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### RESPUESTAS ECOFISIOLÓGICAS DE CUATRO ESPECIES ARBÓREAS DE BOSQUES HÚMEDOS DE MONTAÑA A LO LARGO DE UN GRADIENTE ALTITUDINAL.

### TESIS

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### Resumen

En este estudio se analizó la relación entre la variación ambiental (temperatura del aire, nitrógeno total y disponible en los horizontes orgánicos del suelo, radiación fotosintéticamente activa y ultavioleta [400-700 nm y 280-320 nm; respectivamente]) y las respuestas funcionales (caracteres foliares morfológicos y anatómicos, propiedades ópticas, pigmentos fotosínteticos, nitrógeno foliar, concentraciones de compuestos fenólicos e isótopos de <sup>13</sup>C) de *Ticondendron* incognitum, Drimys granadensis, Podocarpus matudae var. macrocarpus y Vaccinium consanguineum, a lo largo de un gradiente altitudinal en los bosques húmedos de montaña de la Sierra Norte de Oaxaca. Con el propósito de determinar el impacto que tiene la variación ambiental, impuesta por el cambio de altitud, sobre las respuestas funcionales de estas especies, su desempeño y distribución altitudinal. La variación de los factores ambientales evaluados no mostró, con excepción de la temperatura, un comportamiento lineal con la altitud. Por lo que, su impacto sobre las respuestas funcionales fueron locales a lo largo de este gradiente. Las respuestas funcionales evaluadas en las cuatro especies sugieren que el estatus nutricional de los suelos, en particular del nitrógeno disponible en los horizontes orgánicos, tiene un fuerte impacto sobre la variación de estas respuestas a lo largo del gradiente altitudinal, aunque son modulados por el ambiente lumínico y los regímenes de temperatura. A nivel comunitario otras variables ambientales, no ligadas directamente con el cambio altitudinal (la toxicidad de compuestos fenólicos, Mn y Al en los suelos y los disturbios), pueden explicar la variación altitudinal de los atributos de estos bosques. La variación de las respuestas funcionales de las especies estudiadas sugiere que en los bosques húmedos de montaña existe una gran diversidad funcional. Por ello, el análisis de estos razgos en las comunidades arbóreas es útil para entender los factores ambientales que influyen en los cambios de los atributos comunitarios a lo largo del gradiente altitudinal. De manera particular, en estudios comunitarios el uso de la masa seca por unidad de área, como una variable predictora del desempeño de los árboles, puede ayudar a establecer patrones de variación altitudinal de los atributos de las comunidades de estos bosques, así como establecer que factores ambientales que influyen en sus cambios.

### Abstrac

The aim this study was analyzed the relationship between environmental variation (air temperature, total and available nitrogen in organic soil horizons, photosynthetic active and ultraviolet radiation [400-700 nm and 280-320 nm, respectively]) and functional responses (leaf morphological and anatomical traits, optical properties, photosynthetic pigments, leaf nitrogen contens of phenolic compounds and isotopes of <sup>13</sup>C) of *Ticondendron incognitum*, *Drimys* granadensis, Podocarpus matudae var. macrocarpus and Vaccinium consanguineum along an altitudinal gradient in the montane forests of the Sierra Norte de Oaxaca. In order to determine the impact of environmental change, imposed by the change in altitude, on the functional responses of these species, their performance and altitudinal distribution. The variation of environmental factors tested did not show a linear realtionship with elevation, except the temperature. So, their impact on the functional responses were local. Morpho-functional responses assessed in the four species suggested that the nutritional status of soils, particularly avaliable nitrogen at organic horizons, has a strong impact on these traits along the altitudinal gradient, although were modulated by the ambient light and temperature regimes. In the community level, other environmental variables, not directly associated with the change of altitude (the toxicity of phenolic compounds, Mn and Al in soils and disturbances), may explain the altitudinal variation of the attributes of these forests. The variation of functional responses of the study species suggested that in this montane forests have high functional diversity. Therefore, the analysis of functional traits in tree communities is useful to understand the environmental factors that influence changes in community attributes along the altitudinal gradient. In particular, in community studies using the dry mass per unit area as a predictor trait of the performance of trees, can help to establish patterns of altitudinal variation of the community attributes in these forests, and the environmental factors that affect their changes.

Capítulo 1

Introducción General

En las zonas montañosas de las regiones tropicales, en distancias relativamente cortas, a lo largo de gradientes altitudinales, se presentan grandes variaciones en las condiciones ambientales. Esta variación determina la existencia de condiciones de vida muy diversas para las plantas, por lo que las comunidades vegetales de las zonas montañosas son muy heterogéneas en composición florística, fisonomía, estructura y patrones de características foliares. Estos cambios de las propiedades de las comunidades vegetales han sido documentados en estudios que analizan las características de la cubierta vegetal a lo largo de estos gradientes. Un ejemplo de estos cambios es la disminución de la riqueza de familias y especies con el incremento en altitud. (Whitmore 1984, Kappelle y Zamora 1995, Kappelle 1996, Lieberman et al. 1996, Vázquez-G y Givnish 1998). Asimismo, hay una gran heterogeneidad en el predominio de las diferentes formas de vida asociadas a este gradiente (Leigh 1975, Grubb 1977, Lieberman et al. 1996), y cambios en la estructura de la vegetación como la disminución de la talla de los árboles, el aumento en su densidad y un decremento en la productividad (Leigh 1975, Grubb 1977, Lieberman et al. 1996, Williams-Linera et al. 1996). También se registran tendencias de variación en los atributos foliares de los árboles de estas comunidades; en los sitios de mayor altitud las hojas tienden a ser más pequeñas y presentan un cambio en sus características anatómicas (Kapos y Tanner 1985, Velázquez-Rosas et al. 2002).

Algunas de las causas señaladas como determinantes para la variación de las características estructurales y funcionales de los bosques montanos son: la alta precipitación y humedad atmosférica, el decremento en la temperatura que ocurre con el incremento de altitud, la reducción de la radiación fotosintéticamente activa y el decremento de los nutrimentos en los suelos, principalmente de nitrógeno (Bruijnzeel *et al.* 1993, Tanner *et al.* 1998).

Generalmente, el efecto de la variación de los factores ambientales a lo largo del gradiente altitudinal sobre la composición, estructura y productividad de las comunidades vegetales montanas ha sido establecido mediante análisis de correlación entre los valores de los factores ambientales y algunos parámetros estructurales (área basal, altura del dosel, características foliares y concentración de nutrientes en las hojas, tasas de crecimiento de los tallos). Sin embargo, son escasos los estudios que han analizado la relación entre las características funcionales *in situ* y la variación ambiental establecida por el gradiente altitudinal (*e.g.* Tanner *et* al. 1990, Tanner et al. 1992, Cordell et al. 1998, Santiago et al. 2000, Schrumpf et al. 2001, Wilcke et al. 2008, Soethe et al. 2008). A pesar de que estos estudios son necesarios para entender cuáles son los factores determinantes en las repesutas morfofuncionales y en los patrones de cambio altitudinal de las comunidades montanas (Bruijnzeel y Veneklass 1998). Las dificultades técnicas para evaluar las respuestas funcionales in situ (equipos costosos, condiciones ambientales no favorables para la toma de datos de respuestas instantáneas, sitios poco accesibles) y para caracterizar la variación ambiental a lo largo de gradientes altitudinales, aunados a la alta diversidad de estos bosques son parte de los problemas que limitan estos estudios.

El estudio de la diversidad funcional y el análisis de las características funcionales pueden ser herramientas teóricas y prácticas muy útiles para poder entender la influencia de la variación ambiental sobre las respuestas de las especies y, en último término, de las propiedades de las comunidades vegetales de los bosques montanos del trópico a largo de gradientes ambientales, tal como ha sido planteado en otros ecosistemas y regiones del planeta (Díaz *et al.* 1999). En las últimas décadas el estudio de la diversidad funcional se ha fortalecido dentro de la ecología vegetal (Díaz y Cabido, 2001, Lavorel y Garnier 2002, MacGill *et al.* 2006), llegando a

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considerar que la diversidad funcional tiene un papel preponderante en los procesos y la estabilidad de los ecosistemas (Díaz y Cabido, 2001, MacGill et al. 2006, Westoby y Wright 2006, Violle et al. 2007). Actualmente la diversidad funcional se entiende como el tipo, intervalo y abundancia relativa de los caracteres funcionales presentes en una comunidad (Díaz et al. 2002); mientras que los caracteres funcionales se definen como los rasgos morfológicos, fisiológicos o fenológicos, que puede ser determinados a nivel individual, de la célula al individuo completo, sin referencia al medio ambiente o a otro nivel de organización (Violle et al. 2007). Estos caracteres se encuentran relacionados con un efecto sobre uno o más procesos ecológicos o con una respuesta a uno o más factores ambientales (Martín-López et al. 2007, Violle et al. 2007). Dentro de las comunidades, distintos grupos de especies pueden presentar caracteres funcionales similares, presentándose una importante redundancia funcional, que sustenta la resilencia de las comunidades vegetales (MacGill et al. 2006, Westoby y Wright 2006). Esta redundancia también puede tener una utilidad práctica importante en el análisis de la diversidad funcional de una comunidad, ya que éste puede realizarse a través de revisión de los caracteres funcionales que presentan las especies más abundantes en una comunidad. Estos caracteres pueden reflejar cuales son los factores ambientales selectivos más importantes (disponibilidad de agua y nutrientes, radiación, temperatura, herbivoría, eutrofización, etc.), además de indicar la magnitud, la tasa y dirección de los procesos ecosistémicos (Grime 1998, Díaz et al. 2002).

En los bosques húmedos del trópico y en otros ecosistemas el análisis de las características funcionales de las comunidades vegetales ha mostrado ser una herramienta muy útil para entender el papel de los factores ambientales que determinan sus propiedades y funcionamiento (Reich *et al.* 1997, Tanner *et al.* 1998, Reich y Oleksyn 2004, Wrigth *et al.* 2004, Poorter y

Bongers 2006, Pooter *et al.* 2006). De manera particular, la evaluación de los atributos foliares (área foliar, área foliar específica, estructura anatómica, concentraciones de pigmentos fotosintéticos, contenidos de nutrientes, compuestos fenólicos e isótopos estables, propiedades ópticas, asimilación de  $CO_2$  y tiempo de vida de las hojas, entre otros) han sido de los más utilizados para estos fines (Reich *et al.* 1997, Tanner *et al.* 1998, Poorter y Bongers 2006).

El presente estudio tiene como objetivo general evaluar la variación altitudinal (1520 – 2550 m snm) de los caracteres funcionales de las hojas de cuatro especies arbóreas (*Ticondendron incognitum, Drimys granadensis, Podocarpus matudae* var. *macrocarpus, Vaccinium consanguineum*) de un bosque húmedo de montaña de la Sierra Norte de Oaxaca, que difieren en su distribución altitudinal y sus historias de vida. Esto se realizó con el propósito de determinar el impacto que tiene la variación ambiental, impuesta por el cambio de altitud, sobre sus respuestas funcionales, su desempeño y distribución altitudinal. De manera particular, se analizaron las características morfológicas y anatómicas, propiedades ópticas, pigmentos fotosínteticos, nitrógeno foliar, concentraciones de compuestos fenólicos e isótopos de <sup>13</sup>C y se relacionaron con la variación altitudinal de los siguientes factores ambientales: temperatura del aire, nitrógeno total y disponible en los horizontes orgánicos del suelo, radiación fotosintéticamente activa y ultavioleta (400–700 nm y 280–320 nm; respectivamente), los cuales han sido señalados como determinantes en los cambios de las propiedades y funcionamiento de las comunidades de los bosques húmedos de montaña del trópico.

Para desarrollar el objetivo arriba expuesto, este trabajo está estructurado de la siguiente manera: en el capítulo dos se presenta una breve revisión sobre la información que se ha publicado sobre lo variación ambiental a largo de gradientes altitudinales en bosques húmedos de montaña del trópico y sus efectos sobre las plantas. En el tercer capítulo se describen las

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características de los bosques montanos (bosque mesófilo de montaña) del estado de Oaxaca y en particular de las comunidades vegetales de la zona de estudio. En el cuarto capítulo se analiza la variación altitudinal de las reservas de nitrógeno total y disponible de los horizontes orgánicos de los suelos de los sitios de estudio y sus relaciones con las propiedades de las comunidades vegetales y los atributos foliares de las cuatro especies estudiadas. En el quinto capítulo se evalúan las respuestas funcionales de las hojas en relación con la radiación fotosínteticamente activa y la ultravioleta B. En el sexto capítulo se analiza la variación altitudinal de las concentraciones isotópicas de <sup>13</sup>C de las cuatro especies de estudio y sus relaciones con la temperatura del aire, la demanda y abastecimiento de CO<sub>2</sub>, evaluados a través del nitrógeno y la morfología foliar, además de sus implicaciones en el desempeño de las especies en estudio. Por último, en el séptimo capítulo se desarrolla la discusión general sobre las respuestas funcionales de las cuatro especies estudiadas en función de los factores ambientales evaluados, enfatizando los efectos de estas variables ambientales sobre el desempeño y distribución altitudinal de estas especies. Además se revisa la repercusión de estos resultados para entender los cambios en las propiedades de las comunidades vegetales a lo largo del gradiente altitudinal donde se desarrollan los bosques montanos de la Sierra Norte Oaxaca.

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# Capítulo 2

## Variación del ambiente físico en los bosques montanos a lo largo de

## gradientes altitudinales

La información sobre las características ambientales (climáticas, edáficas, etc.) de los bosques húmedos de montaña del trópico es bastante limitada y existen pocos datos que describan con detalle su variación a lo largo de gradientes altitudinales continuos. En nuestro país la información es aún más reducida porque en la mayoría de las zonas donde se distribuyen estos bosques no existen estaciones meteorológicas. A continuación se presenta una breve revisión sobre la variación ambiental que se ha documentado para las comunidades montanas a lo largo de gradientes altitudinales en distintas regiones tropicales.

### 2.1 Radiación solar

La constante nubosidad en las zonas montañosas trae como resultado una consecuente disminución en los niveles de radiación (Whitmore 1984, Nakashizuka *et al.* 1991, Cavelier 1996). La reducción de la radiación en estos bosques puede ser del 16% (Aylet 1985) al 53% del flujo de densidad fotónica (Cavelier y Mejía 1990). Sin embargo, una fracción de ésta, la radiación ultravioleta se incrementa conforme aumenta la altitud (Jones 1992, Cavelier 1996). Esto es relevante porque este tipo de radiación afecta el crecimiento y desarrollo de las plantas. El incremento de la radiación UV-B (280–320 nm) a lo largo de gradientes altitudinales es baja, generalmente de 14–18% por cada 1000 m de altitud, mientras que a lo largo de gradientes latitudinales los cambios son mucho más grandes, llegando a ser del orden de 47% por cada 20° de latitud (Caldwell *et al.* 1989). Estos cambios en la radiación UV-B en ambos gradientes sean diferentes. A pesar de esto, hay evidencias de que las especies y ecotipos originarios de latitudes bajas y de altitudes altas son más resistentes a la radiación de UV-B (Robberecht *et al.* 1980). Barnes *et al.* 1987).

Flenley (1995) propone que la radiación UV-B puede tener importantes repercusiones en las características de los bosques montanos, principalmente en las comunidades ubicadas en las partes altas de las montañas, ya que por las mañanas estas zonas se encuentran rodeadas por los bancos de nubes que pueden reflejar una parte considerable de la radiación UV-B e incrementar las dosis que reciben las plantas de estas comunidades. Los incrementos extras por la reflexión pueden llegar a ser del orden del 70%. Esta hipótesis ayudaría a explicar de manera parcial el fenómeno de elevación de masas en las montañas tropicales, en las que se observa la reducción en talla de los árboles, cambios en la composición y el predominio de características foliares "paquifilas". El incremento en las concentraciones de compuestos fenólicos en las hojas y en la hojarasca de las comunidades montanas apoya la idea de que la radiación UV-B tiene efecto sobre las propiedades de estos bosques (Bruijnzeel *et al.* 1993). En los bosques montanos de Jamaica se ha comprobado que estos compuestos se incrementan en función del aumento de la radiación UV-B, funcionando como sistemas de protección (Rozema *et al.* 1997), ya que pueden funcionar como filtros que absorben UV-B por lo que reducen su flujo hacia los tejidos internos.

Otras respuestas funcionales relacionadas con el incremento en la radiación UV-B, como la reducción del crecimiento, de los tamaños foliares y cambios anatómicos (Teramura 1983, Caldwell *et al.* 1989) se han encontrado en las hojas de los árboles de las comunidades montanas (Tanner y Kapos 1982, Velázquez-Rosas *et al.* 2002). Algunos autores sugieren que el incremento de la radiación UV-B no explica totalmente la reducción de tamaño y la presencia de hojas esclerófilas en comunidades de altitudes inferiores a 2000 m, ya que las intensidades de UV-B altas se presentan entre los 2500 – 3000 m snm; sin embargo, se ha encontrado que las plantas expuestas a bajos niveles de radiación total en condiciones de alta humedad tienden a ser

más sensibles a los incrementos de radiación UV-B (Teramura 1983, Sullivan *et al.* 1997, Ziska *et al.* 1992).

Otro tipo de protección contra la radiación UV-B que ha sido registrado en las hojas de los bosques montanos es el incremento en las concentraciones de carotenos. Estos compuestos pueden funcionar como protectores contra la radiación UV-B, ya que disipan los excesos de energía de excitación en el fotosistema II, evitando que la clorofila transfiera la energía al oxígeno y se formen moléculas de  $O_2$  excitadas que dañan los componentes celulares. Además, los carotenos involucrados en el ciclo de las xantofilas tienen un papel importante en mantener el balance de energía (Bornman *et al.* 1997).

Existen pocos trabajos que describan las respuestas morfológicas y funcionales de los árboles a los ambientes lumínicos que se presentan en los bosques húmedos de montaña a lo largo de gradientes altitudinales. La cantidad y calidad de la radiación solar que reciben las plantas limitan de manera fundamental a la fotosíntesis y al intercambio gaseoso, por lo que las reducciones en flujo fotónico registradas en los bosques montanos pueden influir de manera negativa en las tasas de asimilación de  $CO_2$  (Körner *et al.* 1986, Bruijnzeel *et al.* 1993). Esto podría explicar parcialmente el decremento en la productividad de estas comunidades, aunque es importante considerar que otras variables, como las deficiencias de nitrógeno disponible en suelos, también pueden estar influyendo en los patrones de productividad.

Poorter *et al.* (2000) describen las características ópticas de las hojas de un bosque montano de Venezuela. Estos autores encontraron que las hojas de sol tuvieron una mayor absorbancia de radiación fotosintéticamente activa por unidad de área, mayor peso seco y nitrógeno por unidad de área y bajas concentraciones de clorofila por unidad de masa. Esto determina que las hojas de sol tengan capacidades de cosecha de luz similares a las de las hojas de sombra, aunque las hojas de sombra presentaron una mayor eficiencia de absorbancia por unidad de biomasa. La absorbancia de las hojas de este bosque fue similar a las reportadas para bosques húmedos de tierras bajas y de especies de bosques más estaciónales. Por último, estos autores distinguieron que existían pocas diferencias entre las características foliares de especies primarias y pioneras.

### 2.2 Temperatura.

La temperatura es una de las variables que presenta un cambio más conspicuo a través del gradiente altitudinal y es considerada como uno de los factores más importantes en la determinación de la zonación altitudinal de las comunidades vegetales montanas (Grubb 1977, Whitmore 1984, Jones 1992, Bruijnzeel et al. 1993, Cavelier 1996, Kappelle 1996). En las zonas tropicales la temperatura del aire puede disminuir en una tasa de 0.5-0.6°C por cada 100 m de incremento en altitud (Whitmore 1984, van der Hammen 1984, Jones 1992, Cavelier 1996, Kappelle 1996). Sin embargo, este cambio depende de la época del año, la hora del día y el contenido de vapor de agua en la atmósfera, por lo que también se reportan tasas de aproximadamente 0.97-1.0 C por cada 100 m de incremento de altitud (Cavelier 1996). Otro punto importante de los cambios de temperatura en las zonas montañosas del trópico es que la oscilación de temperatura diurna es considerablemente mayor que la variación térmica durante el año (Ohsawa et al. 1985, Kappelle 1996). Sin embargo, en otras zonas montañosas se ha observado que los patrones de oscilación de temperatura no difieren con la altitud. Los cambios extremos de temperatura influyen directamente en las respuestas funcionales de las especies vegetales (reducción del crecimiento, presencia de características esclerófilas) y pueden determinar patrones característicos de respuesta a la temperatura similares a los reportados en las comunidades alpinas de las zonas templadas (Körner 1999).

Las temperaturas del suelo presentan importantes variaciones a lo largo del gradiente altitudinal y están estrechamente relacionadas con la precipitación y la presencia de las nubes. En la Sierra de Santa Martha se ha registrado que la temperatura del suelo en las partes bajas (500-1300 m snm) es entre 0.4 a 1°C menor que la temperatura del aire, mientras que las zonas altas (3300 - 4100 m) las temperaturas del suelo son entre 1.6 y 2.6°C mayores que las del aire, y ambas temperaturas son similares en las zonas medias (1300–3300 m), en donde se registran altas precipitaciones y nieblas frecuentes (Cavelier 1996). Kappelle (1996) encontró una relación lineal entre la temperatura del suelo (30–60 cm de profundidad) y la altitud en los robledales del Cerro Chírripo, Costa Rica. La temperatura promedio osciló entre 9 y 15°C, y en ninguna de las altitudes consideradas la temperatura del suelo fue menor a 8°C, lo que sugiere que la temperatura crítica del suelo para la distribución de estos bosques es de aproximadamente 12°C, ya que por debajo de los  $8-10^{\circ}$ C se reduce significativamente la capacidad de las raíces para absorber agua (Lauer 1981). Otros efectos que pueden tener las temperaturas del suelo están relacionados con las tasas de descomposición y mineralización de la materia orgánica, esto se discute en una sección posterior.

#### 2.3 Precipitación pluvial

La precipitación, junto con la temperatura, es una variable ambiental determinante para la distribución de los bosques montanos tropicales, ya que tiene importante repercusión en algunos procesos fisiológicos como la transpiración y la fotosíntesis (Grubb 1977, Burckley *et al.* 1980, Tanner y Kapos 1982, Kapos y Tanner 1985, Bruijnzeel *et al.* 1993, Kappelle 1996, Santiago *et al.* 2000). A diferencia de lo que se registra en las zonas templadas, donde la precipitación se incrementa conforme aumenta la altitud, en las montañas tropicales la precipitación pluvial se

incrementa de la base de la montaña hasta una altitud determinada, a partir de la cual disminuye (Marrs *et al.* 1988, Cavelier 1996). La distribución de los bosques húmedos de montaña coincide con la distribución de las zonas de mayor precipitación. En algunos análisis sobre los patrones de precipitación a lo largo de gradientes altitudinales se han reconocido dos intervalos de máxima precipitación, el primero localizado entre 900 y 1400 m snm y el segundo cercano al límite arbóreo (2700–3200 m; Lauer 1981). Sin embargo, estas observaciones deben ser vistas con cautela ya que en las zonas tropicales existen pocos datos recopilados por estaciones meteorológicas a lo largo de gradientes altitudinales que confirmen estos patrones. La precipitación promedio anual que se presenta en las zonas donde se distribuyen los bosques montanos oscilan entre 2000 y 4000 mm (Webster 1995), aunque en algunos sitios es mayor a 5000 mm (Rzedowski y Palacios-Chávez 1977).

Otra fuente importante de abasto de agua para los bosques montanos es la captura de neblina (precipitación horizontal), que en algunos sitios llega a representar entre 40 y 50% del total de agua recibida anualmente (Cavelier y Goldstein 1989). La importancia de esta fuente de agua no es similar en todos los bosques húmedos de montaña, ya que en algunos sitios representa solamente el 3.5 %. Aunque este tipo de precipitación es muy importante para estos bosques, técnicamente ha sido muy difícil determinarla, debido a que los sistemas de evaluación son variados y no incluyen a todos los factores que la determinan (características de la vegetación y factores climáticos; Bruijnzeel y Proctor 1995). Esto ha dado como resultado que las mediciones obtenidas muestren resultados muy variables. En el caso de evaluaciones hechas con colectores de niebla se ha podido observar que hay una correlación negativa entre la precipitación horizontal y la precipitación vertical, es decir la precipitación horizontal disminuye en cuando se incrementa la precipitación vertical. Los valores de precipitación horizontal suelen ser bajos en sitios con

altas precipitaciones anuales y durante la época de lluvias (Bruijnzeel y Proctor 1995). Cavelier y Goldstein (1989) observaron que la precipitación vertical en las montañas costeras de Venezuela se incrementa de este a oeste, mientras que la precipitación horizontal correspondiente decrece.

La presencia constante de niebla y la alta precipitación pueden afectar el intercambio gaseoso de las especies de los bosques húmedos de montaña y con ello la asimilación de  $CO_2$ , ya que estos procesos están íntimamente ligados. Bruijnzeel *et al.* (1993) muestran, en un bosque montano de Malasia, que la transpiración total tiende a decrecer cuando se incrementa la altitud. Estos autores consideran que la reducción de la transpiración con el incremento de altitud es resultado de la presencia frecuente de niebla y de lluvia, las cuales reducen la duración de las horas soleadas que ocurren en los sitios altos. Se han reportado tendencias similares para la conductividad y densidad estomática (Körner *et al.* 1983, Friend y Woodward 1990). Estas tendencias son contrarias a las encontradas para zonas templadas, en donde la conductividad y la densidad estomática con la altitud (Körner *et al.* 1986). Aunque en las zonas tropicales se registra una reducción importante de la conductividad y de la transpiración con la altitud, también se ha reportado que las especies de estos bosques de zonas altas tienen un control estomático eficiente para evitar la pérdida de agua cuando se presentan momentos de alta radiación (Kapos y Tanner 1985, Cavelier 1990).

#### 2.4 Variación de las propiedades edáficas

Los suelos de los bosques húmedos de montaña presentan una gran variabilidad en sus características fisicoquímicas y en las unidades edáficas, por lo que sólo se pueden hacer pocas generalizaciones de sus propiedades (Bruijnzeel y Proctor 1995). A pesar de ello, se postula que en estos bosques existe abastecimiento limitado de nutrimentos, determinado climáticamente por

la reducción en las tasas de transpiración y la consecuente reducción en la absorción, la saturación hídrica, la acidez extrema de los suelos y la baja fertilidad de éstos (Edwards y Grubb 1977, Grubb 1977, Tanner 1977, Whitmore 1984, Bruijnzeel *et al.* 1993).

El pH medido en estos bosques generalmente oscila entre 3 y 7, el carbón orgánico y el nitrógeno total generalmente se incrementan con la altitud (Bruijnzeel y Proctor 1995, Kitayama1995, Wilcke et al. 2002, Soethe et al. 2008); sin embargo, en otros bosques estas variables presentan valores más bajos, entre el 0.05 y 0.3%, respectivamente (Bruijnzeel y Proctor 1995). En pocos trabajos se ha medido la concentración de P total y se ha registrado que los valores de P extraíble se encuentran en intervalos de 1.1 y 78  $\mu$ g g<sup>-1</sup> en suelo seco, sin que se muestre ninguna tendencia de cambio altitudinal, por lo que no es considerado como un elemento que influya negativamente en la productividad de los bosques montanos (Tanner et al. 1998). Las bases intercambiables y el porcentaje de bases de saturación son generalmente bajas y tampoco presentan un patrón de cambio altitudinal (Edwards y Grubb 1977, Grubb 1977, Tanner 1977, Álvarez et al. 2008). Kitayama (1995) registró que en los bosques del Monte Kinabalu, Malasia, no se presentan diferencias nutricionales entre sitios localizados a baja y alta elevación. Los horizontes superficiales de los suelos en ambas altitudes presentan valores altos de fósforo soluble y nitrógeno total. El magnesio y el potasio intercambiable son similares a los registrados en los bosques de tierras bajas.

La baja concentración de nitrógeno disponible en los suelos es considerada como un factor determinante en la productividad de las comunidades de los bosques montanos y generalmente disminuye con el incremento de altitud (Vitousek y Sanford 1986, Tanner *et al.* 1990, Tanner *et al.* 1992, Tanner *et al.* 1998, Cavelier et al. 2000, Soethe *et al.* 2008, Wilcke *et al.* 2008). Esta disminución del nitrógeno disponible está asociada con la reducción en la descomposición y la

mineraliazión que se registra en estos bosques conforme se incrementa la altitud. Marrs et al. (1988) mostraron que la mineralización y la nitrificación disminuyen conforme se incrementa la altitud en el Volcán Barva, Costa Rica. Las tasas de mineralización de nitrógeno pasan de 38 µg  $g^{-1}$  en una altitud de 100 m a 2 µg  $g^{-1}$  en 2600 m. De manera contraria, en los bosques montanos de Jamaica se han registrado tasas relativamente altas de mineralización (Tanner 1977) y en los bosques bajos y altos de Krakatau las tasas de nitrificación son similares (Bruijnzeel y Proctor 1995). Estos resultados contradictorios pueden ser provocados porque en muchos casos no se determinan con precisión a que horizontes corresponden los valores registrados (Roman y Scatena en prensa). La reducción, en la tasa de mineralización y nitrificación con la altitud, ha sido relacionada con la reducción de temperatura conforme se incrementa la altitud y con la gran saturación hídrica de los suelos, lo que establecen condiciones anaerobias que son desfavorables para los organismos descomponedores (Edwards y Grubb 1977, Grubb 1977, Tanner 1977, Whitmore 1984, Marrs et al. 1988, Bruijzeel et al. 1993, Ross et al. 1996, Cavelier et al. 2000, Ushio et al. 2008). Esto determina que exista una reducción en el nitrógeno disponible para las plantas (Marrs et al. 1988, Soethe et al. 2008).

Las evaluaciones del mantillo realizadas en los bosques montanos son escasas y muestran una amplia variación en las concentraciones de nutrimentos. De manera general, la cantidad de mantillo es mayor en comparación con la registrada en los bosques de tierras bajas y presentan contenidos de nutrimentos bajos, especialmente de N (Vitousek 1984, Bruijnzeel y Proctor 1995, Tanner *et al.* 1998). Estos resultados son ocasionados por la reducción de las tasas de descomposición y los bajos contenidos de nutrimentos de la hojarasca. La concentración de N y P en la hojarasca se reduce conforme se incrementa la altitud y con frecuencia los valores de ambos elementos son bajos por encima de los 1500 m (Tanner *et al.* 1998). Las comparaciones hechas

entre los contenidos nutricionales de las hojas maduras de los árboles, la hojarasca y el mantillo sugieren que existe una tasa alta de translocación de N y P de las hojas senescentes a otros órganos y hojas jóvenes de las plantas.

En las zonas tropicales se considera que el N es el elemento que restringe la productividad en los bosques húmedos de montaña a lo largo de gradientes altitudinales, ya que puede influir la asimilación de CO<sub>2</sub> (Tanner et al. 1998). Aunque recientemente Wilcke et al. (2008) encontraron que el P, Ca y Mn de los horizontes orgánicos tienen un efecto sobre el crecimiento de los árboles en un bosque montano de Ecuador. La relación entre la asimilación de CO<sub>2</sub> y la concentración de nitrógeno foliar es una relación positiva, es decir, se registran tasas de asimilación altas cuando se incrementa la concentración de N foliar, este patrón es constante entre diferentes especies y en todas las formas de crecimiento (Medina 1981, Field y Mooney 1986). Por ello, una forma indirecta de estimar la capacidad de asimilación total es a través de la concentración de nitrógeno foliar. Cuando se analiza el contenido de nitrógeno foliar en función de la masa foliar (mg g<sup>-1</sup>) se ha observado que la concentración de nitrógeno tiende a aumentar en la medida que se incrementa la altitud (Vitousek et al. 1992, Cavalier 1996, Tanner et al. 1998, Soethe et al. 2008); este cambio ha sido interpretado como una respuesta al decremento de N disponible que se presenta en los suelos cuando se incrementa la altitud (Marrs et al. 1988, Grubb 1977, Tanner et al. 1998, Soethe et al. 2008, Wilcke et al. 2008). El incremento con la altitud en la concentración de N foliar por unidad de área ha sido confirmado también por otros autores en diversos bosques montanos (Medina 1981, Körner 1989, Morecroft et al. 1992, Vitousek et al. 1992, Cordell et al. 1998), por lo que se podría esperar que ganancia total de  $CO_2$  se incrementará, de acuerdo con lo reportado para zonas alpinas (Körner 1989). Esto no necesariamente puede ocurrir porque en las hojas de las zonas tropicales altas se ha registrado una reducción del nitrógeno que se asigna para la fotosíntesis, debido a que hay una mayor inversión en elementos estructurales de protección contra patógenos (Edwards y Grubb 1982, Field y Mooney 1986). Aunado a esto se ha observado que el peso foliar por unidad de área se incrementa con la altitud; este cambio está asociado con el incremento en la longevidad de las hojas de los bosques montanos, que en algunos casos puede ser mayor de 30 meses (Tanner 1980, Williams-Linera 2000). Tanner *et al.* (1998) proponen que estos cambios pueden dar como resultado que se presente una disminución en la eficiencia instantánea del uso del nitrógeno con el incremento en altitud, debido parcialmente a las bajas temperaturas y a la ineficiencia fotosintética de las hojas esclerófilas.

#### 2.5 Efecto de elementos tóxicos y fenoles en los suelos de los bosques montanos

Los suelos de los bosques montanos pueden tener altas concentraciones de Al, Fe y Mn, pudiendo establecer condiciones de toxicidad que afectan el estatus nutricional de estos suelos y el crecimiento de las plantas (Bruinjzeel y Proctor 1995). La idea de que exista toxicidad por Al está basada en el hecho de que en algunos bosques se han medido altas concentraciones foliares de este elemento; por ejemplo, se registró entre 1640  $\mu$ g g<sup>-1</sup> de peso seco, en un bosque montano de Venezuela (Grimm y Fassbender 1981) y 1040  $\mu$ g g<sup>-1</sup> en Sri Lanka (Werner y Balasubramaniam 1988). Sin embargo, se han registrado valores más bajos en bosques enanos de Gunung Mulu Sarawak (230  $\mu$ g g<sup>-1</sup>, Bruijnzeel y Proctor 1995). Es probable que en los suelos de los bosques montanos se presente también toxicidad de Fe y Mn, la cual generalmente se encuentra asociada a suelos con una constante saturación de agua, aunque en otros bosques esta condición no es predominante, por ello es importante realizar más mediciones de estos elementos para determinar su papel en estos ecosistemas (Bruijnzeel y Proctor 1995).

En los bosques montanos húmedos las altas concentraciones de compuestos fenólicos medidos en las hojas pueden jugar un papel importante en el ciclo de nutrimentos (Bruinjzeel *et al.* 1993). El estrés por nitrógeno y el déficit de agua son los principales factores que afectan la concentración de compuestos fenólicos en los tejidos de las plantas. Se ha demostrado que los polifenoles afectan las tasas fotosintéticas, la división celular en raíces finas, el abastecimiento de iones y la apertura estomática (Hättenschwiler y Vitousek 2000). Aunque para estos bosques no son claras las causas que provocan los incrementos de concentración de los polifenoles foliares, se ha encontrado una relación inversa entre la concentración de N total y los fenoles totales (Bruijnzeel *et al.* 1993). En plantas expuestas a incrementos de radiación UV-B se ha registrado que aumenta la producción de compuestos fenólicos. Estos compuestos pueden funcionar como filtros que absorben y reducen el flujo de UV-B hacia tejidos internos. Sin embargo, de acuerdo con Hättenschwiler y Vitousek (2000), los polifenoles afectan el ciclo de nutrimentos mediante dos tipos de mecanismos, el primero está relacionado con los efectos sobre los organismos del suelo y el segundo con los efectos fisicoquímicos sobre grupos y formas de los nutrimentos.

Los compuestos fenólicos pueden afectar directamente la composición y actividad de los organismos descomponedores y de esta forma influir en las tasas de descomposición y el ciclo de nutrimentos. Sin embargo, los efectos de los compuestos fenólicos sobre los organismos del suelo son variables e incluso hay reportes opuestos. En algunos casos pueden estimular o inhibir la germinación de las esporas y el crecimiento de las hifas en hongos saprófitos y presumiblemente en los hongos micorrizógenos. Los efectos negativos podrían ser mayores, dependiendo del tipo de compuesto fenólico del que se trate (Kuiters 1990). Por ejemplo, en el caso de una micorriza ericoidea se observó que la biomasa se reduce cuando se expone a una mezcla de ácidos fenólicos comunes y se incrementa cuando los fenoles aplicados son los del hospedero (Leake y Read 1989). En bosques boreales se ha demostrado que la infección de micorrizas, la ganancia de nutrimentos y el crecimiento pueden ser afectados por compuestos fenólicos específicos liberados por especies competidoras (Nilsson *et al.* 1993). También se ha sugerido que los fenoles pueden

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inhibir a los organismos nitrificadores, mediante la supresión o estimulación del crecimiento y la actividad de las bacterias simbiontes fijadoras de nitrógeno Frankia y Rhizobium (Kuiters 1990, Schimel 1998). Aunque las altas concentraciones de polifenoles podrían tener efectos negativos sobre la macrofauna del suelo, que influye en la biodegradación y mineralización de la materia orgánica, es difícil demostrarlo ya que otros compuestos de suelo podrían tener efectos similares (Neuhaser y Hortenstein 1978). Los polifenoles pueden regular la disponibilidad de N por la presencia de complejos de polifenol-proteína. Estos complejos se forman durante la senescencia de las hojas cuando en el suelo los polifenoles complejos entran en contacto con las proteínas del mantillo o las enzimas extracelulares de los microorganismos. Estos compuestos pueden representar el 20% del peso seco de las hojas senescentes. Los complejos polifenol-proteína son resistentes para muchos organismos descomponedores, excepto para basidiomicetes que tengan la enzima polifenol oxidasa y las lombrices que pueden utilizar una porción importante de N que se encuentra en estos complejos. Sin embargo, la resistencia de los complejos de polifenol-proteína depende de la calidad de los polifenoles (Hättenschwiler y Vitousek 2000). Las tasas de mineralización decrecen sustancialmente con el incremento de la capacidad de los polifenoles a formar los complejos con las proteínas (Northup 1998). A pesar de que las altas concentraciones de polifenoles pueden disminuir la mineralización, Northup (1998) registró correlaciones positivas entre los polifenoles y la liberación de nitrógeno orgánico soluble en las hojas de pino. Bajo la luz de estos hallazgos los autores consideran que las plantas en ambientes con concentraciones reducidas de N podrían ser favorecidas por un incremento en el cociente N orgánico soluble: N inorgánico. Estos efectos positivos incluirían una reducción en las pérdidas de N por lixiviación y denitrificación (pérdida de  $NO_3$ ). Además, establecen la hipótesis de que la alta producción de polifenoles por plantas de ambientes infértiles podrían representar un

atributo para el control del N en el suelo e influir favorablemente en las plantas para eficientizar su competitividad en la captura del N, limitado en formas orgánicas. Esta hipótesis está sostenida bajo el supuesto de que la inmovilización microbiana de N inorgánico es alta en ambientes limitados por N y porque los organismos del suelo son superiores a las plantas como competidores en la captura de N inorgánico. Los polifenoles pueden interactuar con otros nutrimentos mediante distintos procesos, cuando se unen con minerales arcillosos o con sesquioxidos los polifenoles están protegidos de los ataques microbianos. Estos complejos reducen la toxicidad del aluminio en los suelos con alta acidez y compiten con otros compuestos con carga negativa por cationes intercambiables o por micronutrientes metálicos (Hättenschwiler y Vitousek 2000). Los polifenoles unidos con los sesquioxidos pueden influir en la retención y liberación de fosfatos, por lo que las altas concentraciones de polifenoles podrían contribuir a mantener la disponibilidad de P en suelos muy saturados, con una acidez alta y con altos niveles de sesquioxidos de Fe y Al (Davis 1982). Además, los polifenoles pueden retener cationes inorgánicos (Ca<sup>++</sup>, Mg<sup>++</sup>, y K<sup>+</sup>) en sitios con altos niveles de lixiviación y acidez, manteniendo la disponibilidad de micronutrientes metálicos, aunque existe poca información que confirme este planteamiento (Schnitzer 1984, Zech 1992).

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# Capítulo 3.

## El bosque mesófilo de montaña en Oaxaca y descripción de la zona

de estudio.

## 3.1 El bosque mesófilo en el estado de Oaxaca

El bosque mesófilo de montaña o bosque de niebla es uno de los ecosistemas más frágiles e importantes a escala global (Hamilton et al. 1995) y en peligro de desaparición en México (Challenger 1998). Esta condición de riesgo es debida a que: a) requieren de condiciones climáticas que se presentan en zonas restringidas del país, b) están sometidos a fuertes presiones por las actividades humanas (agrícolas, extracción de madera, asentamientos humanos y de ganadería; Challenger 1998) y c) que actualmente presentan un incremento en la frecuencia de incendios forestales (Absjorsen et al. 2005, Gallardo et al. 2008). Paradójicamente, a pesar de que la extensión de los bosques mesófilo de montaña representan menos del 1% del área total de país, estos ecosistemas albergan entre el 10 y 12 % de la riqueza nacional de plantas, con un 30% de endemismos, esto lo convierte en el tipo de vegetación más diverso del país en función del área que ocupa (Rzedowski 1991, 1996, Challenger 1998). Además de esta gran biodiversidad, estos bosques proveen importantes servicios ambientales, juegan un papel fundamental en la regulación las cuencas hidrográficas. En el estado de Oaxaca dos ejemplos muy importantes de esta función lo constituyen las zonas de bosques mesófilos de la Chinantla alta, en los cuales se forman los afluentes primarios del río Papaloapan y los bosques mesófilos de los Chimalapas que son vitales para las cuencas de los ríos más importantes de esta región como el Usumacinta, Coatzacoalcos, Uxpanapa (en el Golfo de México), Ostuta y Espíritu Santo (vertiente pacífica).

En México el bosque mesófilo de montaña tiene una distribución fragmentada y ocupa una extensión de aproximadamente 17356 km<sup>2</sup>, que corresponde al 0.89% de la superficie total del país (Palacios- Prieto et al. 2000). De acuerdo con el Inventario Nacional Forestal del 2000 (Palacios- Prieto *et al.* 2000), el bosque mesófilo en Oaxaca ocupaba una superficie de 5190 km<sup>2</sup> (5.6%), siendo el segundo estado con mayor superficie de este tipo de vegetación, después de

Chiapas. En Oaxaca las comunidades de bosque mesófilo se ubican principalmente en las partes altas, templadas y húmedas de la vertiente norte de la Sierra Norte de Oaxaca (Sierra Juárez), en los distritos de Teotitlán, Cuicatlán, Ixtlán, Tuxtepec, Villa Alta, Mixe y Tehuantepec (Challenger 1998, Velázquez *et al.* 2001, Torres-Colín 2004). Por su parte, en la vertiente de la Sierra Madre del Sur se distinguen manchones de este tipo de vegetación en los distritos de Tlaxiaco, Juxtlahuaca, Putla, Jamiltepec, Juquila, Sola Vega, Miahuatlán y Pochutla. En la Sierra Atravesada también hay manchones en los distritos de Tehuantepec y Juchitán, sobre todo en la región de los Chimalapas (Challenger 1998, Velázquez *et al.* 2001, Asbjorsen *et al.* 2005, Gallardo *et al.* 2008). Es muy probable que las áreas con mayores extensiones continuas de este tipo de vegetación en el estado sean las ubicadas en la Sierra Norte de Oaxaca, dentro del denominado cordón de Zempoaltepelt (Challenger 1998). De acuerdo con la CONABIO, en la zona prioritaria de conservación Sierra Norte de Oaxaca-Mixe, el 20% de área total de esta zona (3876 km<sup>2</sup>) corresponde a comunidades de bosque mesófilo de montaña (Arriaga *et al.* 2000).

El estado de Oaxaca es uno de los estados de la republica mexicana más diversos en especies vegetales, hasta el 2004 se habían registrado 8,431 especies de flora vascular (García-Mendoza *et al.* 2004) que se desarrollan en los 26 tipos de vegetación que se describen para el estado (Torres Colín 2004). De manera general, se ha estimado que Oaxaca concentra el 40% de la flora del país y el 70% de los tipos de vegetación (García-Mendoza *et al.* 2004). Esta gran biodiversidad es producto de la compleja relación entre los factores ambientales, que a su vez son resultado de su compleja historia geológica, de la variación de su geomorfología, fisiografía, edafología y climas, que se presentan dentro del territorio oaxaqueño. Los bosques mesófilos de montaña son un tipo de vegetación muy relevante en Oaxaca, debido a que presentan la segunda área más importante

del país y en varias zonas su estatus de conservación es alto a lo largo de gradientes altitudinales continuos.

En la Tabla 1 se resumen las características de varios bosques montanos estudiados en el estado de Oaxaca. A pesar de que estos estudios se han realizado con distintas técnicas y utilizando diferentes tamaños de muestra es posible observar algunas tendencias generales de cambio a lo largo del gradiente altitudinal y que son compartidas con otros bosques montanos del trópico. De manera general, las propiedades de las comunidades montanas cambian fuertemente a través de gradientes altitudinales. La riqueza y estructura de los bosques montanos de Oaxaca muestran que las comunidades son muy heterogéneas dentro y entre sitios. A pesar de ello, la disminución de la riqueza conforme se incrementa la altitud se confirma en los sitios de Soyolapan y La Chinantla. En Soyolapan cambia de 55 especies arbóreas en 1750 m a 21 especies en 2750, mientras que en la Chinantla cambió de 52 especies en 1640 m a 22 especies en 2550 m. Aunque en la densidad y área basal no se encuentra una tendencia consistente de cambio lo largo de gradientes altitudinales donde se desarrollan estos bosques. De manera contraria, en los sitios de Chimalapas en un mismo piso altitudinal puede haber importantes variaciones en la composición y estructura de las comunidades montanas. En estos sitios los bosques que se desarrollan en suelos derivados de rocas metamórficas la riqueza, densidad y área basal es más alta que en las comunidades que se desarrollan en suelos derivados de rocas calizas (Gallardo et al. 2008).

Tabla 1. Características estructurales de distintos bosques montanos ubicados en las vertientes del Golfo de México y Pacífica en el estado de Oaxaca. PP= precipitación media anual, TM = tamaño de muestra, R = riqueza de especies, D = densidad y AB = área basal. sa = sin área, nd = no determinado.

Localidad	Referencia	Altitud	PP	ТМ	DAP	R	D	AB
		(m snm)	(mm año <sup>-1</sup> )	(ha)	(cm)		$(I ha^{-1})$	$(m^2 ha^{-1})$
Vertiente del Golfo								
Tiltepec	Arellanes (2000)	1640	> 4,000	1.0	3.3	52	1,738	42.7
Soyolapan	Boyle (1996)	1750	> 4,000	0.1	2.5	55	3,470	63.7
		1750	> 4,000	0.1	2.5	54	4,100	42.2
		2250		0.1	2.5	42	3,900	74.6
		2750	> 4,000	0.1	2.5	21	2,263	64.3
Puerto	Ruiz-Jiménez et	2250	2,100	s/a	3.18		2,716	41.8
Soledad	al. (2000)							97.8
Chinantla	Rincón (2008)	1640	> 5,000	0.1	2.5	52	3,290	70.3
		1830	> 5,000	0.1	2.5	45	2,910	60.0
		2010	> 5,000	0.1	2.5	40	4,300	44.3
		2260	> 5,000	0.1	2.5	27	2,210	62.7
		2430	> 5,000	0.1	2.5	36	8,320	49.3
		2560	> 5,000	0.1	2.5	22	1,610	72.4
Vertiente pacífica								
Chimalapas	Gallardo et al. (2008)	1750	n/d	0.27	10.0	49	689	51.0
		1750	n/d	0.27	10.0	30	500	33.0
Teipan	Mejía-	2400	> 1,400	1.0	2.5	39	1,035	46.41
	Domínguez							
	et al. (2006)							

Dentro de la Sierra Norte de Oaxaca se encuentra ubicada la región de La Chinantla, que corresponde a la porción de bosques húmedos que abarcan la mitad norte del distrito de Choapan, el noroeste de Villa Alta y la porción sur del distrito de Tuxtepec (Lipp 1969). Dentro de la Chinantla se estima que existen aproximadamente 58,073 ha de bosque mesófilo, siendo una de las zonas con mayores extensiones continuas de este tipo de vegetación, condición que cada día es menos común en otras regiones del país (Rincón 2007). Justamente en la porción más sureña del Distrito de Tuxtepec se localiza la población de Santa Cruz Tepetotutla, perteneciente a la Chinantla Alta, en cuyos terrenos se desarrollan bosques mesófilos con un alto nivel de

conservación en un gradiente altitudinal que va de los 1500 a los 2900 m snm. Dentro de los bosques mesófilos de Santa Cruz Tepetotutla se realizó el presente estudio (Fig. 1). En los siguientes apartados se presenta una descripción de las condiciones ambientales y de la vegetación de Santa Cruz Tepetotutla.

### 3.2 Descripción de la zona de estudio

El territorio de Santa Cruz Tepetotutla se encuentra ubicado dentro de la Sierra Juárez, en una unidad ecogeográfica clasificada como Sierra Alta Compleja (Anta y Mondragón 2006), tiene una extensión de 11,241 ha, con una variación altitudinal que va de los 800 a los 2900 m snm. Esta comunidad se encuentra situada en el extremo sureste del municipio de Usila, distrito de Tuxtepec. Sus límites correspondientes a la parte más alta coinciden con el parteaguas de la sierra, ubicado a una altitud aproximada de 2900 m. Sus terrenos forman parte de la cuenca del río Perfume, la cual está delimitada al sureste con la comunidad de La Nopalera, al suroeste con San Francisco la Reforma, hacia el norte con San Antonio del barrio, al noreste con San Pedro Tlatepusco y al noroeste con San Juan Zapotitlan. Esta zona está caracterizada por su alta heterogeneidad topográfica, laderas con fuertes pendientes que varían de 20 a 50° y una historia geológica muy compleja (López-Paniagua y Urbán-Lamadrid 1992).



Figura 1. Mapa de localización de los cinco sitios de estudio dentro de la cuenca del río Perfume, en los terrenos comunales de Santa Cruz Tepetotutla, Sierra Norte, municipio de Usila, Distrito de Tuxtepec, Oaxaca.

De manera general, los suelos de la zona de Santa Cruz Tepetotutla derivan de rocas metamórficas, esquistos micaceos, en las zonas altas, mientras que las zonas bajas derivan de areniscas y rocas calizas (López-Paniagua y Urbán-Lamadrid, 1992; van der Wal 1998). van der Wal (1998) describe algunas características edáficas para los suelos de las zonas ubicadas entre los 700 y 1400 m de Santa Cruz, que corresponde a oxisoles. Los suelos derivados de areniscas y metamórficas son ácidos (3.9 - 5.15 pH), pobres en bases y ricos en materia orgánica (5.94 -6.63 % de carbono), nitrógeno total (0.42 - 49 %) y fósforo (415 -874 ppm). Los suelos correspondientes a la zona de los bosques mesófilos de Santa Cruz Tepetotutla (1500 - 2550 m snm) han sido recientemente estudiados por Álvarez et al. (2008). Los tipos de suelos descritos para las zonas de la parte baja (1500 – 1940 m) son cambisoles fólicos (humic, hyperdystric) y para la zona alta (2040 – 2550 m) podzoles folic-stágnicos y stagnosoles fólicos en depresiones locales. Todos los suelos registrados son extremadamente ácidos (pH 2.7 - 4.7) y presentan un horizonte orgánico grueso en el suelo superficial. Los horizontes minerales de todos los suelos descritos son pobres en bases intercambiables y en las reservas totales, las bases están concentradas principalmente en la parte orgánica de suelo superficial. Algunas de las características que cambian a lo largo del gradiente altitudinal son el grosor del horizonte albico, la profundidad de la concentración máxima de Fe<sup>++</sup> y Al<sup>++</sup> extraíbles y la diferencia de arcillas entre los horizontes eluviales e iluviales; todas estas características se incrementa cuando aumenta la altitud. En los sitios altos, la composición de las arcillas es similar a la del material parental (cloritas y micas), con mezclas de estos minerales en proporción de 2:1, aunque en algunos suelos de la parte alta se presentaron otros minerales como la gibsita y caolinita. En los sitios medios y bajos la gibsita y caolinita fueron los minerales dominantes. Los principales procesos pedogenéticos registrados son la acumulación de humus, la intemperización in situ, la podzolización y la reducción de hierro debida a la saturación de fase mineral del suelo superficial.

Por su ubicación geográfica, en la zona de barlovento de la Sierra Norte, los terrenos de Santa Cruz Tepetotutla están expuestos directamente a las lluvias orográficas producidas por las masas

de aire húmedo provenientes de Golfo de México, lo que determina que sea una zona con precipitaciones muy altas que se han estimado por encima de los 4000 mm anuales (Rzedowski y Palacios-Chávez 1977, van der Wal 1998). Desafortunadamente no existen datos climáticos precisos para la zona, por lo que solo se han podido realizar estimaciones del clima a partir de la distribución de la vegetación o través de datos de estaciones meteorológicas cercanas a la zona de estudio. En los terrenos de Santa Cruz Tepetotutla se presenta la transición de los climas cálidos en las zonas bajas a los climas semicálidos y templados hacia las zonas altas, debido a la gran variación altitudinal que se presenta (Rincón 2007). Para las áreas por debajo de los 1400 m se estima que el clima corresponde a Af(m)(e) y por encima de estas altura se presentan climas Cm o C(fm)b(i) (Rzedowski y Palacios-Chávez 1977, van der Wal 1998). Una buena aproximación al clima de Santa Cruz Tepetotutla (para las zonas ubicadas por encima de los 1400 m) se encuentra en los datos climáticos registrados en la estación meteorológica de Vista Hermosa ubicada a 1440 m (Rzedowski y Palacios-Chávez 1977). La precipitación media anual registrada en esta estación fue de aproximadamente 5800 mm y la temperatura media anual es de 16.7°C. El clima es templado de transición, con lluvias todo el año, precipitación del mes más seco del año del orden de 40 mm, y oscilación anual de las temperaturas mensuales menor de 5°C, de acuerdo con la clasificación de Köppen modificada por García (1973) correspondería a C(fm)b(i).

La temperatura en los cinco sitios seleccionados para este estudio ubicados entre los 1520 y los 2550 m snm se midió entre septiembre de 2004 de octubre de 2005. Las mediciones muestran que la temperatura promedio anual decrece de 15.3°C a 11.5°C de 1520 a 2550 m, respectivamente. La tasa de decremento fue de 0.4° por cada 100 m de incremento en altitud. Esta tasa de cambio es más baja de las observadas para otros bosques montanos del trópico que se ubican entre 0.5 - 0.8°C (Ohsawa *et al.* 1985, Kitayama 1995, Kappelle 1996).

La presencia de niebla en la zona de estudio es constante y puede tener importantes repercusiones en la precipitación horizontal, condensación de agua en los tallos y follaje, y el nivel lumínico en que se desarrollan las comunidades vegetales. La precipitación horizontal es una aportación muy significativa de agua en los bosques montanos (Standmüller 1987). En la estación meteorológica de Vista Hermosa se determinó que el promedio de días despejados al año es menor de 50 días (Rzedowski y Palacios-Chávez 1977).

### 3.3 Vegetación de Santa Cruz Tepetotutla

La comunidad de Santa Cruz Tepetotutla junto con las comunidades de San Antonio de El Barrio, Santiago Tlatepusco, San Pedro Tlatepusco, San Antonio Analco (comunidades del municipio de San Felipe Usila) y Nopalera del Rosario (municipio de San Juan Bautista Valle Nacional), integran el Comité de Recursos Naturales de la Región de la Chinantla Alta (CORENCHI A.C.). Uno de los objetivos más importante de la CORENCHI es la conservación de la biodiversidad y el uso racional de sus recursos naturales, utilizando como herramienta de planeación los ordenamientos territoriales comunitarios (Pérez *et al.* 2006). Entre los resultados más importantes de este comité se encuentra el establecimiento de una estrategia conjunta para la conservación de un macizo forestal de 20000 ha, que comprenden bosques mesófilos y selvas altas de montaña a lo largo de un gradiente altitudinal que va de los 200 a los 2900 m snm (Pérez *et al.* 2006).

De acuerdo con el ordenamiento territorial de Santa Cruz Tepetotutla, realizado por ERA (2000), del total de la superficie, el 77% está cubierto por vegetación primaria. El bosque

mesófilo de montaña ocupa una extensión de 6995.98 ha, que corresponden a un 62.2% de la superficie total de Santa Cruz, seguido de los bosques de encinos con una extensión de 951.1 ha (8.5%), bosque de *Pinus chiapensis* (que podría ser considerado como una de las comunidades que integran el bosque mesófilo) con 544.89 ha (4.8%) y por último la selva mediana perennifolia con 209.59 ha (1.9%). El 23% restante de la superficie de Santa Cruz está integrada por zonas de vegetación secundaria, cafetales, zonas de producción agrícola, potreros y la zona urbana.

La vegetación de Santa Cruz Tepetotutla ha sido la más estudiada, desde distintos enfoques, del área que comprende la zona de conservación de la CORENCHI (Meave et al. 1994, Meave et al. 1996, Gallardo y Meave 1998, van der Wal 1998, Romero-Romero et al. 2000, Velázquez-Rosas 2000, Velázquez-Rosas et al. 2002, Meave et al. 2006, Rincón 2007). En el trabajo de Rincón (2007) se presenta un esquema de clasificación para la vegetación de la microcuenca del río Perfume, basado en un detallado análisis de la composición y estructura de nueve comunidades arbóreas (en este estudio se consideraron todos los individuos con un diámetro a la altura del pecho  $\geq 2.5$  cm), a lo largo de un gradiente de altitudinal que va de los 1120 a los 2560 m snm. De acuerdo con Rincón (2007), las comunidades ubicadas por debajo de los 1500 m son consideradas como bosque premontano, mientras que las comunidades ubicadas entre los 1500 y 2300 m corresponden a bosques montanos bajos y por encima de 2300 m bosque montanos altos. Esta propuesta de Rincón (2007) coincide con los resultados de Velázquez-Rosas et al. (2002) quienes a partir de características morfológicas y anatómicas de las hojas de los árboles más abundantes encontraron una diferenciación dentro los bosques montanos altos y bajos aproximadamente entre los 2300 y 2400 m snm.

En las zonas bajas de Santa Cruz (580 – 1200 m snm) se han realizado estudios de los patrones de sucesión secundaria derivados de las prácticas agrícolas de roza tumba y quema en las selvas

alta perennifolias y en las selvas medianas de montaña (van der Wal 1998, Romero-Romero 1999, Romero-Romero *et al.* 2000). En este último estudio se registraron 493 especies vegetales. Aunque de manera general, los patrones sucesionales registrados en estas áreas se ajustan a los modelos teóricos clásicos, como la disminución del área basal, la densidad y la cobertura y la dominancia conforme avanza el proceso sucesional (van der Wal 1998), los autores observaron algunas diferencias importantes, la riqueza específica no muestra un cambio consistente a través del proceso sucesional (Romero-Romero 1999).

Los estudios de la vegetación húmeda de montaña de Santa Cruz revelan que estas comunidades vegetales son muy heterogéneas en su fisonomía, estructura, composición y atributos foliares a lo largo del gradiente atitudinal en el que se desarrollan (Meave et al. 1994, Velázquez-Rosas et al. 2002, Rincón 2007), siendo este su principal eje de variación tal como se ha distinguido en otros bosques húmedos de montaña (Vázquez-G. y Givnish 1998), lo que sugiere fuertemente que la variación ambiental (clima, características edáficas, influencia de la niebla y las nubes) asociada al cambio de altitud es un factor determinante de estos cambios. La variación de estas comunidades a lo largo del gradiente altitudinal, aproximadamente de 1500 m, resulta muy impresionante ya que se presenta en una distancia horizontal muy corta, una situación prácticamente excepcional en el país. Esto hace de los bosques mesófilos de Santa Cruz un lugar ideal para estudiar la influencia de la variación ambiental sobre las respuestas funcionales de estos bosques y su influencia en la variación de los atributos de la vegetación. Además, la presencia de algunas especies con una historia biogeográfica importante como Cyrilla racemiflora, Ticodendron incognitum y Oreomunnea mexicana realzan la importancia de estos bosques para entender la compleja historia de los bosques húmedos del trópico (Meave et al. 1996, Gallardo et al. 1998).

Para la zona de los bosques húmedos de montaña de Santa Cruz, Rincón (2007) reporta 208 morfoespecies, pertenecientes a 103 géneros y 63 familias. Sin embargo, probablemente la riqueza específica sea más alta (para plantas con un dap  $\geq$ 2.5 cm), ya que sus análisis de acumulación de especies en ninguno de lo sitios muestreados logró alcanzar una asíntota, es decir el total de especies por sitio. La familia Lauraceae es la que presenta el mayor número de especies, otras familias importantes fueron Ericaceae, Cletraceae y Theaceae. De acuerdo con este análisis, la riqueza específica de las comunidades decrece conforme se aumenta la altitud, mientras que la diversidad, el área basal y la densidad de individuos no mostraron una tendencia de cambio consistente a lo largo del gradiente altitudinal.

### 3.4 Sitios de estudio y especies seleccionadas

En la zona de bosques mesófilos de montaña de Santa Cruz Tepetotutla se seleccionaron cinco sitios en función de las especies estudiadas. Los sitios estuvieron ubicados entre los 1520 y 2550 m snm (Fig. 1), los sitios localizados por debajo de los 2300 m correspondieron a bosques montanos bajos y los que se localizan por encima de esta altitud a bosques montanos altos, de acuerdo con la propuesta de clasificación establecida por Rincón (2007). Las especies de estudio fueron seleccionadas de acuerdo con sus intervalos de distribución, que estuvieron basados en la revisión de trabajos previos realizados en la zona y por recorridos en campo. En general, se trato de encontrar especies con intervalos amplios y estrechos de distribución altitudinal, dentro de los bosques montanos. Esto se realizó con el propósito de contrastar sus respuestas ecofisiológicas. Las especies seleccionadas fueron: *Vaccinium consanguineum* Klotzsch (Ericaceae), *Drymis granadensis* L. F. (Winteraceae), *Podocarpus matudae* Lundell var. *macrocarpus* (Podocarpaceae) y *Ticodendron incognitum* Gómez Laur. & L. D. Gómez (Ticodendraceae). Los

principales criterios para la selección de estas especies fueron: 1) que presentaran una distribución altitudinal amplia o restringida dentro de la zona de estudio, 2) que fueran especies importantes en las comunidades donde se desarrollan, 3) que sus poblaciones fueran suficientemente grandes para realización del estudio y 4) que estuvieran ubicadas en sitios accesibles con exposición similar (Norte) dentro de la cuenca del río Perfume. Para el caso de V. consaguineum y P. matudae se seleccionaron tres sitios de estudio; ubicados en las siguientes altitudes: 1520, 2040 y 2550 m; 1520, 1940 y 2380 m, respectivamente. Para D. granadensis y T. incognitum sólo se seleccionaron dos sitios de estudio para cada especie. A pesar de que D. granadensis presenta una amplia distribución altitudinal (1640 - 2560 m snm) se decidió elegir sólo dos sitios porque en las partes bajas presenta densidades muy bajas. Los sitios para D. granadensis se ubicaron a 1940 y 2380 m, mientras que para T. incognitum se localizaron a 1520 y 1940 m. En la Tabla 2 se presentan valores de densidad y área basal para las cuatro especies de estudio, en cada uno de los sitios donde fueron colectadas. Cada especie muestra un patrón particular de variación altitudinal. En el caso de T. incognitum la densidad y área basal disminuyen conforme se incrementa la altitud, de manera contraria en D. granadensis ambas variables aumentan con la altitud. Para P. matudae los valores más altos de densidad y área basal se registran en el sitio de 1940 m. Por último, en V. consanguineum la densidad más alta se registra a 2550 m y el valor de área basal más alto se registró en 2040 m.

Tabla 2. Valores estructurales de *Ticodendron incognitum*, *Drimys granadensis*, *Podocarpus matuda*e var. *macrocarpus* y *Vaccinium consanguineum* en los sitios de estudio para cada especie. Los valores fueron determinados para individuos con un diámetro  $\geq 10$  cm a la altura del pecho.

Especies y altitud (m snm)	Densidad (ind. ha <sup>-1</sup> )	Área basal (m <sup>2</sup> ha <sup>-1</sup> )
Ticodendron incognitum		
1520	370	7.2
1940	60	4.4
Drimys granadensis		
1940	50	0.4
2380	150	0.6
Podocarpus matudae var.		
macrocarpus		
1520	30	0.3
1940	50	0.4
2380	40	0.2
Vaccinium consaguineum		
1520	40	0.6
2040	30	1.6
2550	60	1.1

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# Capítulo 4

# Soil nitrogen pools and tree community features in a montane cloud

## forest along an altitudinal gradient in Mexico

Soil nitrogen pools and tree community features in a montane cloud forest along an altitudinal gradient in Mexico ‡

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### 4.1 ABSTRACT

Nutrient shortages, in particular soil nitrogen (N) has been thought to be a limiting factor for productivity in montane cloud forests (MCFs). Specifically, available nitrogen could have a strong influence on plant growth. We determined the altitudinal patterns of total and available N in the organic horizon (Oe) of a Mexican MCFs soil and their relationship to the vegetation's structural traits (density and basal area), diversity, above-ground biomass, N and C:N ratio in the leaves of four tree species. The study was conducted at five sites (1520, 1940, 2040, 2380 and 2550 m asl). Plant communities at these sites are heterogeneous in terms in composition and structure along altitudinal gradient. Total soil N (118.93–259.59 kg ha<sup>-1</sup>) and ammonium (0.14– 0.31 kg ha<sup>-1</sup>) increased with elevation. In opposite, potentially mineralizable organic N decreased with elevation (14  $10^{-5}$ -59  $10^{-5}$  kg ha<sup>-1</sup>). Nitrates showed no changes (0.068– 0.087 kg ha<sup>-1</sup>). At all sites, there was larger ammonium than nitrates stocks. The altitudinal changes in pools of N in the Oe horizon might be related with adverse environmental conditions such as waterlogging and the lower temperatures commonly occurring in MCFs at high altitudes. Diversity and structural vegetation traits did not show any linear change along the altitudinal gradient, only tree density showed a negative relationship with total N. For the four tree species the mean values of foliar N ranged from 13.8 to 21.1 mg  $g^{-1}$ , and the C:N leaf ratios were > 24:1. Foliar N measurements suggest a shortage of available N and were related to low levels of available N in the Oe horizon, although the species responded differently to growth-limitation.

**Keywords:** ammonium, available nitrogen, foliar nitrogen, nitrate, organic horizon, tree diversity, structural vegetation traits

### **4.2 INTRODUCTION**

Montane cloud forests (MCFs) are second only to tropical rain forests in terms of the diversity of plant species, yet they are significantly less studied than all lowland forests (Webster 1995). Although significant efforts have been made to describe the soils of MCFs, there much to be learned about nutrient availability and the cycling of biogenic elements in this ecosystem (Vitousek 1984, Bruijnzeel & Proctor 1995, Tanner et al. 1998). Soil observations suggest that the reserves of available soil nutrients are limited in higher elevations, a condition associated with high soil moisture content, low temperatures and high acidity (Schrumpf et al. 2001, Wilcke et al. 2008, Roman & Scatena in press). In particular, N is a key limiting factor for productivity of these forests along altitudinal gradients (Vitousek 1984, Tanner et al. 1998). Recently it has been suggested that other macro and micronutrients (P, S, K, Mn, Zn, and Ca) can also have a great influence on MCFs productivity (Wilcke et al. 2002, 2008 Soethe et al. 2008). It has also been suggested that Al, Fe and Mn toxicity might limit the productivity of MCFs because these elements affects N availability (Bruinjzeel & Proctor 1995). Phenolic substances in leaves might play an important role in nutrient cycling in MCFs because, among others, polyphenols affects negatively total soil N (Bruinjzeel et al. 1993).

Previous studies have emphasized an increase in total N content with altitude. Nevertheless available mineral forms tend to decrease (Bruijnzeel & Proctor 1995, Schrumpf et al. 2001, Roman & Scatena in press). However, in Jamaica it was found that the N content of organic litter decreases with altitude, having low values at 1500 m (Tanner et al. 1998). Furthermore, in MCFs at Monte Kinabalu, Borneo no differences between sites at high and low altitudes were recorded regarding total soil N (Kitayama 1992). These studies are difficult to evaluate, because authors do

not specify which soil horizons were studied (Roman & Scatena in press). In many MCFs, especially at the highest elevations, tree roots are distributed principally in the organic layer, presumably because unfavorable conditions in the mineral soil, such as oxygen deficiency (Chiu et al. 1999, Hertel et al. 2003, Soethe et al. 2006, Álvarez et al. 2008). Therefore concentration of nutrients in organic horizons, particularly of available nitrogen forms, could have a strong influence on plant growth (Soethe et al. 2008, Wilcke et al. 2008). Organic horizons generally have 6 and 20 times higher concentration of total N than the mineral horizons (Wilcke et al. 2008). The importance of specifying the soil horizon has been demonstrated in studies along altitudinal gradients carried out in Ecuador (Wilcke et al. 2002, 2008; Soethe et al. 2008). For instance, Wilcke et al. (2002) found that nutrient concentrations were lower at higher elevations in the organic horizons, due to lower mineralization rates. Low nutrient levels were correlated with low productivity in these forests (Wilcke et al. 2008) and with low foliar nutrient concentrations (Soethe et al. 2008). These studies point to the need of further studying pools of soil-available N and their effect on plant community traits along altitudinal gradients.

In this study we recorded the altitudinal distribution of total and mineral N forms (NO<sub>3</sub>-N and NH<sub>4</sub>-N) in the soil's organic horizons and their relationship with some tree and foliar traits in MCFs of Sierra Juárez Mountains, Mexico. We hypothesize that with increasing altitude, the total nitrogen (N) will increase in Oe horizon (intermediately decomposed organic matter), whereas potentially mineralizable nitrogen ( $N_{min}$ ), ammonium (NH<sub>4</sub>-N) and nitrates (NO<sub>3</sub>-N) will decrease. Altitudinal changes of these variables will also be associated with changes in above ground tree biomass, diversity, leaf N concentrations and foliar C:N ratios. To test these hypotheses, in an elevational gradient, we determined the stocks of total N, N<sub>min</sub>, NO<sub>3</sub>-N and NH<sub>4</sub>-N, in Oe horizons of five sites located in a range from 1520 to 2550 m above sea level

(masl). We also determined the species diversity, basal area, density, as well as above-ground biomass in trees with a diameter greater than 10 cm at 1.3 m above soil level (DBH). Nitrogen concentrations and C:N ratios were evaluated in the leaves of four of the tree species selected along the altitudinal gradient: *Vaccinium consanguineum* Klotzsch and *Podocarpus* Lundell *matudae* var. *macrocarpus* J., *Ticodendron incognitum* Gómez Laur. & L. D. Gómez and *Drimys granadensis* L. F.

### 4.3 METHODS

### 4.3.1 Study site

This study was carried out in Santa Cruz Tepetotutla, a small village located in the catchment basin of the Perfume River on the Atlantic slope of the Sierra Juárez Mountains, Tuxtepec district, Oaxaca, Mexico  $(17^{\circ}38' - 17^{\circ}40' \text{ N})$ , and  $96^{\circ}32'-96^{\circ}33' \text{ W})$ . The study area belongs to the physiographic region of the Sierra Madre del Sur. The lithology of the study area consists mainly in mica-chlorite schists (van der Wal 1996). The region's topography is complex, with slopes ranging between  $10^{\circ}$  and  $50^{\circ}$ . The climate of this area is classified as hyper-humid, transitional temperate. Mean annual total precipitation is ca. 5800 mm, with precipitation greater than 50 mm in the driest month of the year. The mean annual temperature is  $16.5^{\circ}$ C (Rzedowski & Palacios-Chávez 1977). Temperature data recorded at the study sites showed that mean temperature decreases from  $15.3 \,^{\circ}$ C to  $11.5 \,^{\circ}$ C in the altitudinal interval from 1520 to 2550 (Álvarez et al. 2008). Temperature decreases at a rate of  $0.4 \,^{\circ}$ C each 100 m in elevation; this rate is lower than rates recorded in others cloud forests,  $0.5 - 0.8 \,^{\circ}$ C (Kappelle 1996).Vegetation along the elevational gradient consists in a mosaic of undisturbed forest communities growing above 1100 m asl, which are classified as premontane ( $1120 - 1500 \,^{\circ}$ ), lower montane ( $1500-2400 \,^{\circ}$ ) and

upper montane forests (over 2400 m) (Rincón 2007). Álvarez et al. (2008) describe the soils in the study area in an altitudinal sequence (1500 - 2550 m asl). Soils are ferrous chlorite shale. The main soil type in lower elevations (1500 - 1950 m asl) is Folic Cambisol, while in upper elevations (2050-2550 m asl) are Folic Stagnic Podzol and Folic Stagnosol. These soils are acidic (2.7-3.5 pH [H<sub>2</sub>O]) and have a thick organic surface horizon (Álvarez et al. 2008). The mineral horizons of all five soils are low in both exchangeable and total base reserves, which are concentrated mainly in the organic horizons. Acidic, base-poor soils characterize the entire ecosystem and do not display an altitudinal trend. As altitude increases, the thickness of albic horizons, the depth of the maximum acid-oxalate-extractable Fe and Al concentrations, and the difference in clay content between the eluvial and iluvial horizons also increase (Álvarez et al. 2008).

### 4.3.2 Structural traits, above-ground biomass and tree diversity

In this study, N pools and structural traits of tree communities were studied in plots placed at the following altitudes 1520, 1940, 2040, 2380, and 2550 m. We selected these sites because they had heterogeneous communities in terms of its composition and structure; and because they exposed the main trends change of MCFs in this area along the altitudinal gradient (Rincón 2007, Velázquez-Rosas et al. 2002). At each study site we established 0.1 ha plot, each plot was divided in ten 2 x 50 m sub-plots (sub-plots were separated one from the other by 10 m). Tree density, basal area, and above-ground biomass were determined for trees > 10 cm DBH. Above-ground biomass was estimate according to Tanner's equation (1980):

 $\log Y = -1.71 + 1.16 \log X$
(where Y = above-ground biomass, and X = basal area). Tree diversity was calculated with the Shannon index (Magurran 2004).

#### 4.3.3 Total and available mineral forms of N at Oe horizon

To assess total N, NO<sub>3</sub>-N, NH<sub>4</sub>-N and N<sub>min</sub>, samples were collected from the Oe horizon after removing the litter layer (Oi horizon) from the soil surface. Soil was collected from five points along a vegetational sub-plot (at 5, 15, 25, 35 and 45 m). Subsamples were collected from the soil layer 5–10 cm depth. However, when the lower limit of the Oe horizon was less than 10 cm deep, the sample was collected from a layer of 5-8 or 5-9 cm depth, care being taken to not mix the sample with the mineral soil. The five subsamples were mixed in order to obtain one composite sample per subplot (only five composite samples were analyzed per plot and elevation). After collection, the samples were kept at 4°C until they were processed. Total N concentration was estimated using the Kjeldahl method (Bremner & Mulvaney 1982) using fresh non-dehydrated samples. Total N was extracted for digestion from 1 g soil samples, which had been previously sifted with a 2 mm sieve, had  $K_2SO_4$ - catalyst mixture and 3 ml of  $H_2SO_4$  added to them, and had been heated in a flask on the digestion stand. After digestion was complete (2 hour), the flask was allowed to cool, and about 200 ml of water and 75 ml of NaOH 32% was added. The amount of NH<sub>4</sub>-N released was assessed in a solution of boric acid and an indicator solution by titration with sulphuric acid  $(H_2SO_4) 0.01M$ .

Mineral nitrogen forms (NO<sub>3</sub>-N and NH<sub>4</sub>-N) were determined by Magnesium oxide-Devarda's alloy method (Keeney and Nelson 1982) using fresh non-dehydrated samples. NH<sub>4</sub>-N was extracted from 10 g samples, previously sieved in a 2 mm sieve to remove coarse organic waste, using a solution 2N KCl and 0.1 g of MgO. The amount of NH<sub>4</sub>-N released was assessed in a

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solution of boric acid and an indicator solution by titration with sulphuric acid 0.005N H<sub>2</sub>SO<sub>4</sub>. After removal of NH<sub>4</sub>-N, we added 0.2 g of Devards Alloy and determined the amount of NO<sub>3</sub>-N released in the distillate by titration with 0.005N H<sub>2</sub>SO<sub>4</sub> (Kenney & Nelson 1982). A rapid steam distillation method of assessing N<sub>min</sub> in soil involves the determination of the ammonia-N produced by steam distillation of the soil sample with pH 11.2 phosphate-borate buffer (Gianello & Bremer 1988). The NH<sub>3</sub>-N produced was trapped with a solution of H<sub>3</sub>BO<sub>4</sub> (2%) and evaluated by titration using H<sub>2</sub>SO<sub>4</sub> (0.005N). To estimate nutrient stocks in soil, average bulk densities of Oe horizon were used (Alvarez et al. 2008).

#### 4.3.4 N<sub>l</sub> and C:N leaf ratios

The concentrations of leaf carbon (C),  $N_1$  and C:N ratios were determined in the four selected species along the altitudinal gradient. The species selected are dominant in several of the tree communities' sampled (Rincón 2007). Species were collected at elevations where they were abundant. Two species were sampled in a narrow altitudinal range. *T. incognitum* was sampled at 1520 and 1940 m asl, *D. granadensis* at 1940 and 2380 m, and two other species in a wider altitudinal range: *P. matudae* var. *macrocarpus* at 1520, 1940 and 2040 and *V. consanguineum* at 1520, 2040 and 2550 m asl, depending on tree accessibility and density. For each species, five individuals were selected at each elevation. For each individual, we collected 10 mature leaves from the upper canopy. The leaves were dried, grounded, and mixed to form one composite sample for each individual. The carbon and nitrogen content of the leaves, from the five composite samples of 13 µg, per elevation and per species were quantified with an Elemental Analyzer (NC 2500, Thermo Quest S.P.A., Rodanao, Milan, Italy). The equipment was calibrated

with a standard curve made with sulfanilamide (C, 41.84%; H, 4.68%; N, 16.27%; O, 18.58% and S, 18.62%) (CE Elantech, Inc., Lakewood, N.J., USA).

## 4.3.5 Statistical analysis

The mean values for N, N<sub>min</sub>, NH<sub>4</sub>-N, NO<sub>3</sub>-N, N<sub>1</sub> and C:N leaf ratio were compared among elevations with an one-way ANOVA; Post hoc tests (Tukey's test) were used to test differences between pairs of means. The homogeneity of variances was confirmed by Bartlett's test. Regression analysis between elevation values and N, N<sub>min</sub>, NH<sub>4</sub>-N, NO<sub>3</sub>-N, community traits (diversity and structural traits); and between N soils pools in Oe horizon (N, N<sub>min</sub>, NH<sub>4</sub>-N, NO<sub>3</sub>-N) community traits (diversity and structural traits) and leaf traits (N<sub>1</sub> and C:N leaf ratios) were performed using Table Curve 2D, v. 3 (AISN Software, Chicago, IL, USA).

# **4.4 RESULTS**

## 4.4.1 Total and mineral nitrogen stocks in Oe horizons

Total N ranged between 0.7– 4.4% in Oe horizons in all five sites. Total mean N values increased linearly with altitude, from 118.93 to 259.59 kg ha<sup>-1</sup>. Significant lower values were found at 1520 m (Fig. 1A;  $F_{4,20} = 4.58$ ; P = 0.0087). The N<sub>min</sub> only showed a tendency to decrease as elevation increased (Fig. 1B). The highest mean value was recorded at 1940 m (59 10<sup>-5</sup> kg ha<sup>-1</sup>), however it was only significantly higher than mean values for the two highest sites ( $F_{4,20} = 3.51$ ; P = 0.027), at 2380 and 2550 m (14 10<sup>-5</sup> and 15 10<sup>-5</sup> kg ha<sup>-1</sup>, respectively). Mean NH<sub>4</sub>-N values increased linearly with elevation (Fig. 1C). The significantly highest NH<sub>4</sub>-N mean value was recorded at 2550 m (0.31kg ha<sup>-1</sup>; ( $F_{4,20} = 8.713$ ; P = 0.00002). In contrast, for NO<sub>3</sub>-N stocks, no significant changes between elevations were found (Fig. 1D); mean values ranged from 0.068 to 0.087 kg ha<sup>-1</sup>. In all sites, NH<sub>4</sub>-N stocks were 1.8–4.1 times higher than NO<sub>3</sub>-N.



Figure 1. Mean values ( $\pm$ SE) for: A) total nitrogen (N), B) potentially mineralizable organic nitrogen (N<sub>min</sub>), C) ammonium (NH<sub>4</sub>-N) and D) nitrates (NO<sub>3</sub>-N), in Oe horizons along an altitudinal gradient. Letters indicate significant differences (p< 0.05) between elevations.

4.4.2 Diversity and structural traits of tree communities,  $N_l$  and C:N leaf ratios along the elevational gradient and their relationship with total and mineral N pools.

The diversity, density, basal area, and above ground tree biomass are summarized in Table 1. Diversity and structural measurements did not show linear changes along the altitudinal gradient. The highest diversity values were recorded at 1940 and 2040 m (2.54 and 2.58, respectively). Tree density was higher only at 1520 m. Tree basal area and above-ground biomass had the highest values at 1520 and 2550 m. There was no significant relationship between the structural traits recorded and total N and its available forms. The exception was tree density, which showed a negative relationship with total soil N ( $r^2 = 0.80$ , n = 5, P = 0.039).

Mean values for N<sub>1</sub> recorded for the four species ranged from 12.6 to 22.9 mg m<sup>-2</sup> (Table 2). The lowest values were recorded in *Vaccinium*. Elevational changes were different for the four species. For *Ticodendron* and *Drimys* the N<sub>1</sub> decreased with increasing altitude. However there were no significant differences between elevations. Contrary for *Vaccinium* the mean values of N<sub>1</sub> increased with elevation, and significant higher values were found on the site of 2550 m (F<sub>2,25</sub> = 4.794; P = 0.0172). In *Podocarpus* the highest value for N<sub>1</sub> was recorded at 1940 m (F<sub>2,25</sub> = 6.118; P = 0.0068). The C:N ratios recorded in the four species ranged from 24.9:1 to 42.4:1. Elevational changes of C:N was opposite to N<sub>1</sub> for all species, in the sites where N<sub>1</sub> values were high the C:N ratio was low (Table 2).

Results of the relationships between leaf traits and N stocks of the Oe horizon are summarized in Table 3. For *Vaccinium* and *Podocarpus* the elevational changes of  $N_1$  and the foliar ratios C: N were significantly related to N and its mineral forms. For *Podocarpus*, the N and  $N_{min}$  were related positively with  $N_1$  and C:N ratios. Similarly, for *Vaccinium* total N, NH<sub>4</sub>-N and NO<sub>3</sub>-N were positively related with  $N_l$ , while the C:N ratio was correlated to  $N_{min}$ . For both species, the relationship of C:N ratios with N and  $N_{min}$  were negative.

Elevation (m asl)	Diversity H'	Density (ind ha <sup>-1</sup> )	Basal area (m <sup>2</sup> ha <sup>-1</sup> )	Above ground biomass (t ha <sup>-1</sup> )	Species
1520	2.45	1210	57.9	316.3	Cyrilla racemiflora L., Ticodendron incognitum Gómez-Laurito & L. D. Gómez, Pinus chiapensis (Martínez) Andresen, Podocarpus matudae Lundell. Zinowiewia sp. Oreomunnag maxiagna (Standl)
1940	2.54	670	56.2	297.0	Coreomannea mexicana (Standi,) Leroy, Ticodendron incognitum Gómez-Laur. & L.D. Gómez., Vaccinium consanguineum Klotzsch, Podocarpus matudae Lundell and Myrsine juergensenii (Mez). Tinowinwia cp. Clethra consenttiana
2040	2.58	930	38.6	200.6	Linowiewia sp., Clethra conzantilana L.M.González, Myrsine juergensenii (Mez) Lundell., Vaccinium consanguineum Klotzsch and Weinmannia pinnata L.
2380	2.47	1030	25.5	112.0	Weinmannia tuerckheimii Engl., Viburnum acutifolium Benth., Symplocos vernicosa L.O. Williams and Ternstroemia oocarpa (Rose) Melch.
2550	2.43	800	69.5	414.1	<i>Quercus ocotetfolia</i> Liebm., Clethra galeottiana Briq., Persea chanissonis Vel. Alt., Ternstroemia oocarpa (Rose) Melch., Cleyera integrifolia (Benth.) Choisy, and Weinmannia tuerckheimii Engl.

 Table 1. Diversity (Shannon index), structural traits and above ground tree biomass in five tree communities in a montane cloud forest growing along an altitudinal in Mexico.

Table 2. Mean values ( $\pm$ SE) of leaf nitrogen (N<sub>1</sub>) and C:N leaf ratios for *Ticodendron incognitum*, *Drimys granadensis*, *Podocarpus matudae* var. *macrocapus* and *Vaccinium consanguineum*. Different letters indicate significant differences between elevations (P < 0.05).

	Elevation	N <sub>1</sub>	
Species	(m asl)	$(\text{mg g}^{-1})$	C:N
T. incognitum	1520	22.9 (0.8)	24.9:1 (0.8)
	1940	21.1 (0.6)	26.1:1 (0.7)
D. granadensis	1940	19.1 (0.8)	31.3:1 (1.1)
	2380	17.9 (0.8)	33.6:1 (1.7)
P. matudae	1520	17.3 (0.4)b	31.8:1 (0.9)a
	1940	20.5 (0.9)a	27.9:1 (1.0)b
	2380	17.9 (0.5)b	30.4:1 (1.0)ab
V. consanguineum	1520	12.6 (0.5)b	39.2:1 (1.3)
	2040	13.8 (0.4)ab	42.4:1 (1.1)
	2550	14.6 (0.4)a	39.4:1 (1.2)

Table 3. Results of the regression analysis carried out to determine the relationships between N soils pools in Oe horizon and leaf traits (N leaf and C:N leaf ratios). Data were fitted to the function y = a + bx and in all the cases the probability was < 0.05, N soil stocks were considered as independent variables. Nitrogen total in Oe horizon = N, nitrates = NO<sub>3</sub>-N, ammonium = NH<sub>4</sub>-N, potentially mineralizable organic nitrogen = N<sub>min</sub>, N foliar = N<sub>1</sub>, carbon:nitrogen foliar ratios = C:N.

Relationship	$R^2$	F	Df	b	а	
V. consanguineum						
N vs. $N_1$	0.46	24.2	29	0.14	11.01	
N <sub>min</sub> vs. C:N	0.36	16.4	29	-211.23	46.1	
$NH_4$ -N vs. $N_1$	0.49	27.9	29	0.07	12.3	
$NO_3$ -N vs. $N_1$	0.40	18.9	29	0.83	7.5	
P. matudae						
N vs. $N_1$	0.62	46.6	29	0.17	15.1	
N vs. C:N	0.53	31.6	29	-0.27	35.3	
$N_{\min} vs. N_1$	0.40	18.89	29	71.58	16.15	
N <sub>min</sub> vs. C:N	0.43	21.35	29	-78.81	33.08	

#### 4.5 DISCUSSION

# 4.5.1 Total and mineral forms of N concentrations in Oe horizon: elevational distribution

Our results showed a linear increase of N with altitude, and relatively high total N in the organic horizon 0.7–4.4%. Our values are similar to those recorded by Wilke et al. (2002) in Ecuador (2.0 to 2.6 %) and to the values reported by Roman & Scatena (in press). The increase in total N results from the accumulation of organic matter due to waterlogging conditions and low temperatures that inhibit the decomposition of organic matter at higher elevations (Marrs et al.

1988, Tanner et al. 1998, Schrumpf et al. 2001, Wilcke et al. 2002). In addition, in these studies carried out in MCFs, total N shows a wide variability, ranging from 0.21 to 2.6%. However, in most studies total N is determined in samples of mixed horizons (organic and mineral horizons), which makes comparisons across studies and sites difficult. Our results suggest that along the gradient the Oe horizons are relatively rich in total N and therefore N might not be a limiting forest productivity, which is also suggested by the low soil C:N (13:1–15.7) ratios recorded previously for this area (García-Calderón et al. 2005). The C:N ratios were also lower than values recorded for organic horizons in Ecuador (19:1–44:1) (Soethe et al. 2008, Wilcke et al. 2008).

Albeit MCFs soils contain large quantities of organic N, most of it is present in forms that either because resistant to microbial degradation of its chemical make-up or because it is fixed in the soil. Only in small portion is mobile, playing a prominent role as a substrate for N mineralization (Wilcke et al. 2008). In MCFs the presence of sclerophyllous species increase the recalcitrant lignin in the organic litter, that as well as the high concentrations of phenolic compounds, reduce the N<sub>min</sub> in soils (Bruinjzeel et al. 1993, Hättenschwiler & Vitousek 2000, Wilcke et al. 2008). In our study the N<sub>min</sub> decreased with increasing altitude (Fig. 1B). At higher elevations we recorded the growth of sclerophyllous species, some of these have high concentrations of phenolic compounds in their leaves (Velázquez-Rosas et al. 2002, Velazquez-Rosas et al. in press), which may partially explain the decreasing found in the N<sub>min</sub> and the consequent reduction in elevation of the mineralization rate and in the concentrations of mineral forms of N (NO<sub>3</sub>-N and NH<sub>4</sub>-N) available for plants. A study conducted at the Barva volcano in Costa Rica (Marrs et al. 1988) showed that nitrification and mineralization rates decreased from 38 mg N kg<sup>-1</sup> at 100 m to 2 mg N kg<sup>-1</sup> at 2600 m. Low mineralization rates at higher altitudes were explained by the effect of anaerobic conditions on micro-organisms caused by the decrease in temperature with altitude and the soil's high-water saturation (Edwards & Grubb 1977, Grubb 1977, Tanner 1977, Marrs et al. 1988, Bruijnzeel et al. 1993, Cavelier et al. 2000).

In addition, we found that the available forms of nitrogen in Oe horizon showed different patterns along the altitudinal gradient. The stocks of NH<sub>4</sub>-N increased with altitude, while NO<sub>3</sub>-N stocks did not change. In all the sampled sites NH<sub>4</sub>-N was higher than NO<sub>3</sub>-N stocks. Ammonization occurs in soils with stagnic properties or unfavorable environment for nitrification (Harmsen & Kolenbranden 1965); such as in the acid and stagnic studied soils; with particular reducing conditions at higher elevations (Álvarez et al. 2008). By contrast, nitrification depends on a relatively narrow group of ammonia-oxidizing bacteria, which are sensitive to low temperatures, soil acidity and lack of oxygen (Loomis et al. 2006). In addition, it has been suggested that an absence of nitrogen-fixing species (e.g. Mimosaceae) at higher elevations contributes to lower NO<sub>3</sub>-N concentrations in soils (Marrs et al. 1988).

In this study, stocks of  $NO_3$ -N at the Oe horizon were not higher than concentrations found in tropical lowlands (Roman & Scatena in press). Our findings are in agreement with other studies in forests. For instance, Marrs et al. (1988) in montane forests of Costa Rica found no difference with the increment in altitud. Likewise Bruijnzeel & Proctor (1995) did not found difference in nitrification rates between the lower and higher sites at the slope of Krakatau volcano (700 m asl).

4.5.2 Relationships between pools of N and traits of tree communities and  $N_l$  and C:N leaf ratios Nutrient shortages, in particular soil N, has been considered to be a limiting factor for productivity of MCFs (Grubb 1977, Tanner et al. 1998), although it has been recognized that productivity is not determined by a single factor (Waide et al. 1998, Cox et al. 2002, Wilcke et al. 2008). In our research, changes in diversity, density, basal area and above-ground biomass were not significantly related to altitude or stocks of N, except density which was significantly related to total N. These results differ in part to those reported for MCFs in Ecuador by Wilcke et al. (2008). These authors founded that static structural traits (tree height, tree diameter and tree basal area) decreased with increasing altitude, but the total N at the organic horizons had no significant effect on the variation of these parameters. In our study, the lack of relationship between above ground biomass and N stocks could be due to other important components of primary productivity which we did not considered, such as the above-ground litter and root production (Tanner et al. 1998, Röderstein et al. 2005).

In MCFs foliar nutrient concentrations have been related to overall plant nutrition. In general, foliar N per unit of mass decreases with increasing altitude, and C:N ratios increase (Vitousek 1984, Tanner et al. 1998, Soethe et al. 2008). In different tree communities of MCFs in Ecuador foliar N decrease with values ranging from 21.7 mg g<sup>-1</sup> at 1900 m to 11.3 mg g<sup>-1</sup> at 3000 m, and C:N increase from 23.3:1 to 44.7:1 in the same elevations (Shoethe et al. 2008). In this study, the mean values of N<sub>1</sub> and C:N ratios at 1940 m and higher altitudes were 13.8- 21.1 mg g<sup>-1</sup> and 26:1-43:1, respectively. This suggests a nitrogen shortage, as has been reported for other MCFs and temperate and tropical forests (7-20 mg g<sup>-1</sup> to N<sub>1</sub> and C:N ratio > 30:1). In this study foliar N deficiency was related to decreasing N available in organic horizons.

Altitudinal variation regarding concentrations of N<sub>1</sub> and C:N leaf ratios showed that species respond differently to growth-limitation. For *Drimys*, *Podocarpus* and *Vaccinium*, concentrations of N<sub>1</sub> and C:N leaf ratios recorded (12.6–20.5 mg g<sup>-1</sup> and 24.9:1–42.4:1, respectively, Table 3) suggest that their growth was limited by N in all the altitudinal gradient (1520–2550 m). These values were similar to those reported by Soethe et al. (2008). Despite of N in the Oe horizon and

 $N_1$  and C:N per species had a significant relationships, in *Vaccinium* and *Podocarpus* (Table 4),  $N_1$  and C:N elevational distribution suggest that nutritional control of N is not directly linked with elevational increase. In *Podocarpus*, leaf  $N_1$  and C:N values show that limitations in N were significantly higher at 1520 and 2380 m than at 1940 m. *Vaccinium* was unique in this respect,  $N_1$  levels were significantly related to altitudinal changes in N, NH<sub>4</sub>-N and NO<sub>3</sub>-N in the Oe horizon along the altitudinal gradient, NH<sub>4</sub>-N had the greatest influence on  $N_1$ , and the relationship with  $N_{min}$  was negative. The two species sampled in a narrow elevational interval did not show significant differences in  $N_1$  and C:N by elevation , but in *Drimys*, these values suggest limitation in N, at difference of *Ticodendron*, which did not show this limitation (Table 3).

Our results suggests that although total N and mineral nitrogen in Oe horizon were not directly related to community properties, as pointed out by Waide et al. (1998), and Roman & Scatena (in press), they may influence some functional traits of species, such as foliar N concentrations. From these results it is necessary to underline how the individual species draw on the N pool. Community properties ought to also be related to other productivity-related factors, such as photosynthetical active radiation, temperature, toxicity of phenolic compounds, Mn and Al, and disturbances (Bruijnzeel et al. 1993, Cox et al. 2002, Waide et al. 1998).

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Capítulo 5

**Optical and morpho-functional traits of the leaves of tree species** 

growing in a mountain cloud forest

Optical and morpho-functional traits of the leaves of tree species growing in a mountain cloud forest‡

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# 5.1 ABSTRACT

The physiological, anatomical and optical leaf properties relative to photosynthetically active (PAR) and ultraviolet (UV-B) radiation were assessed in Ticodendron incognitum, Drimys granadensis, Podocarpus matudae var. macrocarpus and Vaccinium consanguineum, growing along an elevation gradient (1520-2550 m asl) in a montane cloud forest in México. PAR and UV-B absorptance, transmittance and reflectance, UV-B absorptance by foliar compounds, chlorophylls, carotenoids, leaf nitrogen, leaf mass per area, leaf blades, cuticles, epidermis and parenchymas thickness were measured. PAR absorptance efficiencies were calculated. Among the evaluated morpho-functional traits, the studied species displayed different patterns of variation with elevation. Leaf traits could be explained in part by changes in elevation or the distribution of PAR and UV-B in the elevation gradient. *Ticodendron* and *Drimys* leaf traits were likely determined by two cloud banks located at 1940 and 2380 m. In Vaccinium, eight traits were related to elevation and PAR or UV-B. Contrary to this, in *Podocarpus*, most of the nine leaf traits could be explained by only one of these factors. The morphological traits of the studied species were similar to those of species growing in other oligotrophic ecosystems. Significant differences between sun exposed and shade leaves were limited to particular elevations or to particular traits of each species. Vaccinium showed more significant differences between sun and shade leaves than did the other species growing along the gradient. The morpho-functional traits measured in *Podocarpus* and *Vaccinium* showed that, some leaf traits did not change linearly with elevation or PAR. At elevation levels where species co-occur, the species ranking with respect to evaluated traits varied from trait to trait. This indicate that each species copes with light and other environmental factors, that vary with elevation, according to its morpho-functional plasticity and susceptibility to these factors; which may determine the distribution of these species along the gradient.

Keywords: Cloudiness; Drimys granadensis; Elevational gradient; Functional diversity; Light environment; Leaf optical properties; Podocarpus matudae var. macrocarpus; Ticodendron incognitum; Vaccinium consanguineum

## 5.2 Introduction

Montane cloud forests (MCFs) are known for their cloudy weather conditions (Webster, 1995; Cavelier, 1996). In a dry atmosphere, radiation increases with increased elevation (Jones, 1992), but in MCFs, clouds and fog attenuate the increases associated with elevation in photon flux density (PAR, 16% to 53%), and modify light quality. In addition, both of these vary in space and time because of the daily and yearly vertical movements (along the elevational gradient) of the cloud banks and fog (Aylett, 1985). These factors and other variables such as nitrogen availability and temperature could affect the CO<sub>2</sub> assimilation rate of the plants (Körner et al., 1986; Bruijnzeel et al., 1993 Kitayama, 1995; Tanner et al., 1998) and could explain why productivity decreases with increased elevation. Nevertheless, low vapor pressure deficits encourage leaf traits that increase light harvesting without significantly affecting the plant's water balance (Poorter et al., 2000).

Plant adaptation and acclimatization to their growing light conditions (photon flux density and quality) are accurately expressed by leaf traits (Lambers et al. 1998; Sims and Gamon, 2002). Therefore, traits linked to light harvest and/or protection of the photosystems should be indicators of the mean light conditions that plants undergo, as well as the response of the species to environmental factors that change with elevation (Richardson et al., 2001, Richardson and Berlyn, 2002; González et al., 2007). MCFs' species are adapted to shade and sunny conditions, which change in space and time in response to the dynamic movements of clouds and fog (Cavelier and

Goldstein, 1989; Bruijnzeel and Proctor, 1995), and thus, morpho-functional leaf traits might reflect this changing light condition more than the light changes related to elevation do. However, similarly to sun exposed and shade leaves of tropical rain forest species (Poorter et al., 1995), sun exposed and shade leaves of pioneer and primary species of Venezuelan MCF trees growing at 1750 m asl (Poorter et al., 2000) showed significant but reduced differences between them. In these species, photosynthetically active radiation (PAR, 400–700 nm) absorptance is also similar to that found in the shade leaves of species growing in tropical rain forests and in seasonal forests, which also optimize light absorptance by having horizontal leaves with a low specific leaf weight (Bongers and Popma, 1988) and a high chlorophyll mass-based content (Lambers et al. 1998). Consequently, in MCFs where such environmental factors change with elevation, we may expect a reduced, but significant, variation in the morpho-functional leaf traits associated with variations in PAR or other factors such as moisture and temperature that change with elevation and determine nutrient availability.

In general, UV-B increases by 14–18% for each 1000 m of increased elevation (Caldwell et al., 1989; Jones, 1992), but in MCFs, this change may be reduced by the attenuating effects of clouds and fog on UV-B radiation (Dahlback, 1996; Køiskar et al., 2003). Even though levels of UV-B radiation are low, UV-B radiation (280–320 nm) has been reported to determine the leaf traits of MCF species (Bruijnzeel et al., 1993; Flenley, 1995; Rozema et al., 1997). These increases in UV-B are mainly reflected in the leaf traits (Sullivan et al., 1992; Ziska et al., 1992) of plants growing at the highest elevations (Flenley, 1995). Tropical species at high elevations have high concentrations of UV-B absorbing phenolic compounds in the leaves (Robberecht et al., 1980; Barnes et al., 1987; Bruijnzeel et al., 1993; Ziska, 1996). These compounds increase with elevation, as observed in the montane forests of Jamaica (Rozema et al., 1997). Other functional responses related to the increase

in UV-B radiation that have been recorded in plants growing in other ecosystems and in the leaves of trees in montane regions are a reduction in plant growth, a decrease in leaf area, and anatomical changes (Tanner and Kapos, 1982; Caldwell et al., 1989; Antonelli et al., 1997). However, nutrient conditions and temperature may also explain either the decreased leaf size or the presence of sclerophyll leaves in plants growing above 2000 m asl.

In the present work, we hypothesized that, irrespective of the cloudy and foggy environment, morpho-functional leaf traits will reflect the increases in PAR associated with elevational gradients. Specifically, photosynthetic pigments (chlorophylls and carotenoids per unit area) and the leaf mass per area will increase. The leaf content of UV-B screening compounds, the cuticle and the epidermis thickness will increase in response to UV-B. PAR and UV-B absorptance will increase with the elevation gradient, but the PAR absorptance efficiencies, defined as PAR absorptance per unit chlorophyll, carotenoid, biomass or leaf thickness, will decrease. To test these hypotheses in an elevational gradient ranging from 1520 to 2550 m asl, we selected three of the most abundant MCF species located in Santa Cruz Tepetotutla, Oaxaca, México with a wide distribution across different elevations (*Vaccinium consanguineum, Podocarpus matudae* var. *macrocarpus* and *Drimys granadensis*) and one species with a narrow distribution (*Ticodendron incognitum*).

## 5. 3 Materials and methods

#### 5. 3.1. Study zone

This study was conducted in the area surrounding Santa Cruz Tepetotutla, a village in the North of the state of Oaxaca, México  $(17^{\circ}38'-17^{\circ}40' \text{ N}, 96^{\circ}32'-96^{\circ}33' \text{ W})$ . This region has an abrupt topography with slopes between 10° and 50°. The soils are shallow, derived from metamorphic rocks, and have a high content of organic matter. The soils of the upper portion of the toposequence

are Podzols, whereas those of the lower portion of the toposequence are Cambisols (Álvarez et al., 2008). Soils are acidic and lack significant amounts of bases and available nitrogen. Total carbon content decreases as the elevation increases (Álvarez et al., 2008). The climate in the study area is hyper-humid and temperate. The mean annual total precipitation is ~5800 mm, and during the driest month (March), the mean precipitation is 50 mm (Rzedowski and Palacios-Chávez, 1977). Temperature data recorded at the study sites show that the mean temperature decreases from 15.3 °C to 11.5 °C along the altitudinal interval from 1520 to 2550 m (Álvarez et al., 2008; Fig. 1). The temperature decreases at a rate of 0.4 °C per 100 m of elevation; this rate is lower than rates recorded for other cloud forests, which demonstrate rates ranging from 0.5 to 0.8 °C (Kitayama, 1995; Kappelle, 1996). The vegetation along the gradient is a mosaic of forest communities, classified as premontane below 1500 m asl, lower mountain cloud forest between 1500 and 2300 m and upper mountain cloud forest from 2300 to 2550 m asl (Rincón, 2007). These forests are highly diverse, include ca. 200 tree species distributed along the elevational gradient. The study area is covered with an undisturbed and mature forest that is protected under the guidelines of the "farmer's reserve".

## 5.3.2. Species selection, study sites and photon flux density measurements

Four tree species were selected: *Vaccinium consanguineum* Klotzsch (Ericaceae), *Drimys granadensis* L. F. (Winteraceae), *Podocarpus matudae* Lundell var. *macrocarpus* J. Buchholtz et N.E. Gray (Podocarpaceae) and *Ticodendron incognitum* Gómez Laur. & L. D. Gómez (Ticodendraceae). We will refer to these species by their genus. These species have high relative importance values (RIV = relative basal area + relative density + relative frequency) in several communities (Rincón, 2007). Five study sites were established between 1520 and 2550 m asl (1520,

1940, 2040, 2380 and 2550 m). The altitude is followed by a subscript letter that indicates the lowest (L), middle (M) or highest (H) elevation where each species was collected. A small letter stands for a species collected at two elevations only, and capital letters designate species collected at three elevation levels. *Podocarpus, Vaccinium* and *Drimys* grow at 1520 to 2550 m, whereas *Drimys* is very scarce at the lowest and highest elevations. Due to the abrupt topography, the leaves were collected from the species growing on slopes with the same orientation and where the plants were abundant and accessible. *Vaccinium* was collected at 1520<sub>L</sub>, 2040<sub>M</sub> and 2550<sub>H</sub> m, *Podocarpus* at 1520<sub>L</sub>, 1940<sub>M</sub> and 2380<sub>H</sub> m, *Drimys* at 1940<sub>l</sub> and 2380<sub>h</sub> m, and *Ticodendron* at 1520<sub>l</sub> and 1940<sub>h</sub> m. The mean annual temperatures at the collection sites are shown in Figure 1.

In the five study sites, PAR (400-700 nm) and UV-B (280-320 nm) were measured in the dry season (March) and in the rainy season (September) in sun-exposed places using a spectroradiometer (LI-COR, Inc., Nebraska, USA). For each elevation, the measurements were performed on two days with similar weather conditions from 7:00 to 18:00 h. To validate the measurements obtained on different days using the spectroradiometer, PAR measurements were performed simultaneously with five quantum sensors (LI-190, LI-COR, Lincoln, Nebraska, USA), one per elevation level, during 10 days per season. Mean PAR values ( $\pm$  standard error) per elevation, measured with the quantum sensors were: 447.55  $\pm$  49.38, 153.28  $\pm$  15.34, 643  $\pm$  47.63, 317.06  $\pm$  35.26, 510.04  $\pm$  80.79 µmol m<sup>-2</sup> s<sup>-1</sup> (1550, 1940, 2040, 2380, 2550, respectively). In addition, PAR and UV-B data for the dry and rainy seasons were calculated by computing the PAR radiation at the top of the atmosphere for every week (Jones, 1992). The transmissivity of the atmosphere week (Jones, 1992). The transmissivity of the atmosphere was calculated by computing the PAR radiation at the top of the atmosphere on the days when PAR

and UV-B measurements were collected in the field, during the dry and rainy seasons. Calculations were obtained for 26 days per season (dry and rainy).

#### 5.3.3. Functional and anatomical traits

Sun exposed leaves (SL) were collected from sun exposed, south facing branches, while the shade leaves (SHL) were collected inside the crown from north-facing areas. Only healthy, mature and fully expanded leaves were included in the study. Leaves were collected in January of 2004 and 2007 from at least five individuals per sampled site. The leaves of all of these species have a long life span. The degree of canopy openness between these two light environments was assessed using hemispheric photographs taken with a Colpis A Nikon model digital camera equipped with a fish eye lens and analyzed with the Gap Light Analyzer Watch 2.0 program (Frazer et al., 1999). During leaf collection on a cloudy day at noon, PAR was also measured using a quantum sensor. The number of measurements differed between elevations because of variable difficulties associated with climbing the trees (Table 1). After collection, 30 leaves per tree were oven-dried for three days at 70 °C and weighed, and the leaf mass per area (LMA) was calculated. Next, in composed samples, nitrogen content (N) was quantified in five (one per tree) 13 µg samples per elevation and species, with an Elemental Analyzer. (NC 2500, Thermo Quest S.P.A., Rodanao, Milan, Italy). The equipment was calibrated with a standard curve generated for sulfanilamide (C, 41.84%; H, 4.68%; N, 16.27%; O, 18.58% and S, 18.62%; CE Elantech, Inc., Lakewood, N.J., USA).

The transmittance (PAR<sub>*T*</sub>) and the reflectance (PAR<sub>*R*</sub>) of PAR and UV-B (280–320 nm) were measured in leaves that were preserved fresh by immediate placement in water. Optical measurements were conducted after 24 h for five SL and five SHL per species and site, using a spectrophotometer (LAMBDA 19, Perkin Elmer, Boston, USA) with an integrating sphere (B009-

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4012, Perkin Elmer, Boston USA) at intervals of 2 nm. PAR absorptance (PAR<sub>A</sub>) was calculated as follows:  $PAR_A = 1 - (PAR_R + PAR_T)$  (Poorter et al., 2000). UV-B (280-320 nm) absorptance (UV-B<sub>A</sub>) was calculated as UV-B<sub>A</sub> = 1 - UV-B<sub>R</sub>, because the UV-B transmittance of the leaves was below the detection limit of the instrument (photometric accuracy of 0.08% T at 1 A). The optical properties of the entire leaf were measured. To confirm the results, the optical properties were determined for leaves collected in 2004 and in 2007.

To determine the contents of chlorophylls *a* and *b* (Chl<sub>*a*</sub> and Chl<sub>*b*</sub>) and carotenoids (CAR: xanthophylls and carotenes), the pigments were extracted with 4 ml of N,N-dimethylformamide from the leaf disks (0.6 cm in diameter) of 10 SL and 10 SHL per species and site, which were cut from the central part of the leaf blades avoiding the primary vein. The absorptance of the extract was measured with a spectrophotometer at 480, 646.6 and 663.8 nm for CAR, Chl<sub>*a*</sub> and *Chl<sub>b</sub>*, respectively (Porra et al. 1989), using quartz cuvettes. The pigment area-based content was calculated according to Wellburn (1994). The absorptance efficiencies were calculated as the ratios between PAR<sub>*A*</sub> and total chlorophyll (Chl<sub>*a*+*b*</sub>), the CAR area-based contents, the dry leaf mass per area (LMA) and the leaf blade thickness (LBT).

To evaluate UV-B absorptance by screening foliar compounds, leaf disks (0.6 cm in diameter) collected as described for the chlorophyll determinations were obtained from 10 SL and 10 SHL per species and site and placed in screw-on tubes containing 5 ml of extraction medium to assess UV-B screening compounds (79:20:1, v:v:v, ethanol:water:hydrochloric acid). The tubes containing the leaf disks were heated for 2–3 h at 96 °C (boiling water at 2250 m asl) in a water bath until the leaf disks were completely bleached. Leaf extract absorptance was measured at 280 (UV-B<sub>A-280</sub>), 300 (UV-B<sub>A-300</sub>) and 320 nm (UV-B<sub>A-320</sub>) using a spectrophotometer (Rozema et al., 1997).

To relate the anatomical traits to the optical properties in 10 SL and 10 SHL, we measured the leaf blade thickness (LBT), the palisade parenchyma (PP) and the spongy parenchyma thickness (SP), as well as the thicknesses of the adaxial and the abaxial cuticles (ADC and ABC) and epidermis (ADE and ABE). Only in *Podocarpus* did the measurements of the adaxial and abaxial epidermis include the hypodermis (a layer of cells lying immediately beneath the epidermis that may serve as a protective layer). Anatomical traits were measured via transverse cuts in the central part of the leaf blades. These samples were fixed in FAA (formalin 5%, acetic acid 4%, ethylic alcohol 48% and water 43%). After dehydration in a gradual series of ethyl alcohol, the material was infiltrated with paraplast, embedded in the same material, and cut into sections of 10 µm. The sections were stained with safranin and fast green and observed using a photomicroscope Olympus Provis X-70 (Olympus, Tokyo, Japan). To perform anatomical measurements, the images were digitized and analyzed with the Carnoy software (V 2.0, 2001, Peter Schols).

#### 5. 3.4. Statistical analysis

PAR and UV in sun exposed areas of the five elevations were analyzed separately by first using a multifactor ANOVA (elevation and season) and then an ANOVA for each season followed by Tukey's test. The canopy openness and PAR in sunny and shaded areas were analyzed using Student's *t*-test. To assess the morpho-functional responses to elevation and light exposure (SL and SHL), the homogeneity of variances was confirmed by Bartlett's test, and the data were then compared by multifactor ANOVA. Significant differences between SL and SHL (Appendix) were observed for only a few traits at particular elevations. Therefore, to that the results are shown for species, leaf trait, and elevation. In cases for which homogeneity of the variance was not detected,

log transformations were applied. Multiple comparisons were performed using Tukey's test, and the statistical analyses were conducted using a computer program (Statistica 6.1, Statsoft, Inc., 1984-2003, OR, USA). Regression analysis between elevation, PAR or UV-B values (on sunny days) and morpho-functional traits that showed significant differences between elevations (Table 2) were performed for *Vaccinium* and *Podocarpus* using TableCurve 2D, v. 3 (AISN Software, Chicago, IL, USA). For these species, the regression analyses were also conducted separately for sun exposed and shade leaves that showed significant differences in at least one sampled site, per leaf trait and species. These cases were only related to PAR or UV-B (sunny day values). A comparison slope test was carried out to compare the slopes of the curves generated for sun and shade leaves (Zar, 1999). The mean values determined for canopy openness and PAR for sun exposed and shade tree branches were compared for each elevation using Student's *t*-test.

#### 5.4. Results

PAR and UV-B differed significantly between elevations, and the values for the rainy season were significantly lower than those determined for the dry season (P < 0.001). The mean light (PAR and UV-B) values at the sites where the species were collected are shown in Figure 1A-H. PAR and UV-B showed a wide variation along the altitudinal gradient; in both cases, the mean values did not increase linearly with elevation. PAR (Fig. 1I) and UV-B radiation (Fig 1J) varied from 156 to 1260  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and from 0.85 to 4.43  $\mu$ J m<sup>-2</sup> s<sup>-1</sup>, respectively. In the dry season, the lowest PAR values were recorded at 1940 and 2380 m (P < 0.001). At 1520, 2040 and 2550 m, PAR increased with elevation; however, significantly different values were only detected between 1520 and 2550. In the dry season, UV-B at 1940 and 2380 m differed significantly from UV-B at 2550 m (P = 0.0016). At the other elevations, UV-B followed a trend similar to PAR; however, UV-B at 1520 m did not

differ significantly from UV-B at the other elevation levels. In the rainy season, PAR was highest at 2550 m, differing significantly from PAR at 2380 m (P = 0.0073); however, from 1520 to 2380 m, PAR demonstrated no significant differences. During the same season, UV-B did not differ along the elevational gradient (P = 0.068, Fig. 1J). The canopy openness and PAR at the sites and elevations where sun exposed and shade leaves were collected demonstrated significant differences (Table 1); the mean canopy openness was 27.3–29.9 and 12.8–9.3% for sun exposed and shaded sites, whereas the mean PAR values were 118–576 and 24–69  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively.



Figure 1. Mean values ( $\pm$  SE) of environmental data for the elevations where each species was collected. A-D) photosynthetically active radiation (PAR, **circles**) and temperature (squares). E-H) UV-B radiation. I-J) elevational distribution of PAR and UV-B. Sunny (open circles or gray bars) and cloudy days (closed circles or black bars). Letters indicate significant differences inside seasons, small letters for the rainy season and capital letters for the dry season.

Table 1. Mean ( $\pm$  SE) values of canopy openness and photosynthetically active radiation (PAR), in sunny and shaded areas in the study areas, where sun and shade leaves were collected. Letters indicate significant differences (p < 0.05).

Elevation	Canopy openess (%)			PAR ( $\mu \mod m^{-2} s^{-1}$ )		
(m asl)	Sun	Shade	df	Sun	Shade	Df
1520	27.3 (3.1)a	12.8 (2.1)b	10	485 (69.2)a	69 (18.8)b	8
1940	28.7 (2.9)a	14.0 (2.2)b	10	576 (262.7)a	27 (7.5)b	5
2040	36.5 (3.5)a	12.9 (1.2)b	10	179 (47.4)a	24 (4.6)b	5
2380	48.7 (4.7)a	16.3 (1.8)b	10	132 (20.4)a	59 (22.2)b	11
2550	29.9 (4.1)a	19.3 (1.6)b	10	118 (29.3)a	51 (33.4)b	5

No significant differences were found between the optical properties of leaves measured in 2004 and 2007 in all species. The results obtained for leaves collected in 2007 are presented in Fig. 2. At the sites where *Ticodendron* and *Drimys* were sampled, PAR and UV-B followed a negative and positive trend with elevation, respectively (Fig. 1A, B, E, F). In *Ticodendron*, most of the traits followed the same trend observed for PAR and UV-B (inverse trend with elevation): PAR<sub>A</sub> (Fig. 2A), UV-B<sub>A-280</sub> (Fig. 2F), Chls (Fig. 3A-C), Chl<sub>a+b</sub>:N (Fig. 3F), LBT (Fig. 4A), PP (Fig. 4F), and SP (Fig. 4G). In contrast, PAR<sub>T</sub> (Fig. 2B), PAR<sub>R</sub> (Fig. 2C) and PAR<sub>A</sub>:Chl<sub>a+b</sub> (Fig. 5A) increased with increased elevation. In *Drimys*, PAR<sub>R</sub> (Fig. 2K), all the UV-B absorptances by screening foliar compounds (Fig. 2N-P), LBT (Fig. 4I), ADE (Fig. 4L), PP (Fig. 4N) and SP (Fig. 4O) followed the same increasing trends as PAR, UV-B and elevation. Only PAR<sub>A</sub> (Fig. 2I) demonstrated the inverse trend. *Vaccinium* was sampled at altitudes that presented a linear increase in PAR and UV-B with increased elevation (Fig. 1D, H). Thus, the traits that had a significant relationship with



Figure 2. Mean values ( $\pm$  SE) of photosynthetically active radiation absorptance (PAR<sub>A</sub>), transmittance (PAR<sub>T</sub>) and reflectance (PAR<sub>R</sub>), UV-B absorptance (UV-B<sub>A</sub>) and reflectance (UV-B<sub>R</sub>) and UV-B screening foliar compounds absorptance (UV-B<sub>A-280</sub>, UV-B<sub>A-300</sub> and UV-B<sub>A-320</sub>) along elevation. Different letters on the bars indicate significant differences (P < 0.05) between elevations for each species.

elevation demonstrated the same behavior for PAR or UV-B (Fig. 1, Table 2). The relationship was positive for PAR<sub>*R*</sub> (Fig. 2AA), ADE (Fig. 4AB), and all the UV-B absorptances by screening foliar compounds (Fig. 2AD-AF) and negative for CAR (Fig. 3Y) and  $Chl_{a+b}$ :N (Fig. 3AA, Table 2). Exceptions were ADC (Fig. 4Z), which decreased with increased elevation but demonstrated no significant relationship to PAR or UV-B, and N (Fig. 3Z), which showed a significant increase only with PAR (**Table 2**). Other traits that varied significantly between the sites but showed no significant relationships with elevation, PAR or UV-B were as follows: PAR<sub>*A*</sub> (Fig. 2Y), Chl<sub>a</sub> (Fig. 3V), Chl<sub>a+b</sub> (Fig. 3X). LMA (Fig. 3AB), LBT (Fig. 4Y) and ABE (Fig. 4AC) were not related to elevation, PAR or UV-B, but they demonstrated the significantly highest value at 2040<sub>M</sub> m; by contrast, all of the absorptance efficiencies (Fig. 5M-P) at this altitude showed the lowest value.

*Podocarpus* was sampled in an elevational gradient where PAR and UV-B decreased with elevation (Fig. 1C, G); the highest value was recorded at  $1520_{L}$  m. Only PAR<sub>T</sub> (Fig. 2R), ADC (Fig. 4R), PP (Fig. 4V), PP:SP (Fig. 4X) and LBT (Fig. 4Q) showed a positive relationship with elevation (Table 2). In contrast, many traits were related to PAR, related positively to Chl<sub>a+b</sub>:N (Fig. 3T) and PAR<sub>A</sub>:LMA (Fig. 5K), and related negatively to PAR<sub>T</sub> (Fig. 2R), N (Fig. 3S), CAR (Fig. 3R), and LMA (Fig. 3U, Table 2). All UV-B absorptances by screening foliar compounds (Figs. 2V-X) and CAR (3R) showed a negative relationship with UV-B (Table 2). The traits that varied significantly between the sites but had no significant relationship with elevation, PAR or UV-B were ABC (Fig. 4S) and PAR<sub>A</sub>:CAR (Fig. 5J), both of which demonstrated the lowest value at 1940<sub>M</sub> m.

For most of the morpho-functional traits, light exposure (Table 1) induced significant differences between SL and SHL at particular elevations (Appendix). In *Ticodendron*, significant differences were only observed for UV-B<sub>A-280</sub> and UV-B<sub>A-300</sub> at the site with the highest PAR incidence (1520<sub>1</sub> m) and for PAR<sub>R</sub> at the site with the lowest PAR value (1940<sub>h</sub> m, Fig. 1A). In contrast, for *Drimys* at the site with the highest PAR value (2380<sub>h</sub> m, Fig. 1B), differences were detected for several traits: PAR<sub>*T*</sub>, CAR, LBT, ADE and PP. On the other



Figure 3. Mean values ( $\pm$  SE) of chlorophylls (Chl<sub>a</sub>, Chl<sub>b</sub> and Chl<sub>a+b</sub>), carotenoids, leaf nitrogen (N) concentrations, Chl<sub>a+b</sub>:N ratios and leaf mass per area (LMA) for *T. incognitum*, *D. granadensis*, *P. matuda*e and *V. consanguineum* along elevation. Different letters on the bars indicate significant differences (P < 0.05) between elevations for each species.

Table 2– Results of the regression analysis carried out to determine the relationships between morpho-functional traits of *Vaccinium consanguineum* and *Podocarpus matudae* and the PAR or UV-B values registered in sunny days. Data were fitted to the function y = a + bx and in all the cases the probability was 0.00001. (df) degrees of freedom. Only significant relationships are shown. Photosynthetic active radiation transmittance (PAR<sub>T</sub>) and reflectance (PAR<sub>R</sub>); UV-B by screening foliar compounds absorptance (UV-B<sub>A-280</sub>, UV-B<sub>A-300</sub> and UV-B<sub>A-320</sub>); carotenoids (CAR) and leaf nitrogen (N) concentrations; nitrogen investment (Chl<sub>a+b</sub>:N); leaf mass per area (LMA); thickness of: leaf blade (LBT); adaxial cuticle (ADC) and epidermis (ADE); palisade parenchyma (PP); ratio PP:SP; PAR absorptance efficiencies per LMA (PAR<sub>A</sub>:LMA).

Relationship	$\mathbb{R}^2$	F	df	b	Α		
V. consanguineum							
PAR <sub>R</sub> vs ELEVATION	0.70	65.92	29	0.002	5.41		
CAR vs ELEVATION	0.46	48.4	57	-0.046	182.85		
Chl <sub>a+b</sub> :N vs ELEVATION	0.74	81.45	29	-0.0017	8.99		
UV-B <sub>A-280</sub> vs ELEVATION	0.39	37.68	59	0.001	0.49		
UV-B <sub>A-300</sub> vs ELEVATION	0.50	57.96	59	0.001	-0.31		
UV-B <sub>A-320</sub> vs ELEVATION	0.51	60.90	59	0.0009	-0.07		
ADC vs ELEVATION	0.47	53.12	59	-0.0007	-0.49		
ADE vs ELEVATION	0.37	34.75	59	0.002	16.12		
$PAR_{R}$ vs $PAR$	0.72	73.09	29	0.006	4.57		
CAR vs PAR	0.44	43.31	57	-0.1009	210.8		
N vs PAR	0.47	25.31	29	0.063	25.09		
Chl <sub>a+b</sub> :N vs PAR	0.70	66.14	29	-0.005	10.09		
$UV-B_{A-280}$ vs $UV-B$	0.52	62.74	59	1.101	-1.09		
$UV-B_{A-300}$ vs $UV-B$	0.56	73.77	59	1.043	-1.61		
UV- $B_{A-320}$ vs UV-B	0.59	83.07	59	0.743	-0.90		
CAR vs UV-B	0.42	40.39	57	-25.72	184.89		
ADE VS UV-B	0.42	41.99	59	2.4	11.64		
P. matudae							
PAR <sub>T</sub> VS ELEVATION	0.69	63.11	29	0.0007	-0.48		
ADC vs ELEVATION	0.37	34.75	59	0.0012	1.09		
PP vs ELEVATION	0.49	52.08	59	0.025	20.21		
LBT vs ELEVATION	0.5	55.03	59	0.035	248.39		
<b>PP:SP</b> vs ELEVATION	0.41	38.09	59	0.00014	0.12		
$PAR_T$ vs $PAR$	0.6	42.36	29	-0.001	1.90		
CAR vs PAR	0.43	43,48	59	-0.041	147.29		
N vs PAR	0.61	62.54	29	-0.185	248.51		
Chl <sub>a+b</sub> :N vs PAR	0.75	84.32	29	0.011	-0.31		
LMA vs PAR	0.57	37.18	29	-0.062	183.59		
PAR <sub>A</sub> :LMA vs PAR	0.71	69.47	29	0.044	31.53		
UV- $B_{A-280}$ vs UV-B	0.63	95.99	57	-1.360	7.22		
UV-B <sub>A-300</sub> vs UV-B	0.55	109.31	57	-0.663	3.84		
UV-B <sub>A-320</sub> vs UV-B	0.62	57	57	-0.395	2.44		
CAR vs UV-B	0.49	57.76	59	-16.44	162.18		



Figure 4. Mean values ( $\pm$  SE) of anatomical traits of *T. incognitum*, *D. granadensis*, *P. matuda*e and *V. consanguineum* found along elevation. PP:SP = palisade:spongy parenchymas thickness ratio. Different letters on the bars indicate significant differences (*P* < 0.05) between elevations for each species.

hand, all UV-B absorptances by screening foliar compounds demonstrated significant differences between SL and SHL at the site with the lowest PAR value ( $1940_h$  m, Fig. 1B). In *Drimys*, significant differences were only detected for N at both collection sites.

For the two species sampled at three elevations, *Vaccinium* showed significant differences in UV-B<sub>A-320</sub>, LBT, ADC, PP and SP between light exposures at the three sampled sites (1520<sub>L</sub>, 2040<sub>M</sub> and 2550<sub>H</sub> m; Fig. 1D; Appendix). Comparisons of the slope (P < 0.05) showed that *Podocarpus* differed between SL and SHL with respect to  $Chl_{a+b}$ :N (t = 7.4, df = 14) along the PAR gradient. In contrast, *Vaccinium* displayed differences in UV-B<sub>A-320</sub>, N,  $Chl_{a+b}$ :N, ADC and PP (t = 13.8, 3.12, 5.37, 2.76, and 7.8; df = 29, 14, 14, 29 and 29; respectively).



Figure 5. Mean ( $\pm$  SE) photosynthetic active radiation (PAR) absorptance efficiencies expressed per unit of total chlorophylls (PAR<sub>A</sub>:Chl<sub>a+b</sub>), carotenoids (PAR<sub>A</sub>:CAR), leaf mass per area (PAR<sub>A</sub>:LMA) and leaf blade thickness (PAR<sub>A</sub>:LBT) for *T. incognitum*, *D. granadensis*, *P. matudae* and *V. consanguineum* along elevation. Different letters on the bars indicate significant differences (P < 0.05) between elevations for each species.
# 5.5. Discussion

In MCFs, vegetation plant attributes vary widely along elevational gradients (Velázquez-Rosas et al. 2002, Rincón 2007), and the main source of this variation is the altitude and its associated environmental factors (Vázquez and Givnish 1998). Although the sampling sites were not replicated in the present study due to the low tree density of the studied species (for example, the density of V. *consanguineum* varies between 30 and 60 individual  $ha^{-1}$  (between 1520–2550 m) and the similar slope and geographic orientation (exposure facing) required for each sampling site, the results are indicative of the effects of elevation and light on the development of some leaf traits of the studied species (mainly for the two species sampled at three elevations). However, the patterns of variation by elevation for the evaluated morpho-functional traits were not consistent. PAR did not increased linearly with elevation and did not affect species in the same grade. Each species demonstrated a unique pattern for each measured trait; this result may mask a sampling site effect or reflect functional diversity of species to cope with the environmental changes associated with the elevational distribution of MCFs in the study area. In Ticodendron and Drimys, several of the evaluated traits (12 and 9 traits, respectively) followed the changes observed for PAR and UV-B along the elevational gradient; however, differences in PAR or UV-B between the Drymis sampling elevations were not significant. Nevertheless, these species were only sampled at two elevations, which restricts the generation of conclusions regarding their changing patterns along the elevational gradient and comparisons with the other two studied species. The trend demonstrated by light with elevation and the daily observations made by residents suggest the frequent presence of cloud banks (at 1940 and 2380 m, Fig. 1I, J) that seem to be determinants for the contrasting patterns demonstrated by *Ticodendron* and *Drimys*, because these banks cause reductions mainly in PAR. In *Podocarpus* and *Vaccinium*, many of the elevational changes observed in the studied traits could not be explained directly by changes in PAR or UV-B. There were fewer significant relationships between light and the evaluated traits (9 and 8 traits, respectively) compared to the trends in elevation and light demonstrated by *Ticodendron* and *Drimys*. In *Vaccinium*, a greater number of the changes determined by light (PAR and UV-B) were also significantly related to elevation, likely because *Vaccinium* was sampled at three sites where PAR increased with elevation (1520<sub>L</sub>, 2040<sub>M</sub>, 2550<sub>H</sub>; Fig. 1D, H). In contrast, two cloud banks were located at two of the sites where *Podocarpus* was sampled (1940<sub>M</sub> and 2380<sub>H</sub> m, Fig. 1C, G). Therefore, the significant relationships detected between leaf attributes and light were not the same as those related to elevation (9 and 5 traits, respectively), excluding PAR<sub>T</sub> (Table 2).

In the present study, we evaluated 27 leaf traits; however, *Vaccinium* demonstrated only 9 traits that could be explained by elevation or light, and *Podocarpus* had only 13. This result showed that each species had its own respective strategy for coping with light and other biotic and abiotic environmental factors associated with the collection sites and/or changes in elevation (such as nutrients, temperature and pathogens). It is important to note that the causes of leaf trait variation could be due to microclimatic variation at  $\alpha$  and  $\beta$  scales (2 km–200 m and 200–20 m, respectively; Orozco-Segovia and Sánchez-Coronado, 2009) and at lower scales that modulate biotic interactions, soil properties and dynamics, and other factors such as temperature and light properties. Furthermore, these microclimatic scales, microtopography and wind speed could play important roles in determining the physio-morphology of plants (Jones, 1992). These factors are relevant to understand the functional diversity of MCFs that show a wide variability in their biotic, physical and chemical attributes.

PAR was measured simultaneously at the five elevations during the dry and rainy seasons for the studied species, despite the short periods of field observations the optical properties related to PAR were similar to those of other species growing in MCFs.  $PAR_A$  was higher than  $PAR_R$  and  $PAR_T$ (85-95%, 6-12% and 0.4-2% respectively). The leaves of the species growing at lower elevations demonstrated a high mean PAR<sub>A</sub> (90%) and a low mean PAR<sub>R</sub> values (7.7 %; Poorter et al., 1995; 2000) and, consequently, a low mean PAR<sub>T</sub>. Among the species studied herein, the low PAR<sub>T</sub> values (1% to 7–8%) were consistent with that recorded for other species from MCFs and from other tropical forests (Poorter et al., 2000; Vázquez-Yanes et al., 1990). At noon in the study area, PAR varied between 156.0  $\pm$  65.7  $\mu mol~m^{-2}~s^{-1}$  on cloudy days and more than 1260.0  $\pm$  117.6  $\mu mol~m^{-2}~s^{-1}$ <sup>1</sup> on sunny days. These PAR values could saturate the photosynthesis of shade and sun exposed leaves in C<sub>3</sub> plants (200–500 to 1000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively; Chazdon et al., 1996) in lowland tropical forests, for which photosynthesis is saturated at a wide range of PAR values; this variation is related to leaves acclimatization to the heterogeneity of the light distribution beneath the canopy (Vázquez-Yanes et al., 1990). This may also help to explain why many functional traits in Vaccinium and Podocarpus did not show a significant relationship with PAR along the elevation gradient. For example, although Chls did not change with elevation in most of species, in *Ticodendron, Podocarpus*, and *Vaccinium*, the  $Chl_{a+b}$ :N ratio decreased as the elevation increased. This occurs in elevational gradients where soil N falls as the elevation increases and where radiation increases with increased elevation, due to a reduced investment of the species in light harvesting (Evans, 1989; Niinemets, 1997).

Although the present data obtained for PAR indicate a sharp distinction between sun and shade conditions (Table 1), we consistently found few and inconsistent differences between SL and SHL. These changes were confined to particular elevations for most of the species, excluding changes in

N or  $Chl_{a+b}$ :N and a few other traits that showed significant differences between sun exposed and shade leaves along the gradient in some of the species (see Appendix). This small difference has been attributed to the cloudy and foggy environment of MCFs and the environmental heterogeneity (Poorter et al., 1995, 2000). However, the morphological traits of the studied species did not correspond to those reported for many understory shrubs or rain forests trees (e.g., Bongers and Popma, 1988). For various leaf traits (UV-B<sub>A-320</sub>, N, Chl<sub>a+b</sub>:N, ADC and PP), *Vaccinium* presented a linear relationship along the elevational gradient with significant differences between shade and sun exposed leaves. This can be related to the dense and complex architecture of the crown of this species, compared to the architecture of *Podocarpus* (Velázquez-Rosas, 2000), which demonstrated a difference only in Chl<sub>a+b</sub>:N.

The leaves of five canopy tree species growing in the tropical rain forest reflect 26%–90% of the UV-B light at 287 nm (Lee, 1980, Lee and Lowry, 1980). UV-B<sub>R</sub> was homogeneous (4–5%) among the studied species and elevations. In species growing at high elevations in MCFs and in areas with clear skies, increases in the content of screening foliar compounds have been reported (Caldwell et al., 1989; Rozema et al., 1997; Filella and Peñuelas, 1999). The same was observed for the species studied herein, excluding UV-B<sub>A-320</sub> in *Ticodendron. Drimys* and *Vaccinium* showed an increase in UV-B screening foliar compounds as UV-B radiation increased with elevation. In contrast, *Podocarpus* demonstrated a negative relationship with UV-B. Despite the methodological limitations encountered in evaluating the field UV-B, this contrasting response and the fact that clouds attenuate UV radiation (Dahlback, 1996; Køiskar et al., 2003) strongly suggest that in some species, leaf traits, soil N and/or herbivores and pathogens may induce changes in the content of screening foliar compounds (Harborne and Williams, 2000, Kolb and Pfündel, 2005). In the field, more parasitism and herbivory was observed in *Podocarpus* and *Drimys* populations at sites where

the cloud banks were located (1940 and 2380 m asl; N. Velázquez-Rosas, personal observations). In *Podocarpus* and *Vaccinium*, CAR showed negative relationships with PAR and/or UV-B. Previous studies have demonstrated that screening compounds play a more relevant role in young compared to mature leaves (Lee, 1980), this may explain the results obtained in mature leaves used in the present study, which did not increase with elevation.

Anatomical leaf traits partially determine the optical properties of leaves (Vogelman et al., 1996): the adaxial cuticle and epidermis reflect PAR and UV-B (Baltzer and Thomas, 2005). Polyphenols in the cuticles and epidermis play an important role in protection by reflecting and absorbing PAR and UV-B, respectively (Reuber et al., 1996; Solovchenko and Merzlyak, 2003; Rouseaux et al., 2004; Bidel et al., 2007). In the present study, Podocarpus and Vaccinium demonstrated significant elevational changes in ADC and/or ADE, but only Vaccinium showed a significant relationship between ADE and UV-B radiation. In addition, in *Podocarpus*, PP and LBT were not significantly related to UV-B. In contrast, in *Ticodendron* and *Drimys*, the species with a lower LBT followed the same trend observed for PAR with elevation, and the changes in LBT were due to changes in the parenchyma, which may protect the photosynthetic apparatus. Despite these contrasting changes between the species, the LBT values for all of the species studied, and other traits as cuticles, were comparable to those of sclerophyllous species that grow in exposed canopies in tropical areas (Medina et al., 1990) and in oligotrophic environments; they differed from non-sclerophyllous species that grow in these areas (Bongers and Popma, 1988; Pérez-Garcia et al., 1992). These findings confirm that in the studied species, the soil characteristics of MCFs may play a greater role than the light environment (PAR or UV-B) in determining leaf traits.

The studied species did not demonstrate the same response to light or elevation at sites where the populations overlapped, and several traits (such as the PAR absorptance efficiencies in *Vaccinium* 

and *Podocarpus*) did not change linearly with elevation. Non-linear patterns have been previously reported and attributed to variability in species susceptibility to environmental factors linked to elevation (Li et al., 2006), which may modulate the nature and magnitude of plant responses. In the present study, non-linear patterns could be related in part to the PAR distribution in the gradient determined by the presence of the two cloud banks (Figure 1I, K). These banks might also play a role in modifying the temperature and nutrient availability along the gradient and, consequently, the leaf traits.

#### 5.6. Conclusions

With respect to MCFs, the present research contributes to our understanding of some of the causes inherent to individuals, species and environments that pose difficulties in making generalizations about trends for all morpho-functional traits of plants and environmental parameters with variations in light along elevational gradients, as occurs in other forests (Hultine and Marshall, 2000). The main reasons for these challenges might be the environmental variations at macro and microscales that change with elevation and those related to the sampling sites that are involved in plant responses. Leaf traits did not always change linearly with increased elevation, which could be due to changes at the macroscale when cloud banks are present at different elevations. Additionally, each species coped with light and other environmental factors, according to their plasticity and sensitivity to environmental factors that determine the point at which the distribution of each species declines. The morpho-functional diversity discovered among the studied species reflects and likely explains part of the high diversity of species found in MCFs and in the mosaic of forest communities distributed according to the elevation gradient reported for this area (Rincón, 2007). To better understand of the functionality of the MCFs it is necessary to increase our knowledge about leaf traits in different MCFs and to obtain measurements for PAR and UV-B that encompass an entire year, at different elevations to accurately characterize the cloud and fog movements over days and even years.

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**5.9 Appendix**– ANOVA test results for morphological and functional traits of sun and shade leaves (light exposure) of *T. incognitum*, *D. granadensis*, *P. matudae* and *V. consanguineum*, along an elevational gradient. F value and degree freedoms (in parentheses) are indicated. \* <0.05, \*\* <0.01 \*\*\* <0.001 after Tukey's test. Photosynthetic active radiation absorptance (PAR<sub>A</sub>), transmittance (PAR<sub>T</sub>) and reflectance (PAR<sub>R</sub>); UV-B absorptance (UV-B<sub>A</sub>) and reflectance (UV-B<sub>R</sub>); UV-B by screening foliar compounds absorptance (UV-B<sub>A-280</sub>, UV-B<sub>A-300</sub> and UV-B<sub>A-320</sub>); chlorophylls (Chl<sub>a</sub>, Chl<sub>b</sub> and Chl<sub>a+b</sub>); carotenoids (CAR) and leaf nitrogen (N) concentrations; nitrogen investment (Chl<sub>a+b</sub>:N); leaf mass per area (LMA); thickness of: leaf blade (LBT); adaxial and abaxial cuticles (ADC and ABC) and epidermis (ADE and ABE); palisade (PP) and spongy parenchyma (SP); ratio PP:SP, PAR absorptance efficiencies per unit total chlorophylls (PAR<sub>A</sub>:Chl<sub>a+b</sub>); carotenoids (PAR<sub>A</sub>): leaf mass per area (PAR<sub>A</sub>:LMA) and leaf blade thickness (PAR<sub>A</sub>:LBT).

Traits	Factor	Ticodendron	Drimys	Podocarpus	Vaccinium
		incognitum	granadensis	matudae	consanguineum
$PAR_A$	Elevation	58.8 (1,16)***	15.35 (1,16)***	n.s.	5.81 (2,24)***
(%)	Light exposure	n.s.	n.s	n.s.	n.s
	Elevation $\times$ light exposure	11.2 (1,16)**	n.s	n.s.	4.94 (2,24)*
$\mathbf{PAR}_T$	Elevation	6.34 (1,16)**	n.s.	4.73 (2,24)*	10.46 (2,24)***
(%)	Light exposure	n.s.	6.08 (1,16)*	n.s.	n.s.
	$Elevation \times light \ exposure$	n.s.	n.s	n.s.	4.73 (2,24)**
$PAR_R$	Elevation	41.97(1,16)***	20.99(1,16)*** (1,16)**	n.s.	9.61 (2,24)***
(%)	Light exposure	10.59(1,16)**	n.s	8.52 (1,24)**	n.s.
	$Elevation \times light \ exposure$	18.14 (1,16)***	n.s.	n.s.	4.32 (2,24)*
$UV-B_A$	Elevation	n.s.	n.s.	n.s.	n.s.
(%)	Light exposure	n.s.	n.s.	n.s.	n.s.
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
UV-B <sub>R</sub> (%)	Elevation	n.s.	n.s.	n.s.	n.s.
	Light exposure	n.s.	n.s.	n.s.	n.s.
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
UV-B <sub>A-280</sub>	Elevation	9.36 (1,36)**	13.03 (1,34)***	27.42 (2,52)***	34.02 (2,54)***
	Light exposure	23.64 (1,36)***	16.53 (1,34)***	6.03 (1,52)*	17.53 (1,54)***
UV-B <sub>A-300</sub>	$Elevation \times light \ exposure$	n.s.	n.s.	n.s.	n.s.
	Elevation	n.s	7.92 (1,34)**	31.86 (2,52)***	37.70 (2,54)***
	Light exposure	12.18 (1,36)**	23.07 (1,34)***	5.51 (1,52) *	n.s.
	$Elevation \times light \ exposure$	n.s.	n.s.	3.56 (2,52)*	n.s.
$UV-B_{A-320}$	Elevation	n.s.	10.35 (1,34)**	26.52 (2,52)***	18.63 (2,54)***
	Light exposure	n.s.	22.71 (1,34)***	10.47 (1,52)**	10.90 (1,54)*
	Elevation $\times$ light exposure	n.s.	n.s.	4.84 (2,52)*	n.s.
Chl <sub>a</sub>	Elevation	63.34 (1,34)***	n.s.	n.s.	5.30 (2,52)**
$(\mu mol m^{-2})$	Light exposure	n.s.	n.s.	n.s.	5.12 (1,52)*
	Elevation $\times$ light exposure	n.s	n.s.	n.s.	n.s.
Chl <sub>b</sub> (µmol m <sup>-2</sup> )	Elevation	11.57 (1,34)***	n.s.	n.s.	n.s.
	Light exposure	n.s.	n.s.	n.s.	n.s
	Elevation × light exposure	n.s.	n.s.	n.s.	n.s.
$Chl_{a+b}$	Elevation	47.27 (1,34)***	n.s.	n.s.	4.12 (2,52)*
(µmol m⁻²)	Light exposure	n.s	n.s.	n.s.	6.03 (1,52)*
	Elevation $\times$ light exposure	n.s	n.s.	n.s.	n.s.

CAR	Elevation	n.s	n.s.	5.85 (2,54)**	20.72 (2,52)***
(mg m <sup>-2</sup> )	Light exposure	n.s	13.90 (1,16)**	n.s.	n.s.
N (mmol m <sup>-2</sup> )	Elevation $\times$ light exposure	n.s	n.s.	n.s.	n.s.
	Elevation	n.s	n.s.	16.04 (2,24) ***	11.76 (2,24)***
	Light exposure	n.s	7.75 (1,16)*	14.14 (1,24) ***	9.82 (1,24)**
Chl <sub>a+b</sub> :N	Elevation × light exposure Elevation	n.s 10.93 (1,15)**	n.s. n.s.	n.s. 13.72 (2,24)**	3.45 (2,24)* 11.52 (2,24)**
	Light exposure	n.s.	n.s.	13.57 (1,24)***	n.s.
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	3.94 (2,24)*
$(\sigma m^{-2})$	Elevation	n.s.	n.s.	5.54 (2,24)*	18.91 (2,24)****
(g m )	Light exposure	n.s.	n.s.	12.92 (1,24)*	17.14 (1,24)**
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
LBT	Elevation	7.86 (1,36)**	30.49 (1,36)***	3.46 (2,50)*	6.69 (2,54)**
(µm)	Light exposure	n.s.	11.65 (1,36)**	14.34 (1,50)***	41.88 (1,24)***
	Elevation $\times$ light exposure	n.s.	11.74 (1,36)**	n.s.	n.s.
ADC (um)	Elevation	n.s.	n.s.	28.58 (2,50)***	12.64 (2,54)***
(µm)	Light exposure	n.s.	n.s.	n.s.	40.32 (1,54))***
	Elevation $\times$ light exposure	n.s.	7.41 (1,36)***	n.s.	n.s.
ABC	Elevation	n.s.	n.s.	5.05 (2,50)*	n.s.
(µm)	Light exposure	n.s.	n.s.	5.47 (1,50)*	31.12 (1,54)***
	$Elevation \times light \ exposure$	n.s.	n.s.	4.61 (2,50)*	n.s.
ADE	Elevation	n.s.	12.12 (1,36)**	n.s.	5.48 (2, 54)**
(µm)	Light exposure	n.s.	5.38 (1,36)*	n.s.	12.27 (1,54)***
	$Elevation \times light \ exposure$	n.s.	4.32 (1,36)*	n.s.	n.s.
ABE	Elevation	n.s.	n.s.	n.s.	7.46 (2,54)**
(µm)	Light exposure	n.s.	n.s.	n.s.	34.61 (1,54)***
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
PP	Elevation	10.49 (1,36)**	10.87 (1,36)**	7.43 (1,50)**	n.s.
(µm)	Light exposure	n.s.	16.29 (1,36)***	n.s.	4.89 (1,54)*
	Elevation $\times$ light exposure	n.s.	12.88 (1,36)**	n.s.	6.99 (2,54)**
SP	Elevation	7.73 (1,36)**	28.06 (1,36)***	n.s.	n.s.
(µm)	Light exposure	n.s.	n.s	14.85 (1,50)***	34.62 (1,54)***
	Elevation $\times$ light exposure	7.64 (1,36)**	n.s.	n.s.	n.s.
PP:SP	Elevation	n.s.	n.s.	5.64 (2,50)**	n.s.
	Light exposure	n.s.	n.s.	n.s.	n.s
	Elevation $\times$ light exposure	n.s.	n.s.	3.79 (2,50) *	6.97 (2,54)**
PAR <sub>A</sub> :Chl <sub>a+b</sub>	Elevation	24.76 (1,16)***	n.s.	n.s.	4.75 (2,24)*
(% mmol <sup>-1</sup> )	Light exposure	n.s.	n.s.	n.s.	5.75 (1,75)*
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
PAR <sub>A</sub> :CAR	Elevation	n.s.	n.s.	5.08 (2,24)*	18.31 (2,24)***
(% mg <sup>-1</sup> )	Light exposure	n.s.	n.s	n.s.	n.s.
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.

$PAR_A: LMA$ (% g <sup>-1</sup> cm <sup>-2</sup> )	Elevation	n.s.	n.s.	6.64 (2,24)**	20.08 (2,24)***
	Light exposure	n.s.	n.s	13.1 (1,24)**	15.57 (1,24)**
PAR <sub>A</sub> :LBT (% µm <sup>-1</sup> )	Elevation × light exposure Elevation	n.s. n.s.	n.s. n.s.	n.s. n.s.	n.s. 6.85 (2,24)***
	Light exposure	n.s.	n.s.	14.39 (1,24)***	30.72 (1,24)**
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	3.97 (2,24)*

# Capítulo 6

Elevational variation of the isotopic composition of carbon  $(\delta^{13}C)$  in four species of trees from a mountain cloud forest in southeast Mexico

Elevational variation of the isotopic composition of carbon ( $\delta^{13}$ C) in four species of trees from a montane cloud forest in southeast Mexico<sup>\*</sup>

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Running title: Elevational variation of  $\delta^{13}$ C in cloud forest trees

**Keywords:** cloud forest, isotopic composition <sup>13</sup>C, leaf anatomy; N foliar; Vaccinium consanguineum; Podocarpus matudae var. macrocapus; Ticodendron incognitum; Drimys granadensis

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# 6.1 Abstract:

In this study, we examined the elevational variation of  $\delta^{13}$ C in sun and shade leaves of four cloud forest trees (Vaccinium consanguineum, Podocarpus matudae var macrocarpus, Drimys granadensis and Ticodendron incognitum), and evaluated its relationship with temperature and CO<sub>2</sub> supply and demand through foliar nitrogen (N) content and leaf morphology (thickness of leaf blade, palisade and spongy parenchymas and leaf mass per area (LMA). The  $\delta^{13}$ C values registered for all species were within the intervals reported for C<sub>3</sub> plants (-31.8 to -27.4‰). In general, the  $\delta^{13}C$ values were less negative with elevation increase; the  $\delta^{13}$ C change with elevation was linear, except in *Vaccinium*. A significant relationship between temperature and  $\delta^{13}C$  was recorded only in *Podocarpus*. The mean values of the  $\delta^{13}$ C for three species were positively correlated with LMA, N and leaf and parenchyma thickness, but the effects of these traits on  $\delta^{13}$ C among the species were different. In *Ticodendron*, only LMA and N explain the changes in  $\delta^{13}$ C. The LMA was a good predictor for elevational variation of  $\delta^{13}$ C in all species. The consistency of the influence of foliar N on  $\delta^{13}$ C suggests that species have higher carboxylation efficiency at the higher sites, where there is an N deficit in the soil. The difference in radiation level between sun and shade leaves did not change  $\delta^{13}$ C; however, anatomical changes in the leaves could compensate for changes in radiation. The studied species regulated  $\delta^{13}C$  differently, also reflecting functional diversity among the mountain cloud forests species.

### **6.2 INTRODUCTION**

Changes in concentration of the isotopes  ${}^{13}C$  ( $\delta^{13}C$ ) have been noted along elevational gradients (Cordell et al. 1999). In general, species growing at higher altitudes show less depleted of  $\delta^{13}$ C than species growing in lower zones under similar light and moisture conditions (Körner et al. 1991; Marshall and Zhang 1993; Rundel et al. 2003); however, inverse trends with elevation have also been reported (Warren et al. 2001). In some montane species, these changes in  $\delta^{13}$ C have been associated with greater carboxylation efficiency (Körner et al. 1988; Körner et al. 1991). The variations in  $\delta^{13}$ C have also been associated with various abiotic factors, such as light, temperature, nutrients, air moisture and soil water availability, and with morphological and biochemical plant characteristics, which change across different elevations (leaf mass area, stomatal index, foliar N and others) (Cordell et al.1999; Hultine and Marshall 2000; Shi et al. 2006). The decreases in air temperature, atmospheric concentration of  $CO_2$  and partial pressure (P) that occur as elevation increases are positively correlated with an increase in  $\delta^{13}$ C (Kelly and Woodward 1995). These environmental factors change the relationship between the partials pressures at the carboxylation sites (*Pc*) and atmospheric air of CO<sub>2</sub> (*Pa*) as well as Rubisco  ${}^{12}C/{}^{13}C$  discrimination (Morecroft and Woodward 1996; Körner et al. 1991; Kloeppel et al. 1998).

Stomatal density and conductance (*gs*) have been used to explain the relationship between  $\delta^{13}C$  and elevation since both control the CO<sub>2</sub> supply (Dawson et al. 2002). The effect of *gs* on  $\delta^{13}C$  is differential; Vitousek et al. (1990) found a negative relationship between *gs* and  $\delta^{13}C$ , in contrast to the positive relationships or lack of relationship reported for other sites (Lauteri et al. 1997; Hanba et al. 1999; respectively). Among others, this has been attributed to the moisture and radiation

conditions (Cordell et al. 1999) and to leaf N per area that is positively correlated with  $\delta^{13}$ C (Sparks and Ehleringer 1997; Cordell et al. 1999; Sah and Brumme 2003). This relationship results from the increase in CO<sub>2</sub> demand produced by a greater concentration of photosynthetic enzymes and a consequent higher photosynthetic capacity of the leaves (Morecroft and Woodward 1996; Cordell et al. 1999). The high concentrations of leaf N per area are also associated with high values of leaf mass per unit area (LMA) and leaf blade thickness (LBT; Körner and Diemer 1987; Cordell et al. 1999).

At the level of individual plants, changes have been noted in  $\delta^{13}$ C across the vertical axis of the tree. In general,  $\delta^{13}$ C increases from lower to higher leaf strata (Medina and Minchin 1980; Hanba et al. 1997; Le Roux et al. 2001; Geßler et al. 2001). This tendency has been explained by different hypotheses: *i*) the effects on *Pc/Pa* caused by atmospheric humidity, light and water availability, *ii*) reuse of CO<sub>2</sub> and *iii*) long-term acclimation to the light regime (Le Roux et al. 2001).

To understand the elevational variation in  $\delta^{13}$ C in sun and shade leaves of *Vaccinium* consanguineum, Podocarpus matudae var macrocarpus, Drimys granadensis and Ticodendron incognitum and its relation to CO<sub>2</sub> supply and demand, we evaluated leaf morphological and physiological traits. The following questions were considered: (1) Are the patterns of variation in  $\delta^{13}$ C with elevation and intra-canopy in montane cloud forest consistent with those reported in other forests? (2) Do LMA, leaf N and anatomical traits explain the elevational changes in  $\delta^{13}$ C?

#### 6.3 METHODS

6.3.1. Study area and species

This study was conducted in the surroundings of Santa Cruz Tepetotutla, a village located in northern Oaxaca State, Mexico (17°38′–17°40′ N, and 96°32′–96°33′ W). This region is characterized by an abrupt topography with slopes between 10 and 50°. The soils

are shallow, derived from metamorphic rocks and have high organic matter content (van der Wal 1998). In the study area, the soils are mainly Podzol at the highest sites and Cambisol at the lowest sites (Álvarez et al. 2008). The soils are acidic, and the total reserves of nitrogen and carbon increase with elevation (Velázquez-Rosas unpublished data). The climate in the area is hyper-humid and temperate. The mean total annual precipitation is ~5800 mm and the mean annual temperature is 16.5°C (Rzedowski and Palacios-Chávez 1977). At the study site, the temperature decreases 0.4°C per 100 m of elevation; this rate is lower than rates of 0.5–0.8°C recorded in other cloud forests (Kappelle 1996). The vegetation along the gradient is a mosaic of forest communities distributed according to a gradient from 1120 to 2560 m, classified as premontane below 1500 m, as lower montane cloud forest between 1500 and 2300 m and as upper montane cloud forest from 2300 to 2550 m (Rincón 2007).

The species studied were Vaccinium consanguineum Klotzsch (Ericaceae), Drimys granadensis

L. F. (Winteraceae), *Podocarpus matudae* Lundell var. macrocarpus (Podocarpaceae) and *Ticodendron incognitum* Gómez Laur. & L. D. Gómez (Ticodendraceae). These tree species are important elements of the communities of the cloud forest located in the Sierra Norte of Oaxaca, Mexico (Rincón 2007) and grow from 1500 to 2700 m, but their relative importance changes across the elevational gradient (Rincón 2007). *Ticodendron*, whose relative importance is greater in communities located between 1500 and 2000 m, was collected at 1520 and at 1940 m. *Drimys* grows most abundantly at altitudes above 1900 m and was therefore collected at 1940 and 2380 m. *Podocarpus* and *Vaccinium* grow at all elevations within the studied zone, and they are especially important above 1900 m, so for these two species, three collection sites were chosen, at 1520, 1940 and 2380 m for *Podocarpus* and at 1520, 2040 and 2550 m for *Vaccinium*. Mean temperatures for each of the altitudes sampled are 15.37, 14.8, 13.78, 11.42 and 11.5°C at 1520, 1940, 2040, 2380 and 2550 m, respectively.

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# 6.3.2. Isotopic concentrations of $^{13}C$

To evaluate the isotopic concentration of  $\delta^{13}$ C in the four species, we analysed five composite samples of two different light exposures (sun or shade) per tree, from five different individuals. This was done for each elevation and species. Sun leaves (SL) were collected from the crown, from exposed, south-facing branches, whereas shade leaves (SHL) were collected inside the crown, from north-facing branches. The degree of light exposure in these two environments was previously measured through hemispheric photos taken with a Colpis A Nikon digital camera equipped with a fish eye lens and analysed with the Gap Light Analyzer Watch 2.0 programme (Frazer et al. 1999) and also with a quantum sensor (LI-190, LI-COR, Lincoln, Nebraska, USA). The canopy opening above the sun leaves is 33–66% larger than that above the shade leaves on a cloudy day at noon. Mean values of incident light were 118–437  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> on SL and 27–83  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> on SHL. Each of the composite samples was dried for three days at 70°C, ground in an agate mortar and then analysed, using the combustion method, in a mass spectrometer (Thermofinnigan, Delta XL, Conflo III, German-made) with a precision of 0.2‰. The analyses were carried out in the Laboratory of Mass Spectrometry of Stable Isotopes, in the Geology Institute, UNAM. The quotients of  $\delta^{13}C$  were calculated according to PDC standards.

The other leaf samples were measured with a Leaf Area Meter (LI-3000A LI-COR, Lincoln, Nebraska, USA) dried (as above), weighed and subsequently the LMA was determined. The leaf N and the values for the leaf blade thickness (LBT) and of the palisade (PP) and spongy (SP) parenchymas for each species were taken from Velázquez-Rosas et al. (2010).

# 6.3.3. Statistical Analysis

To compare the mean values of  $\delta^{13}$ C, an ANOVA test was performed considering elevation and light exposure as independent variables. Prior to ANOVA, a Bartlett's test was carried out to test homogeneity of variances. When significant differences were found, we proceeded with a Tukey's test. The statistical analyses were carried out with the programme Statistica 7.1 (Statsoft, Inc., 1984-2003, OK, USA). The relationships between  $\delta^{13}$ C, elevation, temperature and morpho-functional traits were determined by linear regression analysis.  $\delta^{13}$ C was considered as a dependent variable, using a Table Curve 2D, v. 5 (AISN Software, Chicago, IL, USA). We also explored the relationships between the LMA and T and leaf N. These analyses were performed in two different ways: (1) relating  $\delta^{13}$ C and the LMA to the other traits, including either the SL and SHL data or (2) separately. The slopes for each individual group (SL and SHL) were compared through a Student's *t*-test (Zar 1999).

#### 6.4. RESULTS

6.4.1. Relationships of  $\delta^{13}$ C to elevation and temperature

The  $\delta^{13}$ C values of the four species varied between -31.8 and -27.4 ‰ (Fig. 1a-d). *Podocarpus* showed the least negative  $\delta^{13}$ C mean value (-27.4). In *Ticodendron, Drimys* and *Podocarpus*, the less negative mean values for the  $\delta^{13}$ C were recorded at the highest elevation at which each species was sampled (1940, 2380 and 2380 m, respectively; Fig. 1a, b, c). A less negative mean value was recorded at 2040 m (Fig. 1d), but this did not occur in *Vaccinium*. Significant differences in the mean values of  $\delta^{13}$ C among SL and SHL were only observed in *Drimys* and *Podocarpus* at 1940 and 2380 m, respectively (Fig. 1b, c). In *Podocarpus*, the relationships of  $\delta^{13}$ C with elevation and mean

temperature were significant ( $r^2 = 0.42$ , slope = 0.002, p < 0.0001;  $r^2 = 0.46$ , slope = 0.43, p < 0.001; respectively). *Vaccinium* did not show relationships between these variables, and this was not tested in *Drimys* and *Ticodendron* because they were sampled at only two elevations. However, in both species  $\delta^{13}$ C followed the temperature trend in the gradient.



**Figure 1.** Relationships between elevation and isotopic composition  ${}^{13}C$  ( $\delta^{13}C$ ) in sun (closed dots), and shade (open dots) leaves of four tree species growing in the montane cloud forest.

# 6.4.2 Leaf mass per unit area

*Podocarpus* and *Vaccinium* showed significant changes in LMA between sites (Fig. 2c, d); *Vaccinium* showed the highest significant value at the middle elevation (2040 m). Although in most cases the values of LMA were higher in SL than in SHL, significant differences were only found at 2380 m for *Podocarpus* and *Drimys* (Fig 2b, c). In all species, the LBT and N were related significantly to the LMA; the thicker leaves had higher values of LMA (Fig. 2e-h) and N per area (Fig. 2i-l).



**Figure 2.** a-d) Mean values ( $\pm 1$  SE) of leaf mass per unit area (LMA) of sun (closed dots) and shade (open dots) leaves for the studied species. Different letters indicate significant differences (p < 0.05). Relationship between elevation and LMA (a-d); leaf blade thickness (LBT) and LMA (e-h); and LMA and N (i-l). \*\* = p < 0.01, \*\*\* = p < 0.001.

6.4.3. Functional relationships of  $\delta^{13}$ C

In all four species, increases in LMA and N had a positive effect on the  $\delta^{13}$ C. LMA explained 42– 70% of the variation in the  $\delta^{13}$ C in these species (Fig. 3a-d), whereas N explained 55–77% (Fig. 3eh). In *Ticodendron* the anatomical traits did not have significant relationships to  $\delta^{13}$ C. In *Podocarpus* and *Vaccinium*, LBT, PP and SP had positive relationships with  $\delta^{13}$ C (Fig. 3 j, 1, o, p), but in *Drimys* only LBT and SP had positive relationships with  $\delta^{13}$ C (Fig. 3 j, r).

In *Drimys* and *Ticodendron*, a comparison of the slopes of the regression lines fitted to SL and SHL data showed that in the relationships between SP and LBT with  $\delta^{13}$ C the slopes were significantly higher in SL than in SHL (Table 1).



**Figure 3.** Relationship between isotopic composition  ${}^{13}C$  ( $\delta^{13}C$ ) and leaf mass per area, foliar nitrogen (N), thickness of leaf blade (LBT), thickness of palisade parenchyma (PP) and thickness of spongy parenchyma (SP). \*\* = p < 0.01, \*\*\* = p < 0.001.

Table 1. Comparison of slopes' functional relationships sun (SL) and shade (SHL) leaves between  $\delta^{I3}C$  and leaf blade tickness (LBT) and spongy parenchyma (SP). Only significant results (p < 0.05) are showed.

Relationship	Exposure	Slope	<i>t</i> -test	Df		
	light					
Ticodendron incognitum						
SP vs $\delta^{13}$ C	SL	0.203	3.62	16		
	SHL	-0.026				
Drimys granadensis						
LBT vs $\delta^{13}$ C	SL	0.042	2.42	16		
	SHL	0.011				

#### 6.5 DISCUSSION

The values of  $\delta^{13}$ C recorded for the four species were within the reported intervals for C<sub>3</sub> plants (21–35‰; Ehleringer 1991; Hultine and Marshall 2000; Li et al. 2006). It has been established that the  $\delta^{13}$ C values in different species generally are less depleted as elevation increases (Cordell et al. 1998; Sah and Brumme 2003; Shi et al. 2006). The results of this study show that these trends are the same for the four species studied; however, the change was not linear for all studied species. In *Ticodendron, Drimys* and *Podocarpus*, the less negative  $\delta^{13}$ C was noted in the samples taken from the highest elevations (1940 and 2380 m, respectively, Fig. 1), whereas in *Vaccinium*, less negative values of  $\delta^{13}$ C were registered at the middle elevation (2040 m). Nonlinear patterns of change in  $\delta^{13}$ C along elevational gradients have been reported for other species (Li et al. 2006). This suggests that the variables associated with the changes in  $\delta^{13}$ C (N, anatomical traits, radiation and moisture) have different influences in the different species along the elevational gradient.

Variations in environmental factors associated with the change in elevation can alter the  $\delta^{13}$ C values. Particularly, the decreases in air and soil temperatures occurring at high elevations can affect partial pressures of CO<sub>2</sub> in the stomatal cavity (*Pc/Pa*) through a reduction in the stomatal conductance, hence affecting the  $\delta^{13}$ C (Morecroft and Woodward 1990; Körner et al. 1991; Li et al. 2006). In the studied area, a linear decrease of 0.3°C/100 m in the air temperature was registered (Alvarez et al. 2008). Of the studied species, only *Podocarpus* showed a significant effect of temperature on  $\delta^{13}$ C. Nonetheless, in *Ticodendron* and *Drimys*, temperature may have the same effect on the  $\delta^{13}$ C levels, which also increased as temperature decreased with elevation.

In three species, the changes in  $\delta^{13}$ C were positively correlated with LMA, N, thickness of the leaf blade and thickness of at least one of the parenchymas, but these traits that regulate the supply and demand of CO<sub>2</sub> had differential effects on  $\delta^{13}$ C among the species, as described in other studies (Cordell et al. 1998, 1999; Li et al. 2006; Li et al. 2007). The positive relationship between LMA and  $\delta^{13}$ C found in other studies has been interpreted as a consequence of the changes in the internal conductance of CO<sub>2</sub> (*gi*) and of an increase in the photosynthetic enzymes, associated with increases in LMA, LBT and N (Vitousek et al. 1990; Lauteri et al. 1997; Cordell et al. 1999; Hanba et al. 1999; Lamont et al. 2002), as we found in the studied species. The effects of the changes in elevation on structural and functional traits are the increases in the demand for and conductance of CO<sub>2</sub>, which reduces discrimination in the absence of a compensatory stomatal adjustment (common in montane cloud forests species Cordell et al. 1999).

The causes of  $\delta^{13}$ C changes along the elevational gradient are different in each of the studied species. In *Drimys*, *Podocarpus* and *Vaccinium*, the relationship of LMA with  $\delta^{13}$ C could be caused both by variables related to the supply of CO<sub>2</sub> (LBT, SP and or PP) and by those related to demand

for CO<sub>2</sub> (N per area) (Fig. 2). In contrast, in *Ticodendron*, a non-sclerophyllous species with a narrow elevational distribution, the  $\delta^{13}$ C could be affected mainly by CO<sub>2</sub> demand; this species did not have significant relationships between  $\delta^{13}$ C and LBT, PP or SP.

Less negative values of  $\delta^{13}$ C were recorded at the highest elevations, where these species had thicker leaves. The increase of leaf thickness are related with an increase in mesophyll thickness and thus mesophyll surface area resulting in greater CO<sub>2</sub> capture and potentially lower Pc/Pa and therefore reduced discrimination in absence of compensatory stomatal adjustment (Cordell et al. 1999). In *Podocarpus*, the elevational change in  $\delta^{13}$ C might be affected more by anatomical traits (LBT and PP) because they explained more of the variation in  $\delta^{13}$ C (48–70%), whereas N explained only 55% of the elevational change in  $\delta^{13}$ C, suggesting that the CO<sub>2</sub> supply determines the change in  $\delta^{13}$ C. On the contrary, in *Drimys* and *Vaccinium*, anatomical traits and N explained the variation in  $\delta^{13}$ C. Despite the finding that leaf thickness may itself explain the changes in CO<sub>2</sub> fixation and therefore in  $\delta^{13}$ C, (*sensu* Cordell et al. 1999) in *Drimys*, *Podocarpus* and *Vaccinium*, SP explained more of the variation in the  $\delta^{13}$ C values (57–70%) than PP (48–49%) probably due to its functional role in internal CO<sub>2</sub> conductance. This result agrees with the findings described by Ivanova and P'yankov (2002) for mesic conditions.

The fact that in *Vaccinium* the highest values of  $\delta^{13}$ C and N were recorded at the middle elevation (2040 m) suggests that at this elevation additional factors ought to be taken in account to explain its CO<sub>2</sub> assimilation and the consequent  $\delta^{13}$ C. In montane forests, a negative correlation between foliar N per unit area and the concentration of N in the soil has been observed (Tanner et al. 1998), which implies that species growing under a N deficit would present the highest concentrations of foliar N and consequently maintain efficient CO<sub>2</sub> assimilation and a positive balance in the assimilation of

 $CO_2$  (Cordell et al. 1999; Sah and Brumme 2003). In previous studies in the study area it has been noted that at sites between 2040 and 2380 m there are decreases in the concentrations of both total N and its available forms (Velázquez-Rosas N., unpublished data), which would explain why foliar N was higher at these sites, as reflected in the  $\delta^{13}C$  of *Vaccinium* and *Drimys*. Although the relationship between N and  $\delta^{13}C$  was significant in *Ticodendron*, the role of N in the  $\delta^{13}C$  is not entirely clear because in this species there are no differences in foliar N between elevations (Velazquez-Rosas, et al. 2010); the temperature decrease might explain the elevational changes in  $\delta^{13}C$ .

Intra-canopy differences in the isotopic discrimination of  $\delta^{13}$ C have been attributed to changes in the partial pressures of CO<sub>2</sub> (*Pc/Pa*) caused by variations in the atmospheric humidity, the light environment and the refixation of respiratory CO<sub>2</sub> (Buchmann et al. 1997; Le Roux et al. 2001). In the studied species, there were no clear intra-canopy differences across the elevational gradient. There were only differences between SL and SHL in *Podocarpus* and *Drimys* at 2380 m (Table 1). This lack of differentiation in  $\delta^{13}$ C between SL and SHL was a constant pattern among the morphofunctional traits analysed. The tree species of mountain forests are characterised by narrow crowns of low depth, in which leaves are generally located at the periphery (Whitmore 1984; Velázquez-Rosas 2000). Despite no significant differences being found in the  $\delta^{13}$ C between SL and SHL in most of the studied species, in *Drimys* the variable related to the conductance of CO<sub>2</sub> (LBT) had a greater influence on the  $\delta^{13}$ C values for SL than on those for SHL. In general, SL have higher photosynthetic rates and use water more efficiently than do SHL, increasing the CO<sub>2</sub> demand and thereby decreasing discrimination of <sup>13</sup>C (Le Roux et al. 2001; Geßler et al. 2007).

In conclusion, the elevational changes in  $\delta^{13}$ C recorded in the studied species were associated mainly the variables related to the supply and demand of CO<sub>2</sub>, although these variables had different

roles in each species. The LMA was a good predictor for elevational variation of  $\delta^{13}$ C in all species because LMA is related with the balance between the supply and demand of CO<sub>2</sub> (Hultine and Marshall 2000). The consistency of the influence of foliar N on the  $\delta^{13}$ C suggests that species have high carboxylation efficiency at the sites where environmental conditions are less favourable, making more efficient the use of limiting nutrient resources (Cordell et al. 1998). Finally, in all studied species, the radiation incident on SL and SHL did not determine changes in  $\delta^{13}$ C. The anatomical changes may compensate for the changes in radiation on the tree crowns to keep CO<sub>2</sub> assimilation rates similar between leaves with different exposures. The studied species regulate  $\delta^{13}$ C differently, which also reflects the functional diversity in the montane cloud forests.

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Capítulo 7

Discusión general

## 7.1 Discusión general

Los estudios observacionales sobre gradientes altitudinales son muy importantes para evaluar las respuestas ecológicas y evolutivas de la biota a la variación ambiental (Körner 2007). En los bosques húmedos de montaña se presenta una gran variación en los atributos de las comunidades vegetales a lo largo de gradientes altitudinales, cambios que han sido asociados a la variación ambiental ligada al cambio altitudinal (Bruijnzeel et al. 1993, Tanner et al. 1998, Wilcke et al. 2008). En este estudio exploramos la variación de las características funcionales de cuatro especies arbóreas a lo largo de un gradiente altitudinal y su relación con la variación de temperatura atmosférica, la radiación fotosintéticamente activa (400-700 nm; RFA), ultravioleta (280-320 nm, UV-B) y las reservas de nitrógeno en los horizontes orgánicos del suelo, características señaladas como factores determinantes en los atributos de estas comunidades (Bruijnzeel et al. 1993). Los resultados obtenidos muestran que las especies estudiadas presentan distintos patrones de variación altitudinal de sus atributos morfo-funcionales y que el impacto de los factores ambientales analizados, es diferencial y pueden influir de manera distinta en su desempeño. La discrepancia en las respuestas morfo-funcionales de las especies estudiadas a la variación ambiental puede estar basada en el hecho de que cada especie presenta distintos niveles de tolerancia a las condiciones ambientales y uso de sus recursos (Soethe et al. 2008), y por lo tanto las diferencias en las respuestas están reflejando la alta diversidad funcional presente en estos bosques.

Un aspecto importante a considerar en el análisis del efecto de la variación altitudinal sobre los atributos de las comunidades vegetales y las características funcionales de las especies, es distinguir los efectos de factores físicos ligados con la altitud (presión atmosférica, temperatura, radiación solar), de los efectos de factores ambientales locales (precipitación, velocidad del viento, exposición de ladera, entre otros), debido a que estos últimos generalmente pueden confundir el papel de la

altitud y los factores ligados directamente a ésta (Körner 2007). En este estudio se confirmó que la diferenciación en el comportamiento altitudinal de los factores ambientales puede ayudar a explicar los patrones de variación no lineales que se registraron en las respuestas morfo-funcionales de las especies estudiadas. Esta diferenciación también permitió inferir su grado de influencia en el desempeño, abundancia y distribución de las especies estudiadas a lo largo del gradiente. Como se describió en los capítulos cuatro y cinco, los contenidos de nitrógeno disponible en los suelos y los niveles de radiación fotosintéticamente activa (RFA) y ultravioleta (UV-B, 280-320 nm) estimados no mostraron los cambios lineales con la altitud que se esperarían, lo que se correlacionó con algunas de las repuestas morfo-funcionales de las especies.

Aunque tradicionalmente se ha considerado que los niveles de radiación (RFA y UV-B) se incrementan conforme aumenta la altitud, considerando mediciones en atmósferas secas Körner (2007) señala que este patrón no es consistente en los sistemas montañosos, debido a que los niveles de radiación que reciben las plantas a lo largo de este gradiente dependen en gran medida de la dinámica de las nubes y la niebla, las cuales modifican la calidad y cantidad de radiación tanto de RFA como UV-B. Esto fue patente en este estudio donde los niveles de radiación de RFA y UV-B estuvieron directamente relacionados con la presencia de dos bancos de nubes ubicados en 1940 y 2380 m snm que influyeron en las respuestas morfo-funcionales registradas en las especies de estudio.

La temperatura media anual fue el único factor ambiental evaluado que si mostró una tendencia lineal de reducción, ligada al incremento en altitud, pasó de  $15.3^{\circ}$ C en 1520 a  $11.5^{\circ}$ C en 2550 m, con una tasa de decremento de  $0.4^{\circ}$ C por cada 100 m de incremento en altitud. Esta tasa de cambio es más baja de las observadas para otros bosques montanos del trópico que se ubican entre  $0.5 - 0.8^{\circ}$ C (Kitayama 1995, Kappelle 1996). La temperatura ambiental es un factor que influye en

distintos procesos, como el intercambio gaseoso y la fotosíntesis. Sin embargo, la reducción de la temperatura de la atmósfera registrada en este estudio no se correlacionó con ninguna de las características morfo-funcionales analizadas, con excepción del <sup>13</sup>C en *Podocarpus matudae*. Una de las razones por las que probablemente no se encontró una correlación entre la temperatura media anual y las características evaluadas, es que los valores de temperatura promedio no reflejan los valores de temperatura que tienen un impacto fisiológico relevante durante la época de crecimiento de las plantas (Körner 2007). Otro aspecto que puede enmascarar las respuestas de la vegetación a la influencia de la temperatura y a otros factores físicos, a lo largo del gradiente altitudinal, es que a la escala espacial en donde tienen efecto (microescalas  $\alpha$  [2 km–200 m] y  $\beta$  [ 200–20 m); Orozco-Segovia y Sánchez-Coronado 2009) pueden interactuar con otros factores de manera conjunta sobre las propiedades foliares. Debido a esto, las respuestas de éstas pueden ser respuestas integrales al conjunto de factores, es decir, hay una respuesta integrada a más de un factor ambiental.

Una de las hipótesis más recurrente para explicar la reducción de la productividad de los bosques húmedos de montaña y sus características foliares está relacionada con la reducción de nutrimentos en los suelos, en particular con la disponibilidad nitrógeno (Grubb 1977, Bruinjzeel *et al.* 1993, Tanner *et al.* 1998). En relación con el estatus nutricional de los suelos, en este estudio los resultados mostraron que el nitrógeno total y mineral de los horizontes orgánicos no se relacionaron con los atributos comunitarios evaluados (diversidad, área basal, densidad, biomasa aérea), pero sí tuvieron relación con la concentración de nitrógeno y la relación carbono-nitrógeno (C:N) foliar de las especies estudiadas. En muchos casos los factores que determinan los atributos de las comunidades no son los mismos que determinan los rasgos funcionales de los individuos (Violle *et al.* 2007). La falta de relación entre el N del suelo y los atributos comunitarios ha sido descrita para otros bosques húmedos de montaña del trópico (Waide *et al.* 1998, Roman y Scatena en prensa) y

remarca que la variación de estos atributos tiene un origen multifactorial, en donde otras variables ambientales como la temperatura, la radiación fotosintéticamente activa, la toxicidad de compuestos fenólicos, Mn y Al en los suelos y los disturbios pueden tener una fuerte influencia (Bruijnzeel *et al.* 1993, Cox et al. 2002, Waide *et al.* 1998, Wilcke *et al.* 2008).

Las relaciones encontradas entre el N foliar y los almacenes de N total y disponible de los suelos pueden tener importantes repercusiones en el desempeño de las especies estudiadas. Es ampliamente reconocido que las concentraciones foliares de nitrógeno afectan directamente la productividad de las comunidades vegetales en distintos ecosistemas (Reich et al. 1997). De manera particular en Drimys granadensis, Podocarpus matudae y Vaccinium consanguineum los valores de C:N foliar indicaron que enfrentan un déficit nutricional para su crecimiento en los sitios de estudio. En los bosques húmedos de montaña se ha registrado que las especies que se desarrollan en suelos con déficits de N presentan altas concentraciones de N foliar por unidad de área (Tanner et al. 1998). Esta respuesta podría favorecer el incremento en la concentración de las enzimas fotosintéticas (como Rubisco; Evans 1989), lo que puede aumentar la eficiencia en la fijación de CO<sub>2</sub> en momentos de condiciones favorables y un uso más eficiente del nitrógeno por unidad de tiempo, características que se reflejan en las hojas de larga vida de estos bosques (Cordell et al. 1999, Sah y Brumme 2003). Esta respuesta al déficit de N es muy probable que ocurra en las tres especies arriba mencionadas, las cuales pueden presentar una mayor eficiencia en la fijación de CO<sub>2</sub> en los sitios con mayor déficit de N, tal como lo sugieren los valores menos negativos de  $\delta^{13}$ C registrados para los sitios de mayor déficit de N de cada especie (Tabla 2, Capitulo 4; Fig. 1A-D, Capítulo 6). En las tres especies los incrementos de  $\delta^{13}$ C estuvieron correlacionados positivamente con las concentraciones de N foliar (Fig. 3, Capitulo 6). Además de esta respuesta fisiológica ante el estrés nutricional de nitrógeno, en las tres especies se registraron cambios anatómicos que se asocian a condiciones oligotróficas, como el incremento en el grosor de sus parénquimas, epidermis y cutículas (Fig. 4, Capítulo 5). En los bosques montanos estos cambios están asociados con la conservación de nutrientes, incrementos en las tasas de asimilación de CO<sub>2</sub> o mecanismos para la defensa contra patógenos o herbívoros y protección contra los incrementos de radiación UV-B (Grubb 1977, Flenley 1995, Bruijnzeel y Proctor 1995, Cordell *et al.* 1998).

Las respuestas obtenidas para la deficiencia de nitrógeno de las cuatro especies estudiadas pueden estar asociadas a otras variables ambientales evaluadas como los niveles de RFA y UV-B, aunque estos factores parecen tener un menor impacto en la determinación de los rasgos morfo-funcionales evaluados. De manera general, las cuatro especies tuvieron patrones similares en sus propiedades ópticas para RFA (absorbancia [85–95%], reflectancia [6–12%] y transmitancia [0.4–2%]) y UV-B (absorbancia [95-96%] y reflectancia [4-5%]) y pocas variables morfo-funcionales estuvieron correlacionadas de manera significativa y exclusiva con los niveles de RFA y UV-B (Tabla 2, Capítulo 5). A pesar de las limitantes técnicas para la evaluación directa de RFA y UV-B, la amplia variación registrada para RFA (65-1260  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) sugiere que estas especies no se encuentran en ambientes limitados por los niveles de radiación. Los niveles de radiación estimados indican que las hojas de sol y de sombra en plantas C3 del sitio de estudio pueden alcanzar el punto de saturación (200–500 hasta 1000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectivamente; Chazdon *et al.* 1996). Otra evidencia que apoya esta idea, es que la anatomía foliar de estas especies no corresponde a los patrones foliares de hojas de sombra que se han reportado en otros bosques húmedos del trópico (Bongers y Popma 1988) e inclusive no se detectó una clara diferenciación morfo-funcional entre hojas de sol y sombra en las especies estudiadas.

Determinar y separar el impacto ecológico de una sola característica morfo-funcional sobre el desempeño individual de las plantas es una tarea complicada (Poorter *et al.* 2009). Por ello, se ha

propuesto el uso de variables que integren las respuestas de distintas características foliares (estructurales y bioquímicas) a la variación ambiental y regulen el desempeño de las plantas. Dentro de estas características el área foliar específica (m<sup>2</sup> kg<sup>-1</sup>) y la masa seca por unidad de área (g m<sup>-2</sup>; LMA –por sus siglas en inglés) pueden ser características que pueden ser utilizadas como un índice del desempeño de las plantas (Lambers y Poorter 1992, Grime 2001, Poorter *et al.* 2009). El uso de el LMA es útil cuando se desea conocer los factores que determinan la variación de estas características (anatómicas y composición química) debido a que muestra un relación lineal con estos factores (Poorter *et al.* 2009), aunque algunos estudios muestran que el LMA no es siempre el mejor predictor del desempeño de las plantas (Pérez-Harguindeguy *et al.* 2003). En este estudio el LMA podría ser una variable que ayude a explicar el desempeño de las especies, en términos del área basal [AB] de las especies y los factores ambientales que lo regulan a lo largo del gradiente altitudinal; sin embargo, es importante considerar las diferencias entre distintos grupos funcionales que se pueden presentar en estos bosques como a continuación se describe.

De manera general, las cuatro especies estudiadas, mostraron incrementos en LMA en los sitios más altos de su distribución (Fig. 3, Capitulo 5), pero el impacto de esta variable sobre el desempeño parece ser diferencial y merecen una exploración profunda que conduzca a la identificación de grupos funcionales. Algunos datos encontrados en las especies estudiadas sugieren la presencia de distintas estrategias que pueden ligarse a la variación ambiental que determina el cambio altitudinal. Por ejemplo, *Ticodendron incognitum* es la especie que presentó los valores más bajos de LMA y el mayor valor de AB de las cuatros especies. *T. incognitum* se desarrolla preferentemente en las zonas bajas (~1200-2000 m snm), en donde existe una mayor disponibilidad de nitrógeno (ver comportamiento de nitrógeno potencialmente mineralizable; Fig. 1b, Capítulo 4) y sus valores foliares de C:N (24.9:1 en 1520 m; 26.1:1 en 1940 m) no indican que esta especie

enfrente una limitación nutricional para su crecimiento. Por ello, valores bajos de LMA serían favorables para una mayor productividad en el sitio de 1520 m, en donde también se registró una menor radiación. Esto sugiere que las especies arbóreas con valores bajos de LMA podrían tener desventajas competitivas en los sitios más altos donde las condiciones nutricionales y de temperatura disminuyen.

En términos del LMA, D. granadensis y V. consanguineum, tuvieron características foliares típicas de especies de ambientes oligotróficos. Estas especies, mostraron un claro déficit nutrimental en relación C:N foliar, en todos los sitios donde fueron colectadas fueron mayores a 30:1. En ambas especies la relación LMA-AB fue positiva, es decir ambas variables cambiaron en el mismo sentido. En ambientes con condiciones de crecimiento adversas, valores altos de LMA pueden representar una ventaja competitiva, ya que conservan y utilizan de manera más eficiente los recursos limitantes (Körner 1989; Tanner et al. 1998, Poorter et al. 2009). Esto podría explicar parcialmente que ambas especies muestran valores más altos de AB en los sitios donde se presenta el mayor déficit nutricional (2040 m para V. consanguineum y 2380 m para D. granadensis). Debido a que la radiación fotosintéticamente activa también puede influir en los incrementos de LMA (Poorter et al. 2009) y por lo tanto afectar su relación con el AB. En ambas especies los valores más altos para el AB fueron registrados en los sitios donde se incrementó la RFA, en 2380 m para Drimys y en 2040 y 2550 m para Vaccinium. En el caso de Podocarpus la relación de LMA-AB no fue clara y la influencia del LMA con el ambiente nutricional y la RFA parece ser más compleja, por lo que es probable que en esta especie el LMA no sea un predictor de su desempeño en términos del área basal; aunque hay que señalar que esta especie es una gimnosperma, lo que puede tener una gran influencia en su respuesta del LMA a las variaciones altitudinales.

## 7.2 Conclusiones.

La variación de los factores ambientales evaluados (temperatura, nitrógeno total y disponible en los horizontes orgánicos de los suelos y niveles de radiación RFA y UV-B), no mostró, con excepción de la temperatura, un comportamiento lineal a lo largo del gradiente altitudinal. Por lo que, su impacto sobre las respuestas funcionales de los árboles son locales a lo largo de este gradiente. Una caracterización que integre la variación espacial y temporal de estas variables, podría ayudar a dilucidar con mayor precisión su impacto sobre los cambios de los atributos de las comunidades de los bosques húmedos de montaña a lo largo del gradiente altitudinal.

La variación de las respuestas morfo-funcionales de las especies estudiadas sugiere que en los bosques húmedos de montaña existe una gran diversidad funcional. La diversidad funcional es un atributo relevante en ambientes con condiciones y recursos muy heterogéneos, tal como sucede en los bosques húmedos de montaña.

Las respuestas morfo-funcionales evaluadas en las cuatro especies sugieren que el estatus nutricional de los suelos, en particular del nitrógeno disponible en los horizontes orgánicos, tiene un fuerte impacto sobre la variación de estas características a lo largo del gradiente altitudinal, aunque son modulados por el ambiente lumínico y los regímenes de temperatura. Sin embargo, a nivel comunitario otras variables ambientales, no ligadas directamente con el cambio altitudinal (la toxicidad de compuestos fenólicos, Mn y Al en los suelos y los disturbios pueden tener una fuerte influencia) y pueden explicar la variación altitudinal de los atributos de estos bosques.

En la presente investigación fue dificil establecer generalizaciones sobre respuestas morfofuncionales a la variación de RFA y UV-B a lo largo del gradiente altitudinal a partir de los resultados de cuatro especies estudiadas. Por ello, para determinar con mayor precisión el impacto de la variación del ambiente lumínico sobre las respuestas y los atributos de los bosques mesófilos es relevante realizar caracterizaciones más completas del ambiente lumínico, que integren la variación temporal y espacial a lo largo del gradiente altitudinal, como sugiere Pearcy (2007) para otros bosques donde el ambiente lumínico es muy heterogéneo.

El análisis de las características funcionales en las comunidades arbóreas de los bosques húmedos de montaña es útil para entender los factores ambientales que influyen en los cambios de los atributos comunitarios a lo largo del gradiente altitudinal. De manera particular, en estudios que incluyan un número más amplio de especies, hay que considerar el uso del LMA como una variable predictora del desempeño de los árboles, que ayude a establecer patrones de variación altitudinal de los atributos de las comunidades de estos bosques, así como establecer las variables ambientales que influyen en sus cambios.

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Anexo 1

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Original article

# Optical and morpho-functional traits of the leaves of tree species growing in a mountain cloud forest

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#### ABSTRACT

The physiological, anatomical and optical leaf properties relative to photosynthetically active (PAR) and ultraviolet (UV-B) radiation were assessed in Ticodendron incognitum, Drimys granadensis, Podocarpus matudae var. macrocarpus and Vaccinium consanguineum, growing along an elevation gradient (1520-2550 m asl) in a montane cloud forest in México. PAR and UV-B absorptance, transmittance and reflectance, UV B absorptance by foliar compounds, chlorophylls, carotenoids, leaf nitrogen, leaf mass per area, leaf blades, cuticles, epidermis and parenchymas thickness were measured. PAR absorptance efficiencies were calculated. Among the evaluated morpho-functional traits, the studied species displayed different patterns of variation with elevation. Leaf traits could be explained in part by changes in elevation or the distribution of PAR and UV-B in the elevation gradient. Ticodendron and Drimys leaf traits were likely determined by two cloud banks located at 1940 and 2380 m. In Vaccinium, eight traits were related to elevation and PAR or UV B. Contrary to this, in Podocurpus, most of the nine leaf traits could be explained by only one of these factors. The morphological traits of the studied species were similar to those of species growing in other oligotrophic ecosystems. Significant differences between sun exposed and shade leaves were limited to particular elevations or to particular traits of each species. Vaccinium showed more significant differences between sun and shade leaves than did the other species growing along the gradient. The morpho-functional traits measured in Podocarpus and Vaccinium showed that, some leaf traits did not change linearly with elevation or PAR. At elevation levels where species co-occur, the species ranking with respect to evaluated traits varied from trait to trait. This indicate that each species copes with light and other environmental factors, that vary with elevation, according to its morpho-functional plasticity and susceptibility to these factors; which may determine the distribution of these species along the gradient.

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#### **1. Introduction**

Montane cloud forests (MCFs) are known for their cloudy weather conditions (Webster, 1995; Cavelier, 1996). In a dry atmosphere, radiation increases with increased elevation (Jones, 1992), but in MCFs, clouds and fog attenuate the increases associated with elevation in photon flux density (PAR, 16%–53%), and modify light quality. In addition, both of these vary in space and time because of the daily and yearly vertical movements (along the elevational gradient) of the cloud banks and fog (Aylett, 1985). These factors and other variables such as nitrogen availability and

temperature could affect the CO<sub>2</sub> assimilation rate of the plants (Körner et al., 1986; Bruijnzeel et al., 1993 Kitayama, 1995; Tanner et al., 1998) and could explain why productivity decreases with increased elevation. Nevertheless, low vapor pressure delicits encourage leaf traits that increase light harvesting without significantly affecting the plant's water balance (Poorter et al., 2000).

Plant adaptation and acclimatization to their growing light conditions (photon flux density and quality) are accurately expressed by leaf traits (Lambers et al., 1998; Sims and Gamon, 2002). Therefore, traits linked to light harvest and/or protection of the photosystems should be indicators of the mean light conditions that plants undergo, as well as the response of the species to environmental factors that change with elevation (Richardson et al., 2001; Richardson and Berlyn, 2002; González et al., 2007). MCFs' species are adapted to shade and sumy conditions, which change in space and time in

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response to the dynamic movements of clouds and fog (Cavelier and Goldstein, 1989; Bruijnzeel and Proctor, 1995), and thus, morphofunctional leaf traits might reflect this changing light condition more than the light changes related to elevation do. However, similarly to sun exposed and shade leaves of tropical rain forest species (Poorter et al., 1995), sun exposed and shade leaves of pioneer and primary species of Venezuelan MCF trees growing at 1750 m asl (Poorter et al., 2000) showed significant but reduced differences between them. In these species, photosynthetically active radiation (PAR, 400-700 nm) absorptance is also similar to that found in the shade leaves of species growing in tropical rain forests and in seasonal forests, which also optimize light absorptance by having horizontal leaves with a low specific leaf weight (Bongers and Popma, 1988) and a high chlorophyll mass-based content (Lambers et al., 1998). Consequently, in MCFs where such environmental factors change with elevation, we may expect a reduced, but significant, variation in the morphofunctional leaf traits associated with variations in PAR or other factors such as moisture and temperature that change with elevation and determine nutrient availability.

In general, UV-B increases by 14-18% for each 1000 m of increased elevation (Caldwell et al., 1989; Jones, 1992), but in MCFs, this change may be reduced by the attenuating effects of clouds and fog on UV-B radiation (Dahlback, 1996; Køiskar et al., 2003). Even though levels of UV-B radiation are low, UV-B radiation (280 320 nm) has been reported to determine the leaf traits of MCF species (Bruijnzeel et al., 1993; Flenley, 1995; Rozema et al., 1997). These increases in UV-B are mainly reflected in the leaf traits (Sullivan et al., 1992; Ziska et al., 1992) of plants growing at the highest elevations (Flenley, 1995). Tropical species at high elevations have high concentrations of UV-B absorbing phenolic compounds in the leaves (Robberecht et al., 1980; Barnes et al., 1987; Bruijnzeel et al., 1993; Ziska, 1996). These compounds increase with elevation, as observed in the montane forests of Jamaica (Rozema et al., 1997). Other functional responses related to the increase in UV-B radiation that have been recorded in plants growing in other ecosystems and in the leaves of trees in montane

regions are a reduction in plant growth, a decrease in leaf area, and anatomical changes (Tanner and Kapos, 1982; Caldwell et al., 1989; Antonelli et al., 1997). However, nutrient conditions and temperature may also explain either the decreased leaf size or the presence of sclerophyll leaves in plants growing above 2000 m asl.

In the present work, we hypothesized that, irrespective of the cloudy and foggy environment, morpho-functional leaf traits will reflect the increases in PAR associated with elevational gradients. Specifically, photosynthetic pigments (chlorophylls and carotenoids per unit area) and the leaf mass per area will increase. The leaf content of UV-B screening compounds, the cuticle and the epidermis thickness will increase in response to UV-B. PAR and UV-B absorptance will increase with the elevation gradient, but the PAR absorptance efficiencies, defined as PAR absorptance per unit chlorophyll, carotenoid, biomass or leaf thickness, will decrease. To test these hypotheses in an elevational gradient ranging from 1520 to 2550 m asl, we selected three of the most abundant MCF species. located in Santa Cruz Tepetotutla, Oaxaca, México with a wide distribution across different elevations (Vaccinium consanguineum, Podocarpus matudae var. macrocarpus and Drimys granadensis) and one species with a narrow distribution (Ticodendron incognitum).

#### 2. Materials and methods

#### 2.1. Study zone

This study was conducted in the area surrounding Santa Cruz Tepetotutla, a village in the North of the state of Oaxaca, México (17°38′ 17°40′ N, 96°32′ 96°33′ W). This region has an abrupt topography with slopes between 10° and 50°. The soils are shallow, derived from metamorphic rocks, and have a high content of organic matter. The soils of the upper portion of the toposequence are Podzols, whereas those of the lower portion of the toposequence are Cambisols (Ålvarez et al., 2008). Soils are acidic and lack significant amounts of bases and available nitrogen. Total carbon content decreases as the elevation increases (Ålvarez et al., 2008).



Fig. 1. Mean values (±SE) of environmental data for the elevations where each species was collected. A D) photosynthetically active radiation (PAR circles) and temperature (squares). E-H) UV-B radiation. I-J) elevational distribution of PAR and UV-B. Sunny (open circles or gray bars) and cloudy days (closed circles or black bars). Letters indicate significant differences inside seasons, small letters for the rainy season and capital letters for the dry season.

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The climate in the study area is hyper-humid and temperate. The mean annual total precipitation is ~ 5800 mm, and during the driest month (March), the mean precipitation is 50 mm (Rzedowski and Palacios-Chávez, 1977). Temperature data recorded at the study sites show that the mean temperature decreases from 15.3 to 11.5 °C along the altitudinal interval from 1520 to 2550 m (Álvarez et al., 2008; Fig. 1). The temperature decreases at a rate of 0.4 °C per 100 m of elevation; this rate is lower than rates recorded for other cloud forests, which demonstrate rates ranging from 0.5 to 0.8 °C (Kitayama, 1995; Kappelle, 1996). The vegetation along the gradient is a mosaic of forest communities, classified as premontane below 1500 m asl, lower mountain cloud forest between 1500 and 2300 m and upper mountain cloud forest from 2300 to 2550 m asl (Rincón, 2007). These forests are highly diverse, include ca. 200 tree species distributed along the elevational gradient. The study area is covered with an undisturbed and mature forest that is protected under the guidelines of the "farmer's reserve".

## 2.2. Species selection, study sites and photon flux density measurements

Four tree species were selected: V. consanguineum Klotzsch (Ericaceae), D. granadensis L. F. (Winteraceae), P. matudae Lundell var. macrocarpus J. Buchholtz et N.E. Gray (Podocarpaceae) and T. incognitum Gómez Laur. & L. D. Gómez (Ticodendraceae). We will refer to these species by their genus. These species have high relative importance values (RIV - relative basal area + relative density - relative frequency) in several communities (Rincón, 2007) Five study sites were established between 1520 and 2550 m asl (1520, 1940, 2040, 2380 and 2550 m). The altitude is followed by a subscript letter that indicates the lowest (L), middle (M) or highest (H) elevation where each species was collected. A small letter stands for a species collected at two elevations only, and capital letters designate species collected at three elevation levels. Podocurpus, Vaccinium and Drimys grow at 1520-2550 m, whereas Drimys is very scarce at the lowest and highest elevations. Due to the abrupt topography, the leaves were collected from the species growing on slopes with the same orientation and where the plants were abundant and accessible. Vaccinium was collected at  $1520_{\rm b}$ ,  $2040_{\rm M}$  and  $2550_{\rm H}$  m, Podocarpus at  $1520_L$   $1940_M$  and  $2380_H$  m, Drimys at  $1940_l$  and  $2380_h$  m, and Ticodendron at 15201 and 1940h m. The mean annual temperatures at the collection sites are shown in Fig. 1.

In the five study sites, PAR (400-700 nm) and UV-B (280-320 nm) were measured in the dry season (March) and in the rainy season (September) in sun exposed places using a spectroradiometer (LI-COR, Inc., Nebraska, USA). For each elevation, the measurements were performed on two days with similar weather conditions from 7:00 to 18:00 h. To validate the measurements obtained on different days using the spectroradiometer, PAR measurements were performed simultaneously with five quantum sensors (U-190, U-COR, Lincoln, Nebraska, USA), one per elevation level, during 10 days per season. Mean PAR values (±standard error) per elevation, measured with the quantum sensors were:  $447.55-49.38,\ 153.28+15.34,\ 643+47.63,\ 317.06-35.26,\ 510.04\ \pm\ 80.79\ \mu mol\ m\ ^2\ s\ ^1$  (1550,\ 1940,\ 2040,\ 2380,\ 2550, respectively). In addition, PAR and UV-B data for the dry and rainy seasons were calculated by computing the PAR radiation at the top of the atmosphere according to the solar geometry and irradiance and taking into account the solar elevation and declination, the angle between the surface and the sun, and the transmissivity of the atmosphere for every week (Jones, 1992). The transmissivity of the atmosphere was calculated by computing the PAR radiation at the top of the atmosphere on the days when PAR and UV-B measurements were collected in the field, during the dry and rainy seasons. Calculations were obtained for 26 days per season (dry and rainy).

#### 2.3. Functional and anatomical traits

Sun exposed leaves (SL) were collected from sun exposed, south facing branches, while the shade leaves (SHL) were collected inside the crown from north-facing areas. Only healthy, mature and fully expanded leaves were included in the study. Leaves were collected in January of 2004 and 2007 from at least five individuals per sampled site. The leaves of all of these species have a long life span. The degree of canopy openness between these two light environments was assessed using hemispheric photographs taken with a Colpis A Nikon model digital camera equipped with a fish eye lens and analyzed with the Gap Light Analyzer Watch 2.0 program (Frazer et al., 1999). During leaf collection on a cloudy day at noon, PAR was also measured using a quantum sensor. The number of measurements differed between elevations because of variable difficulties associated with climbing the trees (Table 1). After collection, 30 leaves per tree were oven-dried for three days at 70 °C and weighed, and the leaf mass per area (LMA) was calculated. Next, in composed samples, nitrogen content (N) was quantified in five (one per tree) 13 µg samples per elevation and species, with an Elemental Analyzer (NC 2500, Thermo Quest S.P.A., Rodanao, Milan, Italy). The equipment was calibrated with a standard curve generated for sulfanilamide (C, 41.84%; H, 4.68%; N, 16.27%; O, 18.58% and S, 18.62%; CE Elantech, Inc., Lakewood, N.J., USA).

The transmittance (PAR<sub>T</sub>) and the reflectance (PAR<sub>R</sub>) of PAR and UV-B (280–320 nm) were measured in leaves that were preserved fresh by immediate placement in water. Optical measurements were conducted after 24 h for five SL and five SHL per species and site, using a spectrophotometer (LAMBDA 19, Perkin Elmer, Boston, USA) with an integrating sphere (B009-4012, Perkin Elmer, Boston, USA) with an integrating sphere (B009-4012, Perkin Elmer, Boston, USA) at intervals of 2 nm. PAR absorptance (PAR<sub>A</sub>) was calculated as follows: PAR<sub>A</sub> = 1–(PAR<sub>A</sub> + PAR<sub>T</sub>) (Poorter et al., 2000). UV-B (280–320 nm) absorptance (IV-B<sub>A</sub>) was calculated as UV-B<sub>A</sub> = 1–UV-B<sub>R</sub>, because the UV-B transmittance of the leaves was below the detection limit of the instrument (photometric accuracy of 0.08% T at 1 A). The optical properties of the entire leaf were measured. To confirm the results, the optical properties were determined for leaves collected in 2004 and in 2007.

To determine the contents of chlorophylls a and b (Chl<sub>a</sub> and Chl<sub>b</sub>) and carotenoids (CAR: xanthophylls and carotenes), the pigments were extracted with 4 ml of N,N-dimethylformamide from the leaf disks (0.6 cm in diameter) of 10 SL and 10 SHL per species and site, which were cut from the central part of the leaf blades avoiding the primary vein. The absorptance of the extract was measured with a spectrophotometer at 480, 646.6 and 663.8 nm for CAR, Chl<sub>a</sub> and Chl<sub>b</sub>, respectively (Porra et al., 1989), using quartz cuvetles. The pigment area-based content was calculated according to Wellburn (1994). The absorptance efficiencies were calculated as the ratios between PAR<sub>6</sub> and total chlorophyll (Chl<sub>a</sub>), the CAR area-based contents, the dry leaf mass per area (LMA) and the leaf blade thickness (LBT).

Table 1						
Mean (J	LSE) values	of canopy	openness	and	photosynthetically a	active radiation
(PAR), in	sunny and	shaded an	eas in the	study	y areas, where sun a	nd shade leaves
were col	lected. Lett	ers indicate	e significan	t diff	erences (p < 0.05).	

Elevation	Canopy openness (%)			PAR (µ.mol <sup>2</sup> 5 <sup>1</sup> )			
(masl)	Sun	Shade	dſ	Sun	Shade	dſ	
1520	27.3 (3.1)a	12.8 (2.1)5	10	485 (69.2)a	69 (18.8)b	8	
1940	28.7 (2.9)a	14.0 (2.2)5	10	576 (262.7)a	27 (7.5)	5	
2040	36.5 (3.5)a	12.9 (1.2)5	10	179 (47.4)a	24 (4.6)5	5	
2380	48.7 (4.7)a	16.3 (1.8)5	10	132 (20.4)a	59 (22.2)h	11	
2550	29.9 (4.1)a	19.3 (1.6)5	10	118 (29.3)a	51 (33.4)b	5	

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To evaluate UV-B absorptance by screening foliar compounds, leaf disks (0.6 cm in diameter) collected as described for the chlorophyll determinations were obtained from 10 SL and 10 SHL per species and site and placed in screw-on tubes containing 5 ml of extraction medium to assess UV-B screening compounds (79:20:1, v:v:v, ethanol:water:hydrochloric acid). The tubes containing the leaf disks were heated for 2-3 h at 96 °C (boiling water at 2250 m asl) in a water bath until the leaf disks were completely bleached. Leaf extract absorptance was measured at 280 (UV-B<sub>A-230</sub>), 300 (UV-B<sub>A-300</sub>) and 320 nm (UV-B<sub>A-320</sub>) using a spectrophotometer (Rozema et al., 1997).

To relate the anatomical traits to the optical properties in 10 SL and 10 SHL, we measured the leaf blade thickness (LBT), the palisade parenchyma (PP) and the spongy parenchyma thickness (SP), as well as the thicknesses of the adaxial and the abaxial cuticles (ADC and ABC) and epidermis (ADE and ABE). Only in Podocarpus did the measurements of the adaxial and abaxial epidermis include the hypodermis (a layer of cells lying immediately beneath the epidermis that may serve as a protective layer). Anatomical traits were measured via transverse cuts in the central part of the leaf blades. These samples were fixed in FAA (formalin 5%, acetic acid 4%, ethylic alcohol 48% and water 43%). After dehydration in a gradual series of ethyl alcohol, the material was infiltrated with paraplast, embedded in the same material, and cut into sections of 10 µm. The sections were stained with safranin and fast green and observed using a photomicroscope Olympus Provis X-70 (Olympus, Tokyo, Japan). To perform anatomical measurements, the images were digitized and analyzed with the Carnoy software (V 2.0, 2001, Peter Schols).

#### 2.4. Statistical analysis

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PAR and UV in sun exposed areas of the five elevations were analyzed separately by first using a multifactor ANOVA (elevation and season) and then an ANOVA for each season followed by Tukey's test. The canopy openness and PAR in sunny and shaded areas were analyzed using Student's t-test. To assess the morpho-functional responses to elevation and light exposure (SL and SHL), the homogeneity of variances was confirmed by Bartlett's test, and the data were then compared by multilactor ANOVA. Significant differences between SL and SHL (Appendix) were observed for only a few traits at particular elevations. Therefore, to that the results are shown for species, leaf trait, and elevation. In cases for which homogeneity of the variance was not detected, log transformations were applied. Multiple comparisons were performed using Tukey's test, and the statistical analyses were conducted using a computer program (Statistica 6.1. Statsoft, Inc., 1984–2003, OR, USA), Regression analysis between elevation, PAR or UV-B values (on sunny days) and morphofunctional traits that showed significant differences between elevations (Table 2) were performed for Vaccinium and Podocarpus using TableCurve 2D, v. 3 (AISN Software, Chicago, IL, USA). For these species, the regression analyses were also conducted separately for sun exposed and shade leaves that showed significant differences in at least one sampled site, per leaf trait and species. These cases were only related to PAR or UV-B (sunny day values). A comparison slope test was carried out to compare the slopes of the curves generated for sun and shade leaves (Zar, 1999). The mean values determined for canopy openness and PAR for sun exposed and shade tree branches were compared for each elevation using Student's t-test.

#### 3. Results

PAR and UV-B differed significantly between elevations, and the values for the rainy season were significantly lower than those determined for the dry season (P < 0.001). The mean light (PAR and UV-B) values at the sites where the species were collected are shown

#### Table 2

Results of the regression analysis carried out to determine the relationships between morpho-functional traits of Vaccinium consanguineum and Podocarpus matudae and the PAR or UV-B values registered in sunny days. Data were fitted to the function y = a + bx and in all the cases the probability was 0.00001. (df) degrees of freedom. Only significant relationships are shown. Photosynthetic active radiation transmittance (PAR<sub>i</sub>) and reflectance (PAR<sub>i</sub>); UV-B by screening foliar compounds absorptance (UV- $B_{A-250}$ , UV- $B_{A-300}$  and UV- $B_{A-320}$ ); carotenoids (CAR) and leaf nitrogen (N) concentrations; nitrogen investment (Chl<sub>a+b</sub>:N); leaf mass per area (LMA); thickness of: leaf blade (LBT); adaxial cuticle (ADC) and epidermis (ADE); palisade parenchyma (PP); ratio PP:SP; PAR absorptance efficiencies per LMA (PARALIMA).

Relationship	R <sup>2</sup>	F	df	b	a
V. consunguineum					
PAR <sub>8</sub> vs ELEVATION	0.70	65.92	29	0.002	5.41
CAR VS ELEVATION	0.46	48.4	57	-0.046	182.85
Chla+U: N vs ELEVATION	0.74	81.45	29	-0.0017	8.99
UV-BA-280 VS ELEVATION	0.39	37.68	59	0.001	0.49
UV BA-300 VS ELEVATION	0.50	57.96	59	0.001	0.31
UV-BA-320 VS ELEVATION	0.51	60.90	59	0.0009	-0.07
ADC vs ELEVATION	0.47	53.12	59	-0.0007	-0.49
ADE vs ELEVATION	0.37	34.75	59	0.002	16.12
PAR <sub>R</sub> vs PAR	0.72	73.09	29	0.006	4.57
CAR VS PAR	0.44	43.31	57	-0.1009	210.8
N vs PAR	0.47	25.31	29	0.063	25.09
Chla+t:N vs PAR	0.70	66.14	29	-0.005	10.09
LIV-BA 280 VS LIV-B	0.52	62.74	59	1.101	-1.09
UV-BA-300 VS UV-B	0.56	73.77	59	1.043	-1.61
ITV-BA 330 VS LIV-B	0.59	8.3.07	59	0.743	-0.90
CAR vs UV B	0.42	40.39	57	25.72	184.89
ADE VS UV-B	0.42	41.99	59	2.4	11.64
P. matudae					
PAR <sub>T</sub> VS ELEVATION	0.69	63.11	29	0.0007	-0.48
ADC vs ELEVATION	0.37	34.75	59	0.0012	1.09
PP vs ELEVATION	0.49	52.08	59	0.025	20.21
LBT vs ELEVATION	0.5	55.03	59	0.035	248.39
PP:SP vs ELEVATION	0.41	38.09	59	0.00014	0.12
PAR <sub>7</sub> vs PAR	0.6	42.36	29	-0.001	1.90
CAR VS PAR	0.43	43.48	59	-0.041	147.29
N vs PAR	0.61	62.54	29	-0.185	248.51
Chla+t:N vs PAR	0.75	84.32	29	0.011	-0.31
LMA vs PAR	0.57	.37.18	29	-0.062	183.59
PARA: LMA vs PAR	0.71	69.47	29	0.044	31.53
UV-BA-280 VS UV-B	0.63	95.99	57	-1.360	7.22
UV BA-300 VS UV B	0.55	109.31	57	0.663	3.84
UV-BA-320 VS UV-B	0.62	57	57	-0.395	2.44
CAR VS LIV-B	0.49	57.76	59	-16.44	162.18

in Fig. 1A-H. PAR and UV-B showed a wide variation along the altitudinal gradient; in both cases, the mean values did not increase linearly with elevation. PAR (Fig. 11) and UV-B radiation (Fig. 1]) varied from 156 to 1260  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and from 0.85 to 4.43  $\mu$ l m<sup>-2</sup> s<sup>-1</sup>. respectively. In the dry season, the lowest PAR values were recorded at 1940 and 2380 m (P < 0.001). At 1520, 2040 and 2550 m, PAR increased with elevation; however, significantly different values were only detected between 1520 and 2550. In the dry season, UV-B at 1940 and 2380 m differed significantly from UV-B at 2550 m (P-0.0016). At the other elevations, UV-B followed a trend similar to PAR; however, UV-B at 1520 m did not differ significantly from UV-B at the other elevation levels. In the rainy season, PAR was highest at 2550 m, differing significantly from PAR at 2380 m (P = 0.0073); however, from 1520 to 2380 m, PAR demonstrated no significant differences. During the same season, UV-B did not differ along the elevational gradient (P = 0.068, Fig. 1J). The canopy openness and PAR at the sites and elevations where sun exposed and shade leaves were collected demonstrated significant differences (Table 1); the mean canopy openness was 27.3-29.9 and 12.8-9.3% for sun exposed and shaded sites, whereas the mean PAR values were 118-576 and 24–69  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively.

No significant differences were found between the optical properties of leaves measured in 2004 and 2007 in all species.

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The results obtained for leaves collected in 2007 are presented in Fig. 2. At the sites where *Ticodendron* and *Drimys* were sampled, PAR and UV-B followed a negative and positive trend with elevation, respectively (Fig. 1A, B, E, F). In *Ticodendron*, most of the traits followed the same trend observed for PAR and UV-B (inverse trend with elevation): PAR<sub>A</sub> (Fig. 2A), UV-B<sub>A-280</sub> (Fig. 2F), Chls (Fig. 3A–C), Chl<sub>a+b</sub>:N (Fig. 3F), LBT (Fig. 4A), PP (Fig. 4F), and SP (Fig. 4G). In contrast, PAR<sub>7</sub> (Fig. 2B), PAR<sub>8</sub> (Fig. 2C) and PAR<sub>4</sub>;Chl<sub>a+b</sub> (Fig. 5A) increased with increased elevation. In *Drimys*, PAR<sub>8</sub> (Fig. 2K), all the UV-B absorptances by screening foliar compounds (Fig. 4D) followed the

same increasing trends as PAR, UV-B and elevation. Only  $\mbox{PAR}_A$  (Fig. 2I) demonstrated the inverse trend.

*Vaccinium* was sampled at altitudes that presented a linear increase in PAR and UV-B with increased elevation (Fig. 1D, II). Thus, the traits that had a significant relationship with elevation demonstrated the same behavior for PAR or UV-B (Fig. 1, Table 2). The relationship was positive for PAR<sub>R</sub> (Fig. 2AA), ADE (Fig. 4AB), and all the UV-B absorptances by screening Ioliar compounds (Fig. 2AD-AF) and negative for CAR (Fig. 3Y) and  $chl_{a+b}$ :N (Fig. 3AA, Table 2). Exceptions were ADC (Fig. 4Z), which decreased with increased elevation but demonstrated no significant relationship to



**Fig. 2.** Mean values ( $\pm$ SE) of photosynthetically active radiation absorptance (PAR<sub>A</sub>), transmittance (PAR<sub>A</sub>) and reflectance (PAR<sub>A</sub>). UV B absorptance (UV B<sub>A</sub>) and reflectance (UV-B<sub>A</sub>) and UV-B<sub>A-300</sub> and UV-B<sub>A-3</sub>

T. incoanitum D. granadensis P. matudae V. consanguineum A Н 0 v a (µmol m<sup>-2</sup>) 750 Chla 500 250 в (Jumol m<sup>-2</sup>) chlb 300 200 100 Chla+b √ m<sup>-2</sup>) ( 0 X 1000 800 (Jumol 600 400 Carotenoids D P 150 (mg m<sup>-2</sup>) 100 50 Leaf nitrogen (mmol m<sup>-2</sup>) 200 В 7 S I. 150 h a ab h 100 b 12 F M AA 10 Chla+b:N h 8 b 6 b 4 200 AB G LMA (g m<sup>-2</sup>) ab 150 h 100 50 1520,940,2040,2380,2550 1520,940,2040,2380,2550 1520,940,2040,2380,2550 1520,940,040,2380,2550 Elevation (m asl)

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Fig. 3. Mean values ( $\pm$ SE) of chlorophylls (Chl<sub>a</sub>, Chl<sub>b</sub> and Chl<sub>a-C</sub>), carotenoids, leaf nitrogen (N) concentrations, Chl<sub>a+C</sub>N ratios and leaf mass per area (LMA) for *T. incognium*. *D. granadensis*, *P. matuda*e and *V. consanguineum* along elevation. Different letters on the bars indicate significant differences (*P*; < 0.05) between elevations for each species.

PAR or UV-B, and N (Fig. 3Z), which showed a significant increase only with PAR (Table 2). Other traits that varied significantly between the sites but showed no significant relationships with elevation, PAR or UV-B were as follows: PAR<sub>A</sub> (Fig. 2Y), Chl<sub>a</sub> (Fig. 3V), Chl<sub>a+b</sub> (Fig. 3X). LMA (Fig. 3AB). LBT (Fig. 4Y) and ABE (Fig. 4AC) were not related to elevation, PAR or UV-B, but they demonstrated the significantly highest value at 2040<sub>M</sub> m; by contrast, all of the absorptance efficiencies (Fig. 5M–P) at this altitude showed the lowest value.

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*Podocarpus* was sampled in an elevational gradient where PAR and UV-B decreased with elevation (Fig. 1C, G); the highest value was recorded at 1520<sub>1</sub> m. Only PAR<sub>7</sub> (Fig. 2R), ADC (Fig. 4R), PP (Fig. 4V), PP:SP (Fig. 4X) and LBT (Fig. 4Q) showed a positive relationship with elevation (Table 2). In contrast, many traits were related to PAR, related positively to Chl<sub>a+b</sub>:N (Fig. 3T) and PAR<sub>4</sub>: LMA (Fig. 5K), and related negatively to PAR<sub>7</sub> (Fig. 2R), N (Fig. 3S), CAR (Fig. 3R), and LMA (Fig. 3U, Table 2). All UV-B absorptances by screening Ioliar compounds (Fig. 2V X) and CAR (3R) showed a negative relationship with UV-B (Table 2). The traits that varied significantly between the sites but had no significant relationship with elevation, PAR or UV-B were ABC (Fig. 4S) and PAR<sub>A</sub>:CAR (Fig. 5]), both of which demonstrated the lowest value at  $1940_{\rm M}$  m.

For most of the morpho-functional traits, light exposure (Table 1) induced significant differences between SL and SHL at particular elevations (Appendix). In *Ticodendron*, significant differences were only observed for UV-B<sub>A-290</sub> and UV-B<sub>A-300</sub> at the site with the highest PAR value (1520<sub>1</sub> m) and for PAR<sub>R</sub> at the site with the lowest PAR value (1940<sub>h</sub> m, Fig. 1A). In contrast, for *Drimys* at the site with the highest PAR value (2380<sub>a</sub> m, Fig. 1B), differences were detected for several traits: PAR<sub>R</sub> CAR, LBT, ADE and PP. On the other hand, all UV-B absorptances by screening foliar compounds demonstrated significant differences between SL and SHL at the site with the lowest PAR value (1940<sub>h</sub> m, Fig. 1B). In *Drimys*, significant differences were only detected for N at both collection sites.

For the two species sampled at three elevations, Vaccinium showed significant differences in UV-B\_A  $_{\rm 3201}$  LBT, ADC, PP and SP



Fig. 4. Mean values ( $\pm$ SE) of anatomical traits of *L* incognitum, *D. granadensis*, *P. matudae* and *V. consonguineum* found along elevation. PPSP = palisade:spongy parenchymas thickness ratio. Different letters on the bars indicate significant differences (P < 0.05) between elevations for each species.

between light exposures at the three sampled sites ( $1520_L$ ,  $2040_M$ , and  $2550_H$  m; Fig. 1D; Appendix). Comparisons of the slope (P < 0.05) showed that *Podocarpus* differed between SL and SHL with respect to Chl<sub>a+0</sub>:N (t = 7.4, df = 14) along the PAR gradient. In contrast, *Vaccinium* displayed differences in UV-B<sub>A 320</sub>, N, Chl<sub>2+b</sub>:N, ADC and PP (t = 13.8, 3.12, 5.37, 2.76, and 7.8; df = 29, 14, 14, 29 and 29; respectively).

### 4. Discussion

In MCFs, vegetation plant attributes vary widely along elevational gradients (Velázquez–Rosas et al., 2002; Rincón, 2007), and the main source of this variation is the altitude and its associated environmental factors (Vázquez and Givnish, 1998). Although the sampling sites were not replicated in the present study due to the low tree density of the studied species (for example, the density of *V. consanguineum* varies between 30 and 60 individual ha<sup>-1</sup> between 1520 and 2550 m) and the similar slope and geographic orientation (exposure facing) required for each sampling site, the results are indicative of the effects of elevation and light on the development of some leaf traits of the studied species (mainly for the two species sampled at three elevations). However, the patterns of variation by elevation for the evaluated morpho-functional traits were not consistent. PAR did not increased linearly with elevation and did not allect species in the same grade. Each species demonstrated a unique pattern for each measured trait; this result may mask a sampling site effect or reflect functional diversity of species to cope with the environmental changes associated with

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**Fig. 5.** Mean ( $\pm$ 5E) photosynthetic active radiation (PAR) absorptance efficiencies expressed per unit of total chlorophylls (PAR<sub>4</sub>:Chl<sub>3</sub>), carotenoids (PAR<sub>4</sub>:CAR), leaf mass per area (PAR<sub>4</sub>:LMA) and leaf blade thickness (PAR<sub>4</sub>:LRT) for *T. incognitum*, *D. granadensis*, *P. monulae* and *V. consanguineum* along elevation. Different letters on the bars indicate significant differences (P < 0.05) between elevations for each species.

the elevational distribution of MCFs in the study area. In Ticodendron and Drimvs, several of the evaluated traits (12 and 9 traits, respectively) followed the changes observed for PAR and UV-B along the elevational gradient; however, differences in PAR or UV-B between the Drymis sampling elevations were not significant. Nevertheless, these species were only sampled at two elevations, which restricts the generation of conclusions regarding their changing patterns along the elevational gradient and comparisons with the other two studied species. The trend demonstrated by light with elevation and the daily observations made by residents suggest the frequent presence of cloud banks (at 1940 and 2380 m, Fig. 11, [] that seem to be determinants for the contrasting patterns demonstrated by Ticodendron and Drimys, because these banks cause reductions mainly in PAR. In Podocarpus and Vaccinium, many of the elevational changes observed in the studied traits could not be explained directly by changes in PAR or UV-B. There were fewer significant relationships between light and the evaluated traits (9 and 8 traits, respectively) compared to the trends in elevation and light demonstrated by Ticodendron and Drimys. In Vaccinium, a greater number of the changes determined by light (PAR and UV-B) were also significantly related to elevation, likely because Vaccinium was sampled at three sites where PAR increased with elevation (1520<sub>L</sub>, 2040<sub>M</sub>, 2550<sub>H</sub>; Fig. 1D, H). In contrast, two cloud banks were located at two of the sites where Podocarpus was sampled (  $1940_M$  and  $2380_\Pi$  m, Fig. 1C, G). Therefore, the significant relationships detected between leaf attributes and light were not the same as those related to elevation (9 and 5 traits, respectively), excluding PAR<sub>1</sub> (Table 2).

In the present study, we evaluated 27 leaf traits; however, *Vaccinium* demonstrated only 9 traits that could be explained by elevation or light, and *Podocarpus* had only 13. This result showed that each species had its own respective strategy for coping with light and other biotic and abiotic environmental factors associated with the collection sites and/or changes in elevation (such as nutrients, temperature and pathogens). It is important to note that

the causes of leaf trait variation could be due to microclimatic variation at  $\alpha$  and  $\beta$  scales (2 km–200 m and 200–20 m, respectively; Orozco-Segovia and Sánchez-Coronado, 2009) and at lower scales that modulate biotic interactions, soil properties and dynamics, and other factors such as temperature and light properties. Furthermore, these microclimatic scales, microtopography and wind speed could play important roles in determining the physio-morphology of plants (Jones, 1992). These factors are relevant to understand the functional diversity of MCFs that show a wide variability in their biotic, physical and chemical attributes.

PAR was measured simultaneously at the five elevations during the dry and rainy seasons for the studied species, despite the short periods of field observations the optical properties related to PAR were similar to those of other species growing in MCFs. PARA was higher than PAR<sub>R</sub> and PAR<sub>T</sub> (85-95%, 6-12% and 0.4-2%, respectively). The leaves of the species growing at lower elevations demonstrated a high mean  $\text{PAR}_{\text{A}}(90\%)$  and a low mean  $\text{PAR}_{\text{R}}$  values (7.7%; Poorter et al., 1995, 2000) and, consequently, a low mean PART. Among the species studied herein, the low PART values (1 to 7-8%) were consistent with that recorded for other species from MCFs and from other tropical forests (Poorter et al., 2000; Vázquez-Yanes et al., 1990). At noon in the study area, PAR varied between  $156 = 65.7 \ \mu\text{mol} \ \text{m}^{-2} \ \text{s}^{-1}$  on cloudy days and more than  $1260 \pm 117.6 \ \mu mol \ m^{-2} \ s^{-1}$  on sunny days. These PAR values could saturate the photosynthesis of shade and sun exposed leaves in C<sub>3</sub> plants (200 500 1000 µmol m<sup>-2</sup> s<sup>-1</sup>, respectively; Chazdon et al., 1996) in lowland tropical forests, for which photosynthesis is saturated at a wide range of PAR values; this variation is related to leaves acclimatization to the heterogeneity of the light distribution beneath the canopy (Vázquez-Yanes et al., 1990). This may also help to explain why many functional traits in Vaccinium and Podocarpus did not show a significant relationship with PAR along the elevation gradient. Per example, although of Chls did not change with elevation in most of species, in Ticodendron, Podocarpus, and Vaccinium, the Chl<sub>atb</sub>:N ratio decreased as the elevation increased.

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This occurs in elevational gradients where soil N falls as the elevation increases and where radiation increases with increased elevation, due to a reduced investment of the species in light harvesting (Evans, 1989: Niinemets, 1997).

Although the present data obtained for PAR indicate a sharp distinction between sun and shade conditions (Table 1), we consistently found few and inconsistent differences between SL and SHL. These changes were confined to particular elevations for most of the species, excluding changes in N or  $Chl_{a+b}$ : N and a few other traits that showed significant differences between sun exposed and shade leaves along the gradient in some of the species (see Appendix). This small difference has been attributed to the cloudy and foggy environment of MCFs and the environmental heterogeneity (Poorter et al., 1995, 2000). However, the morphological traits of the studied species did not correspond to those reported for many understory shrubs or rain forests trees (e.g., Bongers and Popma, 1988). For various leaf traits (UV-BA 320, N, Chl<sub>a+b</sub>:N. ADC and PP), Vaccinium presented a linear relationship along the elevational gradient with significant differences between shade and sun exposed leaves. This can be related to the dense and complex architecture of the crown of this species, compared to the architecture of Podocarpus (Velázquez-Rosas, 2000), which demonstrated a difference only in  $Chl_{a+5}$ :N.

The leaves of five canopy tree species growing in the tropical rain forest reflect 26 90% of the UV-B light at 287 nm (Lee, 1980; Lee and Lowry, 1980). UV-B<sub>R</sub> was homogeneous (4–5%) among the studied species and elevations. In species growing at high elevations in MCFs and in areas with clear skies, increases in the content of screening foliar compounds have been reported (Caldwell et al., 1989; Rozema et al., 1997; Filella and Peñuelas, 1999). The same was observed for the species studied herein. excluding UV-BA-320 in Ticodendron. Drimys and Vaccinium showed an increase in UV-B screening foliar compounds as UV-B radiation increased with elevation. In contrast, Podocurpus demonstrated a negative relationship with UV-B. Despite the methodological limitations encountered in evaluating the field UV-B, this contrasting response and the fact that clouds attenuate UV radiation (Dahlback, 1996; Køiskar et al., 2003) strongly suggest that in some species, leaf traits, soil N and/or herbivores and pathogens may induce changes in the content of screening foliar compounds (Harborne and Williams, 2000; Kolb and Pfündel, 2005). In the field, more parasitism and herbivory was observed in Podocarpus and Drimys populations at sites where the cloud banks were located (1940 and 2380 m asl; N. Velázquez-Rosas, personal observations). In Podocurpus and Vaccinium, CAR showed negative relationships with PAR and/or UV-B. Previous studies have demonstrated that screening compounds play a more relevant role in young compared to mature leaves (Lee, 1980), this may explain the results obtained in mature leaves used in the present study, which did not increase with elevation.

Anatomical leaf traits partially determine the optical properties of leaves (Vogelman et al., 1996): the adaxial cuticle and epidermis reflect PAR and UV-B (Baltzer and Thomas, 2005). Polyphenols in the cuticles and epidermis play an important role in protection by reflecting and absorbing PAR and UV-B, respectively (Reuber et al., 1996; Solovchenko and Merzlyak, 2003; Rouseaux et al., 2004; Bidel et al., 2007). In the present study, Podocarpus and Vaccinium demonstrated significant elevational changes in ADC and/or ADE, but only Vaccinium showed a significant relationship between ADE and UV-B radiation. In addition, in Podocarpus, PP and LBT were not significantly related to UV-B. In contrast, in Ticodendron and Drimys, the species with a lower LBT followed the same trend observed for PAR with elevation, and the changes in LBT were due to changes in the parenchyma, which may protect the photosynthetic apparatus. Despite these contrasting changes between the species, the LBT values for all of the species studied, and other traits as cuticles. were comparable to those of sclerophyllous species that grow in exposed canopies in tropical areas (Medina et al., 1990) and in oligotrophic environments; they differed from non-sclerophyllous species that grow in these areas (Bongers and Popma, 1988; Pérez-Garcia et al. 1992). These findings confirm that in the studied species, the soil characteristics of MCFs may play a greater role than the light environment (PAR or UV-B) in determining leaf traits.

The studied species did not demonstrate the same response to light or elevation at sites where the populations overlapped, and several traits (such as the PAR absorptance efficiencies in Vaccinium and Podocarpus) did not change linearly with elevation. Non-linear patterns have been previously reported and attributed to variability in species susceptibility to environmental factors linked to elevation (Li et al., 2006), which may modulate the nature and magnitude of plant responses. In the present study, non-linear patterns could be related in part to the PAR distribution in the gradient determined by the presence of the two cloud banks (Fig. 11, K). These banks might also play a role in modifying the temperature and nutrient availability along the gradient and, consequently, the leaf traits.

#### 5. Conclusions

With respect to MCFs, the present research contributes to our understanding of some of the causes inherent to individuals. species and environments that pose difficulties in making generalizations about trends for all morpho-functional traits of plants and environmental parameters with variations in light along elevational gradients, as occurs in other forests (Hultine and Marshall, 2000). The main reasons for these challenges might be the environmental variations at macro and microscales that change with elevation and those related to the sampling sites that are involved in plant responses. Leaf traits did not always change linearly with increased elevation, which could be due to changes at the macroscale when cloud banks are present at different elevations. Additionally, each species coped with light and other environmental factors, according to their plasticity and sensitivity to environmental factors that determine the point at which the distribution of each species declines. The morpho-functional diversity discovered among the studied species reflects and likely explains part of the high diversity of species found in MCFs and in the mosaic of forest communities distributed according to the elevation gradient reported for this area (Rincón, 2007). To better understand of the functionality of the MCFs it is necessary to increase our knowledge about leaf traits in different MCFs and to obtain measurements for PAR and UV-B that encompass an entire year, at different elevations to accurately characterize the cloud and fog movements over days and even years.

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## Appendix

ANOVA test results for morphological and functional traits of sun and shade leaves (light exposure) of *E* incognitum, *D*, granadensis, *P* matudae and *V*, consanguineum, along an elevational gradient. F value and degree freedoms (in parentheses) are indicated.  $^{\sim} < 0.05$ ,  $^{\sim} < 0.01$  and  $^{\sim} < 0.001$  after Tickey's test. Photosynthetic active radiation absorptance (PAR<sub>4</sub>), transmittance (PAR<sub>7</sub>) and reflectance (PAR<sub>4</sub>). UV-B<sub>A320</sub>, and reflectance (PAR<sub>4</sub>), transmittance (PAR<sub>7</sub>) and reflectance (PAR<sub>4</sub>). UV-B<sub>A320</sub> and UV-B<sub>A322</sub>; chorophylis (Chl<sub>6</sub>, Chl<sub>6</sub>hs); carotenoids (CAR) and leaf nitrogen (N) concentrations; nitrogen investment (Chl<sub>6+6</sub>hs); leaf mass per area (LMA); thickness of leaf blade (LBT); adaxial and abaxial cuicles (ADC and ABC; and epidemis (ADE and ABE); palisade (PP) and spongy parenchymas (SP); ratio PP.SP. PAR absorptance efficiencies per unit total chlorophylis (PAR<sub>4</sub>; Chl<sub>6+7</sub>); carotenoids (PAR<sub>4</sub>; CAR); leaf mass per area (PAR<sub>4</sub>:LMA) and leaf blade thickness (PAR<sub>4</sub>; LBT).

Traits	Factor	Ticodendron incognitum	Drimys granadensis	Podocarpus matudae	Vaccinium consonguineum
PAR, (%)	Elevation	58.8 (1,16)***	15.35 (1,16)***	n.s.	5.81 (2,24)***
	Light exposure	n.s.	D.S.	n.s.	ns
	Elevation × light exposure	11.2 (1.16)**	n.s	0.5.	494 (2.24)*
PART (%)	Flevation	6 34 (1 16)**	ns	473 (224)*	10.46 (2.24)***
	Light exposure	ns	6.08 (1.16)*	ns	ns
	Elevation × light exposure	ns	ns	ns	273(274)**
PAR. (%)	Flovation	41.97 (1.16)***	20.09 (1.16)***	ns	961 (2 24)***
	Light evposure	10.59 (1.16)**	ns	8 57 (1 74)**	ns
	Elevation v light exposure	18 14 (1 16)***	n.s n.s	ne char,	A 3 7 (7 7A)*
[IV-B. (%)	Floration	ns	n.s. n.c	n c	ns
the off (a)	light exposure	ns	n.c.	0.5	ns
	Elevation v light exposure	n.s.	n.s.	0.5	ns.
(R/ B. (%)	Elevation ~ light exposere	n.s.	D.C.	nes.	D.S.
DV DR(M)	Light exposure	n.s.	n.s.	n.c.	ns.
	Elevation v light exposure	n.s.	n.s.	0.5	n.s.
IN B	Floration	0.26 (1.26)**	12 02 (1 24)511	11.3. 37 43 <b>/3 53</b> \#≈#	24 02 (2 54)***
UV DA-280	Light exposure	336(1,30)	15.03 (1,34)	E 07 (1E7)*	17 53 (1 54)###
	Elevation light oversure	23.04 (1,30)	10.00 (1,04)	0.05 (1,52,	17.33(1,34)
INIE	Elevation × light exposure	n.s.	7.02/1.2/>>*	21.96 (0.50)###	27 70 (2 54) ***
04-DA-300	Light avecause	13	7.52 (1.55)	51.00 (2,02)	57.70 (2,54)
	Elevation light operation	12.18(1,56)	23.07 (1,34)	3.51 (1,52, 3.56 (3.53)*	11.3.
1010	Elevation × light exposure	II.S.	10.75 /1.74388	3.30 (2.52)	10 CD /D E 41385
UV-DA 320	Light and a suga	11.8.	10.33 (1,34)	20.52 (2,52)	10.00 (1.54)
	right expusite	n.s.	22.71 (1,54) <sup>24</sup>	10.47 (1,52)***	10.90 (1,54)*
Children and an 25	Elevation × light exposure	11.5. (1) 1) 4 (1) 1) 4) 8 10	11.5.	4.04 (2,32)"	IIS.
cma (junoi m - j	Light apparents	03.34 (1,34)***	n.s.	n.s.	5.50 (2,52)**
	right exposite	11.5.	0.5.	11.5.	5.12 (1,52)*
(the formula 2)	Elevation × light exposure	II.S	11.5.	n.s.	ns.
cme (mier m )	Light generate	11.37 (1,34)	11.S.	11.5.	113. Df
	Elevation - light exposure	n.s.	11.3. 5.6	n.s.	11.5
$\Box h \downarrow (m a \rfloor m^{-4})$	Elevation	47 37 (1 24)***	11.5. D.C	0.5	113.
canal b (, and in )	light exposure		D.C.	D.S.	603 (1 52)*
	Elevation v light exposure	ns	ns.	0.5	ns
$CAR (mem ^{2})$	Flevation	ns	ns.	5 85 (2 54)**	20 72 (2 52)***
	Light exposure	0.5	13.90 (1.16)**	0.5	05
	Elevation × light exposure	ns	ns	ns	ns
$N (mmcl m^2)$	Elevation	<b>D</b> .5	n.s.	16.04 (2.24) ***	11.76 (2.24)***
an Arter and a c	Light exposure	n.s	7.75 (1.16) <sup>×</sup>	14.14 (1.24) ***	9.82 (1,24)**
	Elevation × light exposure	n.s	n.s.	n.s.	3.45 (2.24)*
Chlads: N	Elevation	10.93 (1.15)**	n.s.	13.72 (2.24)***	11.52 (2.24)**
	Light exposure	n.s.	n.s.	13.57 (1.24)***	ns.
	Elevation × light exposure	n.s.	n.s.	n.s.	3.94 (2.24)*
LMA $(g m^{-2})$	Elevation	n.s.	n.s.	5.54 (2,24)*	18.91 (2,24)***
Second Contraction of the	Light exposure	n.s.	n.s.	12.92 (1,24)*	17.14 (1,24)**
	Elevation × light exposure	n.s.	n.s.	n.s.	n.s.
LBT (µm)	Elevation	7.86 (1,36)**	30.49 (1,35)***	3.46 (2,50)*	6.69 (2,54)**
	Light exposure	n.s.	11.65 (1,36)**	14.34 (1,50)***	41.88 (1,24)***
	Elevation × light exposure	n.s.	11.74 (1,36)**	n.s.	n.s.
ADC (µm)	Elevation	n.s.	n.s.	28.58 (2,50)***	12.64 (2,54)***
	Light exposure	n.s.	n.s.	n.s.	40.32 (1,54)***
	Elevation × light exposure	<b>D.</b> S.	7.41 (1.36) <sup>=**</sup>	n.s.	n.s.
ABC (µm)	Elevation	n.s.	n.s.	5.05 (2,50)*	n.s.
	Light exposure	n.s.	n.s.	5.47 (1.50)*	31.12 (1,54)***
	Elevation × light exposure	n.s.	n.s.	4.61 (2,50)*	n.s.
ADE (µm)	Elevation	n.s.	12.12 (1,36)**	n.s.	5.48 (2,54)**
	Light exposure	<b>n.</b> S.	5.38 (1,36)≊	n.5.	12.27 (1.54)***
	Elevation × light exposure	<b>n</b> .s.	4.32 (1,36)*	n.s.	n.s.
ABE (µm)	Elevation	<b>n.</b> s.	n.s.	n.s.	7.46 (2.54)**
	Light exposure	n.s.	n.s.	n.s.	34.61 (1,54)***
	Elevation × light exposure	n.s.	n.s.	n.s.	n.s.
<b>ΡΡ (μm)</b>	Elevation	10.49 (1,36)**	10.87 (1,36)**	7.43 (1,50)**	n.s.
	Light exposure	n.s.	16.29 (1,36)***	n.s.	4.89 (1,54)*
6 <b>9</b> (	Elevation × light exposure	n.s.	12.88 (1,36)**	<b>n</b> .s.	<b>b</b> .99 (2,54)**
SP (frur)	Elevation	7.73 (1,36)**	28.05 (1,35)***	n.s.	n.s.
	Light exposure	n.s.	n.s	14.85 (1,50)***	34.b2 (1,54)***
	Elevation × light exposure	7.64 (1,36)**	D.S.	n.s.	n.s.

Traits	Factor	Ticodendron incognitum	Drimys granadensis	Podocarpus matudae	Vaccinium consanguineun
PP:SF	Elevation	n.s.	n.s.	5.64 (2,50)**	n.s.
	Light exposure	n.s.	n.s.	n.s.	n.s
	Elevation × light exposure	n.s.	n.s.	3.79 (2,50) *	6.97 (2,54)**
PAR <sub>a</sub> :Chl <sub>a+b</sub> (% mmol <sup>-1</sup> )	Elevation	24.76 (1.16)***	n.s.	n.s.	4.75 (2.24)*
	Light exposure	n.s.	n.s.	n.s.	5.75 (1.75)*
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
PARA: CAR (% mg <sup>-</sup> )	Elevation	n.s.	n.s.	5.08 (2,24)*	18.31 (2.24)***
	Light exposure	n.s.	n.s	n.s.	n.s.
	Elevation $\times$ light exposure	<b>TLS</b> .	D.S.	<b>D.</b> <i>S</i> .	n.s.
PAR <sub>A</sub> : LMA ( $\%$ g <sup>-1</sup> cm <sup>-2</sup> )	Elevation	n.s.	0.5.	6.64 (2,24)**	20.08 (2.24)***
	Light exposure	n.s.	0.5	13.1 (1,24)**	15.57 (1,24)**
	Elevation $\times$ light exposure	n.s.	n.s.	n.s.	n.s.
$PAR_A:LBT (\% \mu m^{-1})$	Elevation	n.s.	n.s.	n.s.	6.85 (2.24)***
	Light exposure	n.s.	0.5.	14.39 (1.24)***	30.72 (1.24)**
	Elevation × light exposure	n.s.	OLS.	n.s.	3.97 (2.24)*

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