data) for a total of 68 studies.

The meta-analysis approach assumes independence between studies (Hedges, Gurevitch & Curtis 1999). Therefore, when studies included multiple time points or soil horizons, only the final time and/or “A horizon” data were considered. Also, measurements within one study from different levels of N-supply, N-chemical forms, and ecosystems, were considered independent. For all variables, three sets of information were recorded for each pair comparison (i.e. control and treatment, hereafter referred to as *k pair*): the statistical set, including sample size, mean, and standard error or deviation; the experimental conditions set, including rate and time of N-supply, chemical form of fertilizer, and experiment kind (i.e. *in situ* or *ex situ*); and the site characteristics set, including the biome, successional status, elevation, mean annual precipitation (MAP), mean annual temperature (MAT), and soil characteristics. Unlabeled error bars were assumed to denote

the standard error and were changed to standard deviation DigitizeIt 1.5 (GeoMem Software, Perthshire, UK) was used to extract data from figures.

From database build, thirty-eight response variables (Table 1) were selected for analysis when containing over three independent k comparisons. In order to detect the effects of experimental conditions and site characteristics on response variables, the rate and time of N-supply, chemical form of fertilizer, MAP, MAT, and site soil C:N ratio were considered as modulator variables (Table 2); these variables were consistently reported across studies. Additionally, an association was found among biome and forest type, relative to elevation, successional status, and experimental type of study. Thus, these variables were combined into a new variable called *Study Site* (Table 2).

Meta-data analysis

The meta-analysis was performed following Hedges, Gurevitch & Curtis (1999) using MetaWin 2.0 software (Rosenberg, Adams & Gurevitch 2000). The natural log of response ratio ($\ln R$) was used to calculate the effect size, which is a proportion of the change resulting from experimental manipulation. The first step in the analysis was to calculate the overall weighted $\ln R$ and its variance for all paired observations. Then, a mixed model approach was used to analyze each variable. Overall $\ln R$ was calculated from the fixed component, in which an significant total heterogeneity (Q_T) indicate a good fit of model, while the statistical significance of modulator variables was calculated from the random component (Hartung, Knapp & Sinha 2011). The response variables were analyzed for categorical and continuous modulator variables when each level had at least three observations and sample size was over 30, respectively. Statistical

significance of the fixed component was achieved when the 95% confidence intervals (CI) did not include 0. The random component was considered as significant when the p-value of between-group heterogeneity (Q_B) of model was < 0.05 . The percentage change of significant variables was calculated from $\ln R - 1$, where a value other than 0 indicated an increment or decrement of the response variable in response to N-supply. Because the meta-analysis had small sample sizes and could violate statistical assumptions, CIs were bias-corrected via bootstrapping (i.e. 999 repetitions) (Adams *et al.* 1997). Finally, a principal component analysis was performed to visualize how the categories of modulator variables influence the significant variables of each nutrient cycle. This analysis was performed with Info Stat software (Di Rienzo *et al.* 2011).

Results

Studies were most frequently in lowland and montane tropical forests (Fig. 1). Total number of k comparisons used in the analysis was 804, in which the most common locality conditions were lowland (< 500 m asl), humid (*c.a.* 2000 mm year⁻¹), and hot (*c.a.* 20 °C) forests, in which soil C:N ratios were nearly 16:1 (Fig. S1a-h). Experimentally, the most common k conditions were of NH_4NO_3 addition and for low rate and long-term N addition (Fig. S1f-g). The k comparisons were frequently taken in moist, lowland, and montane forests (Fig. S1h).

Aboveground macronutrient pools and fluxes

N addition significantly increased variables associated with plant productivity, such as biomass (43 %), diameter at breast height (DBH) (39 %), litterfall mass (30 %), and litter mass (17) (Fig. 2). Moreover, added N was taken up by plants, resulting in increased shoot N (20 %) and foliar N:P

ratios (22 %); however, N uptake did not change the foliar N-concentration (Fig. 2). N addition not only increased the litterfall mass, but also significantly increased its N-concentration (9 %) and in turn enriched litter quality by increasing both N (15 %) and P (10 %) litter concentrations (Fig. 2). N fertilization decreased biological N-fixation in litter (65 %) (Fig. 2).

Belowground macronutrient pools and fluxes

N addition decreased soil pH (2 %) ($\ln R = -0.0233$, $CI = -0.014 - -0.0175$, $n = 34$). The belowground C-cycle was the least sensitive cycle studied for N addition. N addition increased total soil C (6 %) and soil organic C (17 %) concentrations, but it did not change the C-concentration of microbial biomass, soil respiration, litter decomposition, or dissolved organic C-concentration (Fig. 3). The effects of N addition were the strongest on the soil N-cycle compared to other cycles, increasing microbial biomass N (32 %), total soil-N (9 %), NH_4^+ -N (57 %), NO_3^- -N (98 %), total dissolved-N (65 %), dissolved organic-N (23 %), and dissolved inorganic-N (20 %) concentrations (Fig. 3). The overall increase in mineral forms of N as a response to N addition, in turn, showed that N fluxes escalate by increasing net N-mineralization (17 %), N_2O (99 %), NO (by two times) emissions, and NO_3^- -N leaching (7 times); however, N addition did not significantly change net nitrification (Fig. 3). Additionally, extra N supply decreased biological N-fixation in the soil (17 %). Parameters relating to the soil P-cycle did not change as a result of the N-supply (Fig. 3).

Effects of the experimental conditions of N-supply

Categories of N-supply influenced aboveground variables more than belowground variables (Fig. 4a, S2). The $\ln R$ s of the aboveground variables increased in the low N-supply rate category, while

they decreased, increased or caused no change with high N-supply of the foliar and litter N concentrations, as well as the litterfall mass, respectively (Fig. S2). Soil-dissolved organic C-concentrations only had a significant increase with medium amounts of N-supply (Fig. S2).

Categories of N time addition influenced belowground more than aboveground variables (Fig. S3), and also it influence N cycle more than other cycles (Fig. 4b). Nitrogen litter concentration decreased and increased for intermediate and long-term categories, respectively (Fig. S3). Regarding belowground variables, we found no difference between categories for some N loss variables (i.e. N-leaching and NO emissions) (Fig. S2), but they differed for N₂O emission concentration (Fig. S3, 4b). N₂O emission exhibited the same behavior as other variables that change between time N-supply categories do, having the greatest change in the short- and long-term categories and the smallest change in the intermediate-term category (Fig. 4b).

The belowground variables of all cycles responded significantly to type of fertilizer categories (Fig. 4c, S4). The heterogeneity of response in these affected variables can identify two main groups: the C- variables, which typically had a significant positive response to urea addition, while the other group, which includes the soil N mineral forms, N mineralization, and dissolved organic C, had similar significant positive responses to the addition of urea plus NO₃NH₄ (Fig. 4c, S4). Variables with unique responses were total dissolved N, which increased strongly with NH₄NO₃ addition, and available P-concentration, which decreased significantly with urea and urea plus NO₃NH₄ additions, and increased significantly with NO₃NH₄ addition (Fig. 4c, S4).

Effects of site

The *Study Site* categories changed the most for belowground variables of all nutrient cycles included in this study (Fig. 4d and S5). There were some main patterns of variation for some *Study Site* categories. First, the soil pools and fluxes of N and decomposition in the mature montane *Study Site* category had significant positive responses (Fig. 4d, S5). Second, the microbial biomass C and total soil C increased significantly in the mature, dry lowland *Study Site* (Fig. 4d, S5). Finally, available soil P and dissolved total N concentrations significantly increased in the mature, moist lowland *Study Site* (Fig. 4d, S5). The MAT and soil C:N ratio were significant for belowground variables (Table 3).

Discussion

Aboveground nutrient pools and fluxes

N addition increased productivity variables in TFs, giving greater support to their NP co-limitation status than their N-limitation status. This outcome is consistent with findings about N-supply increasing the PPN in TFs (8%) (LeBauer & Treseder 2008). Two other results also support the NP co-limitation in TFs. First, the increase in foliar N:P ratio and the N-shoot concentrations were signs that the added N was used by plants. In this way, even though the N-supply did not increase foliar N-concentration, in contrast to global results (Xia & Wan 2008; Lu *et al.* 2011a), the response appears to be an effect of experimental condition. In fact, foliar N-concentration increased with study lengths over 36 months or with addition rates less than $125 \text{ kg N ha}^{-1} \text{ year}^{-1}$, suggesting that foliar allocation of N does not occur when (1) the N-supply is brief, possibly because there is greater demand for N from the microorganisms than from the plants (Kaye & Hart 1997), or (2)

the N addition rate is high, possibly because it stimulates high rates of N losses (Hall & Matson 2003). Thereafter, control of experimental conditions on the foliar N increase could partially explain the increase in plant productivity due to a positive correlation with photosynthetic rate (Shipley *et al.* 2005). Second, the high N- and P-litter availability did not correspond with a significant change in foliar concentrations, suggesting that plant communities decreased their N and P resorption capacity according to nutrient availability (Sistla & Schimel 2012). Our findings are in line with previous studies of N-supply, which increased the N-concentration in both the litter and litterfall (Lu *et al.* 2011a), and ecosystem P-availability (Marklein & Houlton 2012) across a wide variety of terrestrial ecosystems.

Belowground nutrient pools and fluxes

Responses to N-supply on C, N, and P soil cycling supported the NP co-limitation state better than the N-saturation state in TFs; however, the C-cycle was the least sensitive to N addition. Pursuant to previous results reported for a smaller number of TFs (Liu & Greaver 2010), N-supply did not change the C-concentration of the microbial biomass in this study. This result is contrary to global patterns reported (Liu & Greaver 2010), in which the decrease of microbial biomass C is explained because the N-added increased the synthesis of toxic N-compounds or decreased the soil pH (Fog 1988; Treseder 2008). In our results, N addition caused a pH decrease of 2%, which is similar to the decline found in a global analysis (Liu & Greaver 2010), suggesting that this change, as well a possible increase in toxic N-compounds, does not affect the C microbial biomass in TFs. The lack of change in soil respiration, litter decomposition rate, or dissolved organic C-concentration can be explained by the fact that litter decomposition and soil respiration rates are processes that depend

on microbial activity (Treseder 2008). Furthermore, the N-supply increased the total and organic C-concentrations, validating results reported in a global analysis (Liu & Greaver 2010). Since there are no effects of N-supply on C decomposition, the increase in fine-root biomass, which was found previously under N-supply (Xia & Wan 2008), could be a possible explanation for these results.

The effect of N-supply on the soil N-cycle was the strongest where it increased the dissolved organic N and soil NH_4^+ -N concentrations. These compounds are the input and product of the immobilization and the mineralization processes (Chapin III *et al.* 2011), explaining the microbial N biomass and net N-mineralization increment found. Together these results, as well as the C:N ratio of 16 (the theoretical threshold where microorganisms become N-limited) (Booth, Stark & Rastetter 2005), are evidence that microbial biomass tended to be N-limited in TFs, supporting another recently recorded result (Cusack *et al.* 2010).

N addition changed soil N fluxes in a similar direction to those reported in global reviews. Experimental N addition significantly increased (1) the N mineralization rate, (2) the soil N pool concentrations, (3) the NO_3^- -N leaching, and (4) the N oxides fluxes, while it decreased biological N fixation (Liu & Greaver 2009; Lu *et al.* 2011a). Implications of N enrichment effects have been widely discussed in previous reviews. In summary, N addition accelerates the soil N-cycle, increasing N fluxes to the atmosphere or other ecosystems in line with initial predictions for TFs (Matson, Lohse & Hall 2002), which are based on the N-saturation approach. The most important implication of our results is that they support the N-paradox approach in TFs (Hedin *et al.* 2009) and indicate that increases in N-supply would increase N_2O emissions, contributing to the

greenhouse effect and eutrophication of aquatic ecosystems in TFs (Matson, Lohse & Hall 2002; Bai, Houlton & Wang 2012). Additionally, N-supply did not change soil cycle variables of P. This lack of change is similar to previous meta-analysis results, in which the N-supply increased the phosphatase activity in the rhizosphere but did not change P availability in bulk soil (Marklein & Houlton 2012).

Effects of experimental conditions

Increases in length and rate of N addition raised the nitrification rate, weakly supporting our first experimental hypothesis that soil processes associated with N loss would be greatest in the high rate addition and long-term addition time categories. Additionally, soil N₂O emissions, NH₄⁺-N and NO₃⁻-N concentrations, N mineralization, and N nitrification increased significantly following an unexpected behavior over time. These results indicate that soil microorganisms, including nitrifier and nitrifying bacteria, may have a strong initial response in the N cycle to N addition, as seen in other TFs (Hall & Matson 2003). At the intermediate length of time, N-added is used more by other soil microorganisms than by nitrifying bacteria and finally, the added N is again mainly used by bacteria mediating the N loss (Kaye & Hart 1997; Templer *et al.* 2008). These responses suggest that TFs do not fit the N-saturation approach, where it is predicted that N losses will be accumulative and linear with length and rate of N addition (Aber *et al.* 1998).

The effect of fertilizer type supported our hypothesis that belowground variables would respond more strongly than aboveground variables, because soil microorganisms are more susceptible to soil chemical changes caused by each type of fertilizer. The lack of change in aboveground

variables under different fertilizer added is similar to previous results obtained globally (LeBauer & Treseder 2008). Furthermore, C and P cycle variables tended to respond in different ways to urea and urea plus NH_4NO_3 addition than to ammonium salt, but our data are insufficient in explaining these results.

Effects of site

We expected that experimental N addition would change both above- and belowground variables of nutrient cycles in N-limited TFs. The changes found in both mature montane and lowland successional wet forests demonstrate that added N was used biologically, supporting their N-limited state (Tanner, Vitousek & Cuevas 1998; Davidson *et al.* 2004). However, we also found two unexpected responses. First, N-supply affected the N cycle, increasing the soil N mineral concentrations, the net nitrification rate, and the NO emission in montane tropical forests. These changes were higher than those occurring in mature lowland tropical forests, where the highest N losses were predicted according to N-saturation state. These unexpected results are in line with recent studies reporting high losses of N in montane forests, despite biological use (Brookshire *et al.* 2012). Under the N-saturation approach, both biological N use and high N losses are indicators of two extremes of a gradient from N-limitation to N-saturation, respectively, suggesting the need to reconsider the N-saturation approach in TFs.

Second, we found unexpected differences between mature lowland dry and moist tropical forests. Nitrogen supply increased the microbial biomass C and N net mineralization in dry forests, but decreased the microbial biomass C in wet forests, supporting the idea that soil microorganisms

could be experiencing some N-limitation in dry forests. Data supporting the first point are that dry forests have a lower decomposition rate due to low water availability (Powers *et al.* 2009), higher organic soil C and N concentrations (Jiménez, Lorenz & Lal 2011), and a more open nitrogen cycle than their wet counterparts (Austin & Vitousek 1998), suggesting that soil microorganisms could be more N-limited in dry than in wet forests. The patterns seen in this section suggest that types of TFs can respond differently to N addition. However, due to low representation of some *Study Site* categories, these patterns should be considered as preliminary and can be used to develop new hypotheses for testing.

Our findings suggest that ecosystem cycles of C, N, and P respond to N addition in ways not previously predicted based on the assumption of N-state. Nitrogen addition increased the NPP, the nutrient concentrations in litterfall and litter, and the ecosystem losses of N, evidencing that plants and soil microorganisms use the added N, thus changing C, N, and P cycling. These results provide more support to the NP co-limitation state than the N-saturation state, suggesting the need for a broader understanding of TFs role in future global change. Though supported by few and short-term studies, findings show that future N-deposition increments could have greater impact than previously predicted in C, N, and P ecosystem cycles. However, these results also indicate the need for more research, in which experimental conditions must be realistically planned, in order to better understand TFs changes to future N deposition increments as well as the subjacent mechanisms that control them. Because TFs are an important component of the C and N global cycle, future N deposition increments could have a strong contribution to changing projections of global C cycle feedbacks.

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References

- Aber, J., McDowell, W., Nadelhoffer, K.J., Magill, A. & Bernston, G. *et al.* (1998) Nitrogen saturation in temperate forest ecosystems-hypotheses revisited. *BioScience*, **48**, 921–934.
- Adams, D.C., Gurevitch, J. & Rosenberg, M.S. (1997) Resampling tests for meta-analysis of ecological data. *Ecology*, **78**, 1277–1283.
- Austin, A. & Vitousek, P. (1998) Nutrient dynamics on a rainfall gradient in Hawai'i. *Oecologia*, **113**, 519–529.
- Bai, E., Houlton, B.Z. & Wang, Y.P. (2012) Isotopic identification of nitrogen hotspots across natural terrestrial ecosystems. *Biogeosciences*, **9**, 3287–3304.
- Booth, M.S., Stark, J.M. & Rastetter, E.B. (2005) Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecological Monographs*, **75**, 139–157.
- Brookshire, E., Hedin, L., Newbold, J., Sigman, D. & Jackson, J. (2012) Sustained losses of bioavailable nitrogen from montane tropical forests. *Nature Geoscience*, **5**, 123–126.
- Campo, J. & Vázquez-Yañes, C. (2004) Effects of nutrient limitation on aboveground carbon dynamics during tropical dry forest regeneration in Yucatan, Mexico. *Ecosystems*, **7**, 311–319.
- Chapin, III F.S., Matson, P.A. & Mooney, H.A. (2011) *Principles of Terrestrial Ecosystem Ecology*. 2nd Edition. Springer. New York.
- Cleveland, C.C., Townsend, A.R., Taylor, P., Alvarez-Clare, S., Bustamante, M. & Chuyong, G. *et al.* (2011) Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecology Letters*, **14**, 939–947.
- Cusack, D.F., Torn, M.S., McDowell, W.H. & Silver, W.L. (2010) The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Global Change Biology*, **16**, 2555–2572.
- Davidson, E.A., Reis de Carvalho, C.J., Vieira, I.C.G., Figueiredo, R.O., Moutinho, P. & Ishida, Y. *et al.* (2004) Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecological Applications*, **14**, 150–163.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M. & Robledo, C.W. (2011) *Infostat, version 2011*, Cordoba.
- Elser, J.J., Bracken, M.E.S., Cleveland, E.E., Gruner, D.S., Harpole, W.S. & Hillebrand, H. *et al.* (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, **10**, 1135–1142.
- FAO (2010) *Global Forest Resources Assessment*. FAO. Roma.
- Fog, K. (1988) The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Reviews*, **63**, 433–462.
- Gruber, N. & Galloway, J.N. (2008) An earth-system perspective of the global nitrogen cycle. *Nature*, **451**, 293–296.

- Hall, S.J. & Matson, P.A. (2003) Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecological Monographs*, **73**, 107–129.
- Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150–1156.
- Hedin, L.O., Brookshire, E.N.J., Menge, D.N.L. & Barron, A.R. (2009) The nitrogen paradox in tropical forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 613–635.
- Jiménez J.J., Lorenz, K. & Lal, R. (2011) Organic carbon and nitrogen in soil particle-size aggregates under dry tropical forests from Guanacaste, Costa Rica - Implications for within-site soil organic carbon stabilization. *Catena*, **86**, 178–191.
- Kaye, J.P. & Hart, S.C. (1997) Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology & Evolution*, **12**, 139–143.
- LeBauer, D. & Treseder, K.K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.
- Liu, L. & Greaver, T.L. (2009) A review of nitrogen enrichment effects on three biogenic GHGs: the CO₂ sink may be largely offset by stimulated N₂O and CH₄ emission. *Ecology Letters*, **12**, 1103–1117.
- Liu, L. & Greaver, T.L. (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*, **13**, 819–828.
- Lohse, K.A. & Matson, P. (2005) Consequences of nitrogen additions for soil losses from wet tropical forests. *Ecological Applications*, **15**, 1629–1648.
- Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X. & Chen, J. *et al.* (2011a) Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytologist*, **189**, 1040–1050.
- Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C. & Chen, J. *et al.* (2011b) Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agriculture, Ecosystems and Environment*, **140**, 234–244.
- Marklein, A.R. & Houlton, B.Z. (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, **193**, 696–704.
- Matson, P., Lohse, K.A. & Hall, S.J. (2002) The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *AMBIO*, **31**, 113–119.
- Myrold, D. (1998) Transformation of nitrogen. *Principles and Applications of Soil Microbiology* (eds Sylvia, D., Fuhrmann, J., Harte, P. & Zuberer, D.), pp. 259–294. Upper Saddle River & Prentice-Hall, New Jersey.
- Nason, G.E. & Myrold, D.D. (1992) Nitrogen fertilizers: fates and environmental effects in forests. *Forest Fertilization: Sustaining and Improving Nutrition and Growth of Western Forests* (eds Chappell, H.N., Weetman, G.F. & Miller, R.E.), pp. 67–81. Institute of Forest Resources, Seattle.
- Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powerll, V.N. & Underwood, E.C. *et al.* (2001) Terrestrial ecoregions of the world: A new map of life on earth. *BioScience*, **51**, 933.
- Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E. & Kurz, W.A. *et al.* (2011) A large and persistent carbon sink in the world's forests. *Science*, **333**, 988–993.
- Powers, J., Montgomery, R.A., Adair, E.C., Brearley, F.Q., DeWalt, S.J. & Castanho, C.T. *et al.* (2009) Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient. *Journal of Ecology*, **97**, 801–811.
- Rosenberg, M.S., Adams, D.C. & Gurevitch, J. (2000) *Metawin. Statistical Software for Meta-Analysis, Version 2*, Sunderland.
- Scatena, F.N., Bruijnzeel, L.A., Bubb, P. & Das, S. (2011). Setting the stage. *Tropical Montane Cloud Forests: Science for Conservation and Management*, (eds Bruijnzeel, L.A., Scatena, F.N. & Hamilton, L.S.), pp. 3–13. Cambridge University Press, New York.
- Shipley, B., Lechowicz, M.J., Wright, I. & Reich, P.B. (2006) Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology*, **87**, 535–541.
- Sistla, S.A. & Schimel, J.P. (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. *New Phytologist*, **196**: 68–78.
- Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton.
- Tanner, E.V.J., Vitousek, P.M. & Cuevas, E. (1998) Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology*, **79**, 10–22.
- Templer, P.H., Silver, W.L., Pett-Ridge, J.M., DeAngelis, K. & Firestone, M.K. (2008) Plant and microbial controls on nitrogen retention and loss in a humid tropical forest. *Ecology*, **89**, 3030–3040.
- Thornton, P.E., Doney, S.C., Lindsay, K., Moore, J.K., Mahowald, N.M. & Randerson, J.T. *et al.* (2009) Carbon-nitrogen interactions regulate climate-carbon cycle feedbacks: results from an atmosphere-ocean general circulation model. *Biogeosciences*, **6**, 2099–2190.
- Treseder, K.K. (2008) Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111–1120.
- Vitousek, P.M. (1984) Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology*, **65**, 285–298.

- Vitousek, P.M., Walker, L.R., Whiteaker, L.D. & Matson P.A. (1993) Nutrient limitations to plant growth during primary succession in Hawaii Volcanos National Park. *Biogeochemistry*, **23**, 197-215.
- Vitousek, P.M., Porder, S., Houlton, B.Z. & Chadwick, O.A. (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications*, **20**, 5–15.
- Wania, R., Meissner, K.J., Eby, M., Arora, V., Ross, I. & Weaver, A.J. (2012) Carbon-nitrogen feedback in the UVic ESCM. *Geoscientific Model Development Discussions*, **5**, 67–118.
- Xia, J. & Wan, S. (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, **179**, 428–439.
- Zaehle, S. & Dalmonech, D. (2011) Carbon-nitrogen interactions on land at global scales: current understanding in modeling climate biosphere feedbacks. *Current Option in Environmental Sustainability*, **3**, 1–10.

Table 1. Response variables considered in this study.

Nutrient		Name variable	Observations	
<i>Aboveground</i>				
Pools	C	Biomass	Biomass increment	
		DBH	Diameter breast height	
		Litter mass		
		Litter MBC	Litter microbial biomass C	
	N	Foliar N		
		Shoot N		
		Litter N		
	P	Foliar P		
		Litter P		
		Leaf NP	Leaf NP ratio	
Fluxes	C	Plant respiration		
		Litterfall mass		
		Litter respiration		
	N	Litter BNF	Litter biological N fixation	
		Litterfall N		
<i>Belowground</i>				
Pools	C	Total C		
		Organic C		
		MBC	Microbial biomass C	
		DOC	Dissolved organic C	
		N	Root N	
			Total N	
			MBN	Microbial biomass N
			NH ₄ ⁺ -N	
			NO ₃ ⁻ -N	
	DTN		Dissolved total N	
	DON	Dissolved organic N		
	DIN	Dissolved inorganic N		
	P	Soil C:N ratio		
		Total P		
	Fluxes	C	Labile P	
			Decomposition	
		Soil respiration		
		N	BNF	Biological N fixation
			Net mineralization	
Net nitrification				
N ₂ O emission				

NO emission
Leaching NO_3^- -N

Table 2. Experimental conditions and their categories included as modulator variables in this analysis. *Variables collapsed in the *Study Site* variable (see *Materials and methods* section).

VARIABLES	
<i>EXPERIMENTAL ADDITION</i>	
Rate of N addition (kg N ha year ⁻¹)	
Low (<75)	
Medium (75-125)	
High (>125)	
Time of N addition (months)	
First-term (<13)	
Intermediate-term (13-35)	
Long-term (>35)	
<i>Fertilizer</i>	
Urea	
Urea/NH ₄ NO ₃	
NH ₄ NO ₃	
Others	
<i>SITE CHARACTERISTICS</i>	<i>Study Site</i>
<i>Biome (Olson et al. 2001)*</i>	
Tropical and subtropical broadleaf moist forest	Dry Lowland Mature (DL-M)
Tropical and subtropical broadleaf dry forest	Dry Lowland Successional (DL-S)
<i>Elevation (Cleveland et al. 2011)*</i>	
Lowland (<1000 m asl)	Moist Lowland Ex situ (ML-E)
Montane (> 1000 m asl)	Moist Lowland Mature (ML-M)
	Moist Lowland Successional (ML-S)
	Moist Montane Mature (MM-M)
	Moist Montane Successional (MM-S)
<i>Successional status (according to each paper description)*</i>	
Successional forest	
Mature forest	
<i>Experimental conditions*</i>	
<i>In situ</i>	
<i>Ex situ</i>	

Table 3. Significant continuous variables for nutrient pools and fluxes in tropical forests. Mean annual precipitation (MAT), mean annual temperature (MAP), and soil C:N ratio. Significance: ns, non significant ($p \geq 0.05$); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Covariable	Response	Intercept	Slope	Q_B
<i>Pools</i>				
Elevation	NO ₃ ⁻ -N	0.29	0.0008 ***	13.2 ***
MAT	NH ₄ ⁺ -N	2.23	-0.0863 ***	11.4 ***
MAT	Soil total C	-0.22	0.0130 *	3.60 *
MAT	NO ₃ ⁻ -N	2.39	-0.8880 **	9.69 **
MAP	NO ₃ ⁻ -N	-0.34	0.0050 *	9.29 *
Soil C:N ratio	Soil total C	0.36	-0.2430 *	9.43 *
<i>Fluxes</i>				
Elevation	N net mineralization	-0.08	0.0005 ***	14.5 ***
Elevation	Net nitrification	0.04	0.0005 *	4.67 *
MAT	Soil respiration	0.30	-0.0001 *	5.22 *
MAT	N net mineralization	2.35	-0.0959 ***	31.1 ***
MAT	Net nitrification	3.03	-0.1182 ***	12.7 ***
MAP	Net nitrification	-0.71	0.0050 ***	14.8 ***
Soil C:N ratio	N net mineralization	-0.48	0.0540 **	8.66 **

Figure 1. Localities included in the meta-analysis.

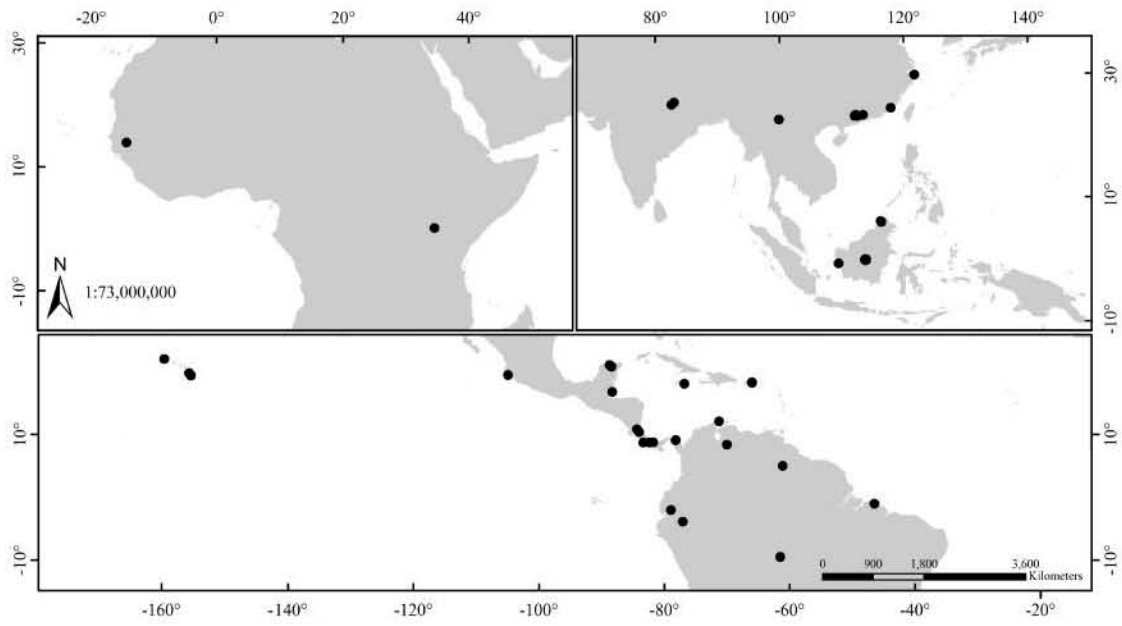


Figure 2. Confidence interval of the response ratio ($\ln R$) for each aboveground variable. n. Number of pair comparison between control and experimental N-supply. Q_T . Total heterogeneity and its associated p-value (***) $p < 0.001$).

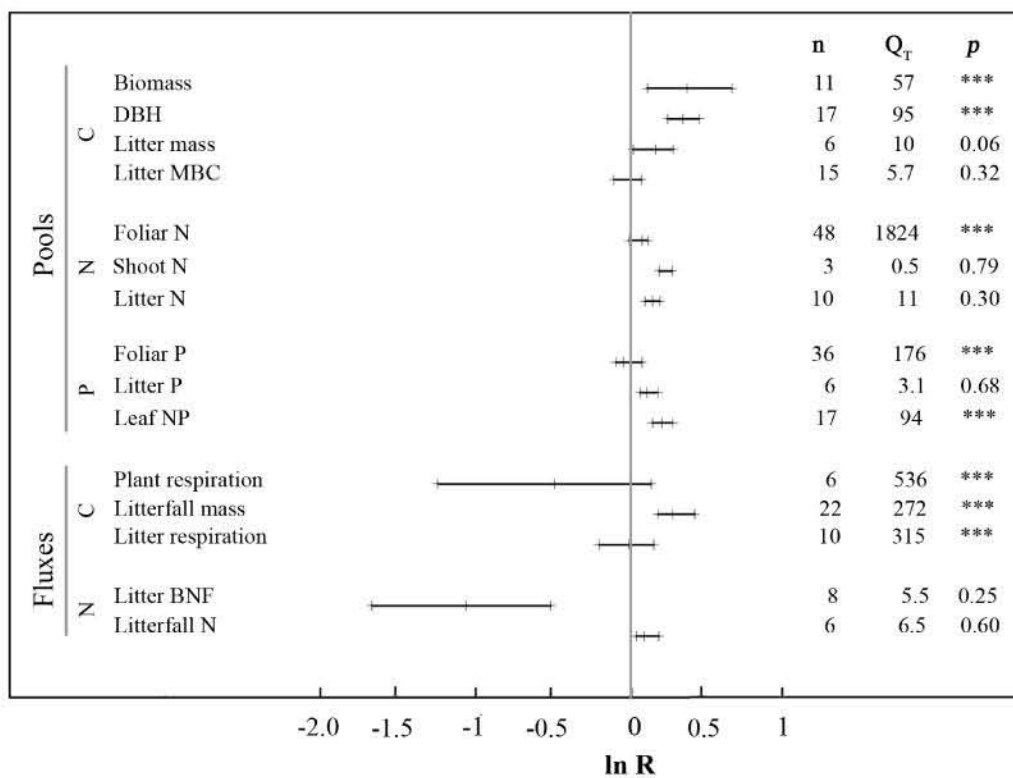


Figure 3. Confidence interval of the response ratio ($\ln R$) for belowground variable. n. Number of pair comparison between control and experimental N-supply. Q_T . Total heterogeneity and its associated p-value (***) $p < 0.001$).

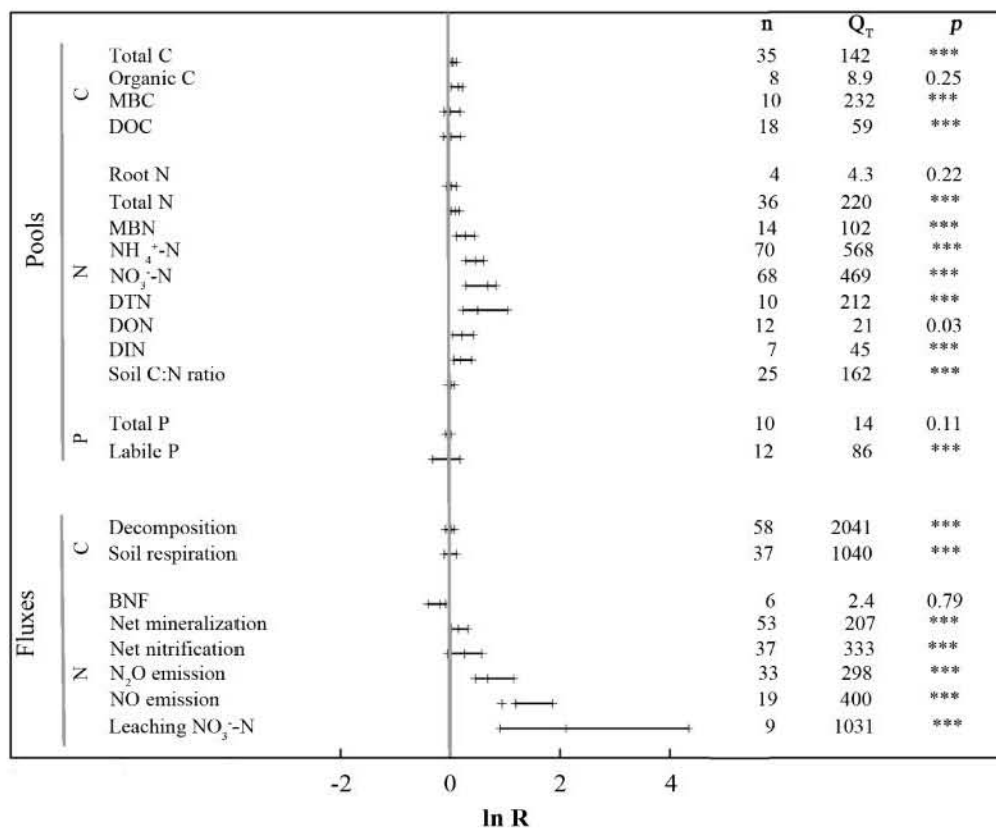
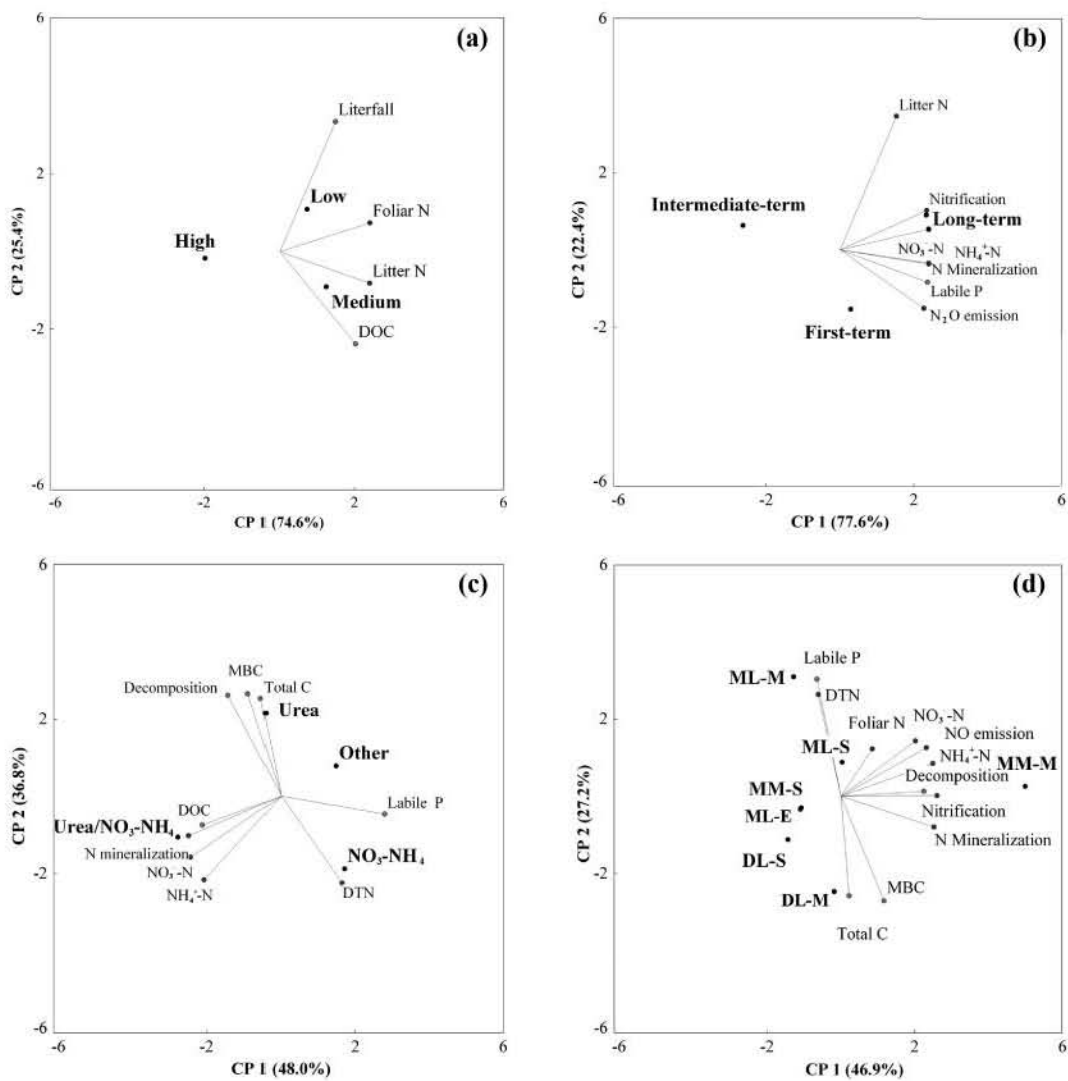


Figure 4. Principal component analyses incorporating the significant variables to experimental conditions. (a) The rate of N addition, (b) the time of N addition, (c) the chemical form of fertilizer and, (d) the Study Site. Study Site abbreviations are in Table 2.



Supporting information

Table S1. A list of 67 papers from which the data were extracted for this meta-analysis.

Adamek M, Corre MD, Holscher D (2009) Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, Panama. *Journal of Tropical Ecology*, **25**, 637–647.

Andersen KM, Corre MD, Turner BL, Dalling JW (2010) Plant–soil associations in a lower montane tropical forest: physiological acclimation and herbivore-mediated responses to nitrogen addition. *Functional Ecology*, **24**, 1171–1180.

Balser TC (2001) The impact of long-term nitrogen addition on microbial community composition in three Hawaiian forest soils. *The Scientific World*, **1**, 500–504.

Bejarano M, Etchevers JD, Ruíz-Suárez G, Campo J (2013) The effects of increased N input on soil C and N dynamics in seasonally dry tropical forests: an experimental approach. *Applied Soil Ecology*, in press.

Cai ZQ, Poorter L, Han Q, Bongers F (2008) Effects of light and nutrients on seedlings of tropical *Bauhinia* lianas and trees. *Tree Physiology*, **28**, 1277–1285.

Campo J, Dirzo R (2003) Leaf quality and herbivory responses to soil nutrient addition in secondary tropical dry forests of Yucatan, Mexico. *Journal of Tropical Ecology*, **19**, 525–530.

Campo J, Solís E, Valencia MG (2007) Litter N and P dynamics in two secondary tropical dry forests after relaxation of nutrient availability constraints. *Forest Ecology and Management*, **252**, 22–40.

Campo J, Vázquez-Yanes C (2004) Effects of nutrient limitation on aboveground carbon dynamics during tropical dry forest regeneration in Yucatán, Mexico. *Ecosystems*, **7**, 311–319.

Cavelier J, Tanner E, Santamaria J (2000) Effect of water, temperature and fertilizers on soil nitrogen net transformations and tree growth in an elfin cloud forest of Colombia. *Journal of Tropical Ecology*, **16**, 83–99.

Cleveland CC, Reed SC, Townsend AR (2006) Nutrient regulation of organic matter decomposition in a tropical rain forest. *Ecology*, **87**, 492–503.

Cleveland CC, Townsend A (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *PNAS*, **103**, 10316–10321.

Corre MD, Veldkamp E, Arnold J, Wright SJ (2010) Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. *Ecology*, **91**, 1715–1729.

Cusack DF, Silver WL, McDowell WH (2009) Biological nitrogen fixation in two tropical forests: ecosystem-level patterns and effects of nitrogen fertilization. *Ecosystems*, **12**, 1299–1315.

Cusack D, Silver WL, Torn MS, McDowell WH (2011) Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. *Biogeochemistry*, **104**, 203–225.

Cusack DF, Torn MS, McDowell WH, Silver WL (2010) The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Global Change Biology*, **16**, 2555–2572.

Davidson EA, Reis de Carvalho CJ, Vieira ICG *et al.* (2004) Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecological Applications*, **14**, 150–163.

Deng Q, Zhou G, Liu J, Liu S, Duan H, Zhang D (2010) Responses of soil respiration to elevated carbon dioxide and nitrogen addition in young subtropical forest ecosystems in China. *Biogeosciences*, **7**, 315–328.

Fang H-J, Mo J, Peng S, Li Z, Wang H (2007) Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China. *Plant and Soil*, **297**, 233–242.

Fang Y, Zhu W, Gundersen P, Mo J, Zhou G, Yoh M (2009) Large loss of dissolved organic nitrogen from nitrogen-saturated forests in subtropical China. *Ecosystems*, **12**, 33–45.

Fang Y-T, Zhu W-X, Mo J-M, Zhou G-Y, Gundersen P (2006) Dynamics of soil inorganic nitrogen and their responses to nitrogen additions in three subtropical forests, south China. *Journal of Environmental Sciences-China*, **18**, 752–759.

Feng Z, Niu J, Zhang W, Wang X, Yao F, Tian Y (2011) Effects of ozone exposure on sub-tropical evergreen *Cinnamomum camphora* seedlings grown in different nitrogen loads. *Trees Structure and Function*, **25**, 617–625.

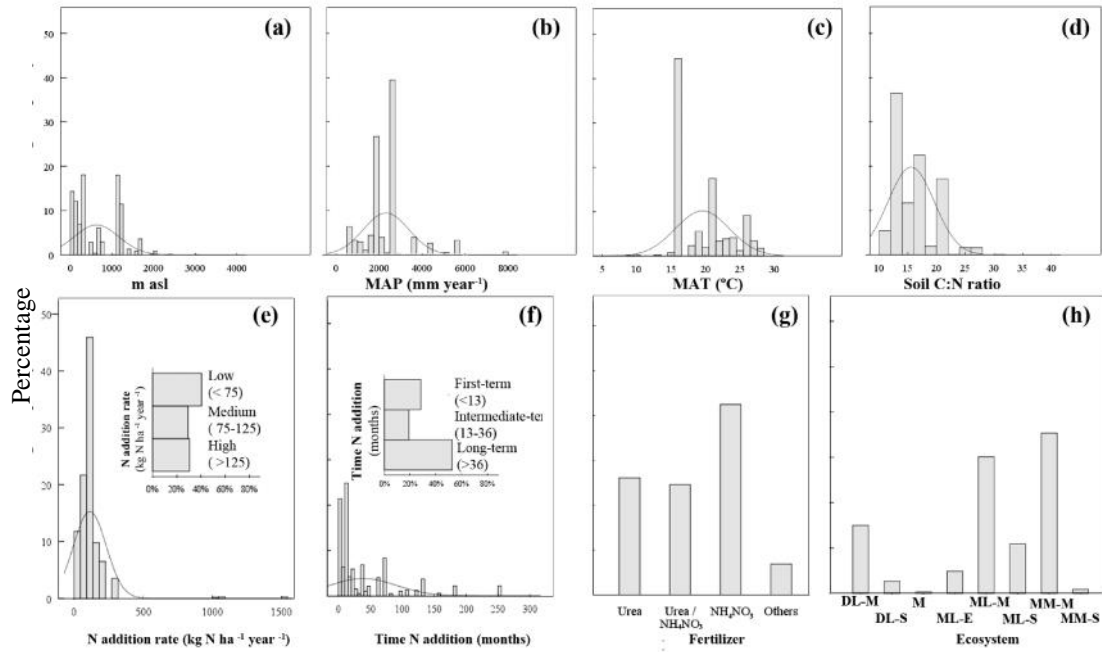
Gamboa AM, Hidalgo C, De León F, Etchevers JD, Gallargo JF, Campo J (2010) Nutrient addition differentially affects soil carbon sequestration in secondary tropical dry forests: Early-versus late succession stages. *Restoration Ecology*, **18**, 252–260.

Hall SJ, Asner GP, Kitayama K (2004) Substrate, climate, and land use controls over soil N dynamics and N-oxide emissions in Borneo. *Biogeochemistry*, **70**, 27–58.

- Hall SJ, Matson PA (1999) Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature*, **400**, 152–155.
- Hall SJ, Matson PA (2003) Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecological Monographs*, **73**, 107–129.
- Harrington RA, Fownes JH, Vitousek PM (2001) Production and resource use efficiencies in N- and P-limited tropical forests: A comparison of responses to long-term fertilization. *Ecosystems*, **4**, 646–657.
- Herbert DA, Fownes JH (1995) Phosphorus limitation of forest leaf area and net primary production on a highly weathered soil. *Biogeochemistry*, **29**, 223–235.
- Kaspari M, Milton N, Harms KE, Santana M, Wright SJ, Yavitt JB (2008) Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters*, **11**, 35–43.
- Khurana E, Singh JS (2004) Impact of elevated nitrogen inputs on seedling growth of five dry tropical tree species as affected by life-history traits. *Canadian Journal of Botany-Revue Canadienne De Botanique*, **82**, 158–167.
- Koehler B, Corre MD, Veldkamp E, Sueta JP (2009) Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest on a decadal time scale. *Biogeosciences*, **6**, 2973–2983.
- Krashevska V, Maraun M, Ruess L, Scheu S (2010) Carbon and nutrient limitation of soil microorganisms and microbial grazers in a tropical montane rain forest. *Oikos*, **119**, 1020–1028.
- Kwabiah AB, Voroney RP, Palm CA, Stoskopf NC (1999) Inorganic fertilizer enrichment of soil: effect on decomposition of plant litter under subhumid tropical conditions. *Biology and Fertility of Soils*, **30**, 224–231.
- Lawrence D (2001) Nitrogen and phosphorus enhance growth and luxury consumption of four secondary forest tree species in Borneo. *Journal of Tropical Ecology*, **17**, 859–869.
- Li D, Wang X, Sheng G, Mo J, Fu J (2008) Soil nitric oxide emissions after nitrogen and phosphorus additions in two subtropical humid forests. *Journal of Geophysical Research*, **113**, D16301.
- Li X, Zheng X, Han S, Zheng J, Li T (2010) Effects of nitrogen additions on nitrogen resorption and use efficiencies and foliar litterfall of six tree species in a mixed birch and poplar forest, northeastern China. *Canadian Journal of Forest Research*, **40**, 2256–2261.
- Liu J, Xu Z, Zhang D *et al.* (2011) Effects of Carbon Dioxide Enrichment and Nitrogen Addition on Inorganic Carbon Leaching in Subtropical Model Forest Ecosystems. *Ecosystems*, **14**, 683–697.
- Liu J, Zhou G, Xu Z, Duan H, Li Y, Zhang D (2011) Photosynthesis acclimation, leaf nitrogen concentration, and growth of four tree species over 3 years in response to elevated carbon dioxide and nitrogen treatment in subtropical China. *Journal of Soils and Sediments*, **11**, 1155–1164.
- Liu JX, Zhou GY, Zhang DQ, Xu ZH, Duan HL, Deng Q, Zhao L (2010) Carbon dynamics in subtropical forest soil: effects of atmospheric carbon dioxide enrichment and nitrogen addition. *Journal of Soils and Sediments*, **10**, 730–738.
- Lohse KA, Matson P (2005) Consequences of nitrogen additions for soil losses from wet tropical forests. *Ecological Applications*, **15**, 1629–1648.
- Lovelock CE, Ruess RW, Feller IC (2006) Fine root respiration in the mangrove *Rhizophora mangle* over variation in forest stature and nutrient availability. *Tree Physiology*, **26**, 1601–1606.
- Lu X, Mo J, Gilliam FS *et al.* (2012) Nitrogen addition shapes soil phosphorus availability in two reforested tropical forests in Southern China. *Biotropica*, **44**, 302–311.
- Mirmanto E, Proctor J, Green J, Nagy L (1999) Effects of nitrogen and phosphorus fertilization in a lowland evergreen rainforest. *Philosophical Transactions of the Royal Society*, **354**, 1825–1829.
- Mo J, Brown S, Xue J, Fang Y, Li Z (2006) Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant and Soil*, **282**, 135–151.
- Mo J, Li D, Gundersen P (2008) Seedling growth response of two tropical tree species to nitrogen deposition in southern China. *European Journal of Forest Research*, **127**, 275–283.
- Mo J, Zhang W, Zhu W, Fang Y, Li D, Zhao P (2007) Response of soil respiration to simulated N deposition in a disturbed and a rehabilitated tropical forest in southern China. *Plant and Soil*, **296**, 125–135.
- Mo J, Zhang WEI, Zhu W, Gundersen P, Fang Y, Li D, Wang H (2008) Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. *Global Change Biology*, **14**, 403–412.
- Nomura N, Kikuzawa K (2003) Productive phenology of tropical montane forests: fertilization experiments along a moisture gradient. *Ecological Research*, **18**, 573–586.
- Ostertag R (2001) Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. *Ecology*, **82**, 485–499.

- Ostertag R (2010) Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. *Plant and Soil*, **334**, 85–98.
- Powers JS, Salute S (2011) Macro- and micronutrient effects on decomposition of leaf litter from two tropical tree species: inferences from a short-term laboratory incubation. *Plant and Soil*, **346**, 245–257.
- Priess JA, Fölster H (2001) Microbial properties and soil respiration in submontane forests of Venezuelan Guyana: characteristics and response to fertilizer treatments. *Soil Biology and Biochemistry*, **33**, 503–509.
- Raich JW, Russell AE, Crews TE, Farrington H, Vitousek PM (1996) Both nitrogen and phosphorus limit plant production on young Hawaiian lava flows. *Biogeochemistry*, **32**, 1–14.
- Reed SC, Cleveland CC, Townsed AR (2007) Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. *Biotropica*, **39**, 585–592.
- Reed SC, Vitousek PM, Cleveland CC (2011) Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence. *Biogeochemistry*, **106**, 323–336.
- Sall SN, Masse D, Bernhard-Reversat F, Guisse A, Chotte JL (2003) Microbial activities during the early stage of laboratory decomposition of tropical leaf litters: the effect of interactions between litter quality and exogenous inorganic nitrogen. *Biology and Fertility of Soils*, **39**, 103–111.
- Solis E, Campo J (2004) Soil N and P dynamics in two secondary tropical dry forests after fertilization. *Forest Ecology and Management*, **195**, 409–418.
- Stuedler PA, Garcia-Montiel DC, Piccolo MC, Neill C, Melillo JM, Feigl BJ, Cerri CC (2002) Trace gas responses of tropical forest and pasture soils to N and P fertilization. *Global Biogeochemical Cycles*, **16**, GB001394.
- Tanner EVJ, Kapos V, Franco W (1992) Nitrogen and phosphorus fertilization effects on venezuelan montane forest trunk growth and litterfall. *Ecology*, **73**, 78–86.
- Tanner EVJ, Kapos V, Freskos S, Healey JR, Theobald AM (1990) Nitrogen and phosphorus fertilization of Jamaican montane forest trees. *Journal of Tropical Ecology*, **6**, 231–238.
- Torn MS, Vitousek P, Trumbore SE (2005) The influence of nutrient availability on soil organic matter turnover estimated by incubations and radiocarbon modeling. *Ecosystems*, **8**, 352–372.
- Tripathi SK, Kushwaha CP, Singh KP (2008) Tropical forest and savanna ecosystems show differential impact of N and P additions on soil organic matter and aggregate structure. *Global Change Biology*, **14**, 2572–2581.
- Vitousek PM, Farrington H (1997) Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry*, **37**, 63–75.
- Vitousek PM, Walker LR, Whiteaker LD, Matson PA (1993) Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry*, **23**, 197–215.
- Wullaert H, Homeier J, Valarezo C, Wilcke W (2010) Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments. *Forest Ecology and Management*, **260**, 1434–1445.
- Xu G, Mo J, Fu S, Gundersen P, Zhou G, Xue J-H (2007) Response of soil fauna to simulated nitrogen deposition: A nursery experiment in subtropical China. *Journal of Environmental Sciences-China*, **19**, 603–609.
- Zhang W, Mo J, Yu G, Fang Y, Li D, Lu X, Wang H (2008) Emissions of nitrous oxide from three tropical forests in Southern China in response to simulated nitrogen deposition. *Plant and Soil*, **306**, 221–236.
- Zhang W, Mo J, Zhou G *et al.* (2008) Methane uptake responses to nitrogen deposition in three tropical forests in southern China. *Journal in Geophysical Research*, **113**, D11116.

Figure S1. Frequency distributions according to modulator variables used in this meta-analysis.



See Site Study abbreviation in Table 2

Figure S2. Confidence interval of the response ratio ($\ln R$) for each significant variable to N addition. n. Number of pair comparison between control and experimental N-supply. Q_B . Between group heterogeneity and its associated p-value (* $p < 0.05$, ** $p < 0.01$).

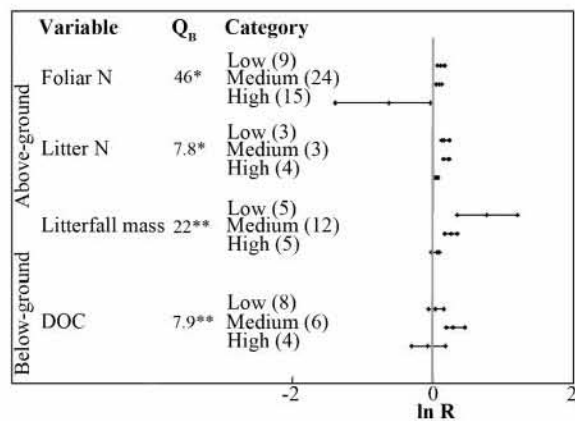


Figure S3. Confidence interval of the response ratio ($\ln R$) for each significant variable to time of the N addition. n. Number of pair comparison between control and experimental N-supply. QB. Between group heterogeneity and its associated p-value (* $p < 0.05$, ** $p < 0.01$).

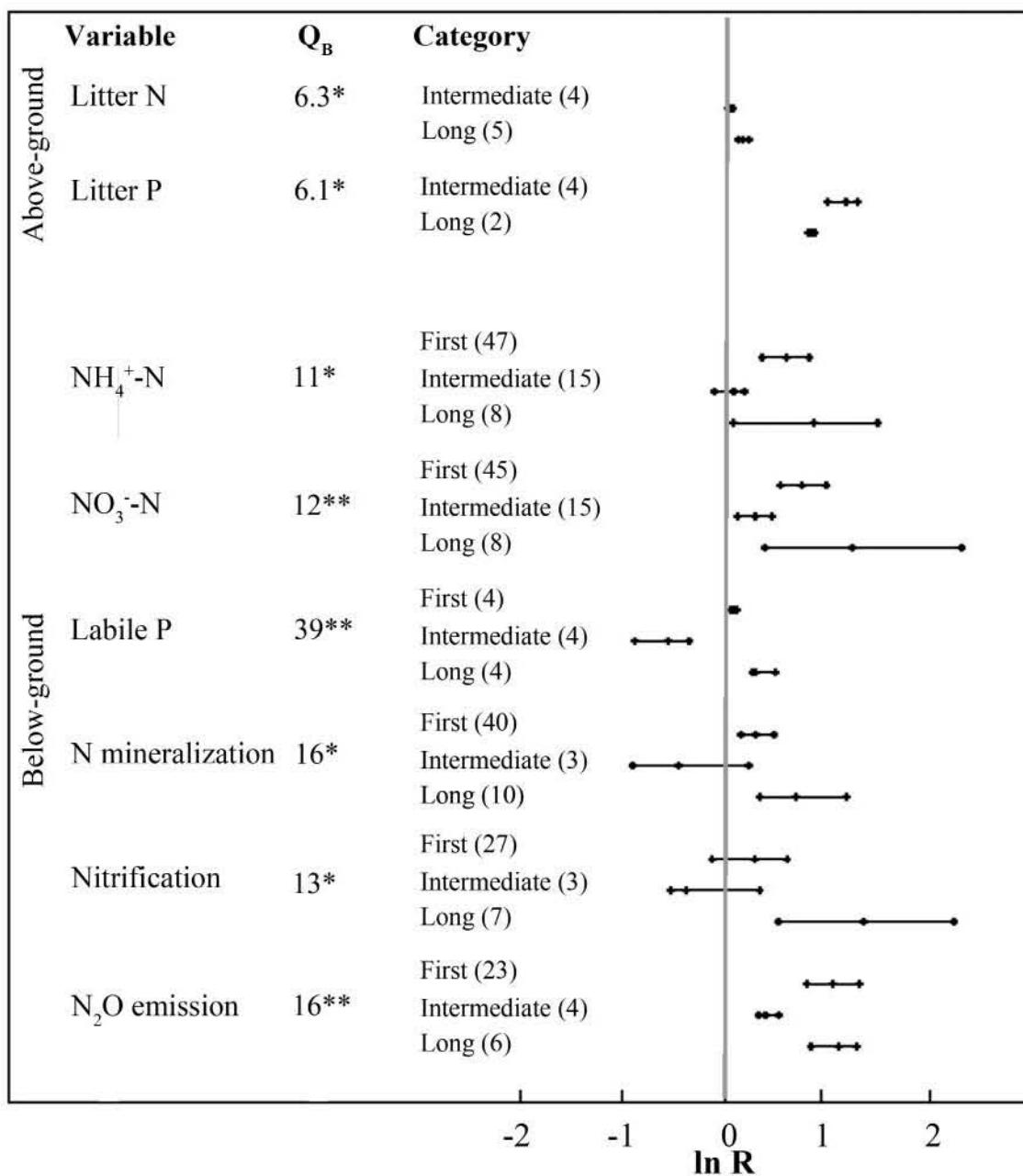
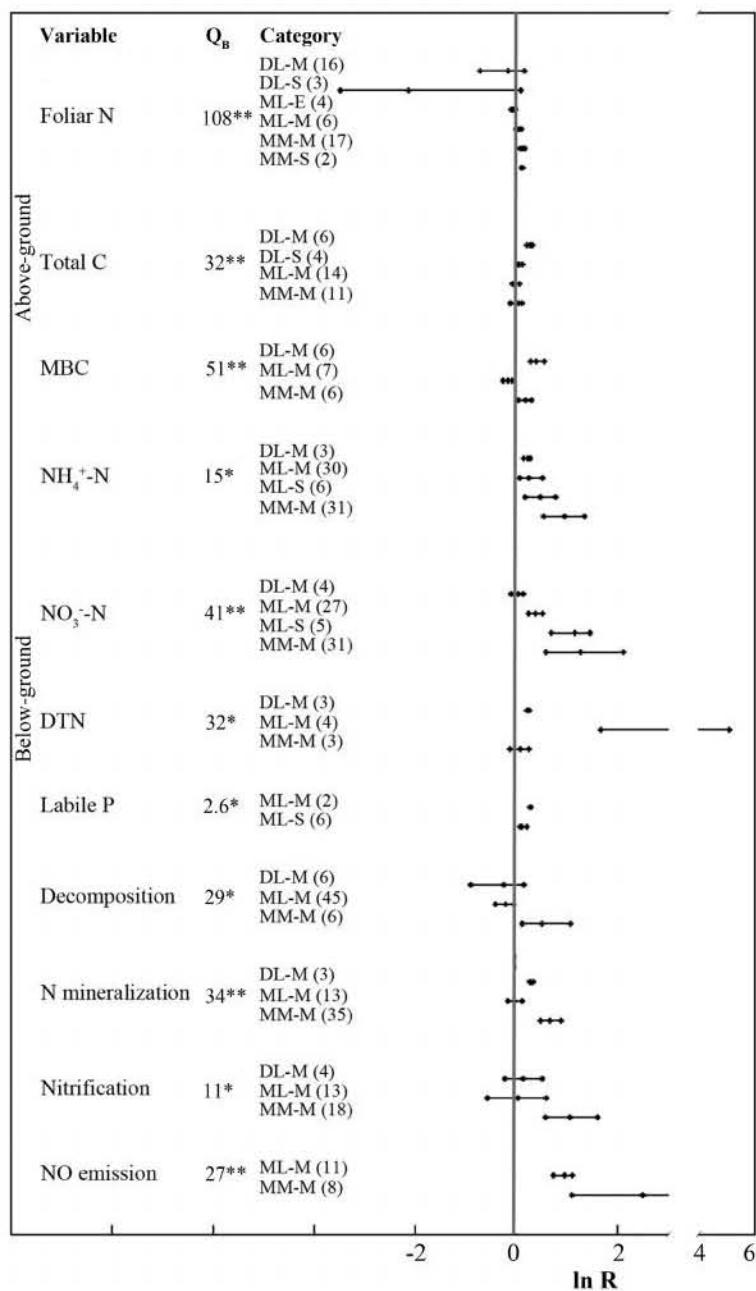


Figure S5. Confidence interval of the response ratio (ln R) for each significant variable to *Study Site* variable. n. Number of pair comparison between control and experimental N-supply. Q_B . Between group heterogeneity and its associated p-value (* $p < 0.05$, ** $p < 0.01$). Acronyms are in Table 2.



CAPÍTULO 2

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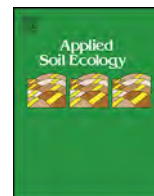
The effects of increased N input on soil C and N dynamics in seasonally dry tropical forests: an experimental approach

Marylin Bejarano^a, Jorge D. Etchevers^b, Gerardo Ruíz-Suárez^c, Julio Campo^a

^a *Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico,* ^b *Colegio de Postgraduados, Montecillos 56230, Mexico,* ^c *Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico*

Resumen

Los bosques tropicales tienen un papel desproporcionado regulando los ciclos globales del carbono (C) y del nitrógeno (N). En estos bosques no ha sido bien estudiado el impacto del incremento en la deposición del N atmosférico. Nuestro objetivo fue determinar si el ingreso del N tiene un efecto en el ciclo del C y del N en bosques tropicales estacionalmente secos (BTES) a través de un gradiente de precipitación (540 – 1040 mm de precipitación media anual). Usamos un experimento de adición de N en microcosmos para explorar cómo responden la dinámica del C y del N en el suelo. Los flujos de NO₂ y la tasa de mineralización y de nitrificación del suelo incrementaron considerablemente con el aumento de la precipitación media anual, mientras que la emisión de CO₂ decreció con el incremento de la precipitación media anual. Las concentraciones de C orgánico en el suelo de los bosques más secos y húmedos decrecieron significativamente con la adición de N. Sin embargo, la biomasa microbiana respondió diferencialmente entre los sitios al incremento de N. En los suelos del sitio más seco, el incremento de N declinó significativamente las concentraciones microbianas de C y N. En contraste, en los suelos del sitio húmedo, la adición de N incrementó las concentraciones microbianas de C y de N. La mineralización de N y las tasas de nitrificación no respondieron ante la adición de N. En el suelo del bosque más seco, la adición N implicó el incremento de las pérdidas de C y N debido a las emisiones de CO₂ y N₂O; sin embargo, en los suelos del bosque más seco el incremento de N no afectó consistentemente las emisiones de CO₂ y N₂O. En los suelos del bosque más húmedo, la adición de N solo incrementó emisiones de CO₂. Concluimos que los BTES pueden reaccionar a la adición de N de manera variable dependiendo de la limitación hídrica de los sitios, rasgo que parece ser el mecanismo regulador del ciclo de N en estos suelos.



The effects of increased N input on soil C and N dynamics in seasonally dry tropical forests: An experimental approach



Marylin Bejarano^a, Jorge D. Etchevers^b, Gerardo Ruíz-Suárez^c, Julio Campo^{a,*}

^a Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

^b Colegio de Postgraduados, Montecillos 56230, Mexico

^c Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

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ABSTRACT

Although tropical forests play a disproportionate role in regulating global C and N cycles, the impact of elevated N-deposition on these ecosystems is not well studied. Our goal was to determine whether elevated N input had an effect on soil C and N cycling in seasonally dry tropical forests along a precipitation gradient (with mean annual precipitation ranging from 540 mm to 1040 mm). We used a microcosm N-fertilization experiment to explore how soil C and N dynamics respond to N addition. NO₂ fluxes and net N-mineralization and nitrification rates in soils increased considerably with mean annual precipitation. In contrast, CO₂ fluxes decreased with increased mean annual precipitation. Organic C concentrations in soil from both the driest and the wettest forest sites decreased significantly with elevated N input. However, microbial biomass responded to N enrichment differently across sites. In soils from the driest site, microbial biomass C and N concentrations declined significantly with elevated N input. In contrast, in soils from the wettest site, microbial biomass C and N immobilization increased. Net N-mineralization and nitrification rates did not respond to elevated N input. In soils from the driest site, elevated N input resulted in an increase in C and N losses due to CO₂ and N₂O fluxes. However, elevated N input to soils from the wettest site did not consistently affect CO₂ and N₂O fluxes. In soils from the wettest site, the main response to N enrichment was an increase only in CO₂ fluxes. We conclude that the reactions of seasonally dry tropical forest soils to increases in N deposition will vary, depending on the details of the site's water limitations, which appear as a regulatory mechanism of the N cycle in these soils.

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1. Introduction

Nitrogen fertilizer use, the cultivation of N-fixing plants, the burning of fossil fuels and biomass burning have changed the global N cycle (Gruber and Galloway, 2008; Davidson, 2009). From 1860 to 2005, the deposition of reactive N increased one order of magnitude, with regional variations reflecting differences in the intensity of agriculture and fossil fuel use (Galloway et al., 2008). In recent decades, the deposition of reactive N has increased in the tropics, and this region may see the most dramatic increases in the coming decades (Lamarque et al., 2005; Dentener et al., 2006). Findings from moist and dry tropical forest studies suggest long-term change in the N cycle in these ecosystems (Hietz et al., 2011).

Evidence regarding the effects of elevated N deposition on terrestrial ecosystem has been obtained in temperate and boreal ecosystems, where net primary production is frequently limited by N availability. Elevated N input decreases the microbial

immobilization of inorganic N and increases N losses through leaching and denitrification (Houlthorn et al., 2006); it also affects the rate of CO₂ emissions from the soil (Treseder, 2008) and the leaching of labile compounds (Sleutel et al., 2009). Although tropical forests represent 44% of global forest area and contribute significantly to global C and N cycles (Malhi and Phillips, 2004; Pan et al., 2011; Zhuang et al., 2011), we have little information about the impact of elevated N deposition on N cycling and C storage in these ecosystems (5% of all studies of global forest ecosystems were performed in tropical regions; Lu et al., 2011a,b). Studies in tropical rain forest ecosystems show that both changes in soil N cycling with elevated N input and the magnitude of N losses depend on soil nutrient status (Hall and Matson, 1999; Koehler et al., 2009b). In these studies, tropical rain forests with N-limited net primary productivity did not exhibit a short-term increase in N losses. However, tropical forest sites with net primary productivity not limited by N showed rapid and large increases in soil N cycling and losses—although ecosystem responses to N enrichment in Panama's forests also depended on the presence of an organic layer in soils (Koehler et al., 2009b; Corre et al., 2010). On the other hand, while N addition did not increase net primary productivity in these N-rich tropical forests, C pools

* Corresponding author. Tel.: +52 55 56229027, fax: +52 55 56228995.

E-mail address: jcampo@ecologia.unam.mx (J. Campo).

Table 1
Characteristics of three climatic sites of Yucatan, Mexico.

	Chicxulub	X'matkuil	Hobonil
Localization	24°14'N, 89°31'W	20°51'N, 89°35'W	19°38'N, 88°59'W
Altitude (m a.s.l.)	4	22	36
Climate ^a	BS1	Aw0	Aw1
Mean annual temperature (°C)	25.8	26.6	26.2
Mean annual precipitation (mm yr ⁻¹)	531	993	1035
Precipitation/Potential evapotranspiration (mm mm ⁻¹) ^b	0.5	0.8	1.0
Soil bulk density (g cm ⁻³) ^b	0.53 ± 0.03b	0.66 ± 0.03a	0.66 ± 0.04a
Soil clays (%)	21.7 ± 1.0b	23.3 ± 1.4b	30.0 ± 2.4a
Soil silt (%)	1.0 ± 0.0b	1.0 ± 0.0b	1.3 ± 0.5a
Soil sand (%)	74.5 ± 1.0a	73.3 ± 1.4a	65.8 ± 2.8b
pH (H ₂ O)	7.4 ± 0.04a	7.5 ± 0.04a	7.4 ± 0.02a
Field capacity (% moisture) ^c	65.6	43.0	33.1
Permanent wilting point (% moisture) ^c	57.7	35.6	26.1
Tree density (stems 144 m ⁻²) ^b	37 ± 5c	104 ± 6a	56 ± 7b
Tree height (m) ^b	3.3 ± 0.2b	3.4 ± 0.1b	4.5 ± 0.2a

Different letters indicate significant differences ($P < 0.05$) among sites.

^a Climatic classification according Köppen adapted by García (2004).

^b Cuevas et al. (2013).

^c Roa-Fuentes et al. (2012).

are sensitive to elevated N deposition in some tropical soils (Cusack et al., 2011). For example, field-fertilization experiments indicate that microbial activity responds positively to elevated N input, with potential effects on the loss of soil C pools via respiration (Cleveland and Townsend, 2006). Responses to elevated N input are likely to vary among tropical forests, which differ significantly in climate (mainly in total amount and distribution of annual precipitation) and in soil nutrient status (Vitousek and Sanford, 1986). The lack of sufficient information on the relative strength of these functional responses in tropical forest regions constitutes one of the greatest uncertainties for the prediction of the future climates (Thornton et al., 2009; Yang et al., 2010; Zaehle and Dalmonech, 2011).

The objective of the present study was to determine the impact of elevated N input on C and N dynamics in soils collected from three mature seasonally dry tropical forests (SDTF) along a precipitation gradient in Yucatan (Mexico). Sites ranged from very dry, with a mean annual precipitation of 540 mm, to a dry site that receives 1040 mm of annual precipitation. SDTFs in the Yucatan Peninsula show high N contents in leaves and litterfall (Campo and Dirzo, 2003; Read and Lawrence, 2003), and soils are both nitrate- and C-rich (Solís and Campo, 2004). Previous N-addition experiments showed that elevated N increased the net primary productivity in some of these Yucatan forests, and consistently increased the N input to soil due to litterfall (Campo and Vázquez-Yanes, 2004; Campo et al., 2007). Soil C and N storage, however, did not change under elevated N-input (Gamboa et al., 2010), suggesting greater C and N losses via soil respiration and denitrification.

Our hypotheses were that: (1) soils from the wettest site should exhibit the most soil C and N cycling, indicated by higher net N transformation rates, soil organic matter decomposition, and C and N losses exemplifying a system with elevated microbial activity due to high water availability; and (2) following N enrichment, soils from the driest site should exhibit the greatest changes in soil C and N cycling, typifying a system with high organic C and N storage due to the reduced availability of water for microbial activity.

2. Materials and methods

2.1. Study sites

The study was carried out at three sites along a gradient of mean annual precipitation (MAP) in the Yucatan Peninsula (Table 1). The landscape consists of flat areas and the predominant lithology includes late Pliocene material, with numerous areas of exposed limestone. Soils are either shallow *black lithosols* surrounding rock

outcrops or deeper *red rendzinas* at slightly lower relief categories (Shang and Tiessen, 2003). In both soils (*black lithosols* and *red rendzinas*), it is common that organic debris and limestone fragments form a large portion of the soil matrix. At least 90% of soil C pools are stored in the Oa soil horizon (Shang and Tiessen, 2003). Dominant soils at study sites (rendzic Leptosols) are mainly shallow (0.2 m in depth) and organic-rich, and directly overlie weathered calcium carbonate.

Long-term climate data from weather stations across the Yucatan Peninsula show that the entire region is characterized by a distinct seasonal period of low precipitation (García, 2004). The climate in the area is hot, ranging from semi-arid to subhumid and supporting tropical dry or very dry forest. Mean temperature is approximately 26 °C (Table 1), with less than a 6 °C difference between the coolest and warmest months. Average annual precipitation increases from 531 to 1035 mm along the gradient; most of it falls during the rainy season (June–October), which contributes 75% of total precipitation (Fig. 1). The natural vegetation at the study sites is SDTF, with mean canopy heights increasing with MAP. Despite this increase in tree size, both aboveground and belowground biomass is very similar across sites (Roa-Fuentes et al., 2012). Although tree species composition varies across study sites, these SDTFs share the same set of dominant tree species (*Acacia gaumeri*, *Bursera simaruba*, *Caesalpinia gaumeri*, *Gymnopodium floribundum*, and *Lonchocarpus yucatanensis*).

2.2. Soil sampling and experiment design

Soils at each forest site were sampled to determine C and N concentrations at the onset of the rainy season (June) of 2010. At each site, 138 soil cores (5 cm × 10 cm deep) were collected on a grid of 6 × 23 points (50 m × 220 m, total area 1.1 ha) in flat areas (i.e. there are no topographic variations within the sampled areas). The cores from each forest were transported to a greenhouse and stabilized for two weeks before the N-addition experiment. A subset of 16 cores collected at this time was used to measure initial soil C and N concentrations. Of the remaining cores, 96 were used in laboratory assays pertaining to net N transformation and CO₂ and N₂O fluxes. The 26 cores with the highest rock content (determined by gravimetry) were discarded.

In the laboratory, samples from each site were randomly assigned to one of two treatments (that is, we used a completely randomized design): control and elevated N input. Three times a week, for four months (the duration of the rainy season at the study sites), we added fertilizers and enough deionized water to keep

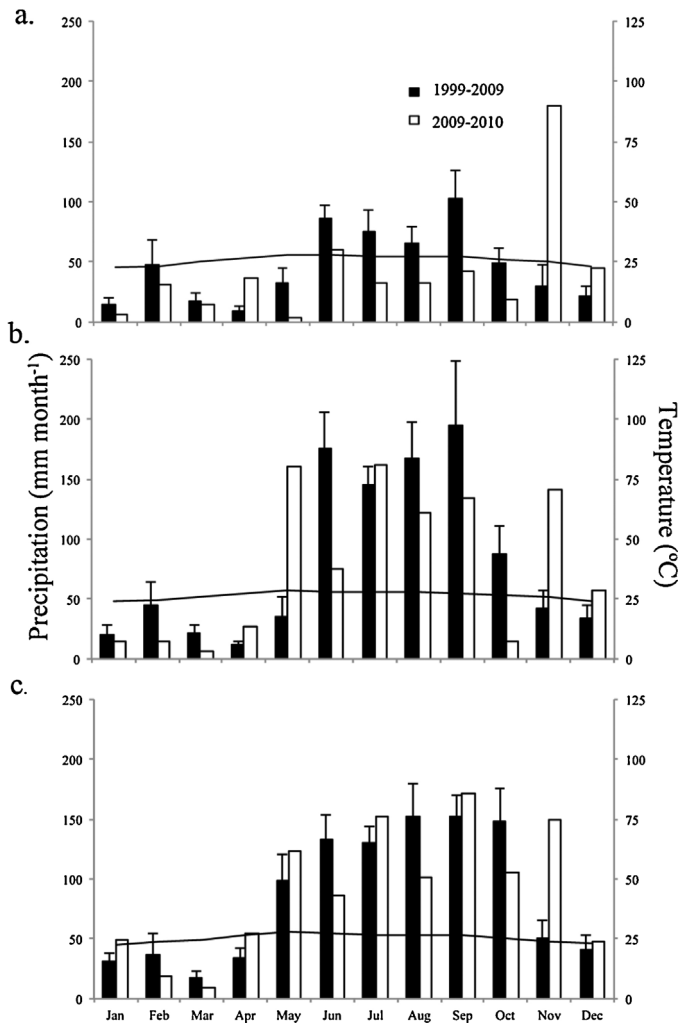


Fig. 1. Mean monthly precipitation (± 1 SE) indicated by bars and temperature indicated by the solid line (June 1999–2009), and monthly precipitation in the year preceding soil cores collection (Jun 2009–2010) in three seasonally dry tropical forest sites at Yucatan Peninsula. Inter-annual variation in monthly temperature is negligible. (a) Chicxulub (531 mm of mean annual precipitation; weather station Progreso 21°17'34"N, 89°36'28"W); (b) X'matkuil (993 mm of mean annual precipitation; weather station Mérida 20°59'00"N, 89°38'00"W); (c) Hobonil (1035 mm of mean annual precipitation; weather station Tantakín 20°01'89"N, 89°02'50"W).

soils at approximately 50% of water holding capacity (100% water holding capacity was determined as the gravimetric water content of water-saturated soil that had been allowed to drain over 6 h). The amount of N added was the same each time. The treatments comprised total additions equivalent to 8 kg N ha⁻¹ for the control and 32 kg N ha⁻¹ for the elevated N input; in both treatments N was added as (NH₄)₂SO₄. Such N additions are similar to the background value in the Yucatan Peninsula in the year 2000—where N concentrations in rainfall are almost four times higher than the background value proposed elsewhere (Cerón et al., 2002)—and the proposed rate for 2030, respectively (Dentener et al., 2006).

Measurements of C and N concentrations were made at 0 (i.e. before incubation), 30, 60 and 120 d of the experiment. At each time, 16 soil cores were removed for C and N measurements and for gas emissions assays. Six soil cores were analyzed for C and N concentrations in microbial biomass and 10 soil cores were incubated for 48 h at 25 °C and 50% of field water holding capacity in a glass container (1 L volume) to collect N₂O and C₂O soil fluxes. Two samples were used to measure gas flux—one at the beginning of incubation and one at the end. After each incubation period, we

separated soil cores into two groups: six cores were analyzed for total and inorganic C and N concentrations in soils, and four cores were used for the remaining analyses (water-extractable C and N). Before each analysis, the soil of each core was sieved (No. 30) and weighed. A 2 g soil subsample from each core was taken to estimate gravimetric water content.

2.3. Sample analyses

2.3.1. Soil C and N concentrations

Total soil C was analyzed using an automatic C analyzer (SHIMADZU SSM-5000A) after grinding a 5 g air-dried subsample and passing it through a 100-mesh screen. Soil inorganic C concentration was estimated from carbonate concentrations in samples; four cores were sampled at each time of incubation, and a subsample of 5 g of dry soil was ground, sieved (No. 100), mixed with 50 mL of 0.5N HCl, and boiled for five minutes. The mixture was filtered (No. 2) and the extracts were separated to a 5 mL aliquot. Aliquots were mixed with two drops of phenolphthalein to titrate the remaining acid with 0.25N of NaOH (Van Reeuwijk, 2002).

The concentration of total N was determined from acid digestion in concentrated H₂SO₄ (Anderson and Ingram, 1993) using an NP element analyzer (Technicon Autoanalyzer II). Microbial biomass C and N concentrations were determined using chloroform fumigation-extraction (Vance et al., 1987) with replicated samples of fresh soil. Fumigated and non-fumigated samples were incubated for 24 h at 24 °C. Microbial biomass C was extracted from both fumigated and non-fumigated samples using 0.5 M K₂SO₄ and Whatman No. 42 filter paper; the concentration of C was measured with an automatic C analyzer (SHIMADZU TOC-Vcsn). Concentrations of C in the non-fumigated extracts were subtracted from concentrations in the fumigated extracts, and microbial C was estimated using a conversion factor (K_C) for microbial C equal to 0.45 (Vance et al., 1987). The inorganic C concentrations in the extracts of microbial biomass were estimated by adding 0.5N HCl to the extracts. Microbial biomass N was extracted in a similar way and filtered through Whatman No. 1 filter paper; the filtrate was digested in acid and the total concentration of N was determined with the NP element analyzer. The method for calculating microbial biomass N concentrations was similar to that employed for microbial C; in this case, a conversion factor (K_N) of 0.57 was used (Jenkinson, 1988).

For each study site we performed a linear regression between total and inorganic C concentrations in soils and in microbial biomass (Supplementary data, Tables S1 and S2). Organic carbon concentrations in soil were estimated by subtracting inorganic C concentration from total C concentration. Also, we proceeded to correct microbial biomass C concentrations for inorganic C concentrations in microbial biomass.

Water-extractable C and N were determined by stirring for 1 h a 10 g soil subsample with deionized water (soil:solution ratio, 1:10, w/v) to estimate the concentration of water-extractable inorganic C and N, water-extractable organic C and N, and water-extractable total C and N. The extracts were vacuum filtered through a membrane (Millipore 45 μm). Water-extractable total and inorganic C concentrations were estimated using high-temperature oxidation (SHIMADZU TOC-Vcsn), and water-extractable organic C concentration was calculated by subtracting water-extractable inorganic C concentration from water-extractable total C concentration. Water-extractable total N concentration was determined by chemiluminescence (TOCV-TNM-1) and water-extractable inorganic N concentration, by colorimetry (Automator II AA Technicon). Water-extractable organic N concentration was calculated by subtracting inorganic N concentration from the total N concentration in water extracts. Finally, water-extractable C:N ratios were calculated by dividing organic C by total N concentration in water extracts.

2.3.2. Net N transformations

We measured inorganic N concentrations ($\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$) and net N-mineralization and nitrification rates using 2 M KCl extraction and aerobic incubation. Inorganic N concentrations were measured by extracting a 15 g sub-sample from each soil sample in 100 mL 2 M KCl (Robertson et al., 1999). The soil KCl solution was shaken for 1 h and allowed to settle overnight. A 20 mL aliquot supernatant was transferred into sample vials and frozen for later analysis (initial inorganic N concentration). Net N-mineralization and nitrification rates were measured for each incubation time (30, 60 and 120 d of aerobic incubation) (Robertson et al., 1999). Another three samples were wetted to field water holding capacity with distilled water, maintained at field capacity moisture (by gravimetry) and incubated at 25 °C for 30, 60 or 120 days before extraction with KCl (final inorganic N concentrations). An analysis of both initial and final inorganic N concentrations was done on an Auto-analyzer system using procedures to determine $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ (which were reported as $\text{NO}_3\text{-N}$; using the sulfanilamide method) and $\text{NH}_4\text{-N}$ (using the salicylate-hypochlorite procedure). Net N-mineralization was calculated as the difference between inorganic N at the beginning and end of each incubation time, and results were expressed in terms of mean daily inorganic N production. Likewise, net nitrification was calculated as the difference between $\text{NO}_3\text{-N}$ at the beginning and end of the each incubation time, and results were expressed in similar units.

2.3.3. CO_2 and N_2O fluxes

Gas samples were stored at 3 °C and were analyzed in a gas chromatograph (Shimadzu GC-2014) with a sample loop injector (1 mL, 120 °C), Supelco column (04162010, chemical phase HayeSep D, 2 m × 1/8 IN SS) methanizer to convert CO_2 to CH_4 (380 °C), and two detectors (electron capture detector, ECD, 325 °C, for N_2O , and flame ionization detector, FID, 250 °C, for CH_4). N_2 was used as a carrier gas (flux 25 mL min⁻¹). Gas concentrations were determined by comparison with a calibration curve and its integrated peak areas. Calibration curve points were determined using standard certified mixed gases and their dilutions in high-purity He (Standard Praxair: 970 ppm CH_4 , 5120 ppm CO_2 , 80 N_2O ppm; Standard Scotty Analyzed Gases: 5 ppm CH_4 , 605 ppm CO_2 , 1 ppm N_2O).

2.4. Statistical analyses

Statistical analyses were performed using R software (R Development Core Team, 2011). For each estimated variable we built two linear models. The initial model (IM) was formulated to test for differences among sites before N addition ($t=0$ d), and the experimental model (EM) to estimate the effect of experimental factors and their interactions (N-addition treatment (N), experimental time (T) and forest site (S)). In both models, residuals were explored for normality and variance homogeneity. Also, EM was explored for temporal correlation among experimental times (30, 60, and 120 d). When the residuals of each model satisfied homoscedasticity and normality assumptions, we employed the least square (LS) and maximum likelihood (ML) methods to fit the models using the *stats* package (R Development Core Team, 2011). When the residuals of some models had heterogeneity of variance among levels of S and/or N factors, we followed the methodology suggested by Zuur et al. (2009) to avoid data transformation and to incorporate heterogeneity into EM. Models were built using the *nlme* package (Pinheiro et al., 2011) with generalized least square (GLS), restricted maximum likelihood (REML) and the *VarIdent* function. These estimation methods have been explained with considerable statistical detail and implemented in ecological studies elsewhere (West et al., 2007; Zuur et al., 2009). Briefly, first we tested whether it was necessary to use an autoregressive correlation structure for the relationship between levels of the T factor

(AR-1). The AR-1 correlation is used to explain temporal data sets that are expected to display a closer correlation between the values of a given time and the time immediately previous (Ives and Zhu, 2006). The second step was to select the best variance structure, and the last step was to model the selection according to the Akaike information criterion (Hoeting et al., 2006) and the lowest p value of likelihood ratio (Zuur et al., 2009) (Supplementary data, Table S3). All final models were checked for normality of the distribution of residuals. When variables or interactions among them were significant in the final model, comparisons between interaction levels were performed using the *contrast* package (Kuhn et al., 2011). The correlations between each pair of estimated variables were calculated using Spearman's rank-order correlation coefficient; p values were corrected using the Holm-Bonferroni method and the *Rcmdr* package (Fox, 2005).

3. Results

3.1. Regional patterns in soil C and N concentrations and fluxes

There were significant differences in organic C and total N concentrations among sites (Table 2). Soils from the driest site had the highest organic C and total N concentrations, whereas soils from the wettest site had the lowest concentrations ($F=13.3$, $P<0.001$, for organic C; $F=6.1$, $P<0.05$, for total N). In contrast, concentrations of inorganic C increased with the MAP ($F=11.2$, $P<0.01$). The soil organic matter quality at the wettest site was high, as indicated by a low soil C:N ratio ($F=10.8$, $P<0.01$). In contrast, soils from drier sites (Chicxulub and X'matkuil), had higher soil C:N ratios (range 19–23), indicating poor-quality organic matter.

Microbial C and N varied significantly among sites, although changes were not consistently related to MAP (Table 2). Soils from X'matkuil had the highest concentrations of microbial biomass C and N, whereas soils from the wettest site, Hobonil, had the lowest concentrations ($F=62.6$ and $F=66.2$, $P<0.001$, for microbial biomass C and N, respectively), reflecting the lower concentrations of organic C and total N in Hobonil soils. Microbial C:N ratios differed considerably among forests. The corresponding ANOVA test showed that soils from Chicxulub and X'matkuil had the highest microbial C:N ratios ($F=5.33$, $P<0.01$).

The microbial C mean contribution to soil C (measured as the ratio between C concentrations in microbial biomass and organic C concentrations in soils) ranged from 0.7% to 2.0%. This mean contribution was highest in X'matkuil soils and lowest in Chicxulub soils. Also, the contribution of microbial N to total soil N was lowest at the driest site (1.2%) and increased at wetter sites (ranging between 1.3 and 1.4%).

As in the case of microbial biomass, water-extractable C concentrations in soils varied significantly among sites (by 75% in the case of total and organic C, and by a factor of 2 in the case of inorganic C), but changes were not consistently related to MAP (Table 2). X'matkuil soils had the highest concentrations of water-extractable total C, organic C and inorganic C, whereas at the driest site, Chicxulub, and the wettest site, Hobonil, soils had the lowest C concentrations in water extracts ($F=6.20\text{--}12.7$, $P<0.01$). In contrast, water-extractable N concentrations in soils varied considerably among the three sites with MAP. All water-extractable N concentrations (i.e. total, organic and inorganic N) in soils from the driest site were approximately three times higher than in soils from the wettest site ($F=11.8$ for total N, $F=8.61$ for organic N, and $F=28.8$ for inorganic N, $P<0.01$). Water-extractable C:N ratios increased with MAP ($F=15.5$, $P<0.001$).

Across sites, inorganic N concentrations (NO_3 and NH_4) in soils varied significantly (Table 2). NH_4 concentrations were highest in soils from X'matkuil ($F=3.61$,

Table 2
Carbon and nitrogen concentrations and carbon:nitrogen ratios in soil cores before nitrogen addition experiment (time 0).

Variable	Sites		
	Chicxulub	X'matkuil	Hobonil
Organic C (mg C g ⁻¹) ^a	273 ± 18a	228 ± 17a	90 ± 7b
Inorganic C (mg C g ⁻¹) ^a	5.9 ± 0.3c	7.7 ± 0.5b	21.3 ± 4.3a
Total N (mg N g ⁻¹) ^a	14.1 ± 1.4a	9.7 ± 1.1b	8.7 ± 0.9b
Soil C:N ratio ^a	19.4 ± 5.9a	23.5 ± 3.0a	10.4 ± 1.8b
Microbial biomass C (μg C g ⁻¹) ^a	1899 ± 332b	4551 ± 309a	925 ± 108c
Microbial biomass N (μg N g ⁻¹) ^a	168 ± 21b	440 ± 28a	110 ± 7c
Microbial C:N ratio ^a	11.3 ± 1.1a	10.3 ± 0.5a	8.4 ± 0.5b
Water-extractable Co (μg C g ⁻¹) ^b	724 ± 116b	1273 ± 114a	861 ± 109b
Water-extractable Ci (μg C g ⁻¹) ^b	292 ± 38b	510 ± 45a	249 ± 33b
Water-extractable N total (μg N g ⁻¹) ^b	290 ± 34a	286 ± 36a	111 ± 18b
Water-extractable No (μg N g ⁻¹) ^b	237 ± 30a	256 ± 33a	97 ± 17b
Water-extractable Ni (μg N g ⁻¹) ^b	43 ± 5a	31 ± 3b	13 ± 1c
Water-extractable C:N ratio ^b	2.4 ± 0.3c	4.5 ± 0.6b	7.8 ± 1.0a
NH ₄ ⁺ -N (μg N g ⁻¹) ^a	21.0 ± 2.2b	32.5 ± 3.5a	26.8 ± 3.3ab
NO ₃ ⁻ -N (μg N g ⁻¹) ^a	52.4 ± 4.7a	39.9 ± 2.5b	35.2 ± 2.1b

^a Values for each site are means ± SE of six soil cores.

^b Values for each site are means ± SE of four soil cores.

Different letters indicate that means are significantly different ($P < 0.05$) among sites.

Variable acronyms: Ci, inorganic carbon; Co, organic carbon; Ni, inorganic nitrogen; No, organic nitrogen; N total, total nitrogen.

$P < 0.05$); however, NO₃ concentrations were highest in soils from the driest site ($F = 7.13$, $P < 0.01$). Also, net N-mineralization and net nitrification rates varied among sites (Fig. 2). Consistently, both N transformation rates were lowest at the driest site, Chicxulub, and highest at the wettest site, Hobonil ($F = 4.39$ for net N-mineralization rates and $F = 4.09$ for net nitrification rates, $P < 0.05$). Net nitrification: net N-mineralization ratios did not change across study sites (0.99 ± 0.02 , 1.04 ± 0.02 , and 1.01 ± 0.03 , for Chicxulub, X'matkuil, and Hobonil, respectively) ($F = 1.25$, $P > 0.05$).

CO₂ fluxes in soils from the northern, driest site, Chicxulub, were significantly higher than those from wetter counterparts (i.e. X'matkuil and Hobonil; Fig. 3a; $F = 3.92$, $P < 0.05$). In contrast, N₂O

fluxes in soils from the southern, wettest site, Hobonil, were significantly higher than fluxes from Chicxulub and X'matkuil soils (Fig. 3b; $F = 5.64$, $P < 0.05$).

3.2. Impact of elevated N input

3.2.1. Total soil C and N, microbial biomass C and N, and water-extractable C and N concentrations

The impact of elevated N input on organic C concentrations varied as a function of site (N*site effect, $P < 0.01$) (Table 3). Organic C concentrations in soils from the driest and wettest sites decreased with elevated N input, but their concentrations did not change in soils from X'matkuil. In contrast, the addition of elevated amounts

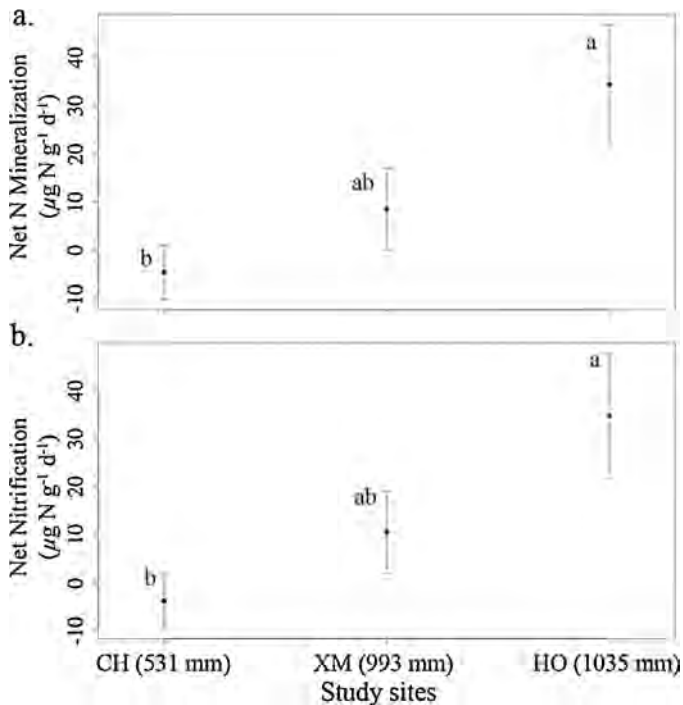


Fig. 2. Net N-mineralization (a), and net nitrification (b) rates in soil cores before to experimental N addition. Values for each site are means ± 1 SE of six soil cores. Different lower cases indicate that means are different statistically ($P < 0.05$) across study.

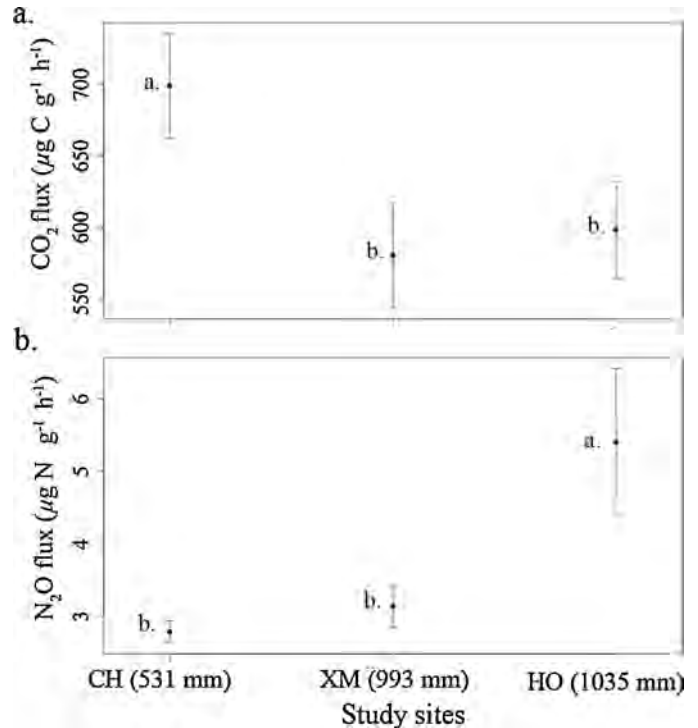


Fig. 3. Fluxes of CO₂ (a), and N₂O (b) in soil cores before to experimental N addition. Values for each site are means ± 1 SE of ten soil cores. Different lower cases indicate that means are different statistically ($P < 0.05$) across study sites.

Table 3
Carbon and nitrogen concentrations and carbon:nitrogen ratios in soil cores under two treatments of nitrogen addition.

Variable	Time (d)	Sites					
		Chicxulub		X'matkuil		Hobonil	
		Control	+N	Control	+N	Control	+N
Organic C ^{C,H} (mg C g ⁻¹)	30	345 ± 42	266 ± 26	233 ± 21	270 ± 30	124 ± 23	81 ± 10
	60	311 ± 27	258 ± 39	240 ± 50	265 ± 24	127 ± 22	69 ± 6
	120	287 ± 28	270 ± 25	227 ± 44	225 ± 18	81 ± 6	91 ± 17
Inorganic C ^{C,H} (mg C g ⁻¹)	30	5.3 ± 0.2	6.2 ± 0.4	7.9 ± 0.4	6.8 ± 0.5	17.2 ± 3.5	22.6 ± 5.7
	60	5.6 ± 0.4	6.3 ± 0.6	7.7 ± 0.8	7.0 ± 0.6	15.5 ± 5.8	29.9 ± 13.9
	120	5.9 ± 0.6	6.2 ± 0.4	8.0 ± 0.7	8.1 ± 1.4	23.0 ± 3.8	27.0 ± 7.3
Total N (mg N g ⁻¹)	30	12.7 ± 2.4	13.7 ± 3.1	9.7 ± 2.9	10.7 ± 2.5	8.6 ± 2.0	6.2 ± 1.7
	60	12.6 ± 1.6	15.7 ± 3.9	12.3 ± 3.6	16.1 ± 3.6	8.8 ± 1.8	8.9 ± 2.3
	120	15.9 ± 1.0	11.0 ± 3.6	8.0 ± 1.9	12.4 ± 2.0	8.8 ± 2.8	9.6 ± 0.9
C:N ratio	30	27.2 ± 13.1	19.4 ± 11.6	24.0 ± 5.8	25.2 ± 7.9	14.4 ± 3.2	13.1 ± 3.5
	60	24.7 ± 7.0	16.4 ± 8.2	19.5 ± 5.0	16.5 ± 5.5	14.4 ± 4.6	7.8 ± 2.1
	120	18.1 ± 1.8	24.5 ± 8.2	28.4 ± 9.8	18.2 ± 2.6	9.2 ± 4.2	9.6 ± 2.8

Values for each site are means ± SE of six soil cores.

Control: addition of 8 kg N ha⁻¹; +N: addition of 32 kg N ha⁻¹.

^{C,H} Significant effect of elevated N addition on the Chicxulub and Hobonil soils.

of N did not have a significant effect on inorganic C and total N concentrations, nor on C:N ratios in soils from any of the three sites ($F=2.28$, $F=0.55$, and $F=1.20$, $P>0.05$; respectively).

The effects of elevated N input on microbial biomass C and N varied as a function of both site and time (N*site effect, $P<0.01$; and N*time-incubation effect, $P<0.05$) (Table 4). Chicxulub soils showed marked declines in microbial biomass C and N concentrations under elevated N input at the beginning (30 d) and at the end of the incubation time (120 d). In contrast, microbial biomass C and N in soils from the wetter sites increased under elevated N input; the increase in microbial biomass C occurred sooner in Hobonil soils than in X'matkuil soils. Effects of elevated N addition on microbial C:N ratios varied as a function of site (N*site effect, $P<0.05$). High input of N decreased the microbial C:N ratios in soils collected at the drier sites (by 58% in the case of Chicxulub, and by 36% in the case of X'matkuil) at 30 d. In contrast, elevated N input increased microbial C:N ratios in the wettest site soils compared to the control soils (by 37%) at 30 d.

Across sites, elevated N input did not affect any water-extractable C concentrations (i.e. total, organic and inorganic C) (Table 5) ($F=0.46$ – 2.44 , $P>0.05$). Also, across sites, water-extractable total, organic and inorganic N concentrations did not change with elevated N input ($F=0.03$ – 0.25 , $P>0.05$). In contrast, elevated N input decreased water-extractable C:N ratios in soils from all three sites compared to the control ($F=8.58$, $P<0.01$).

Table 4
Microbial biomass carbon and nitrogen concentrations and carbon:nitrogen ratios in soil cores under two treatments of nitrogen addition.

Variable	Time (d)	Sites					
		Chicxulub		X'matkuil		Hobonil	
		Control	+N	Control	+N	Control	+N
Microbial biomass C ^{C,X,H} (μg C g ⁻¹)	30	3595 ± 409	1364 ± 194	1496 ± 987	1786 ± 354	602 ± 29	1721 ± 137
	60	4084 ± 217	4615 ± 384	3166 ± 760	2691 ± 211	2781 ± 250	1769 ± 179
	120	6445 ± 1099	3959 ± 1284	2400 ± 289	5010 ± 504	1464 ± 292	2645 ± 567
Microbial biomass N ^{C,X,H} (μg N g ⁻¹)	30	314 ± 41	273 ± 9	174 ± 9	327 ± 22	60 ± 6	122 ± 13
	60	454 ± 95	466 ± 67	357 ± 20	244 ± 44	106 ± 15	93 ± 17
	120	638 ± 138	309 ± 158	335 ± 99	561 ± 93	99 ± 3	151 ± 21
Microbial C:N ratio ^{C,X,H}	30	11.4 ± 1.8	5.1 ± 0.9	8.6 ± 5.7	5.5 ± 1.2	10.1 ± 1.5	14.1 ± 1.1
	60	9.0 ± 2.2	9.9 ± 3.3	8.8 ± 1.9	11.0 ± 2.1	26.2 ± 4.4	19.1 ± 4.3
	120	10.1 ± 1.4	12.8 ± 3.8	7.2 ± 3.4	8.9 ± 2.5	14.8 ± 3.1	17.5 ± 0.9

Values for each site are means ± SE of six soil cores.

Control: addition of 8 kg N ha⁻¹; +N: addition of 32 kg N ha⁻¹.

^{C,X,H} Significant effect of elevated N addition on the Chicxulub, X'matkuil, and Hobonil soils.

Across all sites and N treatments, both water-extractable C concentrations (i.e., organic and inorganic C) changed significantly over time (time effect, $F=11.1$ – 14.0 , $P<0.01$). Water-extractable C concentrations reached their minima at the end of the experiment in 120 d and were at maximum levels in the middle of the experiment, 60 d. In addition, all water-extractable N concentrations increased through time across all sites ($F=16.2$ for water-extractable total N, $F=10.9$ for water-extractable organic N, and $F=31.3$ for water-extractable inorganic N, $P<0.01$). Water-extractable C:N ratios decreased through time (by 50% and 65% in the case of Chicxulub and X'matkuil, and by 75% in the case of Hobonil) ($F=49.6$, $P<0.001$).

3.2.2. Soil inorganic N concentrations and net N transformations

The effects of elevated N input on inorganic N concentrations varied as a function of site in separate analyses (N*site effect, $F=30.2$ for NH₄ and $F=11.6$ for NO₃, $P<0.001$) (Table 6). In samples collected at the driest site, elevated N input increased NH₄ concentrations compared to the control earlier than in samples collected at the wettest site. In contrast, X'matkuil soils showed a decline in NH₄ concentrations under elevated N input during 60 d of incubation and did not vary as much at 120 d. A separate analysis indicates that elevated N increased NO₃ concentrations in soils from the driest and wettest sites ($t=5.39$, $P<0.001$ for Chicxulub soils, and $t=2.98$, $P<0.01$ for Hobonil soils). In contrast, elevated

Table 5

Water-extractable carbon and nitrogen concentrations and carbon:nitrogen ratios in soil cores under two treatments of nitrogen addition.

Variable	Time (d)	Sites					
		Chicxulub		X'matkuil		Hobonil	
		Control	+N	Control	+N	Control	+N
Water-extractable Co ($\mu\text{g C g}^{-1}$)	30	910 \pm 161	709 \pm 269	1044 \pm 102	1461 \pm 134	811 \pm 88	797 \pm 219
	60	1095 \pm 61	894 \pm 199	1181 \pm 273	1082 \pm 201	987 \pm 151	645 \pm 191
	120	637 \pm 81	559 \pm 141	674 \pm 148	641 \pm 185	435 \pm 134	518 \pm 238
Water-extractable Ci ($\mu\text{g C g}^{-1}$)	30	281 \pm 50	304 \pm 75	464 \pm 81	526 \pm 57	280 \pm 75	195 \pm 39
	60	431 \pm 66	181 \pm 57	399 \pm 88	253 \pm 90	349 \pm 53	203 \pm 15
	120	223 \pm 20	223 \pm 37	230 \pm 59	234 \pm 48	188 \pm 29	185 \pm 23
Water-extractable N total ($\mu\text{g N g}^{-1}$)	30	317 \pm 45	243 \pm 61	190 \pm 34	347 \pm 34	93 \pm 13	122 \pm 36
	60	306 \pm 46	380 \pm 75	330 \pm 90	306 \pm 55	152 \pm 12	114 \pm 15
	120	456 \pm 81	559 \pm 46	313 \pm 22	406 \pm 75	210 \pm 18	266 \pm 62
Water-extractable No ($\mu\text{g N g}^{-1}$)	30	269 \pm 28	205 \pm 69	164 \pm 33	311 \pm 34	80 \pm 8	111 \pm 29
	60	260 \pm 21	322 \pm 59	284 \pm 71	381 \pm 43	138 \pm 11	99 \pm 11
	120	377 \pm 61	464 \pm 41	258 \pm 24	338 \pm 63	276 \pm 13	236 \pm 34
Water-extractable Ni ($\mu\text{g N g}^{-1}$)	30	48 \pm 6	38 \pm 7	26 \pm 5	36 \pm 4	13 \pm 0	11 \pm 3
	60	46 \pm 12	58 \pm 12	46 \pm 12	25 \pm 30	14 \pm 1	15 \pm 2
	120	79 \pm 10	95 \pm 5	55 \pm 5	68 \pm 15	34 \pm 9	30 \pm 7
Water-extractable C:N ratio	30	2.9 \pm 0.2	2.9 \pm 0.7	5.5 \pm 0.8	4.2 \pm 0.4	8.8 \pm 0.8	6.5 \pm 1.6
	60	3.6 \pm 0.7	2.3 \pm 0.2	3.7 \pm 0.4	3.5 \pm 1.2	6.4 \pm 0.6	5.7 \pm 0.9
	120	1.4 \pm 0.1	1.0 \pm 0.2	2.1 \pm 0.4	1.6 \pm 0.9	2.1 \pm 0.8	1.9 \pm 0.8

Values for each site are means \pm SE of four soil cores.Control: addition of 8 kg N ha⁻¹; +N: addition of 32 kg N ha⁻¹.

Variable acronyms: Ci, inorganic carbon; Co, organic carbon; Ni, inorganic nitrogen; No, organic nitrogen; N total, total nitrogen.

N input did not have a significant effect on NO₃ concentration in X'matkuil soils ($t=0.26$, $P>0.05$).

Across all sites, elevated N input did not affect net N-mineralization and net nitrification rates (Figs. 4 and 5) ($F=3.02$ – 3.08 , $P>0.05$). Elevated N input, however, increased net nitrification:net N-mineralization ratios compared to the control (by 15%, $F=7.62$, $P<0.01$).

3.2.3. CO₂ and N₂O fluxes

Elevated N input effects on CO₂ and N₂O fluxes varied as a function of site in separate analyses (N*site effect, $P<0.05$). Soils collected at the driest and wettest sites showed more marked increases in CO₂ fluxes under elevated N input compared to the control ($t=5.39$, $P<0.01$ at the driest site; $t=5.84$, $P<0.01$ at the wettest site) (Fig. 6a and c). Elevated N input, however, did not affect the CO₂ flux in X'matkuil soils ($t=0.64$, $P>0.05$) (Fig. 6b). In addition, elevated N input increased N₂O fluxes in dry site soils compared to the control ($t=8.37$, $P<0.001$ for Chicxulub soils; $t=7.35$, $P<0.001$ for X'matkuil soils) (Fig. 7a and b). However, elevated N input did not affect N₂O fluxes in soils from the wettest site ($4.07 \pm 0.34 \mu\text{g}$

N m⁻³ g⁻¹ h⁻¹ in control, and $5.10 \pm 0.61 \mu\text{g N m}^{-3} \text{ g}^{-1} \text{ h}^{-1}$ in elevated N treatment, $t=1.48$, $P>0.05$) (Fig. 7c).

No significant correlations were observed between CO₂ fluxes and organic or inorganic C concentrations ($R=0.34$ – 0.44 , $P>0.05$). Also, none were observed between NO₂ fluxes and net nitrification rates or NO₃ concentrations ($R=0.48$ for net nitrification rates, and $R=-0.40$ for NO₃ concentrations, $P>0.05$).

4. Discussion

4.1. Soil C and N concentrations and losses in seasonally dry tropical forests

Organic C and total and mineral N concentrations in soils at our study sites are higher than those reported for other SDTF soils (between 5 times and an order of magnitude higher, Table 7). The high organic C content was associated with organic matter stabilization by CaCO₃ in Yucatan soils (Shang and Tiessen, 2003). Also, net N-mineralization and nitrification rates in Yucatan soils are high—in fact, they are close to the upper bound of the range reported for SDTF soils (Table 7).

Table 6

Ammonium and nitrate concentrations in soil cores under two treatments of nitrogen addition.

Variable	Time (d)	Sites					
		Chicxulub		X'matkuil		Hobonil	
		Control	+N	Control	+N	Control	+N
NH ₄ ⁺ -N ^{CX,H} ($\mu\text{g N g}^{-1}$)	30	23.9 \pm 3.5	34.7 \pm 3.6	32.1 \pm 4.0	22.7 \pm 2.3	30.2 \pm 4.4	30.3 \pm 2.9
	60	23.2 \pm 2.4	35.4 \pm 3.1	31.7 \pm 4.8	21.8 \pm 4.6	28.9 \pm 3.7	25.7 \pm 5.0
	120	18.8 \pm 6.1	21.7 \pm 3.6	31.2 \pm 6.9	36.8 \pm 2.6	29.3 \pm 3.3	40.4 \pm 2.7
NO ₃ ⁻ -N ^{CH} ($\mu\text{g N g}^{-1}$)	30	54.3 \pm 6.6	60.4 \pm 4.8	45.6 \pm 2.7	46.6 \pm 1.8	32.6 \pm 2.1	37.3 \pm 2.4
	60	40.0 \pm 6.2	70.2 \pm 2.2	51.1 \pm 5.9	41.1 \pm 3.5	35.2 \pm 7.4	34.1 \pm 3.4
	120	76.0 \pm 7.2	68.6 \pm 5.1	49.0 \pm 7.1	57.2 \pm 7.6	31.0 \pm 5.0	36.0 \pm 8.2

Values for each site are means \pm SE of six soil cores.Control: addition of 8 kg N ha⁻¹; +N: addition of 32 kg N ha⁻¹.

CH Significant effect of elevated N addition on the Chicxulub and Hobonil soils.

CX,H Significant effect of elevated N addition on the Chicxulub, X'matkuil, and Hobonil soils.

Table 7
Carbon and nitrogen concentrations in soils of seasonally dry tropical forests (SDTFs).

Variable	This study	SDTFs in Yucatan	Other SDTFs	References
Organic C (mg C g ⁻¹)	89–272	107–116	5–24	1–4
Total N (mg N g ⁻¹)	8–14	1–20	0.2–4.3	3–14
C:N ratio	10–27	11–23	9–23	1,2,5–9,12–16
Microbial biomass C (μg C g ⁻¹)	920–4550	1000–2300	100–500	1–4,7,9,15
Microbial biomass N (μg N g ⁻¹)	100–400		10–70	4,8,14
Microbial C:N ratio	7–10		8–12	4,14,16
NO ₃ ⁻ (μg N g ⁻¹)	35–52	34–50	2–34	1–3,8–10,12,15–16
NH ₄ ⁺ (μg N g ⁻¹)	21–33	73–100	2–41	1–3,8–10,12,15–16
Net N mineralization (μg N g ⁻¹ d ⁻¹)	10–25		2–29	3,10,12,16
Net nitrification (μg N g ⁻¹ d ⁻¹)	10–23		3–17	3,10,12,16

Values are the minimum and maximum results reported in studies. Data for wet season.

References: (1) Gamboa et al. (2010); (2) Häfele (2007), (3) Jara et al. (2009), (4) Srivastava (1992), (5) Campo and Vázquez-Yanes (2004), (6) Campo et al. (2007), (7) Cuevas et al. (2013), (8) Roy and Singh (1994), (9) Anaya et al. (2007), (10) Ellingson et al. (2000), (11) García-Oliva et al. (1999a), (12) García-Oliva et al. (1999b), (13) García-Oliva et al. (2004), (14) Johnson and Wedin (1997), (15) Montañón et al. (2007), (16) Saynes et al. (2005).

At our study sites, soil C and N are strongly associated with the MAP on a regional scale. Although the wettest forests show larger net primary productivity and larger N content in litterfall (J. Campo, unpublished data), organic C and total N concentrations in soils decreased with increased rainfall, suggesting a more rapid C and N turnover with MAP. Consistent with our hypothesis, we found that the mean contents of organic C in the upper 10 cm of soil profile—calculated as the product of organic C concentration and bulk soil density from Tables 1 and 2—also decrease with increased MAP, from approximately 15 kg C m⁻² at the drier

sites (i.e., Chicxulub and X'matkuil) to 7 kg C m⁻² at the wettest site (Hobonil) reflecting the positive effect of MAP on soil organic matter decomposition (Vitousek et al., 1994; Schuur and Matson, 2001). The increase in C content at the driest site was accompanied by the highest soil organic C respired by microbes, as predicted by our first hypothesis. This positive trend matched a stronger trend involving increased labile soil C content with decreased annual rainfall at our study sites (Cuevas et al., 2013) and decreasing water-extractable C and N concentrations with MAP across sites (an indirect consequence of higher microbial demand; Rees and Parker, 2005).

Also, we expected that sites with more precipitation would exhibit higher net N transformation rates and greater N losses because this factor can stimulate internal N-cycling rates. Our results show that internal N-cycling rates (both net N-mineralization and nitrification rates), and N₂O fluxes were higher at the wettest site than at the driest site. This pattern follows a

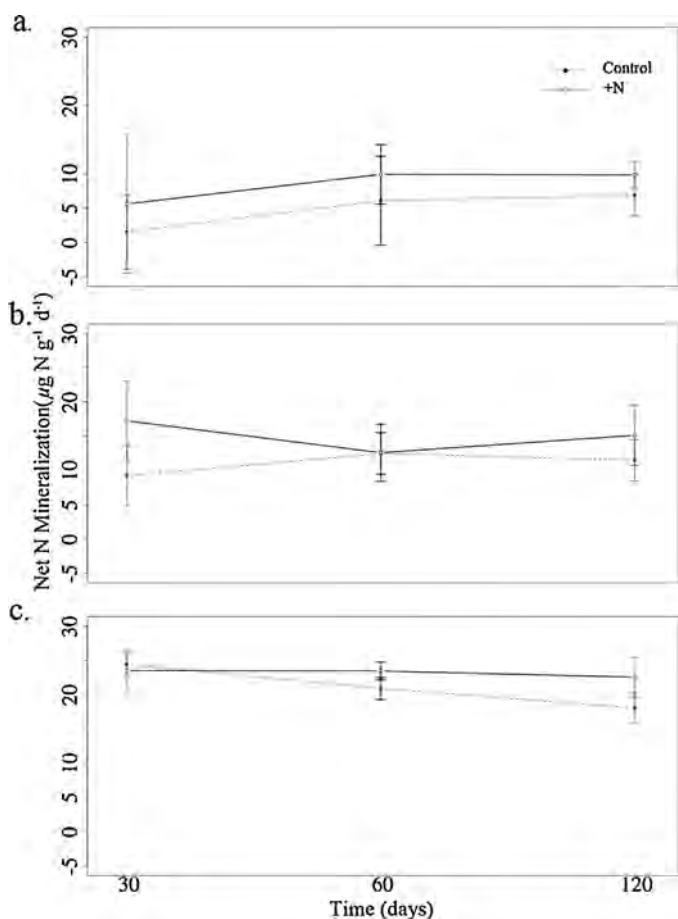


Fig. 4. Net N-mineralization rates in soil cores under two treatments of N addition (+N, N-addition = 32 kg N ha⁻¹; control, N-addition = 8 kg N ha⁻¹). Values for each site are means ± 1 SE of six soil cores. (a) Chicxulub (531 mm of mean annual precipitation), (b) X'matkuil (993 mm of mean annual precipitation), and (c) Hobonil (1035 mm of mean annual precipitation).

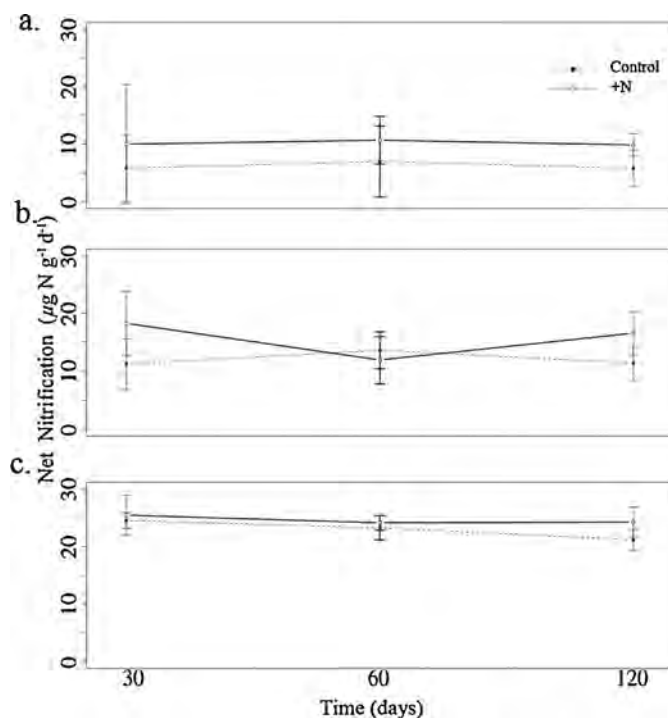


Fig. 5. Net nitrification rates in soil cores under two treatments of N addition (+N, N-addition = 32 kg N ha⁻¹; control, N-addition = 8 kg N ha⁻¹). Values for each site are means ± 1 SE of six soil cores. (a) Chicxulub (531 mm of mean annual precipitation), (b) X'matkuil (993 mm of mean annual precipitation), and (c) Hobonil (1035 mm of mean annual precipitation).

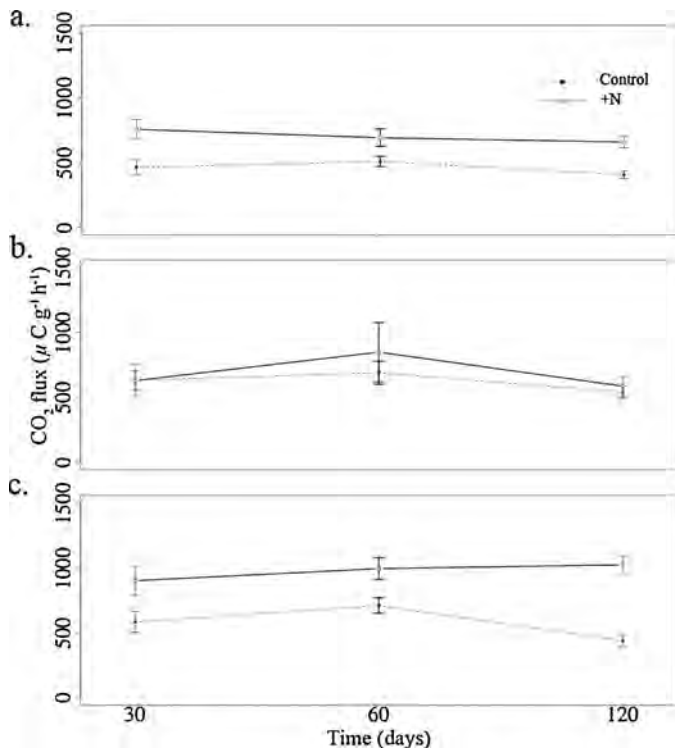


Fig. 6. Fluxes of CO₂ from soil cores under two treatments of N addition (+N, N-addition = 32 kg N ha⁻¹; control, N-addition = 8 kg N ha⁻¹). Values for each site are means ± 1 SE of ten soil cores. (a) Chicxulub (531 mm of mean annual precipitation), (b) X'matkuil (993 mm of mean annual precipitation), and (c) Hobonil (1035 mm of mean annual precipitation).

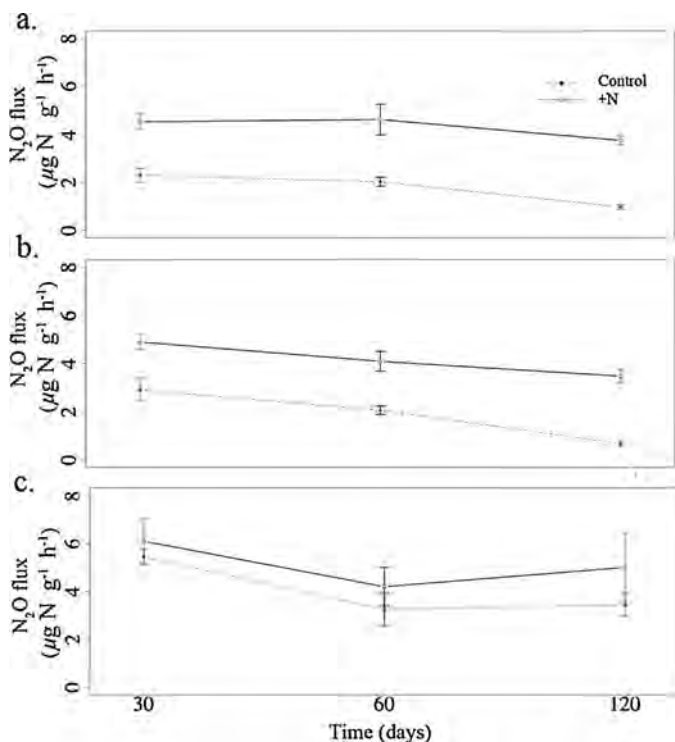


Fig. 7. Fluxes of N₂O from soil cores under two treatments of N addition (+N, N-addition = 32 kg N ha⁻¹; control, N-addition = 8 kg N ha⁻¹). Values for each site are means ± 1 SE of ten soil cores. (a) Chicxulub (531 mm of mean annual precipitation), (b) X'matkuil (993 mm of mean annual precipitation), and (c) Hobonil (1035 mm of mean annual precipitation).

pattern reported for humid tropical forest counterparts: tropical forest soils with high rates of N-cycling have high N-oxide emissions (Davidson et al., 2000; Corre et al., 2010), and could be the key to the observed lowest soil total N storage at the wettest study sites. We cannot determine whether such an increase in N losses via N-oxide fluxes resulted from the higher concentration of NO₃ observed in the soil (thus relaxing a possible direct substrate limitation to denitrification), or whether it reflects more favorable conditions for denitrification in the clay-rich, wettest soils, or both. The lack of a simple gradient effect of rainfall on soil C and N dynamics is also evidenced by the fact that soils collected at X'matkuil had the highest microbial biomass C and N concentrations. Microbial C:N ratios in X'matkuil soils, however, are between those of Chicxulub and Hobonil soils, though they are closer to the ratios observed in Chicxulub soils. Thus, the 500–1050-mm MAP gradient appears to be an important driver of soil C and N status, but the relationship between the turnover of these bioelements and the total annual amount of precipitation observed across sites shows considerable variability, reflecting changes in soil clay content (an indirect consequence of changes in MAP) and water content (Table 1).

4.2. The effects of elevated N input on soil C and N concentrations and losses in seasonally dry tropical forests

Our results are consistent with findings from other studies that show that N addition increases the soil organic matter quality in forest ecosystems (Treseder, 2008; Lu et al., 2011a,b). The increase in substrate quality for microbial activity has been reported as stronger in C-rich soils (such as Yucatan soils) than in C-poor soils (Yang et al., 2007; Cusack et al., 2009, 2011; Koehler et al., 2009a).

We expected that sites with less precipitation would exhibit greater changes in internal C- and N-cycling rates after N enrichment, because less precipitation can limit the microbial activity and nutrient availability, and thereby enhance C and N storage in soils. Our results show that the addition of N increased CO₂ and N₂O emissions from soils collected at the driest site. Soils from the driest site were nutrient-poor and had high C:N ratios, which likely contributed to a relative high demand by microbes. Accordingly, N immobilization increased at the driest site, as indicated by the significant decrease in microbial C:N ratios. The convergence of the results of CO₂ and N₂O flux studies and microbial C:N stoichiometric ratios suggests a system with N limitation. Similarly, our experiment shows that nutrient-rich soils from the higher precipitation site with low C:N (i.e., the wettest site) had higher CO₂ fluxes following N fertilization, compared to unfertilized controls. These patterns of response are similar to those reported by Hall and Matson (2003) for N-limited tropical rain forests, and also for other tropical rain forest ecosystems without evidence of N-limited net primary productivity (Grandy et al., 2008; Cusack et al., 2011). Interestingly, although microbial activity at the wettest site can be limited by N (as the CO₂ fluxes indicate), soil N cycling rates and N losses showed no changes after the increase in N input (as indicated by both net N transformation rates and N-oxide emissions). Overall, these results suggest that higher precipitation at the wettest site could stimulate internal N-cycling rates and thereby intensify N losses. A greater natural abundance of ¹⁵N in the leaves of tree species at the wettest site compared to that found in the leaves of species at the drier sites (Roa-Fuentes, 2013) is consistent with an N cycle that becomes increasingly leaky with increased MAP, considering that foliar ¹⁵N signatures are positively correlated with N losses (Templer et al., 2012). Thus, our experiment demonstrates that a variety of factors influence the potential effects of N inputs on soil C dynamics and loss in these seasonally dry tropical forest ecosystems. There is no data for examining how water availability relates to the indirect influence of N input on C inputs to soil via trees. However, given that the same pattern of CO₂ increase after N

addition occurred in soils from both the driest and the wettest sites, we think this is a robust pattern in microbial response that should stimulate further research on the potential effects of N inputs on C retention and loss in this semiarid forest biome.

This study shows a striking contrast in the consequences of adding high amounts of N at a very dry site (i.e., with a low supply of water) such as the Chicxulub forest ecosystems, as compared to a site with a higher water supply, such as the Hobonil forest ecosystems. Increased N input to the soils of a low-water-supply system leads to a very high increase in N immobilization, much higher than in soils from the site where water is less limiting. This effect is much greater than that observed for net N-mineralization and nitrification at both sites.

Across all sites, elevated N input did not affect net N-mineralization and nitrification rates in soils, as might be expected. However, microbial biomass N concentration in the elevated N-fertilized soils from wetter sites increased with respect to control soils. Additionally, in soils from the driest site, microbial C:N ratios decreased, suggesting a change in microbial biomass composition from fungi, indicated by higher stoichiometric C:N, to the lower stoichiometric ratio of bacteria (Frey et al., 2004; Waldrop et al., 2004; Bradley et al., 2006; Waldrop and Zak, 2006; Cusack et al., 2011). The lack of a concomitant response in terms of increased net N-mineralization and nitrification rates is surprising, as increased N transformation has been found to increase N input rates in tropical rain forest soils (Corre et al., 2010). Thus, although we observed a clear relationship between elevated N input and N-oxide fluxes in the driest site soils, the response of soils from wetter sites was unclear, and only the soils from X'matkuil yielded a similar response to that shown by soils collected at the driest site. An understanding of the lack of responses to elevated N addition in the soils of the wettest site will need to consider differences in microbial composition in very dry and dry climates (considering that the application of N did not have a significant effect on soil pH; data not shown). In any case, our results show that, in addition to the regional variation in microbial C:N ratios, leading to predictable patterns of N cycling in tropical forest soils (Hall and Matson, 1999, 2003), an environmentally induced response may also determine the levels of N-oxide fluxes from the soils.

From an ecosystem perspective, if the increased rates of N inputs from the atmosphere continue as expected (Dentener et al., 2006), the results of our Yucatan studies suggest that all forest sites could exhibit positive feedback due to enhanced N availability resulting from a more rapid return of nutrients to the soil (Campo et al., 2007) and high amounts of N mineralization from high-quality litter (Solís and Campo, 2004). The mechanisms underlying this plant effect could be more complex than a simple increase in N returning to the soil, since it has been observed that fertilization significantly enhances both N availability and net N-mineralization in N-limited ecosystems (Solís and Campo, 2004). Additionally, the possible negative feedback for N availability due to nutrient export from the ecosystem by N-oxide fluxes and N-leaching could be offset by an increase in litterfall production, such as that observed after repeated (3-year) N-fertilization at our study sites (Campo and Vázquez-Yanes, 2004).

Our study on the consequences of regional variation for the impact of elevated N input on the limestone soils of the Yucatan Peninsula allows us to conclude that the reactions of seasonally dry tropical forest ecosystems to increases in N deposition will vary, depending on the details of the site's water limitations that appear as a regulatory mechanism of the N cycle in these soils. Given the potential for tropical forest biogeochemistry research, the strong impact of these ecosystems on global carbon and nitrogen cycles, and the concomitant effect on global warming, the topic of the present study warrants further investigation—for example, on the long-term fates of N in the field, the effects of N-induced

changes on carbon and nitrogen losses, and changes in microbial community composition and function.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsoil.2013.08.015>.

References

- Anaya, C., García-Oliva, F., Jaramillo, V.J., 2007. Rainfall and labile carbon availability control litter nitrogen dynamics in a tropical dry forest. *Oecologia* 150, 602–610.
- Anderson, J.M., Ingram, J.S.I., 1993. *Tropical Soil Biology and Fertility: A Handbook of Methods*, 5th ed. CAB International, Wallingford.
- Bradley, K., Drijber, R.A., Knops, J., 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* 38, 1583–1595.
- Campo, J., Dirzo, R., 2003. Leaf quality and herbivory responses to soil nutrient addition in secondary tropical dry forests of Yucatan. Mexico. *J. Trop. Ecol.* 19, 525–530.
- Campo, J., Vázquez-Yanes, C., 2004. Effects of nutrient limitation on aboveground carbon dynamics during tropical dry forest regeneration in Yucatan. Mexico. *Ecosystems* 7, 311–319.
- Campo, J., Solís, E., Valencia, M.G., 2007. Litter N and P dynamics in two secondary tropical dry forests after relaxation of nutrient availability constraints. *For. Ecol. Manage.* 252, 22–40.
- Cerón, R.M.B., Padilla, H.G., Belmont, R.D., Torres, M.C.B., García, R.M., Báez, A.P., 2002. Rainwater chemical composition at the end of the mid-summer drought in the Caribbean shore of the Yucatan Peninsula. *Atmos. Environ.* 36, 2367–2374.
- Cleveland, C., Townsend, A., 2006. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to atmosphere. *Proc. Natl. Acad. Sci. USA* 103, 10316–10321.
- Corre, M.D., Veldkamp, E., Arnold, J., Wright, S.J., 2010. Impact of elevated N cycling and losses in old-growth lowland and montane forests in Panama. *Ecology* 91, 1715–1729.
- Cuevas, R.M., Etchevers, J.D., Payán, F., Campo, J., 2013. Precipitation influences on active fractions of soil organic matter in karstic soils of Yucatan: regional and seasonal patterns. *Eur. J. For. Res.*, <http://dx.doi.org/10.1007/s10342-013-0703-4>.
- Cusack, D.F., Silver, W.L., McDowell, W.H., 2009. Biological nitrogen fixation in two tropical forests: ecosystem-level patterns and effects of nitrogen fertilization. *Ecosystems* 12, 1299–1315.
- Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92, 621–632.
- Davidson, E.A., 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geosci.* 2, 659–662.
- Davidson, E.A., Keller, M., Erickson, H.E., Verchot, L.V., Veldkamp, E., 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience* 50, 667–680.
- Dentener, F., Drevet, J., Lamarque, J.F., Bey, I., 2006. Nitrogen and sulfur deposition on regional and global scales: a multi-model evaluation. *Global Biogeochem. Cycle* 20, GB4003.
- Ellingson, L.J., Kauffman, J.B., Cummings, D.L., Sanford, R.L., Jaramillo, V.J., 2000. Soil N dynamics associated with deforestation, biomass burning, and pasture conversion in a Mexican tropical dry forest. *For. Ecol. Manage.* 137, 41–51.
- Fox, J., 2005. *The R commander: a basic statistics graphical user interface to R*. *J. Stat. Softw.* 14, 1–42.
- Frey, S.D., Knorr, M., Parrent, J.L., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *For. Ecol. Manage.* 196, 159–171.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892.
- Gamboa, A.M., Hidalgo, C., De León, F., Etchevers, J.D., Gallardo, J.F., Campo, J., 2010. Nutrient addition differentially affects soil carbon sequestration in secondary tropical dry forests: early-versus late succession stages. *Restoration Ecol.* 18, 252–260.

- García-Oliva, F., Sanford, R.L., Kelly, E.a., 1999a. Effect of burning of tropical deciduous forest soil in Mexico on the microbial degradation on organic matter. *Plant Soil* 206, 29–36.
- García-Oliva, F., Sanford, R.L., Kelly, E.b., 1999b. Effect of slash-and-burn management on soil aggregate organic C and N in a tropical deciduous forest. *Geoderma* 88, 1–12.
- García-Oliva, F., Oliva, M., Sveshtarova, B., 2004. Effect of soil macroaggregates crushing on C mineralization in a tropical deciduous forest ecosystem. *Plant Soil* 259, 297–305.
- García, E., 2004. *Modificaciones al Sistema de Clasificación Climática de Köppen*. Universidad Nacional Autónoma de México, México.
- Grandy, A., Sinsabaugh, R.L., Neff, J.C., Stursova, M., Zak, D.R., 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. *Biogeochemistry* 91, 37–49.
- Gruber, N., Galloway, J.N., 2008. An earth-system perspective of the global nitrogen cycle. *Nature* 451, 293–296.
- Häfele, S., 2007. *Land Use Effects on Decomposition of Soil Organic Matter in a Tropical Dry Landscape in Mexico* [Dissertation]. University of Bayreuth, Bayreuth.
- Hall, S.J., Matson, P.A., 1999. Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature* 400, 152–155.
- Hall, S.J., Matson, P.A., 2003. Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecol. Monogr.* 73, 107–129.
- Jara, P., Martínez, E., Campo, J., 2009. N and P dynamics in the litter layer and soil of Mexican semi-arid forests, state of Morelos. *Agricult. Ecosys. Environ.* 130, 164–170.
- Hietz, P., Turner, B.L., Wanek, W., Richter, A., Nock, C.A., Wright, S.J., 2011. Long-term change in the nitrogen cycle of tropical forests. *Science* 334, 664–666.
- Hoeting, J.A., Davis, R.A., Merton, A.A., Thompson, S.E., 2006. Model selection for geostatistical models. *Ecol. Appl.* 16, 87–98.
- Houlthorn, B.Z., Sigma, D.M., Hedin, L.O., 2006. Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proc. Natl. Acad. Sci. USA* 106, 8745–8750.
- Ives, R.A., Zhu, J., 2006. Statistics for correlated data: phylogenies, space and time. *Ecol. Appl.* 16, 20–32.
- Jenkinson, D.S., 1988. The determination of microbial biomass carbon and nitrogen in soil. In: Wilson, J.R. (Ed.), *Advances in Nitrogen Cycling in Agricultural Ecosystems*. CAB International, Wallingford, pp. 368–386.
- Johnson, N.C., Wedin, D.A., 1997. Soil carbon, nutrients, and mycorrhizae during conversion of dry tropical forest to grassland. *Ecol. Appl.* 7, 171–182.
- Koehler, B., Corre, M.D., Veldkamp, E., Sueta, J.P.a., 2009a. Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest on a decadal time scale. *Biogeosciences* 6, 2973–2983.
- Koehler, B., Corre, M.D., Veldkamp, E., Wullaert, H., Wright, S.J.b., 2009b. Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biol.* 15, 2049–2066.
- Kuhn, M., Weston, S., Wing, J., James, F., 2011. Contrast: A Collection of Contrast Methods. R Package Version 0.14. Available from: <http://CRAN.R-project.org/package=contrast>
- Lamarque, J., Kiehl, J., Brasseur, P., Buttler, T., Cameron-Smith, P., Collins, W.D., Granier, C., Hauglustaine, D., Hess, P.G., Holland, E.A., Horowitz, L., Lawrence, M.G., McKenna, D., Merlles, P., Prather, M.J., Rasch, P.J., Rotman, D., Shindell, D., Thornton, P., 2005. Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: analysis of nitrogen deposition. *J. Geophys. Res.* 110, D19303.
- Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., Yang, X., Li, B., 2011a. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol.* 189, 1040–1050.
- Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J., Li, B., 2011b. Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. *Agricult. Ecosys. Environ.* 140, 234–244.
- Malhi, Y., Phillips, O.L., 2004. Tropical forests and global atmospheric change: a synthesis. *Philos. Trans. R. Soc. London Biol.* 359, 549–555.
- Montaño, N.M., García-Oliva, F., Jaramillo, V.J., 2007. Dissolved organic carbon affects soil microbial activity and nitrogen dynamics in a Mexican tropical deciduous forest. *Plant Soil* 295, 265–277.
- Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the World's forests. *Science* 333, 988–993.
- Pinheiro, J., Bates, D., Debroy, S., Sarkar, D., the R Development Core Team, 2011. *Linear and Nonlinear Mixed Effects Models*. R Package Version 3.1–103. Available from: <http://cran.r-project.org/web/packages/nlme/index.html>
- R Development Core Team, 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Read, L., Lawrence, D., 2003. Litter nutrient dynamics during succession in dry tropical forests of the Yucatan: regional and seasonal effects. *Ecosystems* 6, 747–761.
- Rees, R.M., Parker, J.P., 2005. Filtration increases the correlation between water extractable organic carbon and soil microbial activity. *Soil Biol. Biochem.* 37, 2240–2248.
- Roa-Fuentes, L.L., Campo, J., Parra, V., 2012. Plant biomass allocation across a precipitation gradient: an approach to seasonally dry tropical forest at Yucatan Mexico. *Ecosystems* 15, 1234–1244.
- Roa-Fuentes, L.L., 2013. *Variación regional del capital de carbono y características funcionales del bosque tropical estacionalmente seco de Yucatán*. PhD dissertation. Universidad Nacional Autónoma de México, México. 67 pp.
- Robertson, G.P., Wedin, D., Groffman, P.M., Blair, J.M., Holland, E.A., Nadelhoffer, K.J., Harris, D., 1999. Soil carbon and nitrogen availability. Nitrogen mineralization, nitrification and soil respiration potential. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), *Standard Soil Methods for Long-Term Ecological Research*. University Press, Oxford, pp. 258–271.
- Roy, S., Singh, J.S., 1994. Consequences of habitat heterogeneity for availability of nutrients in a dry tropical forest. *J. Ecol.* 82, 503–509.
- Saynes, V., Hidalgo, C., Etchevers, J.D., Campo, J.E., 2005. Soil C dynamics in primary and secondary seasonally dry tropical forest in Mexico. *Appl. Soil Ecol.* 29, 282–289.
- Schuur, E.A.G., Matson, P.A., 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. *Oecologia* 128, 431–442.
- Shang, C., Tiessen, H., 2003. Soil organic C sequestration and stabilization in karstic soils of Yucatan. *Biogeochemistry* 62, 177–196.
- Sluiter, S., Vandenbruwane, J., De Schrijver, A., Wuitz, K., Moeskops, B., Verheyen, K., De Neve, S., 2009. Patterns of dissolved organic carbon and nitrogen fluxes in deciduous and coniferous forests under historic high nitrogen deposition. *Biogeosciences* 6, 2743–2758.
- Solís, E., Campo, J., 2004. Soil N and P dynamics in two secondary tropical dry forests after fertilization. *For. Ecol. Manage.* 195, 409–418.
- Srivastava, S.C., 1992. Microbial C and P in dry tropical soils: seasonal changes and influence of soil moisture. *Soil Biol. Biochem.* 24, 711–714.
- Templer, P.H., Mack, M.C., Chapin III, F.S., Christenson, L.M., Compton, J.E., Crook, H.D., Currie, W.S., Curtis, C.J., Dail, D.B., D'Antonio, C.M., Emmett, B.A., Epstein, H.E., Goodale, C.L., Gundersen, P., Hobbie, S.E., Holland, K., Hooper, D.U., Hungate, B.A., Lamontagne, S., Nadelhoffer, K.J., Osenberg, C.W., Perakis, S.S., Schleppli, P., Schimel, J., Schmidt, I.K., Sommerkorn, M., Spelstra, J., Tietema, A., Wessel, W.W., Zak, D.R., 2012. Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of ¹⁵N tracer field studies. *Ecology* 93, 1816–1829.
- Thornton, P.E., Doney, S.C., Lindsay, K., Moore, J.K., Mahowald, N., Randerson, J.T., Fung, I., Lamarque, J.-F., Feddes, J.J., Lee, Y.-H., 2009. Carbon-nitrogen interactions regulate climate-carbon cycle feedbacks: results from an atmosphere-ocean general circulation model. *Biogeosciences* 6, 2099–2120.
- Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.* 11, 1111–1120.
- Van Reeuwijk, L.P., 2002. *Procedures for Soil Analysis*. International Soil Reference and Information Centre, Wageningen.
- Vance, E.E., Brooker, P.C., Jenkinson, D.S., 1987. An extraction method of measuring soil microbial biomass. *Soil Biol. Biochem.* 19, 703–707.
- Vitousek, P.M., Sanford, R.L., 1986. Nutrient cycling in moist tropical forests. *Annu. Rev. Ecol. Syst.* 17, 137–167.
- Vitousek, P.M., Turner, D.R., Parton, W.J., Sanford, R.L., 1994. Litter decomposition on the Mauna Loa environmental matrix, Hawaii: patterns, mechanisms, and models. *Ecology* 75, 418–429.
- Waldrop, M.P., Zak, D.R., 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. *Ecosystems* 9, 921–933.
- Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol. Biochem.* 36, 1443–1451.
- West, B.T., Welch, K.B., Galecki, A.T., 2007. *Linear mixed models: a practical guide using statistical software*. In: West, B.T., Welch, K.B., Galecki, A.T. (Eds.), *Linear Mixed Models: A Practical Guide Using Statistical Software*. Chapman & Hall/CRC, Boca Raton, pp. 124–167.
- Yang, X., Richardson, T.K., Jain, A.K., 2010. Contributions of secondary forest and nitrogen dynamics to terrestrial carbon uptake. *Biogeosciences* 7, 3041–3050.
- Yang, X.D., Warren, M., Zous, X.M., 2007. Fertilization response of soil litter fauna and litter quantity, and turnover in low and high elevation forest of Puerto Rico. *Appl. Soil Ecol.* 37, 63–71.
- Zaehle, S., Dalmonech, D., 2011. Carbon-nitrogen interactions on land at global scales: current understanding in modeling climate biosphere feedbacks. *Curr. Opin. Environ. Sustainability* 3, 1–10.
- Zhuang, Q., Lu, Y., Chen, M., 2011. An inventory of global N₂O emissions from the soils of natural terrestrial ecosystems. *Atmos. Environ.* 47, 66–75.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. *Dealing with heterogeneity*. In: Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M. (Eds.), *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, pp. 71–100.

Supplementary material

Table S1. Lineal regression between total carbon and inorganic carbon concentrations in soil for each study site.

Study site	Intercept	Slope	R^2	P
Chixchulub	10.2	0.01	0.73	0.02
X'matkuil	14.3	0.02	0.70	0.03
Hobonil	80.7	0.59	0.77	0.01

Table S2. Lineal regression between total carbon and inorganic carbon concentrations in microbial biomass for each study site.

Study site	Intercept	Slope	R^2	P
Chixchulub	10.3	0.01	0.78	<0.001
X'matkuil	0.48	0.02	0.82	<0.001
Hobonil	10.6	0.10	0.86	<0.001

Table S3. Significance test of covariance and variance structures used by fitting models. Models were build to variables of soil carbon and nitrogen dynamic in in soil cores with nitrogen addition.

Variable	Covariance structure		Variance structure		Estimation Method	Final predictor factors
	LR	Φ	Form	LR/R-adj		
Organic C	0.34	-	SxT	26.9 ***	GLS/REML	S + N + SxN
Inorganic C	0.97	-	S	156 ***	GLS/REML	S + N + SxN
Total N	0.01	-	-	0.66 ***	LS/ML	S.
C:N ratio	2.53	-	S	21.0 ***	GLS/REML	S + N
Microbial biomass C	8.58 **	-0.38	SxT	11.3 **	GLS/REML	S + N + T + SxN + SxT + NxT + SxNxT
Microbial biomass N	14.0 **	-0.18	SxT	126 ***	GLS/REML	S + N + T + SxN + SxT + NxT + SxNxT
Microbial C:N ratio	11.4 ***	-0.15	SxT	15.9 **	GLS/REML	S + N + T + SxN + SxT + NxT + SxNxT
Water-extractable Co	1.53	-	-	0.26 ***	LS/ML	S + T
Water-extractable Ci	0.49	-	T	9.46 ***	GLS/REML	S + N + T + SxT + NxT
Water-extractable Ntotal	3.80	-	SxT	33.0 ***	GLS/REML	S + N + T + SxN
Water-extractable No	4.47 *	-0.03	SxT	32.0 ***	GLS/REML	S + N + T + SxN
Water-extractable Ni	0.03	-	SxT	48.7 ***	GLS/REML	S + T + SxT
Water-extractable C:N ratio	1.05	-	SxT	35.7 ***	GLS/REML	S + N + T + SxT
NH ₄ ⁺	1.98	-	S x T	20.5 ***	GLS/REML	S + N + SxN
NO ₃ ⁻	1.92	-	S x T	78.8 ***	GLS/REML	S + N + SxN
Net N mineralization	0.05	-	S x T	27.0 ***	GLS/REML	S + T
Net nitrification	0.06	-	S x T	26.6 ***	GLS/REML	S + T
CO ₂ flux	2.20	-	S x T	52.2 ***	GLS/REML	S + N + T+ SxN

N ₂ O flux	0.59	-	S x T	225	***	GLS/REML	S + N + T + SxN
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Base model was a saturated model (SM). SM: $Y \sim S + N + T + S \times N + S \times T + N \times T + S \times N \times T$

Y, response variable; S, study site; N, N-addition treatment; T, time of incubation; x, interaction among predictor variables

LR, likelihood ratio; Φ , Phi value of covariance structure; GLS, generalized least square; REML, restricted maximum likelihood;

LS, least square; ML, maximum likelihood.

Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

CAPÍTULO 3

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Precipitation Regime and N Addition Effects on Decomposition in Tropical Dry Forests

Marylin Bejarano-Castillo^a, María M. Crosby-Galván^b, Víctor Parra-Tabla^c,

Jorge D. Etchevers^b and Julio Campo^a

^a Instituto de Ecología, Universidad Nacional Autónoma de México, AP 70-275, Mexico D.F. 04510, Mexico, ^b Colegio de Postgraduados, Texcoco 56230, Mexico, ^c Universidad Autónoma de Yucatán, Merida 97000, Mexico

Resumen

Realizamos un experimento en campo usando las hojas senescentes de dos especies dominantes que co-ocurren en un gradiente de precipitación (de 531 hasta 1036 mm de precipitación media anual, durante un año en la Península de Yucatán. El experimento, que combinó la adición de nitrógeno (N), la variación en la precipitación media anual y las medidas de la química inicial del material en descomposición de tres bosques, nos permitió explorar la influencia del agua y de la adición de N en la tasa de descomposición en bosques tropicales estacionalmente secos. Las especies estudiadas fueron *Piscidia piscipula* y *Gymnopodium floribundum*. Cuatro parcelas independientes fueron dejadas intactas (testigos) o fertilizadas simulando dos escenarios de incremento en la deposición de N para el 2030 ((+N1: 2.4 kg ha⁻¹ año⁻¹ o +N2: 24 kg ha⁻¹ año⁻¹) en cada uno de los bosques. La adición de N no tuvo un efecto significativo en la tasa de descomposición de las hojas senescentes de los sitios secos (531 y 993 mm de precipitación media anual). Por el contrario, la adición de N en el sitio más húmedo afectó la descomposición del material. En el sitio húmedo la respuesta general al incremento de N fue un incremento y un decrecimiento en la descomposición de las hojas de *Gymnopodium* y *Piscidia*, respectivamente. En general, encontramos que la tasa de descomposición fue afectada por la adición de N solo cuando el material originado en el bosque más húmedo y con menor contenido de N fue descompuesto en todos los sitios, o cuando el material proveniente de todos los sitios del gradiente fue descompuesto en el bosque más húmedo. Las concentraciones iniciales de los compuestos solubles de carbono ($r^2 = -0.53$, $P < 0.01$) y de lignina ($r^2 = 0.73$, $P < 0.001$) fueron los mejores predictores de la masa final remanente para el material de *Gymnopodium*. En un análisis similar, el porcentaje inicial de compuestos de carbono solubles en agua ($r^2 = -0.49$, $P < 0.01$) y la concentración de N ($r^2 = -0.75$, $P < 0.001$) predijeron la masa final remanente de *Piscidia*. Concluimos que el efecto de la adición de N en el proceso de descomposición podrá variar dependiendo de la cantidad de N disponible en los sitios; este factor, a su vez, es controlado por la precipitación media anual y la identidad de las especies.

Abstract

We conducted a field N experiment over one year, using senescent leaves of two dominant tree species that co-occur along a precipitation gradient (from 531 mm to 1036 mm of mean annual precipitation) in Yucatan Peninsula. This experimental setup, combining N addition experiments, variation in mean annual precipitation, and measurements of the initial litter chemistry of species from three different forest sites, allowed us explore the influence of water and N enrichment inputs on decomposition rate in tropical dry forests. The leaf litter of representative species of each site was studied, including *Piscidia piscipula* and *Gymnopodium floribundum*. Four independent plots (12 × 12 m) at each forest were either left intact (controls) or fertilized with N at two scenarios of increase in N deposition for year 2030 (+N1: 2.4 kg ha⁻¹ yr⁻¹, or +N2: 24 kg ha⁻¹ yr⁻¹). Fertilization with N did not have a significant effect on decomposition rates at the drier sites (531 and 993 mm of MAP sites). In contrast, N addition at the wettest site affects litter decomposition. In this site the main response to N enrichment was an increase in decomposition of *Gymnopodium*'s litter and a decrease in *Piscidia*'s litter. Overall, we observed that, decomposition was affected by the N enrichment only when litter ecotypes from the N-poor wettest site were decomposed in all sites, or when litter from the all sites were decomposed in the wettest nutrient-poor site. Initial water soluble C ($r^2 = -0.53$, $P < 0.01$) and, initial lignin ($r^2 = 0.73$, $P < 0.001$) were the best predictors of observed final mass for litter of *Gymnopodium*. In similar analysis, initial percentage of water soluble C ($r^2 = -0.49$, $P < 0.01$) and N concentration ($r^2 = -0.75$, $P < 0.001$) predicted observed final mass for *Piscidia* litters. We conclude that N addition effects on decomposition processes will vary, depending on the details of the site's N status that results of mean annual precipitation, and the identity of species.

Keywords Carbon compounds, Climate change, Litter quality

Introduction

Tropical forests have a huge influence on the global carbon (C) cycle. These forests exchange more carbon dioxide with the atmosphere than any other ecosystem (Roy et al. 2001; Bond-Lamberty and Thomson 2010). In net ecosystem C balance, litter decomposition is a key process (McGuire and Treseder 2010). Climate, litter quality (particularly initial lignin and nitrogen content) and external nitrogen (N) availability interact to determine decay rate. On the one hand, mean annual precipitation (MAP) has been shown to exert a positive influence on decomposition rates in lowland tropical forests (Powers et al. 2009; Waring 2012). This positive relationship between MAP and decay rate reflects the influence of precipitation regimes on both the leaching of soluble compounds from the litter and the activity of decomposer microorganisms (Berg and McLaugherty 2008). On the other hand, MAP indirectly regulates decomposition processes by controlling carbon quality and the nutrient

content of leaves (both green and senescent). For example, Santiago and Mulkey (2005) showed that there is a negative relationship between MAP and N concentration in both green leaves and litter, while leaf lignin content increases with site precipitation (Austin and Vitousek 2000).

The importance of litter N concentration for decomposition and the effects of increased N input on decomposition have been documented for temperate ecosystems (see Hobbie et al. 2012; and references), but few comprehensive syntheses exist for tropical forests, and the studies that do exist have focused mainly on wet tropical forests (see below). Although the relationship between initial litter N concentration and decomposition rates has been observed in different studies of tropical forests (e.g., Kaspari et al. 2008; Bakker et al. 2011), the relationship between decomposition and external N availability is less clear. It is hypothesized that in terrestrial ecosystems, as N deposition increases, litter quality gains importance as a modulator of decomposition (Knorr et al. 2005). Currently, in tropical forests, N deposition is the second greatest external N input, following biological N fixation (Cleveland et al. 1999). However, biogeochemical global models predict that it could increase four-fold by 2030, reaching the level of current biological N fixation (Dentener et al. 2006). Although productivity in tropical forest is more frequently phosphorus- than N-limited, recent evidence demonstrates that N availability could limit the activity of soil microorganisms (Cusack et al. 2010). Although numerous studies of litter and soil organic matter have explored the effects of N enrichment on decomposition, uncertainties remain about how this process is influenced by N availability. Evidence indicates that applied N may have inconsistent effects on decomposition. Some studies have reported significantly faster decomposition rates in response to increased N availability (Kwabiah et al. 1999; Cusack et al. 2009), while many others have reported either a reduction in decay rates (Mo et al. 2006; Fang et al. 2007; Powers and Salute 2011) or no significant effects (Hobbie and Vitousek 2000). These inconsistencies suggest that the effect of increased N input on decomposition may be very specific to each forest site and experiment. On the one hand, variations in the response to N additions across studies can be explained by differences in litter chemistry—

high plant biodiversity in tropical forests means that local and regional biogeochemistry can be very heterogeneous (Townsend et al. 2008). On the other hand, another source of variation could be the wide range of N addition rates applied across decomposition studies (from 50 to 220 kg N ha⁻¹ yr⁻¹; M. Bejarano and J. Campo, unpublished data). Knorr et al. (2005), in a meta-analysis of the influence of N addition on litter decomposition, showed that the variation in responses can be explained in part by differences in fertilization rates, and in part by differences in litter chemistry (particularly, by lignin content in litters).

One striking aspect of the studies of decomposition and N deposition in tropical forests is the underrepresentation of tropical dry forests (TDF). TDF is the most threatened tropical biome, and current estimates predict that nearly 80% of the area it covers could suffer a decrease in precipitation of at least 50 mm per year by 2055 (Miles et al. 2006). Biogeochemical theory of tropical forest has drawn mainly on wet tropical forest approaches. However, from the perspective of increased N deposition, there are important biogeochemical differences between wet and dry forests, and these differences may mean that increased N deposition leads to greater changes in the latter. Due to the lower water supply, TDFs usually have a lower decomposition rate (Powers et al. 2009), higher soil C and N concentrations (Gamboa et al. 2010; Jiménez et al. 2011), and a more open N cycle than their wet counterparts (Austin and Vitousek 1998). These properties can explain the negative correlation between MAP and soil C:N ratios (Austin and Vitousek 1998) and suggest that microorganisms may be N-limited in TDFs.

Our study sought to elucidate the relationships among litter decomposition, precipitation regime and rate of N addition in TDFs. We conducted a year-long field N experiment using the senescent leaves of two dominant tree species that co-occur along a precipitation gradient (from 531 mm to 1036 mm of MAP) in Yucatan. This experimental setup, which combined N addition experiments, variation in MAP, and measurements of the initial litter chemistry of species from three different forest sites (ecotypes sensu; Ramírez-Valiente et al. 2010),

allowed us explore the influence of water and N enrichment inputs on decomposition rates in TDFs.

We used this natural precipitation gradient to test the following hypotheses: (1) at the wettest site, where the plant growth period is longer and soil mineral N concentrations are lower (Roa-Fuentes et al. 2012), leaf litter should exhibit higher recalcitrant C (lignin) content and lower N concentrations than litter from the driest site; (2) the driest site should exhibit lower decomposition rates than the wettest site when variables other than precipitation regime remain constant; and (3) the wettest site should exhibit higher decomposition rates than the driest and most N-rich site, exemplifying a system with low N availability.

Methods

Study sites

This study was carried out at three sites along a MAP gradient in the Yucatan Peninsula, Mexico. The gradient runs from north/driest (Chicxulub) to south/wettest (Hobonil) (Table 1). The three forest sites studied are similar with regard to temperature, relief, parent material, overstory composition and time. Long-term climate data from weather stations across the Yucatan Peninsula show that the entire region is characterized by a distinct period of low precipitation. The climate in the region is hot and subhumid, and supports either tropical dry or very dry forest in the Holdridge system (Holdridge et al. 1971). Mean temperature in the region is 26.2°C, with less than 1°C difference across sites. Approximately 75% of total precipitation falls between June and October (Fig. 1). The landscape consists of flat areas and the predominant lithology includes late Pliocene material, with numerous areas of exposed limestone. Soils are either shallow black lithosols surrounding rock outcrops or deeper red rendzinas at slightly lower relief (Shang and Tiessen 2003). Although soils at all study sites can be regarded as organic-rich, C concentrations in the forest floor and mineral soil at the driest forest site are 1.7 and 4 times higher than the mean values at the wettest forest. Also, N concentrations in both the forest floor and the mineral soil decrease as MAP increases.

The vegetation at the study sites is TDF, with mean canopy heights increasing with MAP (Table 1). The site with 993 mm MAP has more trees (diameter at breast height, DBH > 2.5 cm) per unit area than its drier and wetter counterparts; however, basal areas do not differ across sites. Despite the increase in tree size, both aboveground and belowground biomasses are very similar across sites (Roa-Fuentes et al. 2012, 2013). Floristically, Leguminosae are the most important family across sites.

Experimental design

At each location, four 12-by-12-m plots received ambient N deposition (control) and eight plots of the same dimensions received ambient N deposition plus low N addition (2.4 kg N ha⁻¹ yr⁻¹, +N1 treatment) or high N addition (24 kg N ha⁻¹ yr⁻¹, +N2 treatment). These N addition rates were selected to obtain the expected N deposition rates for the year 2030 in Yucatan Peninsula and the highest expected N deposition rates in tropical regions for the same year, respectively (Dentener et al. 2006). The N addition was performed at the end of the dry season (May) with a polymer-coated controlled-release urea (Multicote (8) 40-00-00, Haifa Chemical); Multicote fertilizers are expected to release their N contents over four to six months at temperatures greater than 20 °C.

Decomposition experiments

At each of the three study sites, senesced leaves were collected from the middle crown position of two tree species that co-occur and are dominant across sites (*Gymnopodium floribundum* Rolfe, Polygonaceae; and *Piscidia piscipula* (L.) Sarg., Fabaceae) (DBH approximately 10 cm). From this point forward we refer to genus names to avoid confusion. Leaves were collected from the plants before fall, as we were concerned that decomposition of litter and leaching of leaf nutrients would lead to an underestimate of nutrient concentrations in senescent leaves taken from the soil. Senescent leaves of each tree species were collected at each of the three sites at weekly intervals over a period of two weeks. These leaves are easy to identify as they are generally of different color (often yellow) from live leaves. Senesced leaf samples for each species were air-dried, composited and sorted to

remove insect-damaged leaves. Approximately five grams of this homogeneous mixture were sealed in 20 × 20 cm fiberglass mesh (1-mm mesh size) litterbags. Additional senesced leaf samples were dried at 60°C for two days (or to constant weight), weighed to determine dry mass conversions, and analyzed for initial chemistry.

During the first week of June, 2010 (at the start of the rainy season), a total of 1512 litterbags (2 species × 3 ecotypes × 3 decomposition sites × 3 N fertilizer treatments × 4 replicates × 7 periods of litterbag harvesting) were placed on the surface of forest floor layer. Litterbags received one of the following N treatments: control (no nutrient addition), low N addition (+N1, equivalent to 0.24 g N m⁻²), and high N addition (+N2, equivalent to 2.4 g m⁻²); this was done to simulate enrichment over bag area in accordance with plot N treatment. Litterbags were separated in stands by 50 cm. Leaf litter (from this point forward we refer to senescent leaves as leaf litter to avoid confusion) was decomposed at the site where it was collected, to estimate the in situ decomposition rate for each MAP regime, and was decomposed at all sites along the precipitation gradient, to explore the effects of changes in decomposition environment and tissue quality on decomposition rates (common litter experiments). Litterbags were harvested at 15, 30, 60, 90, 120, 180 and 365 days after having been placed in the field.

Analytical methods

Four replicates (approximately 10 g) of the initial oven-dried leaf litter samples were ground in a Wiley mill (40-mesh screen) and used to determine initial litter chemistry. Soil from the litterbags was brushed prior to drying and weighing. Total C concentration in leaf litter was determined by high-temperature combustion using a C analyzer (SHIMADZU SSM-5000A). Total N concentration was estimated using an N elemental analyzer (Technicon Autoanalyzer II) following acid digestion with concentrated H₂SO₄ (Anderson and Ingram 1993). Carbon fractions, including soluble cell components, hemicellulose and bound proteins, cellulose, and lignin and recalcitrant components, were determined using an ANKOM plant fiber analyzer (Ankom Technology, Macedon, New York, USA) and forage fiber methods (Van Soest 1963).

Following collection, decomposition litterbags were air-dried in the field and returned to the laboratory. In the laboratory, adhered debris was removed and litter was dried at 60°C for two days, then weighed to determine remaining mass at each time interval.

Data analysis

Species were not considered as a factor in experimental design, due to the difficulty of determining differences between phylogenetic and experimental effects. To address our first question regarding differences in initial litter quality across sites (i.e., ecotypes) we used one-way analysis of variance (ANOVA). The generalized least square method was used for model estimation with the `gls::nlme` function (Pinheiro et al. 2011) when model residuals did not meet the normality assumptions for ANOVA. No data transformations were needed.

Annual decomposition rates (k-values) were determined using two methods of statistical analysis. First, a linear regression procedure was fitted for each combination of N addition treatment, decomposition site and ecotype to estimate the k annual decay constant. The k-values were calculated as the slope of the log transformation of the exponential function of Olson (1963) and were compared among factor combinations using a Student's t-test. Second, a four-way ANOVA was performed with a linear regression model that included final mass as an explained variable and ecotype, decomposition site and N addition treatment as predictor variables with the `lm::stats` function (R Development Core Team 2011). Models were analyzed for residual normality and homoscedasticity, and no data transformations were needed. Correlations among decomposition rates and initial litter chemistry were performed. Seven factors of initial leaf litter chemistry were tested against decomposition rates: (1) litter N, (2) water-soluble C, (3) cellulose, (4) hemicellulose, (5) lignin concentrations, (6) C:N ratios and (7) lignin:N ratios.

Statistical analyses were performed using R software (R Development Core Team 2011). Statistical significance was set at 95% level for all tests. All ANOVA were fitted by testing

the significance of variables and interactions with likelihood ratio tests (Zuur et al. 2009). When the terms of models were significant, planned orthogonal comparisons were carried using the contrast: contrast package (Kuhn et al. 2011).

Results

Initial leaf litter chemistry

Carbon concentration in litter was very consistent across ecotypes within each species (Table 2). The range of variation across ecotypes was only 1% for *Gymnopodium* leaf litter and 4% for *Piscidia* leaf litter. Also, ecotype did not have a significant effect on leaf litter N concentration in *Piscidia*. In contrast, N concentration in *Gymnopodium* leaf litter was higher in the ecotype from 993 MAP than in other ecotypes. The contrasting concentrations of N among ecotypes resulted in opposing variations in C:N ratios, and thus in distinct stoichiometry among litter ecotypes.

We observed substantial cross-site variation in initial C fractions in *Gymnopodium* leaf litter (Table 2). For example, among ecotypes, initial water-soluble C was highest in the ecotype from the driest site, while initial hemicellulose, lignin and lignin:N ratios were highest in the ecotype from the wettest site. The highest cellulose concentrations were measured in the ecotype from 993 mm MAP, with concentrations decreasing in the following order: 993 mm MAP > 531 mm MAP > 1036 mm MAP.

As in the case of *Gymnopodium* litter, initial water-soluble C concentrations in *Piscidia* litter were highest in the ecotype from the driest site, and content decreased with increases in MAP. In contrast, hemicellulose concentration increased with increases in MAP. Cellulose and lignin concentrations and lignin:N ratios did not vary across sites.

Leaf litter decomposition

Mass loss of *Gymnopodium* litter in the in situ decomposition experiment did not vary across sites (ecotype effect) ($t = -0.6$ to 0.7 , $p = 0.4$ to 0.6) (Fig. 2). In contrast, mass loss of *Piscidia* litter was accelerated with increasing site MAP ($t = -3.1$ to -3.4 , $p < 0.001$) (Fig. 3).

When *Gymnopodium* and *Piscidia* litter ecotypes from 993 mm MAP were decomposed across the precipitation gradient, increased MAP had a positive impact on the k-value at the wettest site (Figs. 2 and 3) ($t = -3.7$ for *Gymnopodium*, and $t = -3.4$ for *Piscidia*, $p < 0.001$). Decomposition rates for the *Piscidia* litter ecotype from the wettest site decreased with decreases in MAP ($t = -5.1$, $p < 0.001$). However, MAP did not influence decomposition rates in the following cases: *Gymnopodium* litter ecotypes from the driest and the wettest sites ($t = -1.4$ – 0.5 , $p = 0.2$ - 0.5), and the *Piscidia* litter ecotype from the driest site ($t = 0.5$, $p = 0.6$).

Fertilization with N did not have a significant effect on decomposition rates for any of the *Gymnopodium* and *Piscidia* litter ecotypes at the drier sites (i.e., 531 and 993 mm of MAP sites) ($t = -0.74$ to 1.1 , $p = 0.2$ to 0.8 , for *Gymnopodium*; Fig. 2) ($t = -1.5$ to 1.1 , $p = 0.2$ to 0.9 , for *Piscidia*; Fig. 3). However, the application of N at the high rate (+N2) significantly increased the decomposition rate of *Gymnopodium* litter from 531 and 1036 mm MAP at the wettest site ($t = 7.1$, $p < 0.001$ and $t = -2.4$, $p = 0.02$, respectively). In contrast, the addition of N at elevated rates decreased the decomposition rate of *Piscidia* litter from 1036 mm of MAP at the wettest site ($t = 7.9$, $p < 0.001$).

The three-way variance analysis indicated that climate had a strong effect on the final mass of litter with the control treatment ($F = 246$ and $F = 266$, $p < 0.001$, for *Gymnopodium* and *Piscidia* litters, respectively), and test showed that decomposition was fastest at the wettest site for litter ecotypes from 993 and 1036 mm MAP (Figs. 2 and 3). The final mass of *Gymnopodium* and *Piscidia* litters varied as a function of both site of decomposition and N addition treatment (site \times N addition effect, $F = 28.5$ and $F = 32.8$, $p < 0.001$, respectively). For *Gymnopodium*, N addition impacted the decomposition of all litter ecotypes at the wettest site, and the decomposition of litter ecotypes at all sites. Although N addition did not

have consistent effects, generally enrichment with N at high rates (+N2) decreased the decomposition of leaf litters. In particular, the addition of N at high rates significantly decreased the decomposition of *Piscidia* litter collected at the 1036 mm MAP site when decomposing at both wetter sites (i.e., in sites with 993 and 1036 mm of MAP), as well the decomposition of litter from 993 mm MAP ecotype when decomposing at the wettest site. We found that control litters generally decomposed faster at their site of origin. Thus, the final mass of the litter ecotype from 531 mm MAP was lower at the driest site than at other sites, while final mass of litter ecotypes from both wetter sites was lower at both 993 and 1036 mm sites (Figs. 2 and 3). Initial water-soluble C ($r^2 = -0.53$, $p < 0.01$) and initial lignin ($r^2 = 0.73$, $p < 0.001$) were the best predictors of observed final mass for *Gymnopodium* litter. In a similar analysis, the initial percentage of water-soluble C ($r^2 = -0.49$, $p < 0.01$) and N concentration ($r^2 = -0.75$, $p < 0.001$) predicted the final mass of *Piscidia* litters.

Discussion

Regional patterns in litter chemistry

Carbon compound concentrations in leaf litter of both dominant species in Yucatan TDFs showed much variation across sites. At the driest site, plants accumulate water-soluble C, while leaf litter from sites with greater precipitation is richer in recalcitrant compounds (e.g., lignin). At our study sites, the duration of the rainy season varies: it lasts four months at Chicxulub, the driest site, five months at X'matkuil and six months at Hobonil, the wettest site. Both results suggest that increases in lifespan and in the period for photosynthetic activity (as might be expected if a longer growing season were involved) favor a greater synthesis of expensive energetic C-chain compounds in the wettest forest.

When the results of *Gymnopodium* and *Piscidia* along this natural rainfall gradient were considered, we observed that leaf litter N concentration did not show consistent responses to precipitation regime. Thus, our results differed from the findings of studies across species in precipitation gradients in tropical regions (Santiago and Mulkey 2005) and on a global scale

(Craine et al. 2009). Those studies show that N concentration in leaf litter decreases with increased MAP. Similar findings were reported from Hawaiian studies with *Metrosideros polymorpha* along a similar precipitation gradient range (i.e., 500 to 1000 mm of MAP; Austin and Vitousek 1998). Thus, our study also indicates a lack of response of leaf litter N concentrations to precipitation regime, and suggests a close N proficiency (sensu Killingbeck 1996) across tropical dry to very dry forests.

Regional variation in litter decomposition

The overall decomposition rates we observed in our study (k-values from 0.4 to 0.8) are in the lower half of the global range for TDFs (0.3-1.7; Martínez-Yrizar 1995). Nevertheless, the low levels of decomposition at our study sites are close to decomposition averages reported for TDFs in the Chicxulub and X'matkuil regions, determined using the mass balance approach (0.7 -0.8 yr⁻¹ in two consecutive years; Campo et al. 2007). Again, decomposition rates appear as similar for the litter of both species. When annual rainfall in the study year was considered, we observed that its value across study sites (698 mm for Chicxulub, 992 mm for X'matkuil, and 1058 mm for Hobonil) was similar to the average reported for longer periods (see Fig. 1). However, no data were available to assess whether our low rates of decomposition were related to other aspects of litter carbon quality (e.g., litter composition poor in accessible C compounds and/or rich in recalcitrant C such as condensed tannins), or to the effect of decomposer fauna. In any case, our results show that, in addition to inter-site variations in resource allocation to recalcitrant C, leading to predictable patterns of decomposition in tropical forest litters, an environmentally induced precipitation variation may also be determinant of the high levels of C accumulation in Yucatan's soil.

Although our hypothesis that accessible C compounds would decrease with MAP and recalcitrant C would increase with precipitation was clearly supported at the regional level, the expected greater decomposition of litter richest in accessible C and relatively poor in recalcitrant C received little support. For example, although the litter of both species (i.e.,

Gymnopodium and Piscidia) from the driest site is relatively rich in water-soluble C and relatively poor in lignin, its decomposition at the wettest site was lower than that of other ecotypes. The opposite result was obtained by Hättenschwiler and Jorgensen (2010) and by Cornwell et al. (2008) regarding the strong control of C quality on decomposition in tropical forests; this suggests that the environment of origin of litter exerts a great influence on its decomposition rate (as suggested by the home-field advantage hypothesis; Gholz et al. 2000). This effect may be caused by a close co-specialization involving the decomposer community and the litter produced at a given site, and is usually reported for litter with a high resistance to decay (Gholz et al. 2000). Although litter ecotypes from 993 and 1036 mm MAP decomposed faster in their respective environments, the final mass for these litter ecotypes decomposing at sites that receive 993 and 1036 mm of MAP constituted a statistically homogeneous group ($p > 0.05$). It is possible but less likely that litter layers at both sites are similar in quality to Gymnopodium and Piscidia litters (under the assumption that decomposer community composition should reflect the average quality-of-litter matrix, likely driven by the most dominant species; Freschet et al. 2012). More detailed analysis of the potential effects of litter matrices (for example, with associated forest floor variation in C quality, stoichiometry, decomposer communities, microclimate and so on) on decomposition is needed to test possible substrate quality-matrix interactions involving specific litters and the ecosystem litter layers at both forest sites.

Nitrogen addition effects on decomposition

The results of this study show a striking contrast between the consequences of adding N to an ecosystem with a relatively low supply of N (where moisture is relatively less limiting), such as the wettest forest, and the consequences of adding it to an ecosystem with a higher N supply (and with a lower water supply), such as the driest forest. Fertilization of low-supply systems with N leads to responses (an increase in the decomposition rates of leaf litter of greater quality, and a decrease in the decomposition of relatively low-quality litter) that are much stronger than those at the site where N is less limiting (as might be expected, considering the greater N concentration in the forest floor and mineral soil), despite similar

N concentrations in the substrate. Our findings suggest that changes in precipitation amount and soil N cycling as well as C quality in the litter, rather than litter N, govern how decomposition processes will react to future increases in N deposition. We cannot determine whether such changes in decomposition resulted from the increased N availability itself (thus relaxing a possible direct nutrient limitation to decomposers at the wettest site), or whether it reflects an increased sensibility in microorganisms (an indirect consequence of an increase in water supply), or both. The first hypothesis (i.e., the existence of N-limitation at the wettest site but not at other sites) could be verified by studying decomposition rates at the ecosystem level and analyzing the way in which each forest along the precipitation gradient responds to an increase in N deposition.

Variation in decomposition rates among litter ecotypes can be explained by two non-exclusive mechanisms associated with enzymatic activity. On the one hand, numerous studies indicate that N addition can stimulate the decomposer guild targeting labile compounds to produce hydrolytic enzymes and/or inhibit the generation of oxidative enzymes by the guild targeting recalcitrant compounds (Carreiro et al. 2000; Sinsabaugh et al. 2002; Cusack et al. 2010). Our results may support this first mechanism since initial soluble C and lignin concentrations are the most important variables for explaining final litter mass. On the other hand, N addition can affect the relationship between oxidative enzymatic responses and the lignocellulose index (i.e., the lignin / lignin plus cellulose ratio) of decomposing material; in this way, a lignocellulose index value lower than 0.4 separates litter types that show net positive or neutral oxidative responses to N enrichment from those that show net negative responses (Sinsabaugh 2010). Our results indicate that lignocellulose values vary from 0.44 to 0.63 for *Gymnopodium* litters, and only from 0.38 to 0.47 for *Piscidia* litters, for the driest and the wettest ecotypes, respectively. Thus, our study also suggests that the responses of decomposition to N enrichment may also depend on the initial lignocellulose proportions in the litter.

From an ecosystem perspective, taking into account that N deposition in the tropics is projected to increase rapidly over the next decades (Dentener et al. 2006; Galloway et al. 2008), our results show that site rainfall and soil N availability may be important indicators of how decomposition processes in TDFs respond to increased N deposition. Our results in the wettest forest support the N addition rate hypothesis that predicts greater changes in decomposition rates under elevated N inputs than under lower ones. However, this study is still ongoing, and it remains to be seen whether under long-term N addition, differences among N enrichment rates are sustained; it is also important to note that previous studies in Yucatan have shown that increased N in these TDFs may favor N uptake by plants (Campo and Dirzo 2003) and litter N fluxes to the soil (Campo et al. 2007). The mechanisms underlying this plant effect could be more complex than a simple increase in N return to soil because it has been observed that N fertilization at low rates enhances N release from the litter, though it also results in negative feedback by increasing losses of N from the ecosystem due to increased N₂O fluxes (Bejarano et al. 2014).

Aside from these short- and long-term scenarios, our study on the consequences of the spatio-rainfall and soil N availability variations for responses to N enrichment in TDFs of the Yucatan Peninsula allows us to conclude that the regulatory mechanisms of litter decomposition in these ecosystems will vary; enhanced N stimulates decomposition rates when litter is rich in soluble C compounds, and it inhibits decomposition when lignin concentrations are higher. Our study also contributes to an understanding of how decomposition in Yucatan forests may respond to the expected reduction in precipitation in tropical dry regions (Dai 2011) under two scenarios of increased N deposition from the atmosphere. Given the potential impact of the massive changes in N deposition and recurring extreme climate events, such as a drought, on ecosystem function, the topic of the present study warrants subsequent investigation.

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References

- Anderson JM, Ingram JSI (1993) Tropical soil biology and fertility. A handbook of methods. CAB International, Wallingford
- Austin AT, Vitousek PM (1998) Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* 113:519–529
- Austin AT, Vitousek PM (2000) Precipitation, decomposition and litter decomposability of *Metrosideros polymorpha* in native forests on Hawai'i. *J Ecol* 88:129–138
- Bakker MA, Carreño-Rocabado G, Poorter L (2011) Leaf economics traits predict litter decomposition of tropical plants and differ among land use types. *Funct Ecol* 25:473–483
- Berg B, McClaugherty C (2008) Plant litter. Decomposition, humus formation, carbon sequestration. 2nd ed. Springer, Berlin
- Bejarano M, Etchevers JD, Ruíz-Suárez G, Campo J (2014) Effects of increased N input on soil C and N dynamic in seasonally dry tropical forests: an experimental approach. *Appl Soil Ecol* 73:105–115
- Bond-Lamberty B, Thomson A (2010) A global database of soil respiration data. *Biogeosciences* 7:1915–1926
- Campo J, Dirzo R (2003) Leaf quality and herbivory responses to soil nutrient addition in secondary tropical dry forests of Yucatán, Mexico. *J Trop Ecol* 19:525–530
- Campo J, Solís E, Valencia MG (2007) Litter N and P dynamics in two secondary tropical dry forests after relaxation of nutrient availability constraint. *For Ecol Manage* 252:33–40
- Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81:2359–2365
- Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems. *Global Biogeochem Cycles* 13:623–645
- Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez - Harguindeguy N, Queded HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, Van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Diaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol Lett* 11:1065–1071
- Craine JM, Elmore AJ, Aida MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLauchlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD,

- Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol* 183:980–992
- Cusack DF, Chou WW, Yang WH, Harmon ME, Silver WL (2009) Controls on long-term root and leaf litter decomposition in neotropical forests. *Global Change Biol* 15:1339–1355
- Cusack DF, Torn MS, McDowell WH, Silver WL (2010) The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Global Change Biol* 16:2555–2572
- Dai A (2011) Drought under global warming: a review. *WIREs Climate Change* 2:45–65
- Dentener F, Drevet J, Lamarque JF, Bey I, Eickhout B, Fiore AM, Hauglustaine D, Horowitz LW, Krol M, Kulshrestha UC, Lawrence M, Galy-Lacaux C, Rast S, Shindell D, Stevenson D, Van Noije T, Atherton C., Bell N, Bergman D, Butler T, Cofala J, Collins B, Doherty R, Ellingsen K, Galloway J, Gauss M, Montanaro V, Müller JF, Pitari G, Rodriguez J, Sanderson M, Solomon F, Strahan S, Schultz M, Sudo K, Szopa S, Wild O (2006) Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Global Biogeochem Cycles* 20:GB4003
- Fang H, Mo J, Peng S, Li Z, Wang H (2007) Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China. *Plant Soil* 297:233–242
- Freschet GT, Aerts R, Cornelissen JHC (2012) Multiple mechanisms for trait effects on litter decomposition: moving beyond home-field advantage with a new hypothesis. *J Ecol* 100:619–630
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892
- Gamboa AM, Hidalgo C, de Leon F, Etchevers JD, Gallardo JF, Campo J (2010) Nutrient addition differentially affects soil carbon sequestration in secondary tropical dry forests: Early- vs. late-succession stages. *Restoration Ecol* 18:252–260
- García E (2004) Modificaciones al sistema de clasificación climática de Köppen. Universidad Nacional Autónoma de México, Mexico
- Gholz HL, Wedin DA, Smitherman SM, Harmon ME, Parton WJ (2000) Long - term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biol* 6:751–765
- Hättenschwiler S, Jorgensen HB (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *J Ecol* 98:754–763
- Hobbie SE, Eddy WC, Buyarski CR, Adair EC, Ogdhal ML, Weisenhorn P (2012) Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. *Ecol Monogr* 82:389–405
- Hobbie SE, Vitousek PM (2000) Nutrient limitation of decomposition in Hawaiian forests. *Ecology* 81:1867–1877
- Holdridge LR, Grenke WC, Hatheway WH, Liang T, Tosi JA (1971) Forest environments in tropical life zones: A pilot study. Pergamon Press, New York
- Jiménez JJ, Lorenz K, Lal R (2011) Organic carbon and nitrogen in soil particle-size aggregates under dry tropical forests from Guanacaste, Costa Rica - Implications for within-site soil organic carbon stabilization. *Catena* 86:178–191
- Kaspari M, García MN, Harms KE, Santana M, Wright SJ, Yavitt JB (2008) Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecol Lett* 11:35–43
- Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology* 77:1716–1727

- Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology* 86:3252–3257
- Kuhn M, Weston S, Wing J, Forester J (2011) Contrast: A collection of contrast methods. R package version 0.14. <http://www.cran.r-project.org/package=contrast/>
- Kwabiah AB, Voroney RP, Palm CA, Stoskopf NC (1999) Inorganic fertilizer enrichment of soil: effect on decomposition of plant litter under subhumid tropical conditions. *Biol Fertil Soils* 30:224–231
- Martínez-Yrizar A (1995) Biomass distribution and primary productivity of tropical dry forests. In: Bullock S, Mooney HA, Medina E (eds) *Seasonally dry tropical forests*. Cambridge University Press, Cambridge, pp 326–345
- McGuire KL, Treseder KK (2010) Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biol Biochem* 42:529–535
- Miles L, Newton AC, DeFries RS, Ravilious C, May I, Blyth S, Kapos V, Gordon JE (2006) A global overview of the conservation status of tropical dry forests. *J Biogeogr* 33:491–505
- Mo J, Brown S, Xue J, Fang Y, Li Z (2006) Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant Soil* 282:135–151
- Olson JS (1963) Energy storage and the balance of producers and decomposers in ecology systems. *Ecology* 44:331–332
- Pinheiro J, Bates D, DebRoy S, Sarkar D (2011) nlme: Linear and nonlinear mixed effects models. R package version 3.1-102
- Powers JS, Montgomery RA, Adair EC, Brearley FQ, DeWalt SJ, Castanho CT, Chave J, Deinert E, Ganzhorn JU, Gilbert ME, González-Iturbe JA, Bunyavejchewin S, Grau HR, Harms KE, Hiremath A, Iriarte-Vivar S, Manzane E, De Oliveira AA, Poorter L, Ramanamanjato JB, Salk C, Varela A, Weiblen GD, Lerdau MT (2009) Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient. *J Ecol* 97:801–811
- Powers JS, Salute S (2011) Macro- and micronutrient effects on decomposition of leaf litter from two tropical tree species: inferences from a short-term laboratory incubation. *Plant Soil* 346:245–257
- Ramírez-Valiente JA, Sánchez-Gómez D, Aranda I, Valladares F (2010) Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiol* 30:618–627
- R Development Core Team (2011) R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, <http://www.R-project.org/>
- Roa-Fuentes LL, Campo J, Parra V (2012) Plant biomass allocation across a precipitation gradient: an approach to seasonally dry tropical forest at Yucatán, Mexico. *Ecosystems* 15:1234–1244.
- Roa-Fuentes LL, Hidalgo C, Etchevers JD, Campo J (2013) Effects of precipitation regime on soil carbon pools on the Yucatan Peninsula. *J Trop Ecol* 29:463–466
- Roy J, Saugier B, Mooney HA (2001) *Terrestrial global productivity*. Academic Press, San Diego
- Santiago LS, Mulkey SS (2005) Leaf productivity along a precipitation gradient in lowland Panama: patterns from leaf to ecosystem. *Trees* 19:349–356
- Shang C, Tiessen H (2003) Soil organic C sequestration and stabilization in karstic soils of Yucatan. *Biogeochemistry* 62:177–196
- Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol Biochem* 42:391–404

Sinsabaugh RL, Carreiro MM, Alvarez S (2002) Enzyme and microbial dynamics of litter decomposition. In: Burns RG, Dick RP (eds) *Enzymes in the environment: Activity, ecology, and applications*. Marcel Dekker Inc, New York, pp 249–265.

Van Soest PJ (1963) Use detergents in the analysis of fibrous feeds. A rapid method for the determination of fiber and lignin. *J Assoc Off Agr Chem* 46:829–835

Townsend AR, Asner GP, Cleveland CC (2008) The biogeochemical heterogeneity of tropical forests. *Trends Ecol Evol* 23:424–431

Waring BG (2012) A meta-analysis of climatic and chemical controls on leaf litter decay rates in tropical forests. *Ecosystems* 6:999–1009

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York

Figure 1. Mean monthly precipitation (± 1 SE) indicated by bars and temperature indicated by solid line (1999-2010), and monthly precipitation in the study year (June 2010 to May 2011) in the three tropical dry forest sites at Yucatan Peninsula. Inter-annual variation in monthly temperature is negligible. (A) Chicxulub (531 mm of mean annual precipitation; weather station Progreso 21°17'34'' N, 89°36'28'' W), (B) X'matkuil (993 mm of mean annual precipitation; weather station Mérida 20°59'00'' N, 89°38'00'' W); (C) Hobonil.

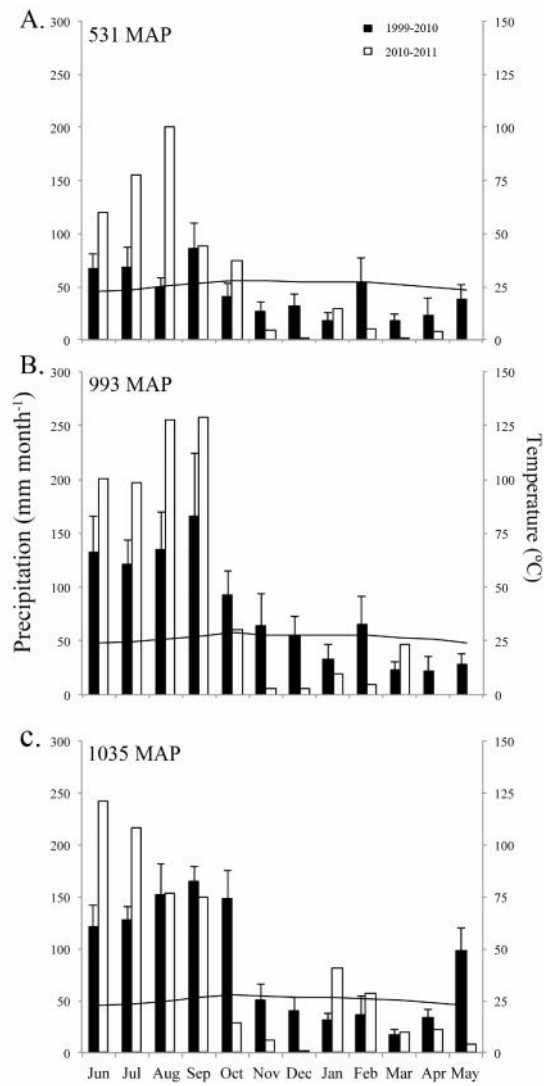


Figure 2. Changes in remaining mass (mean \pm 1 SE), and decomposition rates of *Gymnopodium* leaf litter under three treatments of N addition (control, without N addition; +N1, addition of 2.4 kg N ha yr⁻¹; +N2, addition of 24 kg N ha yr⁻¹) in three tropical dry forest sites at Yucatan Peninsula. Within litter ecotype at each decomposition site, different lowercase letters indicate means are significant different ($p < 0.05$) for N addition treatments; and different numbers indicate means are significant different across decomposition sites within each N addition treatment. Within decomposition site, different uppercase letters indicate means are significant different across ecotypes within each N addition treatment. The r^2 -adjusted for k decomposition rates were significant at the level of $p < 0.001$.

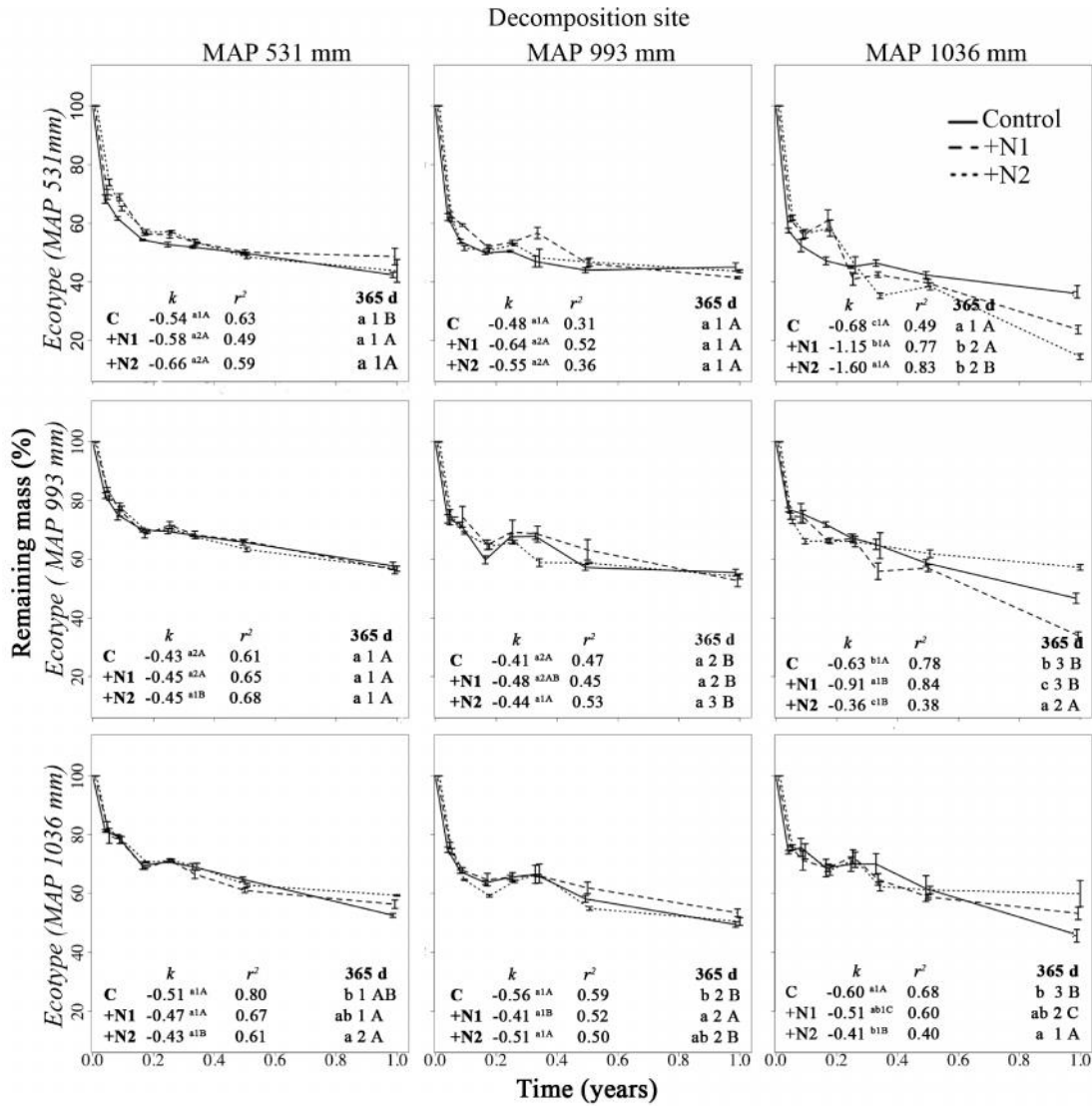


Figure 3. Changes in remaining mass (mean \pm 1 SE), and decomposition rates of *Piscidia* leaf litter under three treatments of N addition (control, without N addition; +N1, addition of 2.4 kg N ha yr⁻¹; +N2, addition of 24 kg N ha yr⁻¹) in three tropical dry forest sites at Yucatan Peninsula. Within litter ecotype at each decomposition site, different lowercase letters indicate means are significant different ($p < 0.05$) for N addition treatments; and different numbers indicate means are significant different across decomposition sites within each N addition treatment. Within decomposition site, different uppercase letters indicate means are significant different across ecotypes within each N addition treatment. The r^2 -adjusted for k decomposition rates were significant at the level of $p < 0.001$.

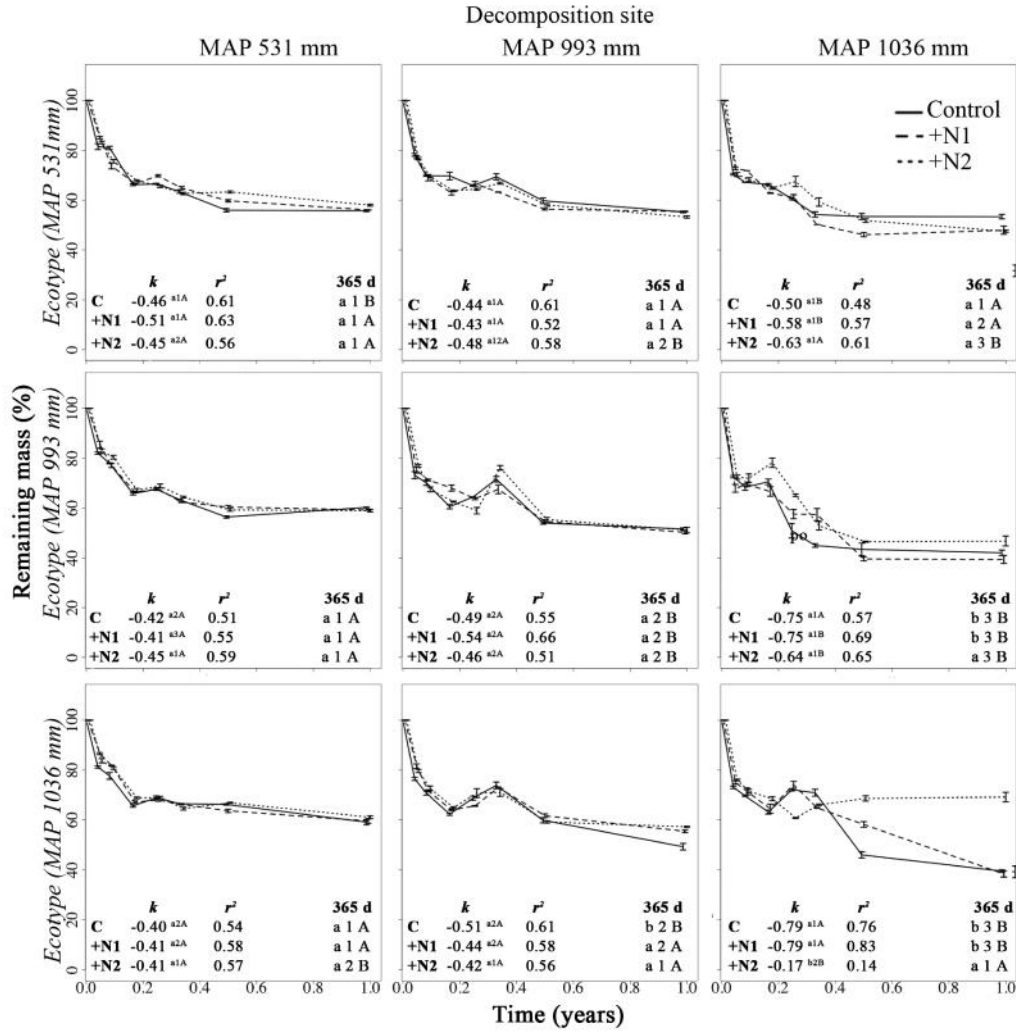


Table 1. Characteristics of three climatic sites of Yucatan Peninsula, Mexico

	Site		
	Chicxulub	X'matkuil	Hobonil
Coordinates	24°14'N 89°31'W	20°51'N 89°35'W	19°38'N 88°59'W
Altitude (m asl)	4	22	36
Climate ¹	BS1	Aw0	Aw1
Mean annual temperature (°C)	25.8 ± 0.2a	26.6 ± 0.1a	26.2 ± 0.3a
Mean annual precipitation (mm yr ⁻¹)	531 ± 43b	993 ± 73a	1036 ± 81a
Precipitation / Potential evapotranspiration (mm mm ⁻¹)	0.5	0.8	1.0
Vegetation ²			
Tree density (stems ha ⁻¹)	2598 ± 338b	7210 ± 391a	3923 ± 441b
Basal area (m ² ha ⁻¹)	30 ± 5a	34 ± 4a	31 ± 5a
Tree height (m)	3.3 ± 0.2b	3.3 ± 0.1b	5.3 ± 0.2a
Soil (0-10 cm in depth)			
Bulk density (g cm ⁻³)	0.70 ± 0.03c	0.78 ± 0.03b	0.91 ± 0.04a
Clays (%)	21.7 ± 1.0c	28.3 ± 1.4b	38.1 ± 2.4a
Silt (%)	4.2 ± 0.2b	7.5 ± 0.3a	4.3 ± 0.5b
Sand (%)	74.1 ± 1.0a	64.3 ± 1.4b	57.6 ± 2.8b
pH (H ₂ O)	7.4 ± 0.04a	7.5 ± 0.04a	7.4 ± 0.02a
Total C (mg C g ⁻¹)	207 ± 7a	135 ± 16b	89 ± 4c
Total N (mg N g ⁻¹)	12.6 ± 0.6a	8.6 ± 0.7b	5.5 ± 0.5c
C:N	16 ± 1.0a	16 ± 0.9a	16 ± 1.0a

¹ Climatic classification according Köppen adapted by García (2004)

² Data from Roa-Fuentes et al. (2012)

Different letters indicate that means are significant different ($p < 0.05$) across sites

Table 2. Initial litter chemistry for ecotypes of *Gymnopodium* and *Piscidia* used in decomposition experiment

	531 mm MAP	Ecotype 993 mm MAP	1036 mm MAP	Model estimator		
				F	<i>p</i>	Model‡
<i>Gymnopodium</i>						
C (mg C g ⁻¹)	481 ± 4a	478 ± 10a	484 ± 4a	0.22	ns	GLS
N(mg N g ⁻¹)	25.1 ± 0.6a	19.9 ± 0.8b	24.9 ± 1.1a	10.7	**	LS
C:N	19.2 ± 0.1b	24.0 ± 0.9a	19.5 ± 0.7b	13.3	**	LS
C fractions (%)						
Water soluble	45.7 ± 1.2a	32.8 ± 2.9b	38.1 ± 0.6b	9.78	**	LS
Hemicellulose	25.5 ± 3.8a	33.2 ± 3.6a	34.3 ± 0.1a	3.61	ns	LS
Cellulose	16.0 ± 0.9b	21.1 ± 0.6a	10.1 ± 1.3c	25.3	***	LS
Lignin	12.8 ± 0.9b	12.9 ± 1.3b	17.5 ± 0.2a	8.21	*	GLS
Lignin:N	5.10 ± 0.37b	6.45 ± 0.67ab	7.03 ± 0.3a	7.61	*	GLS
<i>Piscidia</i>						
C (mg C g ⁻¹)	462 ± 8a	476 ± 6a	484 ± 2a	3.41	ns	GLS
N (mg N g ⁻¹)	21.6 ± 1.2a	21.9 ± 1.0a	22.2 ± 1.3a	0.82	ns	GLS
C:N	21.4 ± 1.0a	21.7 ± 1.3a	21.8 ± 1.2a	0.03	ns	GLS
C fractions (%)						
Water soluble	52.1 ± 1.4a	44.9 ± 0.6b	41.3 ± 1.3a	15.8	**	GLS
Hemicellulose	17.9 ± 1.0c	23.7 ± 0.7b	29.0 ± 0.7a	39.5	***	GLS
Cellulose	19.0 ± 0.9a	17.7 ± 1.4a	15.9 ± 0.8a	4.09	ns	GLS
Lignin	11.0 ± 1.4a	13.7 ± 0.4a	13.8 ± 0.8a	0.73	ns	LS
Lignin:N	5.09 ± 1.85a	6.26 ± 0.44a	6.22 ± 0.95a	0.43	ns	GLS

MAP, mean annual precipitation. Different letters indicate that means are different among litter ecotypes

ns, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Method of model estimation: GLS, Generalized least squares; LS, Least square

DISCUSIÓN Y CONCLUSIONES

Conclusión Objetivo 1: *El incremento esperado en la disponibilidad de N afecta los ciclos del carbono, del nitrógeno y del fósforo de los bosques tropicales.*

La teoría biogeoquímica de los BTs se ha desarrollado a partir de los estudios realizados en BTs húmedos maduros (Cleveland *et al.*, 2011). En estos bosques, la adición experimental de N estimula tasas altas de pérdidas de N (Hall & Matson, 1999, 2003) mientras que en las plantas y en los microorganismos del suelo (excepto los desnitrificadores) no genera cambios (Vitousek *et al.*, 1995, 1993), soportando así la hipótesis de que los BTs están saturados de N (Matson *et al.*, 2002). Sin embargo, los BTs incluyen ecosistemas que difieren en el nutriente que limita la producción primaria neta de acuerdo con la edad del material parental en el que se desarrollan (Vitousek, 1984) y con el estado sucesional (Davidson *et al.*, 2004).

Adicionalmente, existen dos líneas de evidencia que apoyan más fuertemente la existencia de un gradiente de limitación de N a P que sólo la saturación de N en los BTs. La primera de éstas, que es conocida como la paradoja de N, demuestra que en un bosque húmedo existen ambientes de baja disponibilidad de N (en los que la fijación biológica de N es mantenida) y ambientes de alta disponibilidad de N (en los que se registran altas pérdidas de N) (Hedin *et al.*, 2009). La segunda evidencia se basa en datos globales para los ecosistemas terrestres, ésta demuestra que la colimitación N-P tiende a ser más común que la limitación por solo uno de estos bioelementos (Hedin, 2004).

De acuerdo con el gradiente de limitación N-P, la hipótesis central de este objetivo fue que los BTs podían reaccionar a la adición de N utilizándolo biológicamente y cambiando los ciclos del C, N y P. La alteración de estos ciclos se fundamenta en el hecho de que los nutrientes conservan relaciones estequiométricas en los ecosistemas (Sternner & Elser, 2002).

Los resultados del *Capítulo 1* soportan la predicción de que la adición de N, además de incrementar las pérdidas de N, también afecta significativamente la dinámica del C y del P. Específicamente, la adición de N incrementó la productividad primaria neta, la concentración de bioelementos de la hojarasca y del mantillo y las pérdidas de N de los BTs (Figuras 2 y 3). Estos resultados indican que a pesar de que los BTs son ambientes ricos en N (Craine *et al.*, 2009), tanto la comunidad de plantas como la de microorganismos del suelo pueden utilizar el N adicionado. En conjunto, estos resultados demuestran que (1) los supuestos basados en el modelo de saturación de N en BTs (Matson *et al.*, 2002) deben ser reevaluados y (2) que el enfoque de colimitación por N y P para ecosistemas terrestres a nivel mundial (Elser *et al.*, 2007) también es válido para los BTs.

Adicionalmente, los datos del *Capítulo 1* sugieren que las condiciones experimentales de adición de N afectan más fuertemente a la comunidad de microorganismos del suelo que a la comunidad vegetal (Figuras 4, S2, S3, S4). Estos resultados son consistentes con los estudios previos en los que se ha registrado una alta sensibilidad de los microorganismos a cambios climáticos o a la disponibilidad de recursos (Myrold, 1998), porque sus cortos ciclos de vida permiten la reestructuración y adaptación de la comunidad microbiana ante nuevas condiciones (Balsler *et al.*, 2006). En los resultados también se encontró que la adición de N generó un mayor cambio en los ciclos del C y del N en los BTES y en los bosques tropicales montanos que en los otros BTs (Figura S5), sugiriendo así una mayor limitación por N en estos bosques. En los BTES's la limitación puede ser explicada por el desacople temporal que ocurre entre las épocas de mayor disponibilidad de N (época seca) y las de mayor demanda biológica del mismo (época de lluvias) (Austin *et al.*, 2004; Solis & Campo, 2004), así como por las bajas tasas de mineralización de bioelementos en los bosques tropicales montanos como efecto de las bajas temperaturas (Schaberg *et al.*, 1997; Tanner *et al.*, 1990).

Conclusión del Objetivo 2: *La adición de N cambia las dinámicas del C y del N en los suelos de bosques tropicales estacionalmente secos, sin embargo el*

efecto de la adición es modulado por el régimen de precipitación y el ciclo interno de N de cada bosque en particular.

Consistentemente con otros estudios realizados en la Península de Yucatán (Cuevas *et al.*, 2013; Maldonado, 2013), en el *Capítulo 2* se evidenció que los suelos de la región tienen altas concentraciones de C en comparación con los suelos de otros BTES (Tabla 7). Sin embargo, entre los suelos del gradiente de precipitación encontramos que las concentraciones de C orgánico y N, así como la relación C:N se relacionaron negativamente con la precipitación media anual. En condiciones naturales el suelo del sitio más seco tiene menor calidad en su materia orgánica (relación C:N=23), posiblemente porque la mayor limitación hídrica a lo largo del año constriñe la mineralización de la materia orgánica del suelo debido a que la existencia de la película de agua en los poros del suelo es indispensable para permitir el acceso biológico al N (Orchard & Cook, 1983). De esta manera la hipótesis central de este capítulo fue que las dinámicas del C y del N en el suelo del bosque más seco podrían responder más fuertemente a la adición de N que los otros bosques del gradiente.

La adición de N disminuyó la concentración de carbono orgánico, la relación C:N en la biomasa microbiana y aumentó la emisión de CO₂ y el flujo de N₂O hacia la atmósfera (Tablas 3-4, Figuras 6-7), corroborando la hipótesis central acerca de una mayor respuesta a la adición de N en el sitio más seco del gradiente. Sin embargo, también se encontró una fuerte respuesta ante la adición de N en el bosque más húmedo del gradiente. En este bosque disminuyó la concentración de carbono orgánico e incrementó la emisión de CO₂, indicando que el N adicionado fue utilizado por la biomasa microbiana (Tablas 3-4, Figuras 6-7). El descenso de la relación C:N en el suelo procedente del bosque más seco sugiere que la mayor emisión de CO₂ también podría ser debida a un cambio en la comunidad de microorganismos del suelo de hongos a bacterias (Bradley *et al.*, 2006).

El bosque más húmedo del gradiente tiene materia orgánica en la que el N es más disponible (menor relación C:N) para los microorganismos que en los otros suelos del gradiente (Tabla 2); este suelo también presentó las menores concentraciones totales de C y de N y de N mineral (Tabla 2). En este sentido, las respuestas ante la adición de N son explicadas porque mayores valores de precipitación media anual han sido asociados con una dinámica más rápida de C y de N (Austin *et al.*, 2004; Roa-Fuentes, 2013; Solis & Campo, 2004), en especial el N es más fácilmente perdido por emisión de óxidos a la atmósfera o su lixiviación hacia otros ecosistemas (Hall & Matson, 2003).

En general, la adición de N a los suelos del gradiente de precipitación estudiado generó un cambio en las concentraciones de C y N, así como de la relación C:N en la biomasa microbiana, sin embargo este cambio dependió de la interacción entre la adición de N y el tiempo de incubación de los núcleos del suelo (Tabla 4); estos resultados sugieren que la biomasa microbiana de los BTES's estudiados es sensible al ingreso extra de N, soportando la evidencia encontrada en el *Capítulo 1* acerca de que los microorganismos en el suelo son susceptibles a los cambios en la disponibilidad de N (Cusack *et al.*, 2011; Balsler, 2001).

Conclusión Objetivo 3. *El régimen de lluvia y calidad del material en descomposición modulan el efecto de la adición experimental de N en la tasa de descomposición foliar de los BTES de la Península de Yucatán.*

El efecto de la deposición de N en la descomposición de BTs es poco claro, algunos estudios han reportado un efecto positivo (Kwabiah *et al.*, 1999; Cusack *et al.*, 2009), otros no han encontrado un efecto (Hobbie & Vitousek, 2000) y otros han encontrado un efecto negativo (Fang *et al.*, 2007; Mo *et al.*, 2006). Los estudios realizados en otros ecosistemas terrestres han demostrado que el efecto de la deposición de N es modulado por la identidad de los compuestos de carbono en descomposición (Knorr *et al.*, 2005).

La hipótesis central del *Capítulo 3* es que el bosque más húmedo del gradiente podría tener una mayor tasa de descomposición como respuesta a la adición experimental de N, porque este bosque presenta las más bajas concentraciones de N disponible biológicamente (*Capítulo 2*). Los resultados encontrados soportan esta hipótesis (Figura 1 y 2). Sin embargo, la dirección del efecto de la adición de N es definida por la calidad del C al que tienen acceso los microorganismos. En este capítulo la precipitación media anual se relacionó positivamente con la síntesis de compuestos recalcitrantes en las hojas senescentes de ambas especies estudiadas (Tabla 1). De esta manera la adición de N en el material en descomposición proveniente del bosque más seco se descompuso más rápido, mientras que el material del bosque más húmedo se descompuso más lento (Figuras 1 y 2).

Aunque en este trabajo no se alcanzan a explorar los mecanismos subyacentes que permitan explicar el porqué de la dirección de cambio en cada uno de los materiales en descomposición, evidencias previas sugieren que estos estarían asociados a la actividad enzimática. Brevemente, la adición de N a material de alta calidad (*i.e.*, C lábil) ha demostrado que incrementa la acción de las enzimas hidrolíticas, mientras que la adición del bioelemento a material de baja calidad (*i.e.*, C estructural como la lignina y la hemicelulosa) incrementa la acción de enzimas oxidativas (Sinsabaugh & Follstad, 2011; Sinsabaugh *et al.*, 2002).

Un resultado interesante en los experimentos en los que el material se descompuso en el mismo bosque donde se originó, es que la tasa de descomposición del material no se relacionó positivamente con la precipitación media anual, esta relación ha sido demostrada para BTs. En este capítulo se reporta una mayor tasa de descomposición en el bosque más seco del gradiente que en los bosques más húmedos, evidenciando que en estos bosques la precipitación media anual deja de ser un predictor de la descomposición y sugiriendo un mayor efecto de la matriz (*i.e.*, características bióticas y abióticas del suelo y del mantillo del lugar), que de la precipitación media anual en la descomposición de BTES. El fuerte efecto de la matriz es consistente con la hipótesis de ventaja de la hojarasca nativa (*“home field*

advantage hypothesis”) (Gholz *et al.*, 2000), sugiriendo que la comunidad de microorganismos descomponedores es específica a cada uno de los sitios estudio.

PERSPECTIVAS

Los modelos actuales de cambio climático que incluyen la interacción entre los ciclos globales de C y de N aún no incorporan la variabilidad de respuesta de los diferentes tipos de BTs ante el incremento futuro de deposición de N atmosférico (Solokov, 2008). Los resultados del *Capítulo 1* describen patrones de cambio como respuesta a la adición experimental de N en BTs que pueden servir como hipótesis para estudios que busquen el entendimiento de los mecanismos que generan dichos cambios. Hasta el momento los datos disponibles para BTs no son útiles para generar modelos de predicción confiables debido al sesgo en los estudios hacia BTs húmedos montanos y de tierras bajas, y a la alta variabilidad en las condiciones experimentales y corta duración de los ensayos (< 5 años). El significativo papel modulador de las condiciones experimentales y del tipo de bosque en los resultados encontrados, así como los cortos tiempos de adición de N que han sido reportados en el *Capítulo 1*, indican la necesidad de realizar investigaciones a más largo plazo para incrementar el entendimiento de los mecanismos que modulan más fuertemente la respuesta de los BTs ante el incremento de N. Teniendo en cuenta que los BTs se localizan en países en los que el financiamiento de la investigación básica es limitado, la mejor manera de generar información con alto grado de certeza es el uso de parcelas experimentales a largo plazo en pocos pero representativos BTs (Vargas *et al.*, 2012).

Si el incremento en la deposición de N alcanza las tasas pronosticadas para el 2030 (Dentener *et al.*, 2006), los estudios previamente realizados indican que el retorno de N desde la vegetación al suelo será más rápido (Campo *et al.*, 2007), incrementando el ingreso de N extra al suelo (Solis & Campo, 2004) y disminuyendo la limitación de N en el suelo de estos bosques (Capítulo 2). Sin embargo, debido a que en los BTES se predice el descenso de la precipitación en 50 mm por año para el 2055 (Miles *et al.*, 2006), la capacidad de utilizar este N será constreñido por la limitación hídrica. No obstante debido a que el descenso en el régimen de precipitación ha sido predicho en la región tropical (Dai, 2011), entre el 18 y el

80% de los bosques en un régimen másico de precipitación tenderán a transformarse en BTES (Huntingford *et al.*, 2008). En estos nuevos bosques, en donde la limitación hídrica sera similar al bosque más húmedo del gradiente estudiado, el incremento de deposición atmosférica de N podrá generar cambios significativos en la tasa de descomposición de estos ecosistemas (*Capítulo 3*). En este sentido, es importante incorporar los cambios en la distribución espacial de los BTs como efecto del cambio climático dentro de los modelos que incorporan la interacción de los ciclos de C y N a escala global.

REFERENCIAS GENERALES

- Aerts R, Chapin III FS. 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*. 30:1–67.
- Anaya C, García-Oliva F, Jaramillo VJ. 2007. Rainfall and labile carbon availability control litter nitrogen dynamics in a tropical dry forest. *Oecologia*. 150:602–610.
- Austin A, Vitousek P. 1998. Nutrient dynamics on a rainfall gradient in Hawai'i. *Oecologia*. 113:519–529.
- Austin A, Vitousek PM. 2000. Precipitation, decomposition and litter decomposability of *Metrosideros polymorpha* in native forests on Hawai'i. *Journal of Ecology*. 88:129–138.
- Austin A, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia*. 141:221–235.
- Austin A. 2002. Differential effects of precipitation on production and decomposition along a rainfall gradient in Hawaii. *Ecology*. 83:328–338.
- Bai E, Houlton BZ, Wang YP. 2012. Isotopic identification of nitrogen hotspots across natural terrestrial ecosystems. *Biogeosciences*. 9:3287–3304.
- Balbontín C, Cruz CO, Etchevers J. Soil Carbon Sequestration In Different Ecoregions Of Mexico. En: Rattan L, Follett F, editores. *Soil Carbon Sequestration And The Greenhouse Effect*. Mexico; SA Special Publication 57; 2009. pp. 71–96.
- Balser TC, McMahon KD, Bart D, Bronson D, Coyle DR, Craig N, Flores-Mangual ML, Forshay K, Jones SE, Kent a. E, *et al.*. 2006. Bridging the gap between micro- and macro-scale perspectives on the role of microbial communities in global change ecology. *Plant and Soil*. 289(1-2):59–70.
- Bautista F, Palma-López D, Huchin W. Características Y Manejo De Los Suelos De La Península De Yucatán. Implicaciones Agropecuarias, Forestales Y Ambientales. En: Bautista F, Palacios AG, editores. *Actualización De La Clasificación De Los Suelos Del Estado De Yucatán*. México: UAC, UADY, INE; 2005. pp. 105–122.
- Bautista-Zuñiga F, Estrada-Medina H, Jiménez-Osornio J, González-Iturbe JA. 2004. Relación entre el relieve y unidades de suelo en zonas cársticas de Yucatán. *Terra Latinoamericana*. 22(3):243–254.
- Bradley K, Drijber RA, Knops J. 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*. 38(7):1583–1595.
- Van Breemen N, Buurman P. Textural Differentiation. En: Van Breemen N, Buurman P, editores. *Soil formation*. Dordrecht; Springer Netherlands; 2002. pp. 193–214.
- Campo J, Dirzo R. 2003. Leaf quality and herbivory responses to soil nutrient addition in secondary tropical dry forests of Yucatán, Mexico. *Journal of Tropical Ecology*. 19(5):525–530.
- Campo J, Solís E, Valencia MG. 2007. Litter N and P dynamics in two secondary tropical dry forests after relaxation of nutrient availability constraints. *Forest Ecology and Management*. 252(1-3):33–40.
- Campo, J, Jaramillo, J., & Mass, J. 1998. Pulses of soil phosphorus availability in a tropical dry forest: effects of seasonally and level of wetting. *Oecologia*, 115;167–172.
- Campo, J, Maass, J. M., Jaramillo, V. J., & Yrizar, A. M. 2000. Calcium, potassium, and magnesium cycling in a Mexican tropical dry forest ecosystem. *Biogeochemistry*, 49(1);21–36.
- Campo, J, Maass, M., Jaramillo, V. J., Martínez-Yrizar, A., & Sarukhán, J. 2001. Phosphorus cycling in a Mexican tropical dry forest ecosystem. *Biogeochemistry*, 53(2): 161–179.

- Campo, J., & Vázquez-Yanes, C. 2004. Effects of nutrient limitation on aboveground carbon dynamics during tropical dry forest regeneration in Yucatan, Mexico. *Ecosystems*, 7(3); 311–319.
- Ceccon, E., Sánchez, S., & Campo, J. 2004. Tree seedling dynamics in two abandoned tropical dry forests of differing successional status in Yucatán, Mexico: a field experiment with N and P fertilization. *Plant Ecology*, 170(2); 277–285.
- Challenger A, Soberón J. Prioridades De Conservación De Los Bosques Tropicales En México: Reflexiones Sobre Su Estado De Conservación Y Manejo. En: Koleff P, Sarukhán J, editores. *Capital Natural De México. Vol. I. Conocimiento Actual De La Biodiversidad*. México: CONABIO; 2008. pp. 87–108.
- Clark DA, Piper SC, Keeling CD, Clark DB. 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984–2000. *PNAS*. 100(10):5852–5857.
- Cleveland CC, Townsend AR, Taylor P, Alvarez-Clare S, Bustamante MMC, Chuyong G, Dobrowski SZ, Grierson P, Harms KE, Houlton BZ, *and others*. 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecology Letters*. 14(9):939–47.
- Craine JM, Elmore AJ, Aida MPM, Bustamante M, Dawson TE, Hobbie E a, Kahmen A, Mack MC, McLaughlan KK, Michelsen A, *et al.*. 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *The New Phytologist*. 183(4):980–92.
- Crutzen PJ, Andreae MO. 1990. Biomass burning in the tropics-impact on atmospheric chemistry and biogeochemical cycles. *Science*. 250:1669–1678.
- Cuevas RM, Hidalgo C, Payán F, Etcheveres JD, Campo J. 2013. Precipitation influences on active fractions of soil organic matter in seasonally dry tropical forests on the Yucatan: regional and seasonal patterns. *European Journal of Forest Research*. doi 10.1007/s10342-013-0703-4.
- Cunningham SA, Summerhayes B, Westoby M. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs*. 69(4):569–588.
- Cusack DF, Chou WW, Yang WH, Harmon ME, Silver WL. 2009. Controls on long-term root and leaf litter decomposition in neotropical forests. *Global Change Biology*. 15(5):1339–1355.
- Cusack DF, Silver WL, Torn MS, Burton SD, Firestone MK. 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology*. 92(3):621–632.
- Cusack DF, Torn MS, McDowell WH, Silver WL. 2012. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Global Change Biology*. 16(1):400–403.
- Dai A. 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change*. 2(1):45–65.
- Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*. 440(7081):165–173.
- DeFries R, Hansen A, Newton AC, Hansen MC. 2005. Increasing isolation of protected areas in tropical forests over the past twenty years. *Ecological Applications*. 15(1):19–26.
- Dentener F, Drevet J, Lamarque JF, Bey I. 2006. Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Global Biogeochemical Cycles*. 20:GB4003.
- Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ. 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist*. 186(3):593–608.
- Fang H-J, Mo J, Peng S, Li Z, Wang H. 2007. Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China. *Plant and Soil*. 297(1):233–242.

- Fierer N, Schimel JP. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*. 34(6):777–787.
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry*. 70(2):153–226.
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli L a, Seitzinger SP, Sutton M. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*. 320(5878):889–92.
- Gamon JA, Kitajima K, Mulkey SS, Serrano L, Wright SJ. 2005. Diverse optical and photosynthetic properties in a neotropical dry forest during the dry season: implications for remote estimation of photosynthesis. *Biotropica*. 37(4):547–560.
- Gentry AH, Mooney HA, Medina E. Diversity And Floristic Composition Of Neotropical Dry Forests. En: Bullock SH, Mooney HA, Medina E, editores. *Seasonally Dry Tropical Forests*. Cambridge: Cambridge University Press; 1995. pp. 146–194.
- Gholz HL, Wedin DA, Smitherman SM, Harmon ME, Parton WJ. 2000. Long term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology*. 6 (7):751–765.
- Hall SJ, Matson PA. 1999. Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature*. 400(6740):152–155.
- Hall SJ, Matson PA. 2003. Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecological Monographs*. 73(1):107–129.
- Hatakka, A. Biodegradation of Lignin. *Biopolymers. Biology, Chemistry, Biotechnology, Applications*. Vol 1. Lignin, Humic Substances and Coal . Hofrichter, M. & Steinbüchel, A. (eds.). Weinheim: Wiley-VCH, 2001 pp. 129-180.
- Harris NL, Brown S, Hagen SC, Saatchi SS, Petrova S, Salas W, Hansen MC, Potapov P V, Lotsch A. 2012. Baseline map of carbon emissions from deforestation in tropical regions. *Science*. 336(6088):1573–1576.
- Hedin LO. 2004. Global organization of terrestrial plant–nutrient interactions. *Proceedings Of The National Academy Of Sciences Of The United States Of America*. 101(30):10849–10850.
- Hedin LO, Brookshire ENJ, Menge DNL, Barron AR. 2009. The nitrogen paradox in tropical forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics*. 40:613–635.
- Hobbie SE, Vitousek PM. 2000. Nutrient regulation of decomposition in Hawaiian montane forests: do the same nutrients limit production and decomposition. *Ecology*. 81:1867–1877.
- Holdridge LR, Grenke WC., Hatheway W, Liang T, Tosi JA. *Forest environments in tropical life zones: a pilot study*. Oxford; Pergamon Press. 1971.780 pp.
- Huntingford C, Fisher RA, Mercado L, Booth BBB, Sitch S, Harris PP, Cox PM, Jones CD, Betts RA, Malhi Y. 2008. Towards quantifying uncertainty in predictions of Amazon “dieback”. *Philosophical Transactions Of The Royal Society B: Biological Sciences*. 363(1498):1857–1864.
- IPCC. *The Physical Science Basis. Contribution Of Working Group I To The Fourth Assessment Report Of The Intergovernmental Panel On Climate Change*. Soloman S, Quin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, editores. Cambridge and New York: Cambridge University Press; IPCC; 2007. 996 p.
- Jackson LE, Strauss RB, Firestone MK, Bartolome JW. 1988. Plant and soil nitrogen dynamics in California annual grassland. *Plant and Soil*. 110(1):9–17.

- Jaramillo VJ, Sanford RL. Nutrient Cycling In Tropical Deciduous Forests. En: Bullock S, Mooney HA, Medina E, editores. *Seasonally Dry Tropical Forests*. Cambridge: Cambridge University Press; 1995. pp. 346-361.
- Kim YS, Imori M, Makoto W, Hatano R, Yi MJ, Koike T. 2012. Simulated nitrogen inputs influence methane and nitrous oxide fluxes from a young larch plantation in northern Japan. *Atmospheric Environment*. 46:34-44.
- Kwabiah AB, Voroney RP, Palm CA, Stoskopf NC. 1999. Inorganic fertilizer enrichment of soil: effect on decomposition of plant litter under subhumid tropical conditions. *Biology And Fertility Of Soils*. 30(3):224-231.
- Lamarque J, Kiehl J, Brasseur P, Butler T, Cameron-Smith P, Collins W, Granier C, Hauglustaine D, Hess P. 2005. Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: analysis of nitrogen deposition. *Journal Of Geophysical Research*. 110:D19303.
- LeBauer D, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*. 89(2):371-379.
- Liu L, Greaver TL. 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*. 13:819-828.
- Liu L, Greaver TL. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO₂ sink may be largely offset by stimulated N₂O and CH₄ emission. *Ecology Letters*. 12:1103-1117.
- Losos EC, Leigh EG. *Tropical Forest Diversity And Dynamism: Findings From A Large-Scale Network*. Chicago; University of Chicago Press; 2004. 463 pp.
- Lu M, Yang Y, Luo Y, Fang C, Zhou X, Chen J, Yang XD, Li B. 2011a. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytologist*. 189:1040-1050.
- Lu M, Zhou X, Luo Y, Yang Y, Fang C, Chen J, Li B. 2011b. Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agriculture, Ecosystems And Environment*. 140:234-244.
- Luyssaert S, Inglis I, Jung M, Richardson AD, Reichstein M, Papale D, Piao SL, Schulze E, Wingate L, Matteucci G. 2007. CO₂ balance of boreal, temperate, and tropical forests derived from a global database. *Global Change Biology*. 13(12):2509-2537.
- Mahistein I, Daniel J, Solomon S. 2013. Pace of shifts in climate region increases with global temperature. *Nature Climate Change*. doi: 10.1038/nclimate1876.
- Maldonado, V. 2013. *Fracción Activa De La Materia Orgánica Del Suelo En Bosques Tropicales Estacionalmente Secos: Variación Sucesional Y Edafoclimática*. Universidad Nacional Autónoma de México. Instituto de Ecología. Tesis de Maestría: 90 p.
- Malhi Y, Phillips OL. 2004. Tropical forests and global atmospheric change: a synthesis. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. 359(1443):549-555.
- Malhi, Y. 2012. The productivity, metabolism and carbon cycle of tropical forest vegetation. *Journal Of Ecology*, 100(1):65-75.
- Martínez-Yrizar AB, Mooney HA, Medina E. Biomass Distribution And Primary Productivity Of Tropical Dry Forests. En: Bullock S, Mooney HA, Medina E, editores. *Seasonally Dry Tropical Forests*. Cambridge: Cambridge University Press; 1995. pp. 245-326.
- Matson P, Lohse KA, Hall SJ. 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *AMBIO*. 31(2):113-119.
- Matson PA, Vitousek PM. 1987. Cross system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. *Global Biogeochemical Cycles*. 1(2):163-170.

- McGroddy ME, Silver WL. Biogeochemical Cycling In Tropical Forests. En: Busch, M, Flenley, J and Golsing, W. Tropical Rainforest Responses To Climatic Change. Berlin. Springer Berlin Heidelberg; 2011. pp. 315–341.
- Menge DNL, Levin SA, Hedin LO. 2009. Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. *The American Naturalist*, 174(4):465–477.
- Miles L, Newton AC, DeFries RS, Ravilious C, May I, Blyth S, Kapos V, Gordon JE. 2006. A global overview of the conservation status of tropical dry forests. *Journal of Biogeography*. 33(3):491–505.
- Miranda-Hernández X. 1963. Los tipos de vegetación en México. *Boletín De La Sociedad Botánica De México*. 28:29–179.
- Mo J, Brown S, Xue J, Fang Y, Li Z. 2006. Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant And Soil*. 282(1):135–151.
- Myrold D. Transformation of Nitrogen. En: Sylvia D, Fuhrmann J, Harte P, Zuberer D, editors. *Principles and Applications of Soil Microbiology*. Upper Saddle River: Prentice-Hall; 1998. pp. 259–294.
- Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'amico J a., Itoua I, Strand HE, Morrison JC, *et al.*. 2001. Terrestrial ecoregions of the world: A new map of life on earth. *BioScience*. 51(11):933.
- Orchard V, Cook F. 1983. Relationship between soil respiration and soil moisture. *Soil Biology and Biochemistry*. 15:447–453.
- Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG. 2011. A large and persistent carbon sink in the world's forests. *Science*. 333(6045):988–993.
- Parton WJ, Mosier AR, Ojima DS, Valentín DW, Schimel DS, Weier KL. 1996. Generalized model for N₂ y N₂O production from nitrification and denitrification. *Global Biogeochemical Cycles*. 10:401–412.
- Pasquini, S. C., & Santiago, L. S. 2012. Nutrients limit photosynthesis in seedlings of a lowland tropical forest tree species. *Oecologia*, 168(2), 311–319.
- Powers J, Montgomery RA, Adair EC, Brearley FQ, DeWalt SJ, Castanho CT, Chave J, Deinert E, Ganzhorn JU, ME G, *et al.*. 2009. Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient. *Journal of Ecology*. 97:801–811.
- Richter DD, Babbar LI. 1991. Soil diversity in the tropics. *Advances in Ecological Research*. 21:315–389.
- Roa-Fuentes LL, Campo J, Parra-Tabla V. 2012. Plant biomass allocation across a precipitation gradient: an approach to seasonally dry tropical forest at Yucatán, Mexico. *Ecosystems*. 15(8):1234–1244.
- Roa-Fuentes LL, Hidalgo C, Etchevers JD, Campo J. 2013. The effect of precipitation regime on soil carbon pools on the Yucatan Peninsula. *Journal of Tropical Ecology*. doi: 10.1017/s0266467413000552.
- Roa-Fuentes, L. L. 2013. Variación Regional Del Capital De Carbono Y Características Funcionales Del Bosque Tropical Estacionalmente Seco De Yucatán. Universidad Nacional Autónoma de México. Instituto de Ecología. Tesis de Doctorado: 89p.
- Rzedowski J, Rabinovich J, Halffter G. Los Bosques Secos y Semihúmedos de México con Afinidades Neotropicales. En: Rabinovich J, Halffter G, editores. *Tópicos de la Ecología Contemporánea*. México: Fondo de Cultura Económica; 1979. pp. 46-78.
- Rzedowski J. 1991. El endemismo en la flora fanerogámica mexicana: una apreciación analítica preliminar. *Acta Botánica Mexicana*. 105:47–64.
- Sánchez GA A, Portillo-Quintero C. Extent And Drivers Of Change Of Neotropical Seasonally Dry Tropical Forests. En: Dirzo, R., Hillary, SY., Mooney, HA., Ceballos, G. editores. *Seasonally Dry Tropical Forests*. Cambridge: Cambridge University Press; 2011. pp. 45–57.

- Santiago LS, Kitajima K, Wright SJ, Mulkey SS. 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. *Oecologia*. 139(4):495–502.
- Santiago LS, Mulkey SS. 2005. Leaf productivity along a precipitation gradient in lowland Panama: patterns from leaf to ecosystem. *Trees: Structure and Function*. 19:349–356.
- Santiago LS, Schuur EAG, Silvera K. 2005. Nutrient cycling and plant-soil feedbacks along a precipitation gradient in lowland Panama. *Journal of Tropical Ecology*. 21:461–470.
- Saynes, V., Hidalgo, C., Etchevers, J. D., & Campo, J. E. 2005. Soil C dynamics in primary and secondary seasonally dry tropical forest in Mexico. *Applied Soil Ecology*, 29:282–289.
- Saxe H, Cannell MGR, Johnsen Ø, Ryan MG, Vourlitis G. 2001. Tree and forest functioning in response to global warming. *New Phytologist*. 149(3):369–399.
- Schaberg PG, Perkins TD, McNulty SG. 1997. Effects of chronic low-level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce. *Canadian Journal of Forest Research*. 27:1622–1629.
- Schuur EAG, Chadwick OA, Matson PA. 2001. Carbon cycling and soil carbon storage in mesic to wet Hawaiian montane forests. *Ecology*. 82(11):3182–3196.
- Schuur EAG, Matson P. 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian mountain forest. *Oecologia*. 128:431–442.
- Schuur EAG. 2003. Productivity and global climate revisited: The sensibility of tropical forest growth to precipitation. *Ecology*. 84(5):1165–1170.
- Shang C, Tiessen H. 2003. Soil organic C sequestration and stabilization in karstic soils of Yucatan. *Biogeochemistry*. 62:177–196.
- Sinsabaugh RL, Carreiro MM, Alvarez S. 2002. Enzyme And Microbial Dynamics Of Litter Decomposition. En: Marcel, D. editor. *Enzymes in the Environment, Activity, Ecology, and Applications*. New York; Basel. 2002. pp. 249–265.
- Sinsabaugh RL, Follstad JJ. 2011. Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. *Biogeochemistry*. 102:31–43.
- Solis, E., Campo, J. (2004). Soil N and P dynamics in two secondary tropical dry forests after fertilization. *Forest Ecology and Management*. 195; 409–418.
- Solokov AP. 2008. Consequences of considering carbon-nitrogen interactions on the feedbacks between climate and terrestrial carbon cycle. *Journal of Climate*. 21:3776–3796.
- Sterner RW, Elser JJ. *Ecological Stoichiometry: The Biology Of Elements Form Molecules To The Biosphere*. Princeton University Press; 2002 p. 439 p.
- Stark JM, Firestone MK. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*. 61(1):218–221.
- Tanner, E. V. J., Kapos, V., Freskos, S., Healey, J. R., & Theobald, A. M. 1990. Nitrogen and phosphorus fertilization of Jamaican montane forest trees. *Journal of Tropical Ecology*. 6:231–238.
- Townsend, A. R., Asner, G. P., & Cleveland, C. C. 2008. The biogeochemical heterogeneity of tropical forests. *Trends in Ecology and Evolution*. 23(8); 424–431.
- Townsend AR, Cleveland CC, Houlton BZ, Alden CB, White JWC. 2011. Multi-element regulation of the tropical forest carbon cycle. *Frontiers in Ecology*. 9:9–17.
- Trejo I, Dirzo R. 2000. Deforestation of seasonally dry tropical forests a national and local analysis in Mexico. *Biological Conservation*. 94(2):133–142.

- Trejo I. Distribución y diversidad de selvas bajas de México: relaciones con el clima y el suelo. Facultad de Ciencias. Tesis. 1998: 210 p.
- Van Der Putten WH, Bardgett RD, De Ruiter PC, Hol WHG, Meyer KM, Bezemer TM, Bradford MA, Christensen S, Eppinga MB, Fukami T. 2009. Empirical and theoretical challenges in aboveground–belowground ecology. *Oecologia*. 161(1):1–14.
- Van Gestel M, Merckx R, Vlassak K. 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast-and slow-growing microorganisms in soils from different climates. *Soil Biology and Biochemistry*. 25(1):109–123.
- Vargas R, Loescher HW, Arredondo T, Huber-Sannwald E, Lara-Lara R, Yépez EA. 2012. Opportunities for advancing carbon cycle science in Mexico: toward a continental scale understanding. *Environmental Science & Policy*. 21:84–93.
- Veldkamp E, Keller M, Nunez M. 1992. Effects of pasture management on N₂O and NO emissions from pasture soils in the humid tropics of Costa Rica. *Global Biogeochemical Cycles*. 12:71–79.
- Vicente-Serrano SM, Gouveia C, Camarero J, Beguería D, Trigo R, López-Moreno J, Azorin-Molina C, Pasho E, Lorenzo-Lacruz J, Revuelto J, *et al.*. 2013. Response of vegetation to drought time-scales across global land biomes. *PNAS*. 110(1):52.57.
- Waring BG. 2012. A Meta-analysis of climatic and chemical controls on leaf litter decay rates in tropical forests. *Ecosystems*. 6:999–1009.
- White DA, Hood CS. 2004. Vegetation patterns and environmental gradients in tropical dry forests of the northern Yucatan Peninsula. *Journal of Vegetation Science*. 15:151–161.
- Wilson JB, Peet RK, Dengler J, Pärtel M. 2012. Plant species richness: the world records. *Journal of Vegetation Science*. 23:796-802.
- Vitousek PM, Gerrish G, Turner DR, Walker LR, Mueller-Dombois D. 1995. Litterfall and nutrient cycling in four Hawaiian montane rainforests. *Journal of Tropical Ecology*. 11(02):189–203.
- Vitousek PM, Walker LR, Whiteaker LD, Matson PA. 1993. Nutrient limitations to plant growth during primary succession in Hawaii Volcanos National Park. *Biogeochemistry*. 23(3):197–215.
- Vitousek PM. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology*. 65:285–298.
- Wright IJ, Westoby M, Reich PB. 2002. Convergence towards higher leaf mass per area in dry and nutrient poor habitats has different consequences for leaf life span. *Journal of Ecology*. 90(3):534–543.
- Yokelson, R. J., Crouse, J. D., DeCarlo, P. F., Karl, T., Urbanski, S., Atlas, E., Campos, T., *et al.*, 2009. Emissions from biomass burning in the Yucatan. *Atmospheric Chemistry and Physics*. 9:5785–5812.