THÈSE

PRÉSENTÉE À

L'UNIVERSITÉ PIERRE ET MARIE CURIE (PARIS VI)

ÉCOLE DOCTORALE DIVERSITÉ DU VIVANT

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POUR OBTENIR LE GRADE DE

DOCTEUR

Traits fonctionnels de la diversité végétale et faunistique affectant l'herbivorie et la décomposabilité des feuilles dans une forêt pluvieuse Néotropicale (Parc National Yasuní - Équateur)

Soutenue le 29 novembre 2013

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THESIS

PRESENTED AT

PIERRE AND MARIE CURIE UNIVERSITY (PARIS VI)

DIVERSITY OF THE LIVING WORLD DOCTORAL SCHOOL

by **Rafael Enrique CÁRDENAS MUÑOZ**

TO OBTAIN THE DEGREE OF

DOCTOR OF PHILOSOPHY

Plant and fauna functional trait diversity affecting leaf herbivory and decomposability in a Neotropical rainforest (Yasuní National Park - Ecuador)

Defended on November 29th 2013

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for a ‘sumak kawsay’
(living well) world

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ACKNOWLEDGEMENTS

To my advisor Olivier Dangles for his true and enthusiastic commitment with my thesis project. Thank you Olivier for believing in me, for always being totally available, for making problems smaller than they really were, for your judicious guidance, for unreservedly transferring all your expertise and experience, and most important than anything, for your friendship.

To Adriana Argoti for her job in field and laboratory the first two years. For her endless energy and for taking seriously and with responsibility such a big work. If I could share part of my grade to you, be sure I'll do it!

To Renato Valencia for providing total accessibility to the 50-ha Yasuní Dynamic Plot, for all the wise advices that helped planning and ensured the achievement of the project, and for sharing key data for macroecological and spatial analyses.

To Pablo Alvia, Milton Zambrano and Anelio Loor (*pura sazón Manaba*) for their botanist expertise in field. This study would have been logistically impossible to carry out without them.

My colleagues and co-authors of the papers, especially to Renato Valencia, David Donoso, and Nathan Kraft for their insightful comments in previous versions of each of the chapters.

To David Giron, president of my thesis committee, and Jean-François Silvain (IRD BEI) for their support and advises during the elaboration of the project, and for accepting being part of the jury.

To Hervé Jactel, Stephan Hättenschwiler and Nicolas Loeuille for accepting being part of the jury and examine this manuscript.

To all the staff of the Yasuní Research Station, in particular the administrative personnel, David Lasso, Pablo Jarrín, Carlos Padilla, Miguel Rodríguez and Karla Rivadeneira for constant support during the stays at the Station.

To the *Pontificia Universidad Católica del Ecuador*, *Facultad de Ciencias Exactas y Naturales* and *Escuela de Biología*, for financial support that made possible this research. Particular aknowledgements to Hugo Navarrete (Faculty dean) for his constant and uninterested help on solving all the administrative issues.

To the French *Institut de Recherche pour le Développement* for research grants that made possible this research. Special thanks to Aída Melgarejo, Alex Terrazas, Bernard Francou and Jacques Gardon for their efficiency in administrative support.

To all the people that gave a hand in the field and laboratory in Ecuador: Patricio Andino, Rodrigo Espinosa, Mario Herrera, Pamela Arias, Mayra Buenaño, Fernanda Samaniego, Simón Lobos and Gabriela Torrens. Your help was decisive for finishing field and lab work in time.

To Jim Self and his technical personnel who provided full guidance for leaf analyses at Soil, Water & Plant Testing Laboratory at Colorado State University at Fort Collins, USA.

Special acknowledgements to Mónica Páez for patiently supporting me in many ways during the first four years of graduate program. For her help in field and laboratory, and warm hospitality in most of the travels to the USA.

To Sol Cintrón, Martín Asmat and Betty Shoemaker for their hospitality and good temper during my stay in Fort Collins (CO, USA).

My family: mom, dad and sister (Paquita Muñoz, Patricio and Salomé Cárdenas), for their constant and unconditional support since the beginning of my career in Biology. All my love and gratitude for them.

My brother-in-law (Daniel Mancero), for creating such a beautiful music that keeps us linked with our cultural roots. I always brought your melodies with me during all my trips.

All my friends in Ecuador, with special affection to Olivia, Estela and Eva Costa, Manolo Guerra, Pedro Ruales, Roberto Sáa, Alex Escudero and Sofía Mantilla for being constantly aware of me and my work during all these intense years.

The QCAZ Museum of Zoology, Laboratory of Entomology at the *Pontificia Universidad Católica del Ecuador* (Quito) for always providing a nice place to work, and part of the material used in the experiments and laboratory analyses. Special acknowledgements to Florencio Maza, Álvaro Barragán and Fernanda Salazar.

Nanda Studt for her beautiful energy and warm hospitality during my stays in Costa Rica.

To the friends during my stay in Bolivia. For their support and understanding while writing the manuscript. Especially to Sophie Cauvy, Katy Escobar, Émile Faye, Elena Filomeno, Benjamin Lehmann, Isabel Moreno, Pablo Ruiz, Gilda Tapia and Claire Veale. Thanks guys for permanently keep trying to unplug me from research!

The Laboratory of Limnology (presided by Julio Pinto) at the *Instituto de Ecología, Universidad Mayor San Andrés* (La Paz, Bolivia) for their hosting during the last year of manuscript writing. Special acknowledgements to Karina Gonzales and Estefi Quenta for their amity.

ABSTRACT

Plant and fauna functional trait diversity affecting leaf herbivory and decomposability in a Neotropical rainforest (Yasuní National Park - Ecuador)

The main objective of this thesis project is to understand plant-insect interactions in tropical forests, especially those involved in the process of leaf herbivory and decomposition. The study was divided into three main parts that seek to reveal the main factors controlling herbivory and decomposition, and whether there was a relationship between both processes.

In a first step we aimed to identify the leaf physico-chemical factors, and tree phenological and ecological characteristics predicting leaf herbivory in the Ecuadorian Amazon. For this we quantified over one year the leaf herbivory damage of 28 common tree species of the Yasuní forest dynamic plot. Using linear regressions, pairwise- and multivariate analyses we correlated leaf and tree traits to the herbivory damage results obtained in the survey. We found that leaves are defended against herbivores using a combination of physical (toughness), chemical (toughness-related elements) and phenological (tree leaf replacement and potentially conspecifics tree spatial clustering) characteristics that do not necessarily present trade-offs amongst each other. Conventional strategies such as condensed tannins or latex do not seem to be strongly involved as a defense against herbivores.

In a second step we examined the association between leaf herbivory and leaf litter decomposability in the same study area. For this, we assessed whether leaves of 17 tree species with different levels of herbivory damage differed in their subsequent decomposition rates in soil, due to possible differences in leaf litter quality as a consequence of induced plant defense against herbivory. We also measured and correlated the physico-chemical leaf traits to the decomposition rates to elucidate the leaf traits controlling it. We found that herbivory by canopy herbivores influence major changes in some chemical leaf traits (lignin and ash content), but not to its subsequent soil decomposition. We additionally found that chemical (condensed tannins, lignin and Mn:Cu ratio), but not physical traits, were significantly correlated to decomposition rates.

We finally explored the effect of soil detritivore biodiversity on the decomposition process in Yasuní. For this we first provide a detailed description of soil fauna community structure and function in the study area, and second, we set an exclusion experiment where we manipulated the accessibility of soil detritivore size-classes to eight types of plant leaf litter resources. Our results showed that the different detritivore size-classes have a complementary effect on the decomposition process in this ecosystem suggesting that the theoretical extinction of larger invertebrates may not necessarily represent a challenge for the decomposition process in Yasuní.

We conclude that (i) canopy herbivory and soil decomposition in the tropics are two independent processes governed by different factors, and (ii) both leaf and leaf litter consumption depends on its nutritious quality and consumer functional capabilities.

Key words: above- belowground interactions, Amazonia, biodiversity, decomposition, detritivores, herbivory, leaf litter, tropical rain forest.

RESUMEN

Diversidad funcional de plantas y animales que afectan la herbivoría y descomposición de hojas en un bosque lluvioso neotropical (Parque Nacional Yasuní - Ecuador)

Este proyecto de tesis tuvo como objetivo principal entender las interacciones planta-insecto en los bosques tropicales, especialmente aquellas involucradas en el proceso de herbivoría y descomposición. El estudio se divide en tres partes principales que buscan develar los principales factores que controlan la herbivoría y la descomposición de las hojas, y si existe una relación entre ambos procesos.

En una primera etapa se tuvo como objetivo identificar los factores físico-químicos de hojas, y las características fenológicas y ecológicas de los árboles que podrían estar prediciendo la tasa de herbivoría en la Amazonía ecuatoriana. Para esto, durante un año se cuantificó la proporción de daño foliar por herbivoría de 28 especies de árboles comunes del Parque Nacional Yasuní dentro de una parcela de estudios de dinámica forestal. Utilizando regresiones lineales, análisis pareados y multivariados se correlacionaron los rasgos físico-químicos de las hojas y los rasgos fenológicos de los árboles con los valores de herbivoría obtenidos en el muestreo. Se encontró que los árboles amazónicos se defienden contra los herbívoros utilizando una combinación de defensas físicas (resistencia foliar), químicas (elementos relacionados con la dureza de las hojas) y fenológicas-ecológicas (renovación foliar y potencialmente aglomeración de árboles conespecíficos), características que no presentaron ser excluyentes entre sí. Las estrategias convencionales tales como altos contenidos de taninos condensados o látex no demostraron estar fuertemente implicados en la defensa contra los herbívoros.

En una segunda etapa se analizó la asociación entre la herbivoría y la descomponibilidad de la hojarasca en la misma área de estudio. Para ello, se evaluó si las hojas de 17 especies de árboles con diferentes niveles de herbivoría difirieron en sus tasas de descomposición en el suelo, por posibles diferencias en la calidad de la hojarasca como consecuencia de la defensa inducida de las plantas contra la herbivoría. Así mismo se midieron y correlacionaron las características físico-químicas de las hojas con las tasas de descomposición para dilucidar qué rasgos foliares la controlan. Se encontró que la herbivoría influye significativamente sobre ciertas características químicas de las hojas (contenido de lignina y elementos minerales relacionados con la dureza de las hojas), pero no sobre su posterior descomposición en el suelo. Encontramos además que ciertos rasgos químicos (taninos condensados, lignina y la relación Mn:Cu), pero no rasgos físicos, se correlacionan significativamente con las tasas de descomposición.

Finalmente exploramos el efecto de la diversidad de invertebrados detritívoros del suelo en el proceso de descomposición en el Parque Nacional Yasuní. Para esto, se realizó primeramente una descripción detallada de la diversidad y funcionalidad de las comunidades de invertebrados del suelo en el área de estudio. En segundo lugar, instalamos un experimento de exclusión en el que manipulamos la accesibilidad de detritívoros del suelo por clases de tamaño para ocho tipos diferentes de recurso ‘hojarasca’. Nuestros resultados mostraron que las diferentes clases de tamaño de detritívoros tienen un efecto complementario en el proceso de descomposición en este ecosistema lo que sugiere que una teórica extinción de la clase de invertebrados de tamaño más grande puede no representar necesariamente una amenaza para el proceso de descomposición en el Yasuní.

De esta tesis se concluye que (i) en los trópicos, tanto herbivoría como descomposición son dos procesos independientes regidos por factores diferentes, (ii) y que el consumo de hojas y hojarasca depende directamente de su calidad en términos nutricionales, y de los rasgos de vida consumidores.

Palabras clave: Amazonía, biodiversidad, bosque tropical, descomposición, detritívoros, herbivoría, hojarasca, relación dosel-sotobosque.

RÉSUMÉ

Traits fonctionnels de la diversité végétale et faunistique affectant l’herbivorie et la décomposabilité des feuilles dans une forêt pluvieuse Néotropicale (Parc National Yasuní - Équateur)

La relation entre la biodiversité et le fonctionnement des écosystèmes est devenue une question centrale dans la recherche en écologie au cours des 20 dernières années. Diverses études ont montré que la réduction de la diversité génétique, taxonomique et de groupes fonctionnels réduit l’efficacité des communautés à capturer les ressources essentielles (aliments, eau, lumière, proies) et leur conversion en biomasse. Les effets positifs de la biodiversité semblent être remarquablement cohérents entre différents groupes d’organismes, niveaux trophiques, et écosystèmes étudiés. Une telle cohérence suggère qu’il existe des principes généraux contrôlant la façon dont l’organisation des communautés influence le fonctionnement des écosystèmes. Les preuves empiriques de cette relation ‘biodiversité-fonctionnement des écosystèmes’ (*B-EF*) se sont très généralement basées sur des organismes à croissance rapide comme espèces végétales des prairies ou des expérimentations contrôlées en laboratoire, dans des microcosmes ou mésocosmes. Pour élargir les connaissances actuelles des effets de la biodiversité sur le fonctionnement des écosystèmes, des expériences en milieu naturel dans des écosystèmes plus complexes et diversifiés tels que les forêts tropicales sont nécessaires. Cela est d’autant plus important que l’on considère que, par rapport aux espèces de latitudes plus hautes, les organismes tropicaux sont particulièrement vulnérables au changement climatique en raison de leurs tolérances physiologiques étroites.

La relation *B-EF* prendrait plus d’importance si les approches futures considereraient les connexions potentielles *above- belowground*. Ceci, du à que les écosystèmes terrestres se composent de deux sous-systèmes, un de surface (*aboveground*) et un souterrain (*belowground*), dont les rétro-alimentations entre les deux compartiments jouent un rôle déterminant dans la

régulation de la structure des communautés et le fonctionnement des écosystèmes. Dans le sous-système de surface, l'herbivorie est un processus clé à la base de la diversification et la maintenance des communautés de plantes, notamment à travers l'évolution de stratégies de défense contre les herbivores. Dans le sous-système souterrain, la décomposition est un processus clé qui transforme la matière organique morte en éléments inorganiques disponibles pour les plantes, assurant ainsi le recyclage des nutriments dans les écosystèmes à travers trois processus majeurs: la lixiviation, la fragmentation et l'altération chimique.

Étudier la relation *B-EF - above- belowground* serait donc essentielle pour comprendre comment les différentes espèces interagissent et influencent l'efficacité parmi laquelle l'énergie est transmise entre les différents organismes d'un écosystème (par exemple, le cycle des nutriments). Du fait que les espèces végétales diffèrent quant à la quantité et la qualité des ressources qu'elles apportent au sol, la composition des communautés de plantes peut avoir des effets importants sur la composition des communautés du sol et des processus associés. Une question clé à ce sujet est d'étudier comment la réponse des plantes à l'herbivorie (par exemple à travers de défenses physico-chimiques) influence l'efficacité des organismes décomposeurs. Autrement dit, les caractéristiques des plantes peuvent affecter la palatabilité des feuilles et la decomposabilité de la litière en modifiant la qualité de l'apport de litière végétale au sol. Alors que les herbivores ont montré un effet positif sur les taux de décomposition de la litière dans de nombreux écosystèmes non tropicaux, les relations entre herbivorie et décomposition dans les régions tropicales restent controversées, suggérant une faible association entre les deux processus.

Ce projet de thèse a pour objectif de mieux comprendre les interactions plantes-insectes dans les forêts tropicales, notamment celles impliquées dans les processus d'herbivorie et de décomposition des feuilles. Il s'articule autour de trois parties principales : les traits fonctionnels affectant l'herbivorie, la relation entre herbivorie et décomposition, et le rôle de la diversité faunistique du sol dans le processus de décomposition.

Traits fonctionnels affectant l'herbivorie dans une forêt pluvieuse Néotropicale

Dans les forêts tropicales les relations co-évolutives entre les herbivores et les plantes ont donné lieu à une impressionnante variété d'adaptations et d'interactions. La pression exercée par l'herbivorie a conduit à l'évolution des défenses chimiques, physiques (e.g. mécaniques) et phénologiques des plantes, et de résistance des herbivores. Ces relations intimes, plantes-insectes, affectent les réseaux trophiques, le cycle des nutriments et la diversité des communautés dans les forêts tropicales.

Cette partie du projet vise à (1) élucider les facteurs biotiques (traits fonctionnels physico-chimiques des plantes) qui affectent le taux d'herbivorie des feuilles des arbres dans l'Amazonie équatorienne, (2) déterminer s'il existe une corrélation entre les taux de croissance des arbres et leur investissement sur les défenses physico-chimiques (objectif qui cherche à tester ponctuellement cette prediction de l'Hypothèse de Disponibilité des Ressources) et (3) déterminer si l'endommagement des feuilles par l'action des herbivores est liée à la distribution spatiale des arbres (densité et agroupement). Pour cela nous avons quantifié mensuellement pendant un an le pourcentage d'endommagement de 53 espèces communes d'arbres dans une parcelle d'études de la dynamique forestière du Parc National Yasuní dans l'Amazonie équatorienne. Notre étude nous a permis d'obtenir des données sur plusieurs aspects de l'écologie des arbres susceptibles d'affecter l'herbivorie tels que leur croissance ou leur distribution spatiale. Parmi les 53 espèces, nous avons ensuite sélectionné 28 espèces pour lesquelles nous avons mesuré 7 traits chimiques, 7 traits physiques et 4 traits écologiques. En utilisant une combinaison d'analyses multivariées et des modèles linéaires généralisés, nous avons évalué les relations entre les caractéristiques physico-chimiques des plantes et leurs effets des traits sur l'endommagement des feuilles.

Nos résultats montrent que l'endommagement exercé par les herbivores est très variable au sein des 28 espèces d'arbres étudiées, avec une valeur moyenne de 13,4% (de 2,49 à 29,46%). Nous n'avons pas trouvé un *trade-off* significatif entre l'investissement en défenses physiques et chimiques pour ces 28 espèces. Dans l'ensemble, la résistance au cisaillement, la teneur en cendres (mesure de la dureté des feuilles), la taille des feuilles, et l'interaction entre la résistance au cisaillement × C:N sont les meilleurs indicateurs de l'endommagement des feuilles par les herbivores. Les métabolites secondaires tels que les tanins condensés ou le latex, n'ont pas confirmé leur rôle déjà reconnu en tant que défenses chimiques contre les herbivores. Nous n'avons pas non plus trouvé de relation entre l'endommagement des feuilles et les taux de croissance ou la distribution spatiale des arbres. Cependant, nous avons constaté un effet *c.a.* significatif de l'agroupement des arbres, et fortement significatif de l'effet de la production de feuilles, sur les taux d'endommagement foliaire.

En conclusion, dans l'Amazonie occidentale, les feuilles des arbres sont défendues contre les herbivores en utilisant une combinaison de défenses physiques (résistance), chimiques (éléments liés à la dureté des feuilles) et phénologiques (remplacement des feuilles et potentiellement regroupement spatial des congénères). Ces caractéristiques ne présentent pas de *trade-off* en termes d'investissement énergétique. Des stratégies comme des teneurs élevées en tanins condensés ou le latex ne semblent pas être fortement impliquées en tant que moyens de défense contre les herbivores.

Relation entre l’herbivorie et la décomposition des feuilles dans une forêt Néotropicale

Les interactions biotiques à l’interface des compartiments de la canopée et du sol jouent un rôle fondamental dans la régulation de la structure et le fonctionnement des écosystèmes terrestres. Plusieurs études ont montré que les herbivores peuvent jouer un rôle important dans la décomposition de la litière en affectant l’activité des décomposeurs et détritivores du sol par la modification de la qualité de la matière organique apportée. Cependant, différents écosystèmes répondent différemment aux effets de l’herbivorie. Par exemple, dans les écosystèmes tempérés, il a été démontré que l’herbivorie augmente le taux de décomposition de la litière. Les rares expériences au niveau des tropiques suggèrent par contre une faible association entre ces deux processus. Dans ce contexte, cette deuxième partie du projet vise à (1) évaluer au niveau inter-spécifique s’il existe ou non une relation significative entre l’herbivorie des feuilles et la décomposabilité de la litière, (2) examiner si les traits foliaires qui contrôlent l’herbivorie sont les mêmes contrôlant la décomposition, (3) quantifier la variabilité intra-spécifique de l’endommagement des feuilles et les taux de décomposition de la litière, (4) vérifier expérimentalement si la variabilité intra-spécifique de l’endommagement affecte la décomposabilité des litières en répondant aux questions suivantes : est-ce que l’action des herbivores de la canopée affecte la qualité des feuilles? Est-ce-que les feuilles (mécaniquement) endommagées par les herbivores se décomposent à un rythme différent que les non endommagées? Est-ce-que la décomposition des feuilles entières et endommagées est contrôlée par différents facteurs?

Pour aborder ces questions, nous avons réalisé une expérience pilote dans une forêt de nuages dans les Andes occidentales en Équateur suivie d’une expérience à grande échelle en Amazonie équatorienne. Dans la première expérience nous avons comparé les taux de décomposition des feuilles (entières vs. endommagées) d’une espèce commune d’arbre, et les taux de décomposition de disques de cellulose (entiers vs. modifiés physiquement). Dans la deuxième expérience, en utilisant les feuilles de 17 espèces d’arbres nous avons abordé expérimentalement cette question en évaluant si les feuilles avec différents niveaux d’endommagement dans la canopée montreraient différences dans leurs le taux de décomposition. Pour cette expérience nous avons également mesuré plusieurs paramètres physico-chimiques des feuilles qui pourraient affecter les processus d’herbivorie et de décomposition.

Nos résultats montrent que les herbivores pourraient avoir un effet positif sur la décomposition dans les premiers 30+ jours du processus de décomposition potentiellement du fait de la modification de certains traits végétaux - tels que la lignine et la teneur en cendres - dans les feuilles endommagées par les herbivores. Par contre, sur le long terme nous n’avons pas trouvé de relation significative (linéaire ou curviligne) entre le pourcentage d’endommagement des feuilles et le taux de décomposition. Nos résultats montrent donc que les herbivores de la canopée ne facilitent pas la décomposition de la litière dans le sol, suggérant ainsi que ces deux compartiments (canopée et sol) sont indépendants sur les aspects analysés dans cette étude. Par ailleurs, bien que les traits physiques puissent constituer une barrière efficace contre l’herbivorie, les traits chimiques semblent être les principaux facteurs contrôlant la décomposition de la litière dans les sols. L’analyse de résultats obtenus au dernier moment (et présentés uniquement dans la Discussion générale de ce manuscrit) montre que le manganèse (Mn), le cuivre (Cu) et le ratio Mn:Cu sont de bons indicateurs de la décomposition de la litière pour les deux traitements (i.e. feuilles entières et endommagées). Par ailleurs, les tanins condensés, la lignine (+ leur interaction), et le ratio lignine:N ont montré une corrélation négative avec la décomposition de la litière, ce qui est en accord avec d’autres études dans des écosystèmes tropicaux. En conclusion, les analyses basées sur la variabilité au niveau inter- et intra-spécifique n’ont pas montré d’association forte entre l’herbivorie des feuilles et la décomposabilité de la litière. L’herbivorie au niveau des feuilles de la canopée et la décomposition des litières dans les sols sont deux processus indépendants contrôlés par différents traits fonctionnels.

Structure et rôle fonctionnel des communautés d’invertébrés du sol dans une forêt tropicale pluvieuse

L’étude du rôle fonctionnel de la biodiversité est devenue une thématique centrale dans la recherche en écologie au cours des 20 dernières années. Les données empiriques dans les systèmes naturels sont encore rares, et la plupart des études ayant été généralement menées dans des écosystèmes simples et à croissance rapide comme les prairies, ou en laboratoire. Pour élargir les connaissances actuelles sur les effets de la biodiversité dans le fonctionnement des écosystèmes, des expériences en milieu naturel dans des écosystèmes plus complexes et diversifiés tels que les forêts tropicales sont nécessaires. Cette partie du projet a pour objectif, à travers des expériences de terrain, d’évaluer le rôle de la biodiversité des détritivores dans la décomposition des litières.

Cette troisième partie du projet vise ainsi à (1) décrire la structuration de la diversité fonctionnelle des invertébrés du sol dans une forêt tropicale amazonienne, (2) évaluer si la diversité fonctionnelle affecte ou non le taux de décomposition des litières, et (3) élucider si le processus de décomposition est assuré par des espèces redondantes, complémentaires, ou espèces clés (d’un point de vue fonctionnel). Afin d’étudier directement l’effet de la diversité des détritivores sur le processus de décomposition, (1) nous avons réalisé une description détaillée de la structure et du rôle fonctionnel des communautés de la faune du sol, et (2) nous avons établi une expérience d’exclusion où nous

avons manipulé l'accessibilité de différentes classes de taille de détritivores du sol à huit différentes espèces de litière dont nous avons suivi le taux de décomposition.

Nos résultats ont révélé que les fourmis et les collemboles (deux groupes avec fort impact dans la fragmentation des litières) ont été les taxons du sol le plus abondants dans le Parc National Yasuní. Les changements graduels dans le nombre d'espèces et groupes fonctionnels ont affecté significativement et négativement les taux de décomposition de deux des huit traitements de litière utilisées dans l'expérience. Lorsque l'on considère les données regroupées, nous avons trouvé une relation linéaire positive à peine significative (*P* = 0,058) entre la taille des détritivores et le pourcentage de perte de masse de la litière, suggérant que les différentes classes de taille de détritivores ont un effet complémentaire sur le processus de décomposition dans cet écosystème.

Nous concluons que l'extinction des grands invertébrés détritivores ne représente pas forcément un défi pour assurer le processus de décomposition dans le Yasuní, même si ceux-ci jouent certainement d'autres rôles écologiques importants dans le fonctionnement de cette forêt.

En conclusion, cette thèse montre que les processus d'herbivorie des feuilles dans la canopée et de décomposition des litières au sol ne sont pas associés. La consommation de la ressource 'feuille' et de la ressource 'litière' dépend directement (i) des caractéristiques intrinsèques tels que la qualité de la matière organique (propre à chaque espèce et individu) et (ii) des traits de vie physiologiques fonctionnels des consommateurs (spécialistes, généralistes).

Mots clés : Amazonie, biodiversité, décomposition, détritivores, forêt tropicale, herbivorie, litière, relation canopée-sol.

LIST OF PAPERS

1. Cárdenas R. E., Valencia R., Argoti A., Kraft N. J. B. & Dangles O. Plant traits predicting herbivory in a highly diverse Neotropical rain forest. (Submitted to *Journal of Ecology*).

2. Cárdenas R. E. & Dangles O. 2012. Do canopy herbivores mechanically facilitate subsequent litter decomposition in soil? A pilot study from a Neotropical cloud forest. *Ecological Research*, 27: 975–981.

3. Cárdenas R. E., Valencia R., Argoti A. & Dangles O. Weak association between leaf herbivory and decomposability at both inter and intra-specific levels in a tropical rainforest tree community. (Manuscript. Target journal: *New Phytologist*).

4. Cárdenas R. E., Argoti A., Donoso D. A., Herrera M. & Dangles O. Structure and function of soil detritivore communities in a lowland tropical rainforest. (Manuscript. Target journal [potentially as twin papers]: *Oecologia*).

—INTRODUCTION—

1. General background

1.1. Biodiversity and ecosystem processes

Since the industrial revolution, human actions have become the main drivers of global environmental change pushing the Earth system to the edges of a stable environmental state with catastrophic consequences for many regions in the world (Rockström *et al*. 2009). Humans' activities –powered by its demography– have principally led to serious biodiversity losses in recent decades (Larsen *et al*. 2005; May 2010) and consequently losses in the services provided by ecosystems and natural processes (e.g. decomposition, pollination, pest control; see Plate 1; Millenium Ecosystem Assessment 2005). Several review papers indicate that a diversity of species is important to maintain the stability and productivity of ecosystems, and hence to human wellbeing (Hooper *et al*. 2005; Díaz *et al*. 2006; Cardinale *et al*. 2012). Recent analyses have even shown win–win synergies between biodiversity conservation and poverty alleviation, and suggest biodiversity conservation may be a fundamental component of sustainable economic development (de Koning *et al*. 2011; Turner *et al*. 2012). Biodiversity loss is with no doubt the single most significant challenge facing contemporary ecology and the single most general impediment to achieving environmental health and sustainable development (Naeem *et al*. 2009; May 2010).

The relationship between biodiversity and ecosystem functioning has emerged as a central issue in ecological and environmental research during the past 20 years (Loreau 2010; Cardinale *et al*. 2012). As a general rule, meta-analyses published since Hooper *et al*. (2005)'s last consensus paper on biodiversity ecosystem function (BEF) research have shown that reductions in the number of genes, species and functional groups of organisms reduce the efficiency by which whole communities capture biologically essential resources (nutrients, water, light, prey), and convert those resources into biomass (Cardinale *et al*. 2012). Biodiversity effects seem to be remarkably consistent across different groups of organisms, among trophic levels and across the various ecosystems that have been studied (Balvanera *et al*. 2006; Cardinale *et al*. 2006; Reiss *et al*. 2009). Such a consistency suggests that there are general principles that dictate how the organization of communities influences the functioning of ecosystems (Cardinale *et al*. 2012).

While a plethora of theoretical models have hypothesized the effect of changes in biodiversity (invasions and extinctions) in the ecosystem functioning (Naeem *et al*. 2009), empirical evidence in natural ecosystems is however still scarce as studies have usually focus on simple fast-growing ecosystems such as grasslands or laboratory bacteria microcosms (Hooper *et al*. 2005; Wittebolle *et al*. 2009). To widen the current knowledge of the effects of biodiversity in ecosystem functioning, natural experiments into more complex and diverse ecosystems such as tropical forests are necessary (Dangles *et al*. 2012). This is remarkably true when considering that tropical organisms may be particularly more vulnerable to global climate change due to their narrower physiological tolerances comparing to higher-latitude species (Deutsch *et al*. 2008; Tewksbury *et al*. 2008).

1.2. Trophic food webs and biodiversity ecosystem function

Incorporating a trophic food web perspective on biodiversity and ecosystem functioning is essential for understanding how does the number of species interacting within a food web influence the efficiency and reliability by which energy is transmitted (Cardinale *et al*. 2009). Indeed interactions of species across trophic levels can have cascading impacts that influence the diversity and biomass of organisms at numerous levels in a food web, thus affecting ecosystem processes (Cardinale *et al*. 2009). For example top-down/bottom-up control of herbivores and detritivores populations may probably help explaining why herbivory and decomposition processes are positively associated in some environments and not in others. Understanding the functional interactions among natural subsystems is therefore a timely issue. By undertaking these kinds of investigations it is expected that ecological research in the tropics will help to better understand and quantify ecosystem services and natural processes while making ecology more applicable to conservation and restoration strategies (Byers *et al*. 2006; Mooney 2010; Kardol & Wardle 2012).

2. The above– belowground interactions (herbivory and decomposability)

Among coupled ecological sub-systems requiring further attention in the current B-EF debate, the above- and belowground subsystems have a central place as their feedbacks play a crucial role in regulating community structure and ecosystem functioning of terrestrial ecosystems (Bardgett & Wardle 2010). Plant characteristics might be strongly influenced by interactions with aboveground and belowground higher trophic-level organisms (Bezemer & van Dam 2005). For example, root herbivores and decomposers can alter plant biomass production and chemical composition, thereby stimulating herbivore and parasitoid densities aboveground, as well as flower visitation and seed production (Poveda *et al*. 2005). Because plant species differ in both the quantity and quality of resources that they return to soil, individual plant species may have important effects on components of the soil biota and the processes that they regulate (Wardle *et al*. 2004; Poveda *et al*. 2005). For example in forests, differences in the quality of litter produced by coexisting tree species explains the patchy distribution of soil organisms and process rates that result from “single tree” effects (Saetre & Bååth 2000). However, effects of plant composition on decomposer communities appear to be context-dependent (Wardle *et al*. 2004). Studies indicate that aboveground trophic interactions have indirect effects on soil biota by affecting the quantity and quality of resources that plants produce (Van der Putten *et al*. 2001). One key question in this topic has led to investigate how plant responses to foliar herbivory (e.g. plant defenses) influence soil decomposer organisms fitness (Bardgett *et al*. 1998). That is, plant traits may affect leaf palatability and leaf litter decomposability through altering the quality of input of plant litter.

While herbivores have shown to increase litter decomposition rates in many non-tropical ecosystems (e.g. Belovsky & Slade 2000; Chapman *et al*. 2003), herbivory-litter decomposition relationships in the tropics remain controversial, with scarce studies suggesting a weak association between both processes. For example, Kurokawa & Nakashizuka (2008) concluded that herbivory may not generate positive feedback for nutrient cycling in a Malaysian tropical rainforest, and Cárdenas & Dangles (2012) found that leaves damaged by herbivores decomposed significantly faster than entire leaves, but only in early stages of decomposition in an experiment in an Ecuadorian cloud forest. Hence, the association between leaf herbivory and litter decomposition in the tropics remains very scarcely explored, despite its importance on estimating the herbivory effects on carbon and nutrient cycling of ecosystems (Kurokawa & Nakashizuka 2008). In an attempt to fill the gaps, Wardle *et al*. (2004) stated that "*positive effects arise when herbivores promote compensatory plant growth, returning organic matter to the soil as labile fecal material (rather than as recalcitrant plant litter), inducing greater concentrations of nutrients in remaining plant tissues and impairing plant succession, thereby inhibiting ingress of plant species with poorer litter quality. Negative effects arise through impairment of plant productivity by tissue removal, induced production of secondary defenses, and promotion of succession by favoring the dominance of unpalatable plant species with poor litter quality*". They described and explained the above-belowground interactions in two different contexts: fertile *vs*. infertile ecosystems (Plate 2), although the discriminatory characteristics they used for defining both scenarios do not necessarily fits perfectly to many types of ecosystems such as the Amazonian tropical rainforest.

3. Herbivory

3.1. General background

Herbivory is the process whereby an animal eats a plant organism (Begon *et al*. 2006). Up to 20% of global plant production is consumed by herbivores (Cebrian 1999; Agrawal 2011). Because herbivory has a much larger impact than leaf senescence in terms of nutrients loss (nitrogen and phosphorous particularly, Chapin *et al*. 2002), herbivory has been responsible to the evolution of chemical, mechanical, and phenological defenses in plants (Agrawal 2007). Plants investment in defenses (physical and chemical) is particularly strong in many poor-resource habitats such as deserts and several tropical forests to protect nutrients (Wardle *et al*. 2004). Herbivores in turn have evolved to survive with food plants that are trying to starve or poison them. These relationships affect food webs, nutrient cycling, and community diversity, and thus every organism of an ecosystem (Coley & Barone 1996). In ecosystems, herbivores have been recognized as regulators of plant community composition, the total amount of plant biomass in the ecosystems, and the magnitude of ecosystem functional processes such as primary production, decomposition, and nutrient recycling (Hillebrand *et al*. 2007; Gruner *et al*. 2008). Herbivory is performed by a wide range of animal sizes and physiological capacities, from nematodes and protists (~ 3µm) to big mammals (up to 6 tons in the case of larger elephants) as such as those found in the African savannahs (Begon *et al*. 2006). It seems obvious that, at least in terms of biomass consumption, some herbivores will have a greater impact than others. However this could be also evidenced when comparing impact at a similar scale. In some insect–plant interactions, some species need as much as 140 g, and others as little as 3 g of plant tissue to produce 1 g of insect tissue (Gavloski & Lamb, 2000). Positive or negative impact of plant biomass consumption may be context dependent. For example Gruner *et al*. (2008) showed that herbivore removal generally increased primary producer biomass in both freshwater and marine systems, but effects were inconsistent on land.

3.2. Main factors affecting herbivory

In a recent review of the current trends of the evolutionary ecology of plant defense research Agrawal (2011) stated that historically there have been two important failures: a widely held belief that plant resistance traits (i) act singularly, and many researchers continue to search for or measure single plant traits and (ii) should trade-off against each other. Latest advancements in ecological and evolutionary plant defense research have shown that herbivory is actually controlled by at least four no mutually exclusive biotic factors: physical, indirect, phenological and chemical plant defense strategies.

3.2.1. Plant physical defenses

Toughness (sclerophylly) has been widely considered as the most effective defense (Lucas *et al*. 2000) because it takes longer for herbivores to chew and process tougher plant material (Choong *et al*. 1992; Laca *et al*. 2001). The force to punch has largely been the most widely used method for testing leaf toughness resistance (Sanson *et al*. 2001). However other measurements such as the force to shear and the force to tear (Onoda *et al*. 2011) may complement the analyses of leaf resistance to mechanical damage. Toughness may also be related to the leaf thickness and the specific leaf area index (SLA, that corresponds to the ratio of fresh leaf area in cm^2 to dry weight). The thicker a leaf is, the smaller SLA index presents, and the harder for herbivores to consume it.

Reduced leaf size is another physical characteristic that has been associated to lower amounts of herbivory because smaller leaves may be less attractive to herbivores (Garibaldi *et al*. 2011). Moreover, it has been suggested that very small or highly divided and dissected leaves may reduce feeding efficiency in part because, from a mechanical point of view, invertebrate herbivores find difficulty to stabilize over the leaf for consuming it (Brown *et al*. 1991).

Structural traits, such as spinescence, pubescence and raphides (i.e. Calcium oxalate crystals aggregates in plant cells) play an important role in protecting plants from herbivore attack (reviewed by Hanley *et al*. 2007). Spinescence is a term used to describe the plant structures such as spines, thorns and prickles that have evolved as a defense against herbivores. Spinescence is generally considered to be more effective against vertebrates than invertebrates, due to the size relations of the herbivore-plant interactions (Hanley *et al*. 2007). Pubescence refers to the layer of hairs (trichomes) on stems, leaves, or even fruits. It is thought to have evolved primarily as a physiological barrier against desiccation and UV radiation, but it has also been proven to have a role in anti-herbivore defense. Herbivores that feed internally, such as leaf miners and leaf-gallers, or other tiny insects like some grasshoppers, whose size helps them avoid the effects of pubescence, are much less affected by its presence (Andres & Connor 2003). Many species of terrestrial and aquatic plants deposit minerals in leaf and stem tissues (e.g. raphides). Calcium oxalate crystals occurs in most plant families and is the most abundant insoluble mineral in plant tissue, accounting for 3–80% of plant dry mass (Franceschi & Nakata 2005; Korth *et al*. 2006). Structural defenses may however decline with leaf age. Matsuki *et al*. (2004) showed how trichome density and toughness decreased with age in two Japanese birch species. Choong (1996) reported a similar directional change in leaf toughness in a South-east Asian tree species, as did Kursar & Coley (2003) for five Panamanian rainforest tree species, while calcium oxalate concentration was inversely related to leaf age in five Central American rainforest species (Finley 1999).

3.2.2. Indirect and phenological plant defenses

Indirect plant defenses are those features that protect the plant by increasing the probability of attracting the natural enemies of herbivores such as predators or parasitoids (Kessler & Heil 2011). There are two main strategies to attract herbivore enemies: by a mutualistic relationship predator-plant or by airborne signals that operates as an alarm for predators and parasitoids. Ant-plants are major examples of a predator-plant symbiotic mutualism in which plants provide resources to ant species that may be specialized to nest and feed on them (Fiala & Maschwitz 1990; Heil & McKey 2003). The ants consume extrafloral nectar and food bodies, and may nest in specialized plant parts in return for a protection of the plant from attackers and competitors. Airborne signals consist on the emission of herbivory-induced volatile organic compounds (HIVOC) to attract predators and parasitoids to herbivore damaged plants (Paré & Tumlinson 1999). However HIVOC role goes beyond it was originally thought. Classic examples of HIVOC-mediated indirect resistance include the attraction of arthropods for controlling arthropods (see Begon *et al*. 2006 for illustrative examples). However, a study has shown that birds can also be attracted by HIVOCs and use them to find their prey (Mäntylä *et al*. 2008). The composition and abundance of volatiles are species-dependent and can be dramatically altered in response to herbivory (Kessler & Heil 2011) to the point that HIVOCs released from resistance-expressing plants, can even trigger specific defensive responses in neighboring plants of various species (Heil & Karban 2010). A study in Peruvian Andes has also shown that solitary bee pollinators of wild tomato use floral HIVOCs as cue to avoid inflorescences on damaged plants (Kessler *et al*. 2011).

Phenological defense strategies are all those plant life cycle events that have possibly evolved as a function of herbivory pressure and could roughly be seen as a partial compensation strategy for ineffective chemical defenses (Kursar & Coley 2003). Because of the higher nutritional quality and soft texture of new-born leaves most of the damage occurs when they are young and expanding (particularly in the tropics, Coley & Barone 1996). This is important to take into account because most of the phenological strategies rely on protecting young nutritious leaves. For example, rapid leaf expansion minimizes the window of vulnerability. Delayed greening implies that energetic investment in costly photosynthetic compounds is not lost. Leaf production patterns such as (i) synchronous leaf flushing has the intention to satiate starving herbivores, while (ii) leaf production when they are less abundant (the dry season in most tropical forests) has the purpose to avoid them. Studies on plant defense that focused on phenological aspects of plants are not new, however the recognition of these kind of traits as efficient defense runs against the existing paradigm that the ecology and evolution of secondary metabolites represent the 'most important' plant defense against herbivores (Carmona *et al*. 2011).

3.2.3. Plant chemical defenses

The plant kingdom is very rich in chemicals that are not involved in the essential photosynthetic and metabolic activities: they have been classically classified as 'secondary metabolites' (Begon *et al*. 2006). It is widely accepted that herbivory pressure has led to the evolution and selection of a variety of chemical plant defenses (Agrawal 2007) ranging from simple (oxalic acid and cyanide) to complex molecules (alkaloids, terpenoids, saponins, flavonoids, anthocyanins and tannins) to discourage potential consumers. However, there is practically no consensus of its general functionality and toxicity impact on herbivores (Agrawal 2011; Moles 2013). It has been recently suggested that secondary metabolites could have evolved to be important defensive mechanisms not because they have the largest effect on herbivores, but because the constraints on their evolution are the weakest (Carmona *et al*. 2011). Because physical, structural and phenological plant defenses have demonstrated to perform better against herbivory, the same authors suggest that in a microevolutionary context, secondary metabolites are perhaps relics of co-evolutionary interactions in the sense that herbivores may have evolved counter-adaptations to efficiently overcome chemical defenses (Carmona *et al*. 2011) nullifying their biological function (Jones *et al*. 1991) or even benefitting from them (Becerra *et al*. 2001; Després *et al*. 2007). This may explain in part the reasons why effectiveness of secondary metabolites on deterring herbivores has shown to differ among species, ecosystems and biomes (Coley & Barone 1996). For example, alkaloids have shown to be more common and more toxic in the tropics (35% of species) comparing to the temperate regions (16% of species) (Coley & Barone 1996). Condensed tannins in mature leaves have shown to be three times more concentrated in the tropical forests comparing to temperate ones (Turner 1995), but total phenolics leaf concentrations in temperate, tropical dry, and tropical wet forests (averaged 6.9%; range: 6.5%–7.4%) did not show significant differences between forest types (Coley & Aide 1991). It is now well accepted that chemical compounds play a secondary role in plant defense and they shall be regarded as complementary to physical or phenological strategies for deterring herbivores (Carmona *et al*. 2011).

4. Decomposition

4.1. General background

Decomposition is a key ecosystem process which transforms dead organic matter into inorganic nutrients available to plants, thus ensuring ecosystems nutrient cycling (Chapin *et al*. 2002). Although the term ‘decomposition’ refers to the actual mineralization of organic compounds, it has been consistently used to describe its mass loss (Hättenschwiler *et al*. 2011). In the context of this PhD, we will focus on the decomposition processes over the leaf litter plant material in terrestrial ecosystems which results from three types of processes with different controlling factors and consequences: leaching, fragmentation and chemical alteration. (1) Leaching is the physical process by which mineral ions and small water-soluble organic compounds dissolve in water and move through the soil (Chapin *et al*. 2002). It actually begins when leaves are still alive during their senescence as many of the compounds are broken down and transported (resorbed) to other plant parts (Bazzaz *et al*. 1987). After leaf fall, microbes (fungi and bacteria) absorb labile material, and rainfall and/or water courses help diffusing remaining dissolvable substances (Begon *et al*. 2006). (2) Fragmentation is carried out by soil animals, mainly invertebrates (detritivores), leading to chemical (Coulis *et al*. 2009) and physical transformations (Lavelle *et al*. 1997; David & Handa 2010). By breaking the dead organic matter detritivores produce smaller particles providing food source for other organisms and create greater fresh surfaces for microbial colonization, a process considered as ecosystem engineering (Lavelle *et al*. 1997; Jonsson *et al*. 2002). For example, fungi having access to new areas facilitate the penetration of bacteria in leaf litter tissue, allowing both sets of taxa to gain access to resources, increasing decomposition rates (de Boer *et al*. 2005). Detritivory is performed by a wide range of animal sizes and physiological capacities, from nematodes and protists ~ 3µm long to giant tropical cockroaches > 8cm long (Swift *et al*. 1979). (3) Chemical alteration is primarily a consequence of the activity of bacteria and fungi accounting for 80–90% of the total decomposer biomass and respiration (Begon *et al*. 2006). In contrast to bacteria, fungi have enzyme systems capable of breaking down virtually all classes of plant compounds including tissues with very low nutrient concentrations making them more efficient decomposers comparing to bacteria (Chapin *et al*. 2002). Such energy investment to break down lignin or cellulose with specific enzymes (Coûteaux *et al*. 1995) serves primarily to gain access to the more labile substances of the interior of leaf litter cells. The ‘coordinated’ action of fungi (including mycorrhizae) and bacteria leads to the actual decomposition of organic compounds into mineral substances available to plants (Chapin *et al*. 2002).

4.2. Main factors affecting decomposition

Litter decomposition is controlled by three main factors: climate, litter quality, and the nature and abundance of the decomposing organisms (Swift *et al*. 1979; Coûteaux *et al*. 1995).

4.2.1. Climate

Temperature and moisture have been considered the main climatic drivers in terrestrial decomposition (Chapin *et al*. 2002). In a general perspective, temperature affects decomposition by promoting microbial activity and by altering soil moisture. Moisture is also critical as water films on soil surfaces allow the transportation of decomposers microflora and diffusion of substrates (Stark & Firestone 1995). Microbial soil respiration for example increases exponentially at higher temperatures speeding up the mineralization of organic carbon to CO_2 (Lloyd & Taylor 1994). This is because enzyme activity normally increases with temperature, although rapidly falls as the temperature rises above an optimum value (Coûteaux *et al*. 1995). It has been documented that decomposition *k* rates (constant that characterizes the decomposition rate based on an exponentially matter mass loss, see Levins 1968) are significantly different among biomes (Olson 1963) suggesting, for example, that decomposition in the tropics (warmer and wetter) occurs in a faster rate comparing to temperate ecosystems. These assumptions have been confirmed by studies based on large-scale decomposition experiments (Parton *et al*. 2007; Wall *et al*. 2008; Powers *et al*. 2009). The paradigm of a faster decomposition rates in the tropical ecosystems has however recently been challenged by Hättenschwiler *et al*. (2011) who compared leaf litter mass losses between one tropical and one temperate site (with temperature averages differences of 17.6° C, see Hättenschwiler & Gasser 2005 and Hättenschwiler & Bracht Jørgensen 2010) using exactly the same protocol (i.e. experimental design, time of experimentation). They found significant lower mass loss in the tropical site when adjusting data to Q_{10} values. In other words, for every 10° C increase in temperature (e.g., from 11° C to 21° C), the decomposition rate increment of the temperate site was significantly higher than that of the tropical site (Hättenschwiler *et al*. 2011). The assumed slower decomposition rate in the temperate forests may thus rely on the lower temperatures (annual averages) found on these regions.

4.2.2. Leaf litter quality

Leaf litter quality refers to the (1) chemical composition and (2) mechanical (physical or structural) properties of fallen leaves.

(1) Chemical compounds could be roughly categorized as (i) labile metabolic compounds, such as sugars and amino acids; (ii) moderately labile structural compounds such as cellulose and hemicellulose; and (iii) recalcitrant material such as lignin, cutin (Chapin *et al*. 2002), and herbivore-defense residues such as condensed tannins and other phenolic compounds (Handley 1961; Zucker 1983). As a simple model, litter with higher amounts of labile metabolic compounds compared to recalcitrant material for example (i.e. better quality) is predicted to decompose at faster rates (Coûteaux *et al*. 1995). Major elements correlation to decomposition processes have also been subject of intensive research: carbon (C), nitrogen (N), phosphorous (P) and the C:N, N:P ratios are typically used to correlate with decomposition because they are important for ensuring life. C is a major element present in organic molecules such as sugars, lignins, chitins, alcohols, fats, aromatic esters, carotenoids, terpenes, alkaloids among others; N is integral to the proteins, of photosynthetic machinery in plants, and detritivores and decomposers functionality; P is found in organisms nucleic acids, lipid membranes and bioenergetic molecules such as ATP (Wright *et al*. 2004). Deeper analyses have focused their attention on other secondary, but important elements. In a preliminary study in a Neotropical rain forest, Kaspari *et al*. (2008) found an increment of 82% on

decomposition rates in the presence of micronutrients (B, Ca, Cu, Fe, Mg, Mn, Mo, S, Zn). Which of those elements (or combinations) create such an effect, is currently unknown.

(2) Mechanical properties correspond to the structural resistance of leaves to protect from herbivores, and other risks of physical damage (Kitajima & Poorter 2010) that can be influencing subsequent decomposition process (Choong *et al*. 1992; Santiago 2007). Leaf mechanical resistance can be expressed per unit ‘fracture length’ or per unit ‘fracture surface area’, and these quantities are known as ‘structural resistance’ and ‘material resistance’, respectively (Onoda *et al*. 2011). This relies on the physical properties of materials where thicker leaves are more structurally resistant than thinner leaves when leaves are made from the same material for example. But higher structural resistance can also be achieved via tougher material or denser tissue, without changing thickness (Onoda *et al*. 2011). To quantify leaf resistance, three methods have been used: (i) work to shear, (ii) force to punch and (iii) force to tear. Shearing tests measure the work (force × displacement) to traverse a leaf. Punch tests measure the maximum force to punch out the leaf lamina. Tearing tests measure the maximum force to tear a leaf strip. Other typical leaf mechanical traits include the ‘leaf mass per area’ (LMA) or its analogous ‘specific leaf area’ (SLA), and leaf thickness.

Although all these chemical and mechanical leaf traits influence the feeding preferences and rates of leaf consumption (Pérez-Harguindeguy *et al*. 2000), their relative importance is not yet well understood and predictors of decomposition rates seem to be highly context-dependent. That is, specific litter decomposability is correlated to the species ecological strategy within different ecosystems as litter quality seems to contribute much more to the overall variability in decomposition than climate (Cornwell *et al*. 2008). In the tropical rainforests for example, C quality (i.e. lignin, cellulose, hemicellulose, non-structural carbohydrates, condensed tannins and/or phenolics, see Chapin *et al*. 2002; Kurokawa & Nakashizuka 2008; Wieder *et al*. 2009; Coq *et al*. 2010; Hättenschwiler & Bracht Jørgensen 2010) and mechanical traits (i.e. LMA or SLA and/or toughness, see Santiago 2007; Kurokawa & Nakashizuka 2008) have been considered the best predictors of leaf litter decomposition. As a global pattern however, lignin:N has concurrently shown to be a factor negatively correlated to leaf litter decomposition in a wide range of ecosystems (Melillo *et al*. 1982; Moore *et al*. 1999; Taylor *et al*. 1989; Kurokawa & Nakashizuka 2008; Wieder *et al*. 2009).

4.2.3. Soil fauna (detritivore communities)

Detritivore communities have been classically classified into four groups according to their contribution on litter matter mass fragmentation which is directly correlated to its size and volume: mega-, macro-, meso- and micro-fauna (Plate 3; Swift *et al*. 1979). The representation of each group changes dramatically along a latitutinal gradient from the poles to the tropics, with a direct impact on the litter breakdown rates and soil organic matter accumulation (Plate 4, Swift *et al*. 1979). In the tropical ecosystems macro- and meso-fauna play a crucial role in fragmenting dead organic matter. Cárdenas & Dangles (2012) for example, found a decline of 50% on decomposition rates when preventing the access of both groups in a study in Ecuador. Likewise, Coq *et al*. (2010) found a decline of 17.4% in French Guiana, and Yang & Chen (2009) of 40% in tropical China. These values are not directly comparable because differences may rely on the size of the holes of microcosms (coarse- and fine-meshes) used in experiments, and on the intrinsic diversity and physiological capabilities of the soil fauna communities to exploit any particular resource. They confirm however how important is the contribution of organic matter fragmentation of macro- and meso-fauna in tropical rainforests soils (see also González & Seastedt 2001). In a recent study, García-Palacios *et al*. (2013) found climate and litter quality differently modulated the effects of soil fauna on decomposition rates between biomes and suggested the inclusion of biome specific soil fauna effects on litter decomposition large-scale models.

Studies suggest that changes on the interactions between individual species, or functionally distinct organisms, can have profound effects on ecosystem processes (see Wall & Moore 1999 and references therein). Detritivore diversity has proven to be critical to the biogeochemical and ecological functioning of ecosystems having consequences in fertility, plant growth, environmental structure and carbon storage (Brussaard *et al*. 1998). But, how detritivore biodiversity loss may affect decomposition and other ecosystem processes remains unresolved (Wall *et al*. 2010). Factors such as land use, nitrogen enrichment, acidification and climate change have been reported to alter soil and streams detritivore diversity (Gessner *et al*. 2010). Moreover, the loss of key species and trophic groups, such as symbionts, predators, shredders, fungivores, root feeders and bioturbators, may have far-reaching and unpredictable consequences for ecosystem functioning (Wall *et al*. 2010).

Compared to streams, where species richness has been regularly associated with faster decomposition, empirical evidence for the role of terrestrial detritivore diversity in leaf litter decomposition is much scarcer (Gessner *et al*. 2010). Zimmer *et al*. (2005)'s laboratory experiments manipulating temperate detritivore diversity suggest that complementary effects on decomposition vary with resource quality as the synergistic effect disappeared when the detritivores were offered recalcitrant litter (instead of high quality litter). De Oliveira *et al*. (2010) observed a positive complementarity effect on litter relative consumption rates between one species of Mediterranean snail and one of centipedes, but results were relative to the leaf litter species mixtures and litter state (i.e. freshly fallen or pre-decomposed). Other studies however found no influence of species richness on litter decomposition (Cragg & Bardgett 2001; Heemsbergen *et al*. 2004). In the tropics, studies testing the effect of detritivore diversity on leaf litter decomposition are virtually non-existent.

It is important to note that the role of the above mentioned three main factors on organic matter decomposition can be influenced by co-factors such as leaf litter diversity (Hättenschwiler *et al*. 2005), soil physico-chemical properties and soil disturbance (Chapin *et al*. 2002). Additionally, (canopy) herbivory may present a causal connection between plants anti-herbivore defense and the decomposition rate of leaves in soil (Grime *et al*. 1996), an ecological feature which is part of the vast ecological research domain of above– belowground interactions.

5. Thesis justification

Interactions among biotic and abiotic factors and their control over ecological processes such as leaf herbivory and leaf-litter decomposition, remains a hot debate in ecological research (Gessner *et al*. 2010; Carmona *et al*. 2011). This is particularly relevant in the tropics where (1) the potential role of above– belowground interactions is notoriously poorly investigated (Schmid *et al*. 2009), (2) the amount of undescribed canopy and especially belowground diversity complicates our understanding of soil fauna functionality at individual and at community levels (Coleman 2008; Wall *et al*. 2010) –although, some significant efforts have been helpful on filling these enormous gaps (e.g. Moreira *et al*. 2006)–, and (3) invertebrates undergo high anthropogenic pressures that are leading to the extinction of communities with unprecedented and unpredictable consequences on the ecosystem processes and the Earth functioning (Fonseca 2009). For these reasons it is of upmost importance to carry out manipulative experiments *in situ* in tropical systems in order to understand ecological mechanisms to a deeper level –that may challenge established paradigms– (Naeem 2002; Zuidema *et al*. 2013).

In the following, we will first present the study region and study site were our experiments were set up, and then expose the main objectives of our work.

6. The Amazon: study area and study plot

6.1. The Amazonian tropical rainforest

The Amazon represents the most species-rich terrestrial ecosystem in the world (Peres 2005), and its multiple interactions between organisms are yet to be revealed. It originated in the late Cretaceous (primarily as savannahs, Plate 5) leading to the evolution of unprecedented life explosion and complexity (Hoorn *et al*. 2010). It has been a permanent feature of South America, and an important and continuous part of Earth system functioning for at least the last 55 million years (Maslin *et al*. 2005). The Amazon Basin is one of the major 'engines' of the global atmospheric and hydrological circulation, and changes in the hydrological regime of Amazonia (evaporation and condensation) may change rainfall patterns 'downstream', changing rainfall patterns in mid-latitude regions of North America and Eurasia (Gedney & Valdes 2000; Werth & Avissar 2002). Recent research has shown seasonal swings in leaf area are the actual responsible for the initiation of the transition from dry to wet season (Myneni *et al*. 2007). This leaf seasonal cycle is actually timed to the seasonality of solar radiation –which fluctuates between both sides of the *equator* line at opposite time intervals of the year– suggesting that plants anticipate physiologically the 'light-rich' and the 'cloudy' seasons (Myneni *et al*. 2007). Since its origin, this ecosystem has survived the high temperatures of the Early Eocene climate optimum, the gradual Cenozoic cooling, and the drier and lower carbon dioxide levels of the Quaternary glacial periods (Burnham & Johnson 2004; Maslin *et al*. 2005).

The Amazonia accounts for about 15% of global terrestrial photosynthesis (Field *et al*. 1998), it hosts perhaps 25% of the world's terrestrial species (Dirzo & Raven 2003), and the western equatorial portion which has been directly influenced by the Andes Cordillera uplift over millennia (Hoorn *et al*. 2010), shows extraordinary levels of species concentration and endemicity (Myers *et al*. 2000; Bass *et al*. 2010; Jenkins *et al*. 2013). In its original extent, the forests of

Amazonia covered about 6.2 million km^2 (Malhi *et al*. 2008), and are currently contained within 9 countries: 62% in Brazil, with minor amounts in Bolivia, Colombia, Ecuador, French Guiana, Guyana, Suriname, Peru and Venezuela (Plate 6).

Human pressure (primarily deforestation) over this extraordinary species-rich ecosystem has been fragmenting and degrading a rate of ~25000–38000 km^2 of primary forest each year (Skole & Tucker 1993; Malhi *et al*. 2008). As a consequence of ecological changes in fragmented communities, its auto-sustainability and recover capacity (Ferraz *et al*. 2007), and the ecosystem services once provided (Laurance *et al*. 2002), have been altered. Eighty percent of deforestation has occurred in Brazil (Soares-Filho *et al*. 2006) and rates have fluctuated widely over the last twenty years (Ewers *et al*. 2008). Seventy percent of deforestation is provoked by cattle ranching and roughly 6% of deforested land has remained in cropland, 62% in pastures, and 32% in regrowing vegetation (Ramankutty *et al*. 2007). Though it has long been suggested that rapid forest loss and degradation in the tropics could ultimately precipitate a wave of species extinctions (Maslin *et al*. 2005; Malcolm *et al*. 2006), perhaps comparable to mass extinction events in the geological history of the Earth (Laurance *et al*. 2011), Wright & Muller-Landau (2006) challenged the idea of a mega-extinction crisis in the tropics, at least over the next century. Based on socio-economic projections from 45 tropical countries in America, Africa and Asia, the authors concluded that future deforestation will slow, regeneration will accelerate, and mass extinction of tropical forest species will be avoided. Although this idea has been widely criticized –refer to Laurance (2006) for concluding remarks on this topic and references therein– none of the cited analyses however have taken into account the enormous efforts of several tropical countries to efficiently avoid forests fragmentation and degradation (for detailed examples see Nepstad *et al*. 2009; Finer *et al*. 2010; de Koning *et al*. 2011).

6.2. The Yasuní National Park (Ecuador)

The Yasuní National Park (YNP) is a major protected area within the western Amazon. It occupies a unique location at the intersection of the Andes (100 km from the Andean foothills), the Amazon (near the western phytogeographic limit of the Amazon Basin (Peres & Terborgh 1995), and the *equator* (~1° S) (Plate 7). Created in 1979, YNP covers approximately 9,820 km^2 (MAE 2013) (1.1 times the Yellowstone National Park in the USA), and is surrounded by a 10 km buffer zone in all directions except to the east, where it meets the Ecuador-Peru border (Albacete *et al*. 2004). The Park overlaps ancestral Waorani territory, and is inhabited by at least two clans living in voluntary isolation (Pappalardo *et al*. 2013). In 1989, the Yasuní National Park and much of the adjacent area which is now the Waorani Ethnic Reserve were designated as a UNESCO ‘Man and the Biosphere Reserve’ (UNESCO 2013). Both, the Yasuní National Park (YNP) and the adjacent Waorani Indigenous territory cover 16,000 km^2 of forest and form the largest protected area in Amazonian Ecuador (~17.7% of the Ecuadorian territory; Valencia *et al*. 2004a) harboring the world’s most diverse tropical forest (Bass *et al*. 2010, Plate 8).

YNP is an evergreen lowland wet forest ranging in altitude from 200 m to 300 m above sea level. It is covered with primary *terra firme* forests and the canopy normally reaches a height of 30 m (Pitman 2000). Rainfall and temperature are aseasonal with a mean annual rainfall of 2826 mm (none of the 12 calendar months averaging <100 mm) and a mean monthly temperature ranging from 22 to 32° C (min: 16.9; max: 38.9° C) (see Valencia *et al*. 2004a).

YNP belongs to a big block of still virtually intact Amazonian lowland rainforest defined as ‘Napo’ endemicity Region (Ron 2000). *Terra firme* formations in the region are characterized by an extraordinarily high density of old emergent trees. Various authors explain this observation with the high maturity and integrity of these forests (Korning & Balslev 1994; Pitman, 2000). Along the Tiputini river *terra firme* formations are replaced by narrow strips of frequently inundated floodplain forests. Furthermore, small patches of palm swamps dominated by *Mauritia flexuosa* L. (Arecaceae) occur locally. The tree species richness in the Yasuní area is among the highest of the world. Pitman (2000) found a mean of 248 tree species (diameter at breast height - d.b.h. ≥10 cm) in a series of 1-ha plots. Valencia *et al*. (2004a) found 1104 tree species in a 25 ha plot (d.b.h. ≥1 cm). Romoleroux *et al*. (1997) recorded 825 species of woody plants (d.b.h. ≥1 cm) on 2 ha and Nabe-Nielsen (2001) 96 liana species in a 0.2-ha plot, the highest number so far registered. Bass *et al*. (2010) synthesized the vertebrate species richness in YNP area and reported 150 species of amphibians, 121 species of reptiles, 596 species of birds, up to 204 species of mammals and up to 499 species of fishes (the two latter represent estimated values). Ryder Wilkie *et al*. (2010) found a total of 489 ant species comprising 64 genera in 9 subfamilies in 16 ha area of the YNP, they estimated a total of 647–736 ant species, and concluded this region of western Amazonia may support the most diverse ant fauna ever recorded.

How such a diversity of plant species could be coexisting in a relatively small area? Lots of hypotheses have been propounded to attempt explaining the mechanisms of organisms' coexistence (see Palmer 1994 for a general review), and have been possible in part, thanks to the existence of long-term monitored plots (see below point 6.3.). For tropical ecosystems, four mutually compatible hypotheses are currently strongly supported to explain plant coexistence (Wright 2002): (1) niche differentiation which is evident from non-random spatial distributions along micro-topographic gradients and from a survivorship growth trade-off during regeneration (e.g. Silvertown 2004; Kraft *et al*. 2008; Tuomisto 2006); (2) the Janzen-Connell effect that explains host-specific pests reduce recruitment near reproductive adults as a consequence of disease spreading from perennial plants to seedlings of the same species growing close to them (e.g. Cintra 1997; Gilbert 2002; but see Sedio & Ostling 2013 for a new perspective); (3) the negative density dependence that occurs over larger spatial scales among the more abundant species and may regulate their populations (Peters 2003;

and see Volkov *et al*. 2005 for a model application at local scale); (4) the suppression of understory plants making them to rarely come into competition with one another (Wright 2002). Niche differences, Janzen-Connell effects, and infrequent competition among suppressed understory plants, may facilitate the coexistence of the many rare plant species found in tropical forests, while negative density dependence may regulate the few most successful and abundant species (Wright 2002).

6.3. The Yasuní 50 ha dynamic plot

In 1995, the *Pontificia Universidad Católica de Ecuador* (PUCE), the University of Aarhus in Denmark, and the Smithsonian Tropical Research Institute in Panamá (CTFS-STRI) initiated a Forest Dynamics 50 ha Plot (500 × 1000 m) in the northwest portion of the Park in lowland, aseasonal, old-growth rainforest inside the Yasuní Research Station (YRS, http://www.yasuni.ec) managed by this University since 1994 (0° 41' S, 76° 24' W, Plate 9). The plot is part of a worldwide network of permanent forest dynamics plots (around 20 plots), whose primary objective is to describe the long-term demography of thousands of plant species, and explain their dynamics with ecological theories (Condit 1995). Every five years, expert botanists and ecology researchers conduct a census in each plot, georeferencing, measuring and identifying all trees ≥1 cm d.b.h. These data describe the diversity and structure of the community, certain characteristics at the species level such as spatial distributions and rates of recruitment, growth and mortality, and also complement a variety of ecological, evolutionary, taxonomical, and ecophysiological studies (Valencia *et al*. 2004b) (Plate 10). Dozens of papers have been published since 1995 (some of them could be find at http://www.puce.edu.ec/sitios/yasuni/publicaciones.php) where the role of topography and soil properties on the plants distribution, competition, facilitation, species coexistence, niche partitioning, seedling mortality, seed dispersion, carbon quantification, among others, have been the main topics of botanical research. These data are of extremely importance for facilitating studies on plant-animals interactions, such as pollination, parasitism, specialism and generalism in insect herbivory, food-webs, bottom-up/top-down control of ecological processes, etc.

7. Objectives

The overall objective of this thesis aims to evaluate the role of invertebrates in the tropical ecosystem functioning through the biotic and abiotic factors driving herbivory and decomposition, and elucidate the link between both canopy and soil sub-systems (Plate 11). This overall objective is divided into four main specific objectives, each of them corresponding to a chapter of the manuscript. We hypothesized that herbivory and decomposition are two weakly associated processes which are independently controlled by different factors.

7.1. Plant traits affecting herbivory in a highly diverse Neotropical forest

The first part of the thesis aims to describe the role of intrinsic plant traits (i.e. leaf quality) and ecological factors (e.g. tree growth rate, spatial distribution) on the herbivory damage in the Yasuní National Park. A better understanding of the plant strategies for escaping herbivory in a specific ecosystem is important for having the full panorama of subsequent leaf-litter quality that may be affecting its decomposition in soil. More specifically our objectives were (1) to elucidate the biotic factors (physico-chemical plant traits) that are predicting herbivory damage in the study area, (2) to test whether there is a significant correlation of plant growth rates with plants physico-chemical defenses (resource availability hypothesis - RAH, see Endara & Coley 2011) and (3) to examine whether herbivory damage is related to tree spatial distribution. For this, we first performed a leaf litter collection of one-year census using leaf litter traps in the study plot and then quantified the percentage of herbivory damage area for each leaf (Plate 12). Then, we measured leaf physical (Plate 13) and chemical traits (Plate 14). Leaf physical analyses were performed *in situ* (i.e. YRS) just after collection, and consisted on measuring the thickness and toughness (punching, shearing and tearing resistance) of leaves. For this we used a digital dual-range force sensor (Vernier Software & Technology, 2010, Beaverton, Oregon, USA) fixed to a handmade steel instrument that allowed to perform standard movements for all the three tests. Fabrication of this instrument was designed and performed by RC and engineers of the *Servicio Ecuatoriano de Capacitación Profesional* (SECAP). Including proof and error tests, the time for its fabrication took about two months. Because of astonishing cheap prices and facilities (i.e. full equipped laboratory), leaf chemical analyses were performed at the Water, Soil and Plant testing laboratory (Colorado State University, Fort Collins, CO, USA) and comprised the following elements: condensed tannins (using Porter Butanol-HCl methodology), lignin, cellulose and ash contents (using ADF-ADL Acid Detergent Fiber - Acid Detergent Lignin methodology, and incineration for ash), C, N estimation (using infrared and thermal conductivity detection system), and micronutrients estimation (using ICP-AES: Inductively Coupled Plasma - Atomic Emission Spectroscopy). Most of the analyses (equivalent to ~80% of the total analyses to be done) were performed by RC in five weeks of laboratory work between February and March 2013. Note that due to a delay in the reception of remaining results, non-structural carbohydrates and total phenols were not included in the present manuscript. Remaining micronutrients data were received in the last moment reason why they were analyzed in the discussion part only. Both, physical and chemical traits were correlated to herbivory damage results. Herbivory damage was additionally correlated to tree growth rates, leaf production and trees spatial distribution which would have not been possible without the access to the tree community database of 50-ha plot survey (see full description of study plot above).

7.2. Pilot study

The second chapter of this thesis corresponds to a pilot study where Cárdenas & Dangles (2012) tested the idea of a mechanical facilitation of damaged leaves by the action of herbivores on its subsequent decomposition. Actually, this study was performed at the very beginning of this thesis in the lower altitudinal limits of what in Ecuador it is considered a 'cloud forest' (900 masl, see Sierra 1999). The study site (Otongachi) is located in the north-western Andean slopes at ~1.5 hours from the capital, Quito. The results obtained in this study led to thinking on a bigger-scale experiment. We chose the YNP and the YRS of PUCE because of the facilities in terms of logistics, and the Yasuní 50-ha plot because of the precise knowledge on trees identity, spatial distribution, phenology and forest dynamics in general.

7.3. Above- belowground linkages: association between leaf herbivory and decomposability in a Neotropical rain forest

The third part of the project aims to test the effect of leaf damage by canopy herbivores to subsequent leaf decomposition in soils. More specifically our objectives were (1) to quantify whether herbivory damage drives significant changes in the physico-chemical properties of leaves, (2) to test whether there is plant species specific association between herbivory damage and decomposition rates (3) to test whether higher leaf herbivory in the canopy implies higher decomposability in soils (and vice versa), and (4) to examine whether damaged and undamaged leaves decomposition are differently correlated to physico-chemical leaf traits. This study is an extension of Cárdenas & Dangles (2012) pilot study where they found herbivory damaged green leaves of one *Ficus* species decomposed at faster rates only the first month of decomposition process (Chapter 2 of this manuscript). Here, we tested whether senescent leaves of 17 common tree species of Yasuní National Park that presented different levels of physico- chemical traits and damaged proportions (damaged, undamaged and artificially punched) decomposed at different rates. For this, senescent leaves were collected, discriminated in laboratory and let to decompose for 100 days. Finally decomposition rates were correlated to herbivory damage and physico-chemical traits (Plate 15).

7.4. Detritivores diversity loss and its consequences on the leaf-litter decomposition process in an Amazonian forest

The fourth part of the project aims to unravel the soil invertebrate functional diversity of this part of the Amazonian region, and reveal their relative role in the leaf-litter decomposition process. More specifically, (1) we describe for the first time how soil functional invertebrate diversity is structured in the Yasuní tropical forest, (2) we evaluate whether such functional biodiversity enhances or not leaf litter decomposition, and (3) we try to elucidate whether decomposition process is driven by redundant, complementary, or keystone species. We took advantage of an existing collection of soil fauna using two different methodologies and collecting design to unravel the fauna diversity of the study plot (David A. Donoso, *Universidad de Loja*, Ecuador, unpublished data). Specimens from this unprocessed collection were cleaned and sorted, measured, separated into morphospecies and classified into functional groups (Plate 16). Parallel to this, senescent leaves of 8 common tree species of YNP were let to decompose in microcosms of five different mesh-size holes. Decomposition rates of different mesh-treatments were correlated to faunal diversity (abundance and functionality) that had access to the leaf resource (Plate 17).

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—PLATES—

Plate 1. The Millennium Ecosystem Assessment (2005) was an international scientific panel that evaluated and reported the present condition of our planet Earth and provided guidelines for decision-makers related to the environmental crisis such as climate change, biodiversity loss, food security etc. The panel identified four ecosystem services that guarantee life and from which humans directly benefit: supporting, regulating, provisioning and cultural services (see also Powledge 2006 for a synthesis). ©Photos by Rafael E. Cárdenas / biographica.com.ec.

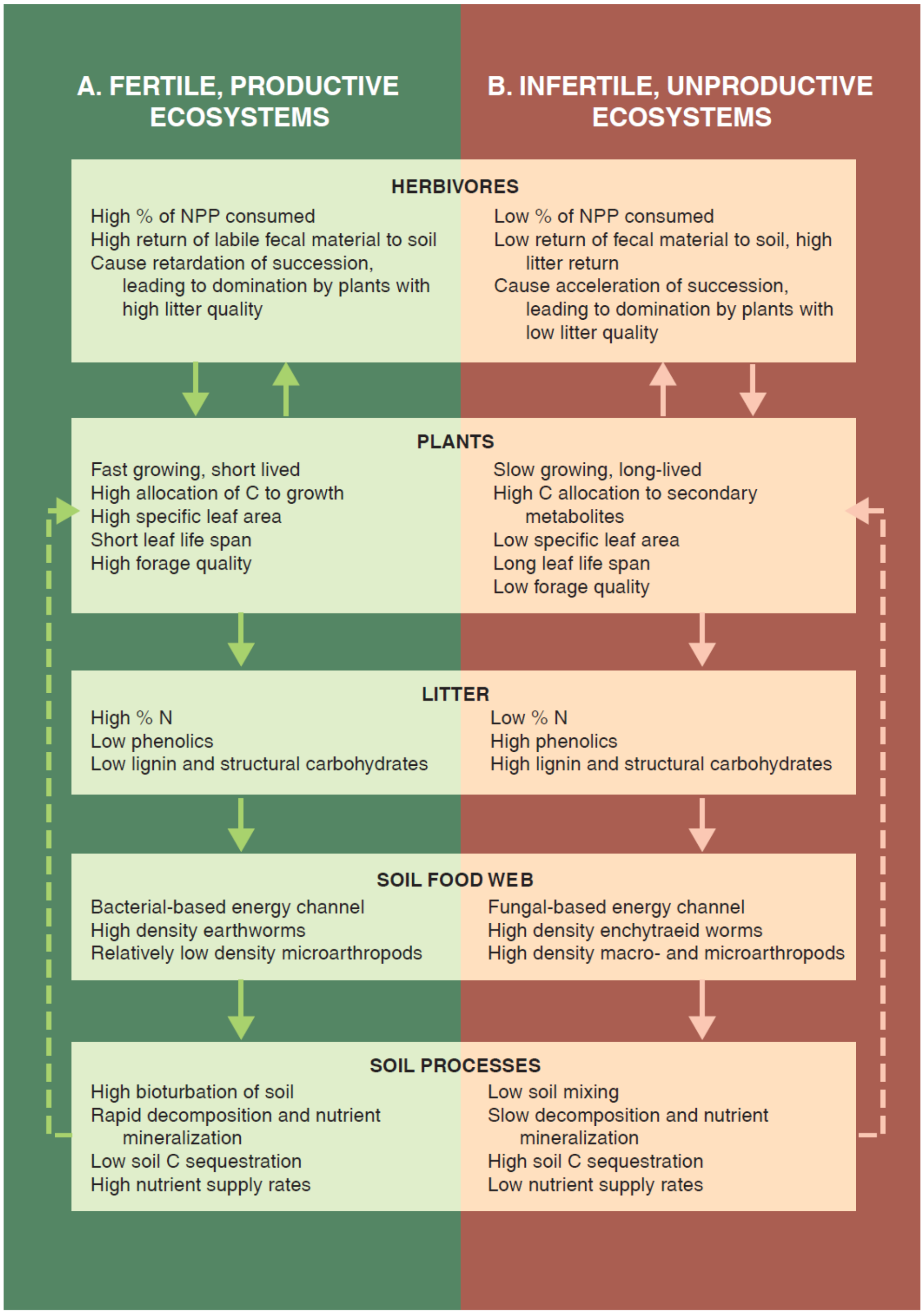


Plate 2. Above- and belowground interactions differ among ecosystems and general patterns have not been easy to reveal, mainly because of the scarcity of studies on this topic, especially in the regions outside the temperate zone. A simple model suggests that differences may rely on the fertility (and productivity) of ecosystems which differ on their capacities to support herbivory rates. This may directly influence on the quality of organic matter that returns to soil, and finally on the soil food web and processes such as nutrient cycling (Figure taken from Wardle *et al*. 2004).

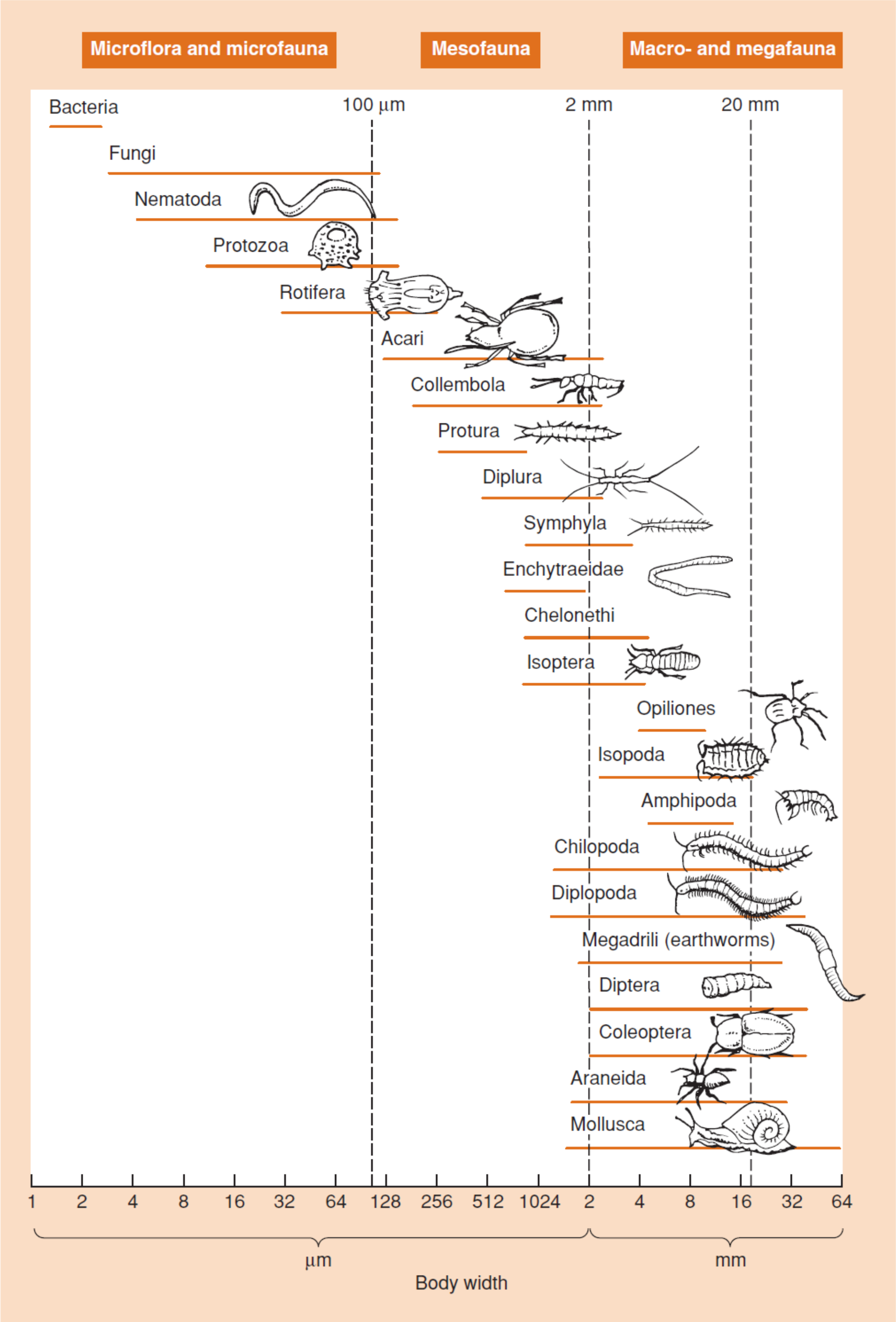


Plate 3. Size classification of taxonomic groups present in terrestrial decomposer food webs (including carnivores). (Swift *et al*. 1979) (Figure taken from Begon *et al*. 2006).

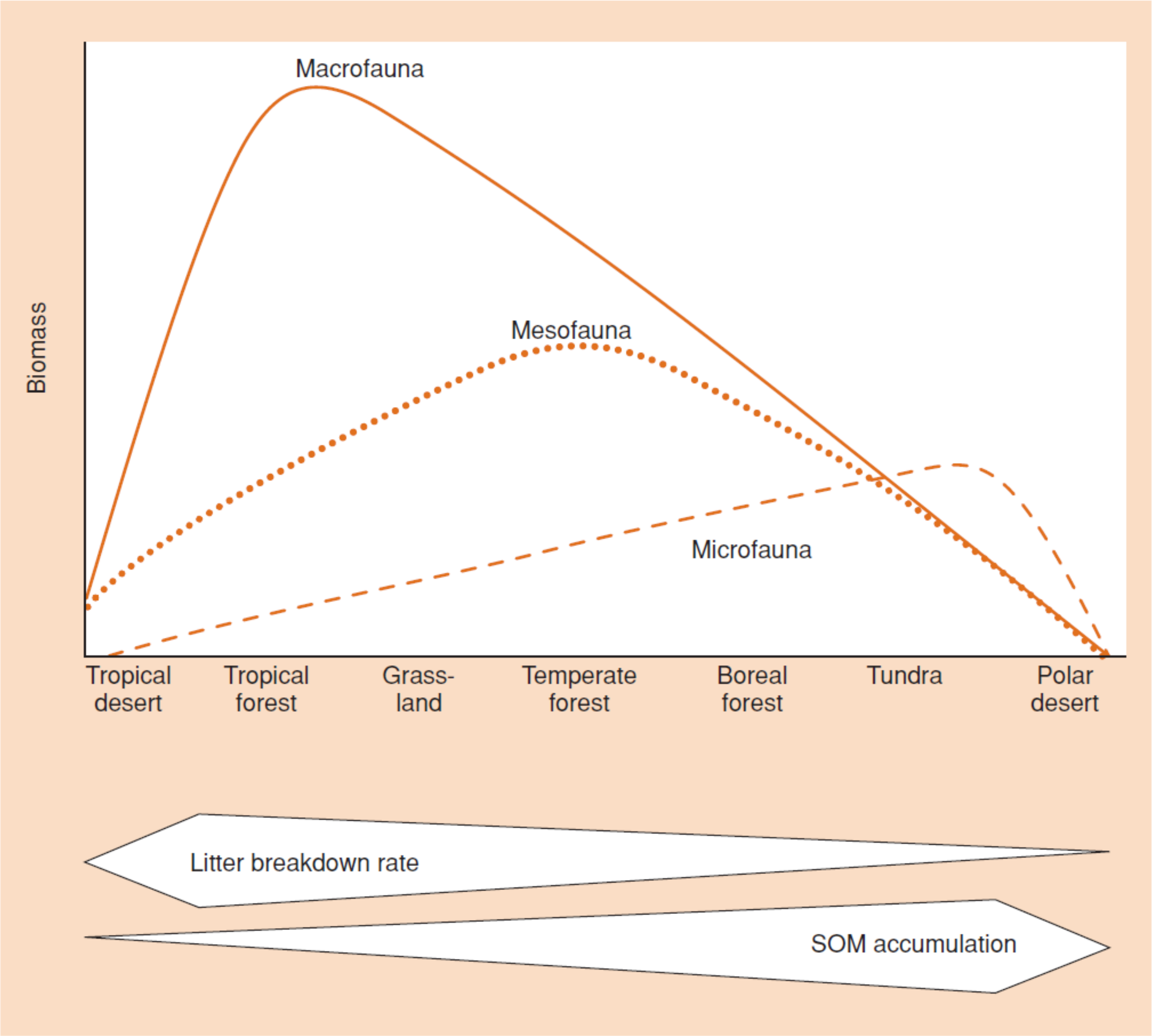


Plate 4. Patterns of latitudinal variation in the contribution of the macro-, meso- and microfauna to decomposition in terrestrial ecosystems, and the relative soil organic matter (SOM) accumulation which is inversely related to litter breakdown rate (Swift *et al*. 1979) (Figure taken from Begon *et al*. 2006).

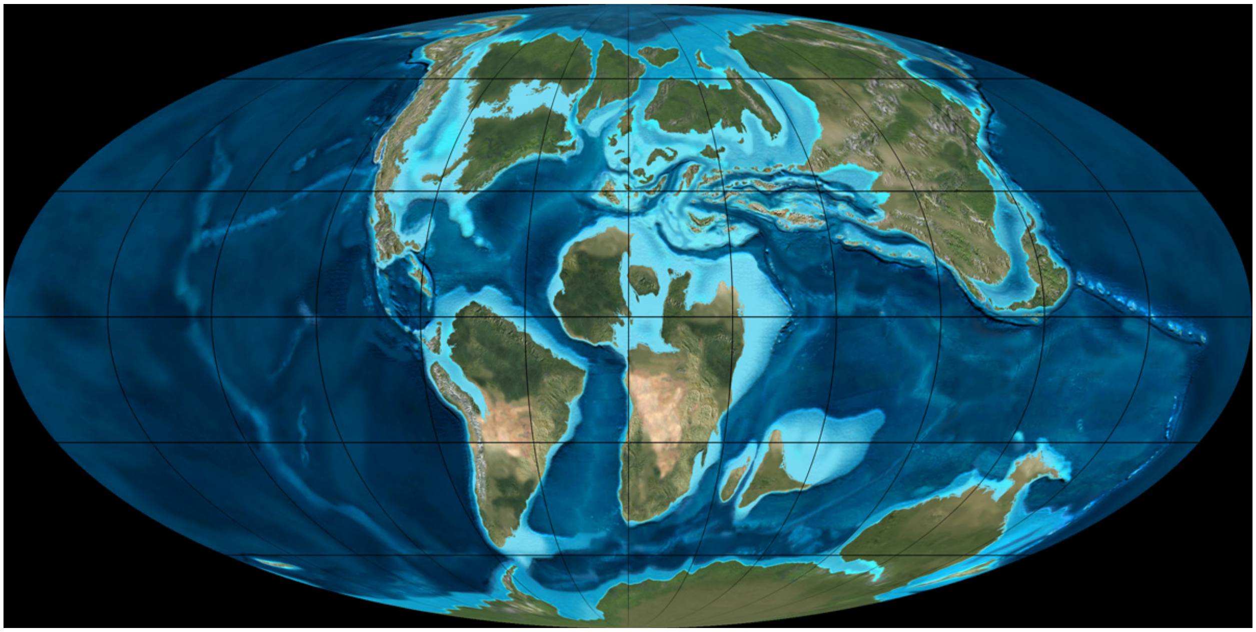


Plate 5. During the Cretaceous Period (145.5–65.5 million years ago), the Americas were gradually moving westward, causing the Atlantic Ocean to expand. The tropics became restricted to equatorial regions and northern latitudes experienced markedly more seasonal climatic conditions. In South America the Andes Cordillera and the Amazon River were incipient (Image taken from http://en.academic.ru/dic.nsf/enwiki/3039).

Plate 6. After its formation the forests of Amazonia originally covered about 6.2 million km^2 and are currently contained within 9 countries: Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Suriname, Peru and Venezuela (Image modified and taken from http://earthobservatory.nasa.gov/IOTD/view.php?id=7705).

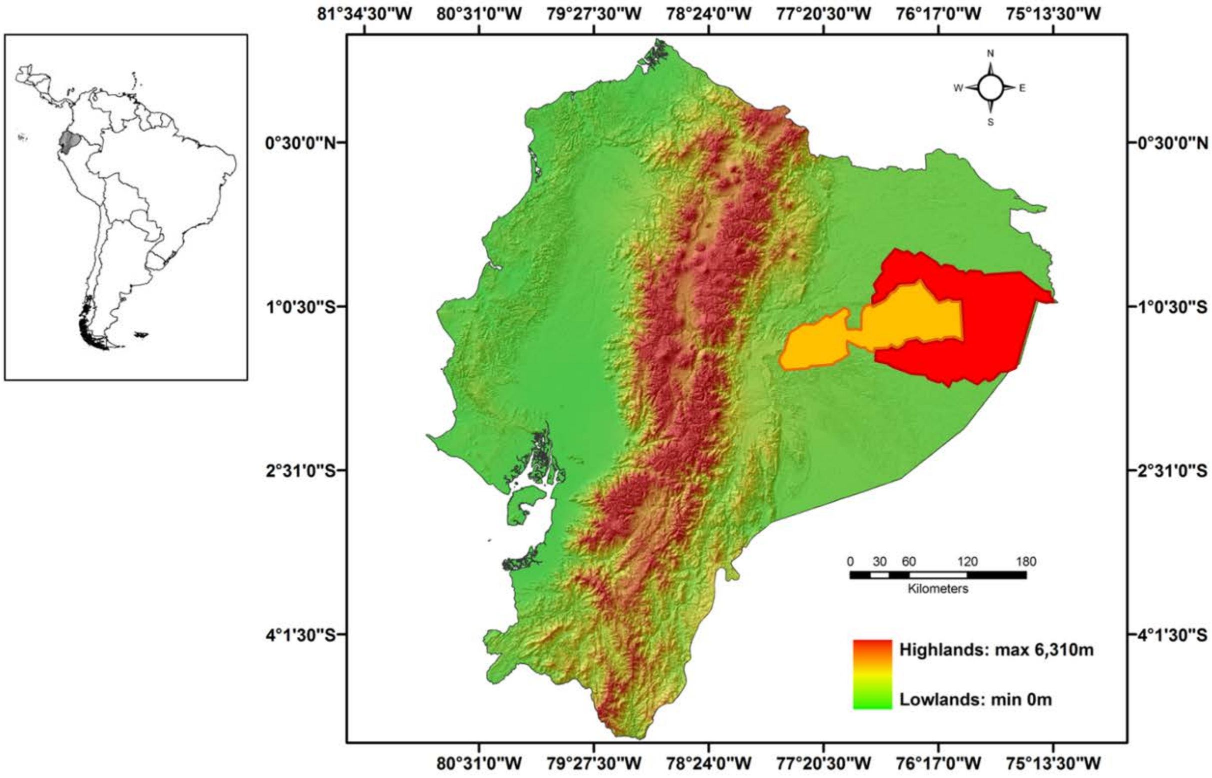


Plate 7. Physical map of the Republic of Ecuador (not including the Galapagos Islands). Yasuní National Park (red polygon) and the adjacent Waorani Ethnic Reserve (orange polygon) cover 16,000 km^2 of forest and form the largest protected area in Amazonian Ecuador (~17.7% of the Ecuadorian territory; Valencia *et al*. 2004a). The map was performed in ArcGIS 9.1 using data from the *Almanaque Electrónico Ecuatoriano* (2002).

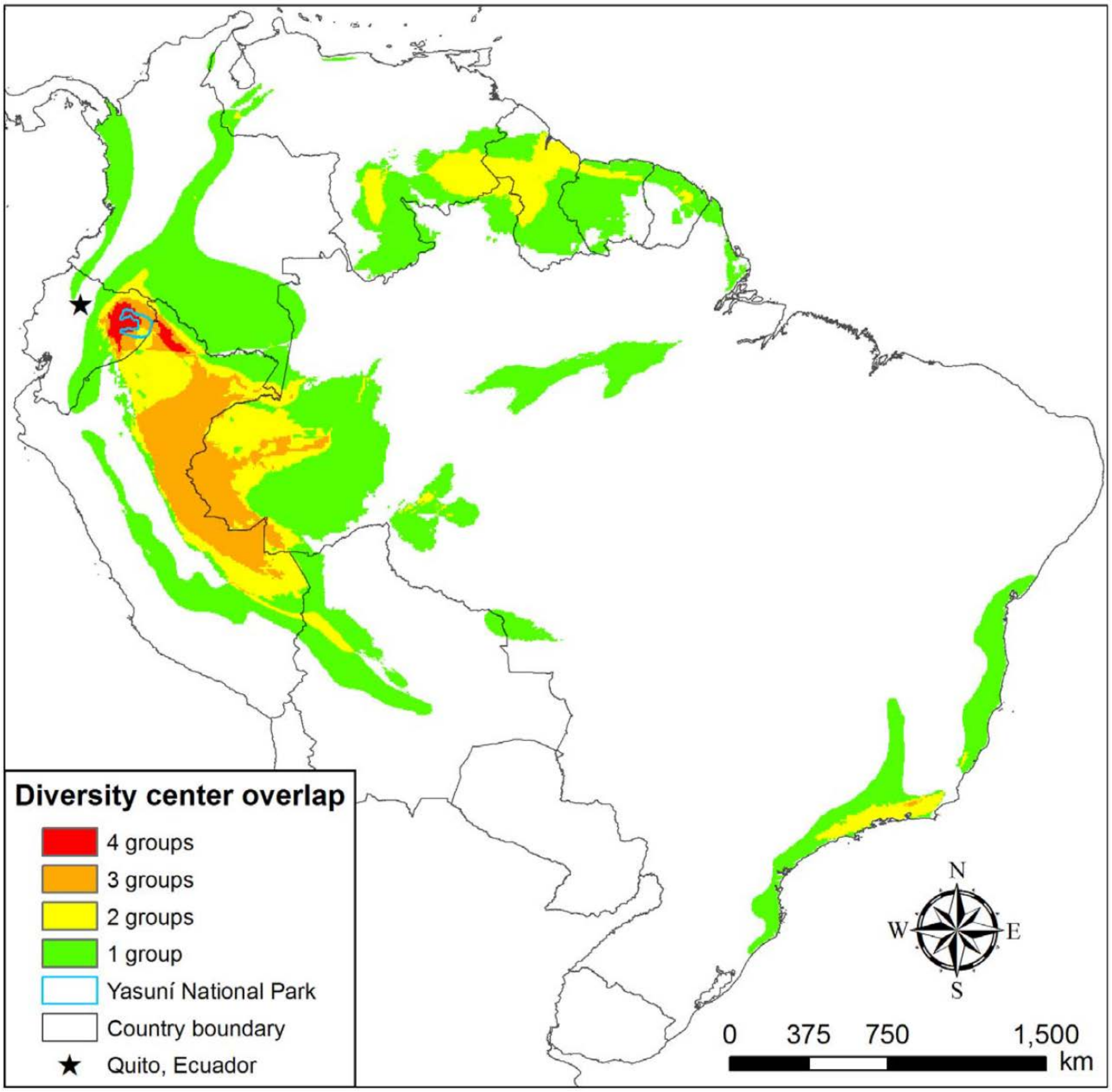


Plate 8. The Yasuní National Park has an outstanding global conservation significance due to its extraordinary biodiversity. This figure shows richness center overlap of four key focus groups: amphibians, birds, mammals and vascular plants. 4 groups = area where richness centers for all four groups overlap; 3 groups = richness centers for three groups overlap; 2 groups = richness centers for two groups overlap; 1 group = richness center for just one group occurs; 0 = richness center for none of the four groups (Figure and information taken from Bass *et al*. 2010).

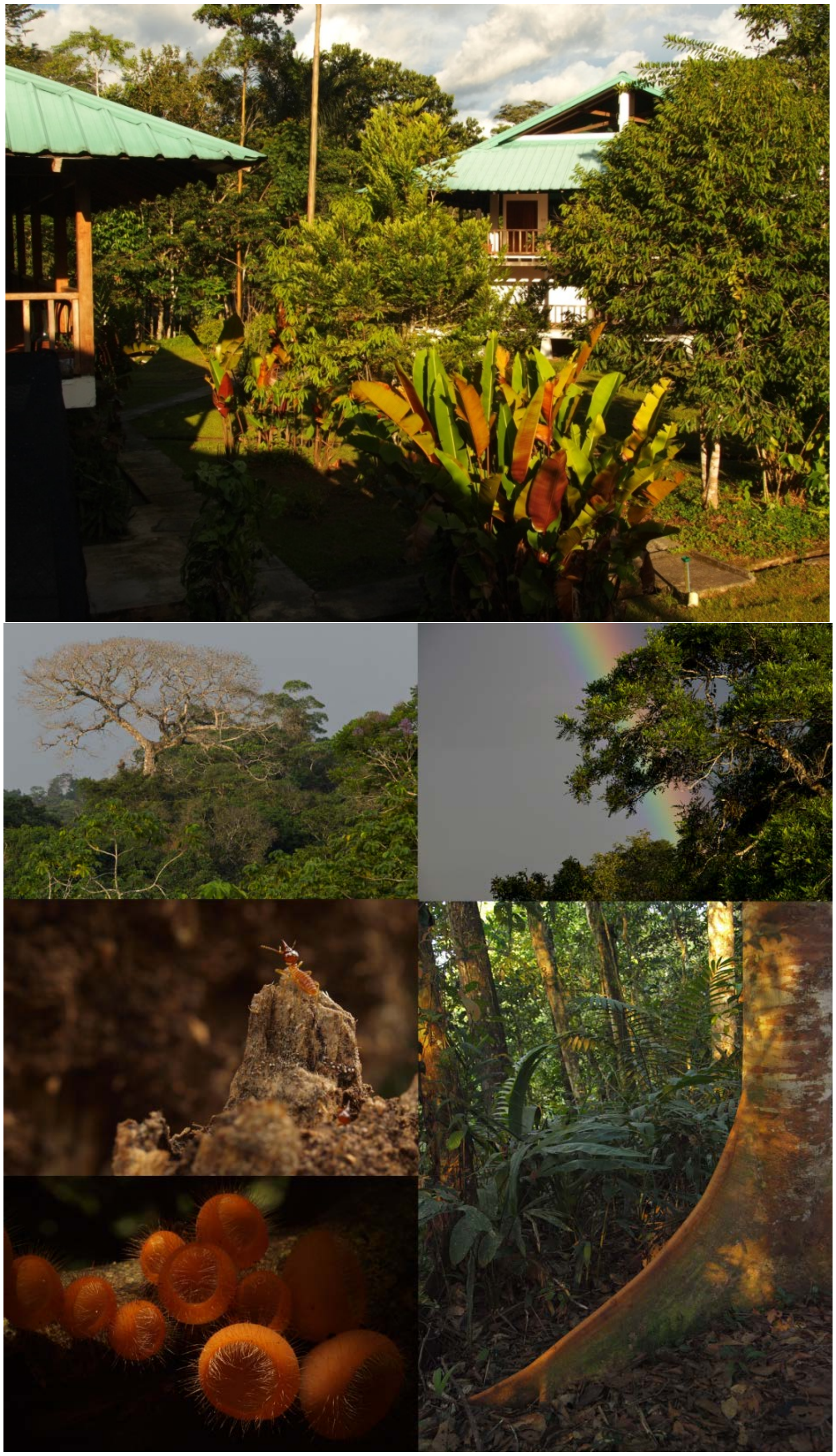


Plate 9. The Yasuní Research Station (YRS, image above) was created on August 25th, 1994 by the Republic of Ecuador and conceded for its management to the School of Biological Sciences of the *Pontificia Universidad Católica del Ecuador* (PUCE) since then.

Bottom images show a small preview of the forest canopy and the understory, and two groups of organisms essential for ensuring the nutrient cycling in this type ecosystem (termites and fungi). ©Photos by Rafael E. Cárdenas / biographica.com.ec.

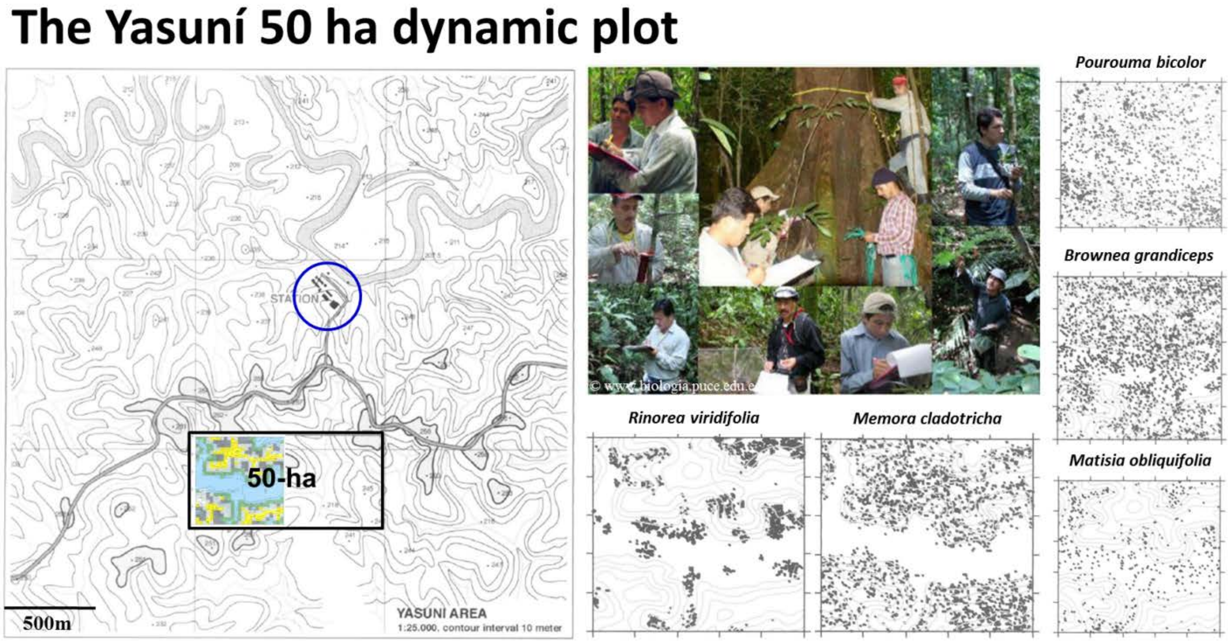


Plate 10. The Yasuní 50 ha dynamic plot (the most diverse worldwide) is located ~1 km South of the YRS. (A) The plot is part of a worldwide network of permanent forest dynamics plots (around 20 plots), whose primary objective is to describe the long-term forest dynamics. Every five years, expert botanists conduct a census georeferencing, measuring and identifying all trees ≥1 cm in diameter. In the plot, permanent leaf litter traps are used for measuring production of organic matter biomass, as well as seed dispersion analyses (Figures taken from Valencia *et al*. 2004a). © Photos from http://www.puce.edu.ec.

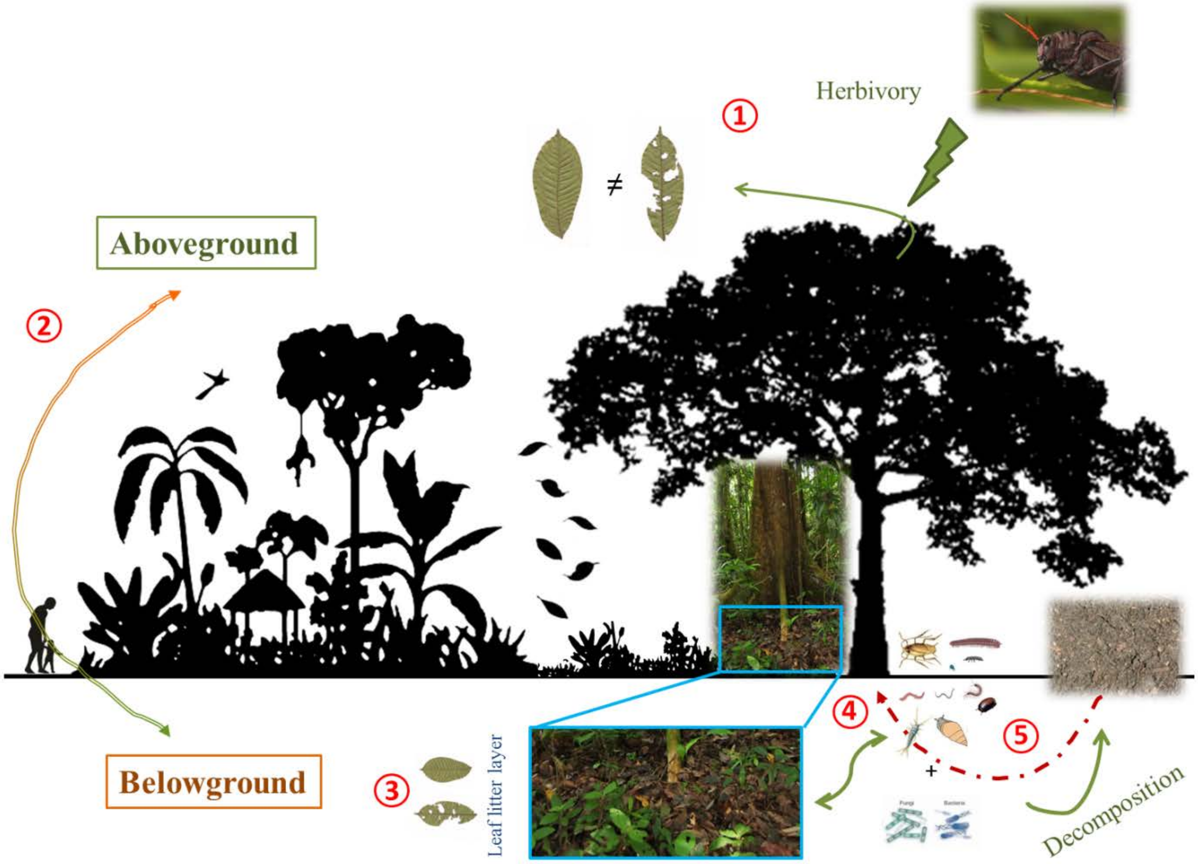


Plate 11. Schematic representation of the main objectives of the present work. In the nutrient cycling context, the above- belowground interaction refers to the potential effect of canopy herbivory of leaves on the soil decomposability of leaf litter. The aims of this thesis project is (1) to quantify whether herbivory is correlated to leaf quality properties, and if herbivory damage drives significant changes in the physico-chemical properties of leaves; (2) to test whether there is plant species specific association between herbivory damage and decomposition rates; (3) to examine whether damaged and undamaged leaves decomposition are differently correlated to physico-chemical leaf traits; (4) to test whether higher leaf herbivory in the canopy implies higher decomposability in soils (and vice versa) due to changes in the leaf litter quality (i.e. more palatability) and detritivores mechanical accessibility; and (5) to reveal whether large, medium and small detritivore species are complementary in terms of efficiency on exploiting a particular resource. Green arrows correspond to the interaction of detritivores and decomposers on the fragmentation and mineralization of the dead organic matter. Red arrow corresponds to the plant reabsorption of nutrients. © Photos by Rafael E. Cárdenas.

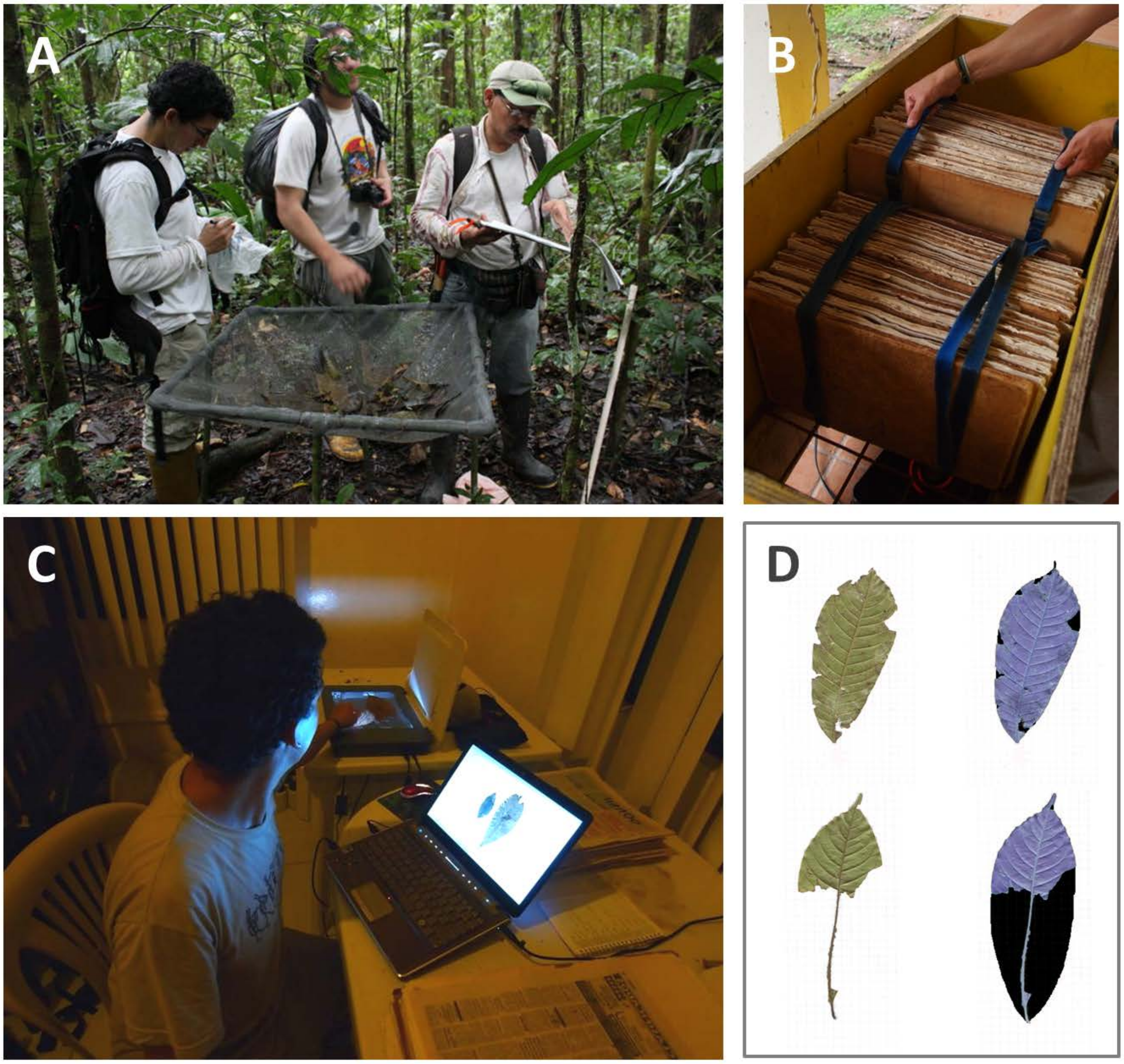
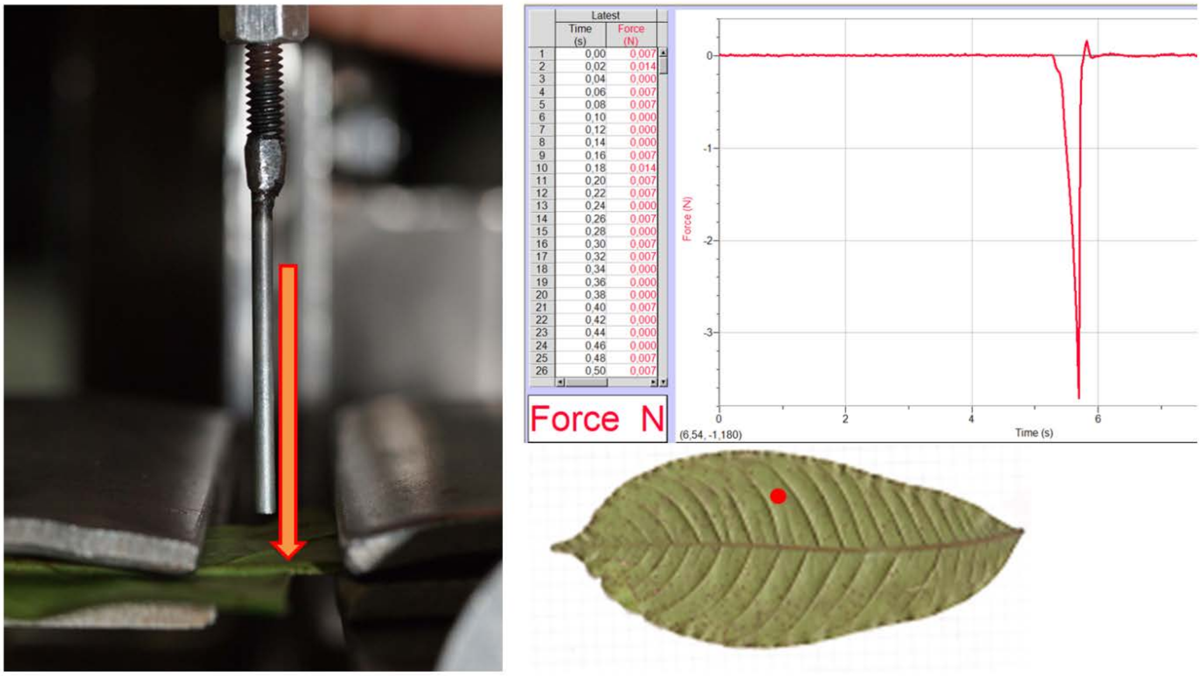


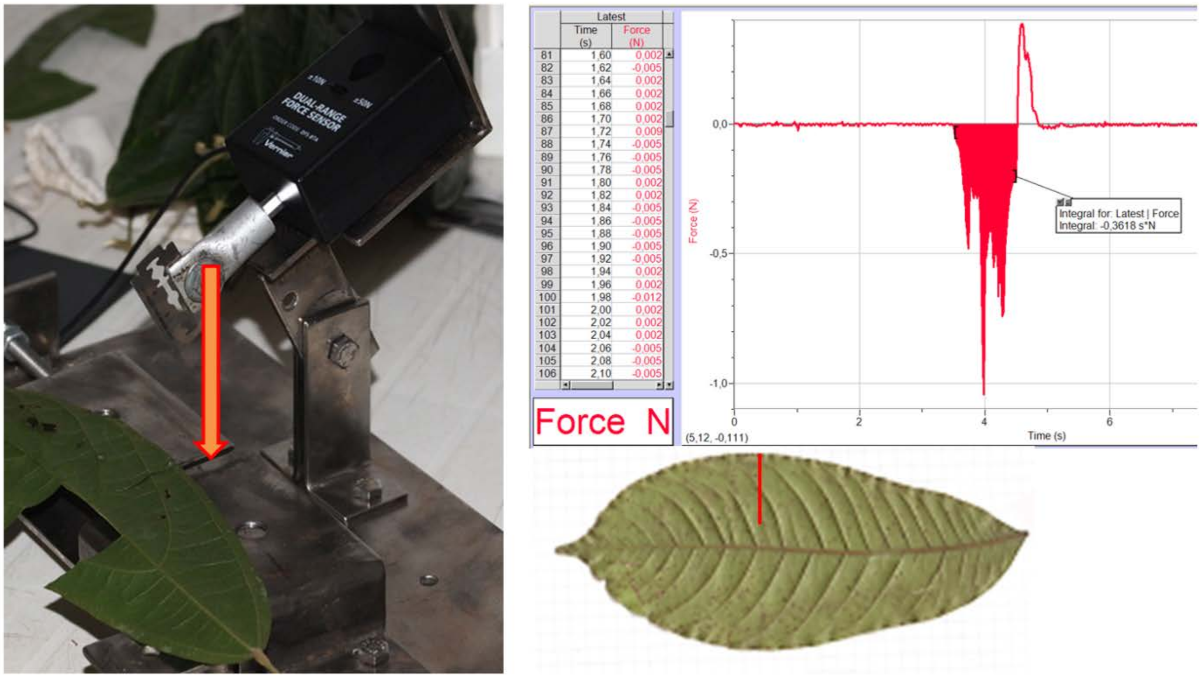
Plate 12. For one year, leaves of the 75 more common tree species were collected from (A) one hundred leaf litter traps (see Chapter 1 for their location within the study plot). (B) Leaves were pressed, dried and weighted, then (C) scanned and finally (D) quantified the damage proportion caused by herbivores. ©Photos by Rafael E. Cárdenas.

A. Punch test



	Latest	
	Time (s)	Force (N)
1	0,00	0,007
2	0,02	0,014
3	0,04	0,000
4	0,06	0,007
5	0,08	0,007
6	0,10	0,000
7	0,12	0,000
8	0,14	0,000
9	0,16	0,007
10	0,18	0,014
11	0,20	0,007
12	0,22	0,007
13	0,24	0,000
14	0,26	0,007
15	0,28	0,000
16	0,30	0,007
17	0,32	0,007
18	0,34	0,000
19	0,36	0,000
20	0,38	0,000
21	0,40	0,007
22	0,42	0,000
23	0,44	0,000
24	0,46	0,000
25	0,48	0,007
26	0,50	0,007

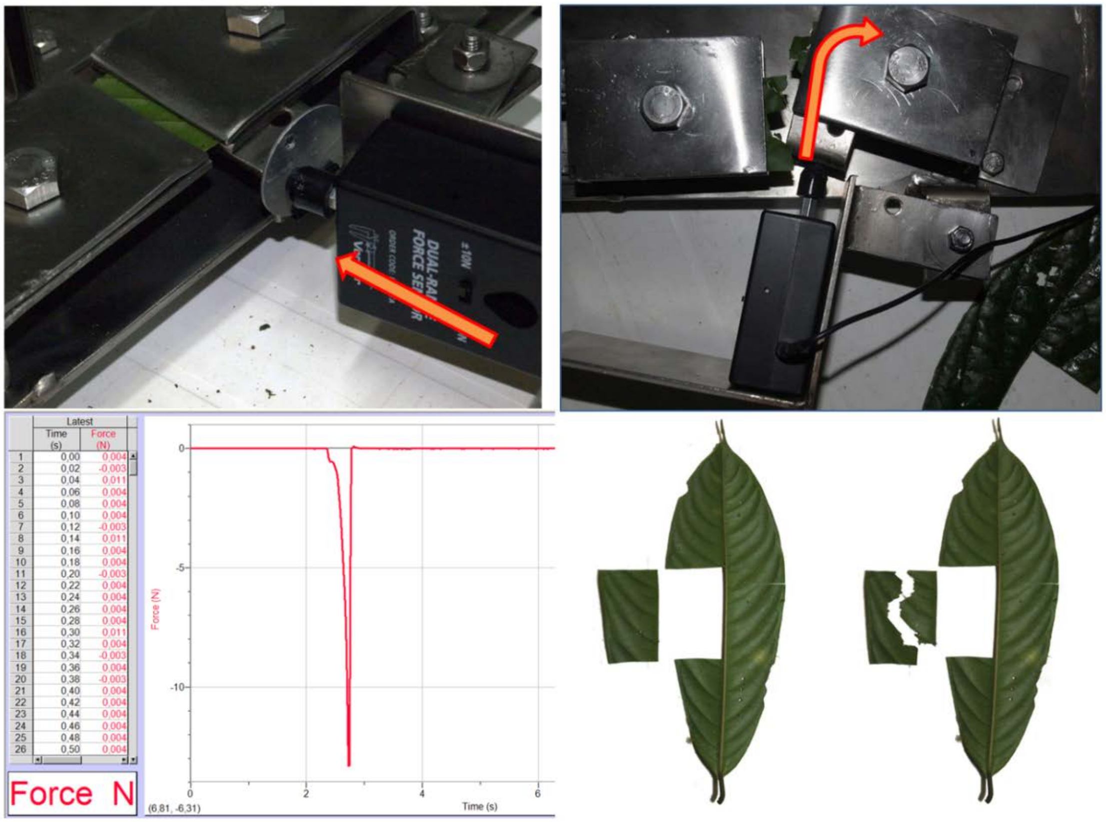
B. Shearing test



	Latest	
	Time (s)	Force (N)
81	1,60	0,002
82	1,62	-0,005
83	1,64	0,002
84	1,66	0,002
85	1,68	0,002
86	1,70	0,002
87	1,72	0,009
88	1,74	-0,005
89	1,76	-0,005
90	1,78	-0,005
91	1,80	0,002
92	1,82	0,002
93	1,84	-0,005
94	1,86	-0,005
95	1,88	-0,005
96	1,90	-0,005
97	1,92	-0,005
98	1,94	0,002
99	1,96	0,002
100	1,98	-0,012
101	2,00	0,002
102	2,02	0,002
103	2,04	0,002
104	2,06	-0,005
105	2,08	-0,005
106	2,10	-0,005

Integral for: Latest | Force
Integral: -0,3618 s*N

Plate 13. (continues on the next page)



C. Tearing test

	Latest	
	Time (s)	Force (N)
1	0,00	0,004
2	0,02	-0,003
3	0,04	0,011
4	0,06	0,004
5	0,08	0,004
6	0,10	0,004
7	0,12	-0,003
8	0,14	0,011
9	0,16	0,004
10	0,18	0,004
11	0,20	-0,003
12	0,22	0,004
13	0,24	0,004
14	0,26	0,004
15	0,28	0,004
16	0,30	0,011
17	0,32	0,004
18	0,34	-0,003
19	0,36	0,004
20	0,38	-0,003
21	0,40	0,004
22	0,42	0,004
23	0,44	0,004
24	0,46	0,004
25	0,48	0,004
26	0,50	0,004

Plate 13. Literature shows that physical traits are measured using different procedures and devices (Onoda *et al* 2011). Thickness was measured avoiding primary and secondary veins using an analog 0–25 mm micrometer caliper at 0.005 mm precision (not shown in images). For resistance to damage measurements we used a digital dual-range force sensor connected to an interface and LoggerPro3 program (Vernier Software & Technology, 2010, Beaverton, Oregon, USA) fixed to a handmade steel instrument (*Servicio Ecuatoriano de Capacitación Profesional* - SECAP fabrication) that allowed to perform standard movements for all the three tests. (A) For the punching test we screwed on the force sensor a 1.68 mm diameter Aluminum flat-ended rivet (area: 2.22 mm^2) and measured the maximum force per unit of fracture (N_{max}/mm^2) to perpendicularly punch the leaf avoiding primary and secondary veins. (B) For the shearing test we screwed on the force sensor a peg-like folded steel sheet that supported a super thin Gillette blade (Gillette®, Procter & Gamble Co., Brazil). The leaf was fixed between two under pressure plaques leaving a space for shearing. Measures were recorded as the force per unit of time (s × N; the area under the curve) and normalized to force per unit of fracture (s × N/mm). (C) For tearing tests we cut a leaf segment from the central part of the leaf, in parallel to its main axis and avoiding the midrib. Length and width were measured (mm) using a digital caliper and the leaf strip was fixed between two under-pressure plaques –one fixed, the other mobile– leaving a space for tearing. Force Sensor was attached to an arm-like that performed a horizontal movement. A rubber bumper directly screwed to the sensor pushed the mobile plaque until the leaf strip was ripped. We measured the maximum force to tear and normalized the data per unit of fracture (N_{max}/mm). ©Photos by Rafael E. Cárdenas.


Nitrogen %
Carbon %
Ballast 813 mm Hg
Furnace 951 C
Flow Medium
000000 g
Sulfuric Acid
Nitric Acid

Plate 14. All chemical analyses were performed using standardized methodologies at Colorado State University certified 'Water, Soil and Plant testing laboratory' (see Chapter 1 and Chapter 4 Material and Methods sections for more details). Work included the analysis of plants condensed tannins (using Porter Butanol-HCl methodology), lignin, cellulose and ash contents (using ADF-ADL Acid Detergent Fiber - Acid Detergent Lignin methodology, and incineration for ash), C, N estimation (using Infrared and thermal conductivity detection system), and micronutrients estimation such as Boron, Calcium, Copper, Iron, Magnesium, Manganese, Molybdenum, Sodium, Sulfur, Phosphorous, Potassium and Zinc (using ICP-AES: Inductively Coupled Plasma - Atomic Emission Spectroscopy). Non-structural carbohydrates and total phenolics were analyzed by qualified personnel in the laboratory. ©Photos by Rafael E. Cárdenas.

A. Leaf collection

© Pablo Alvia

B. Leaf preparation

© Rodrigo Espinosa

C. Microcosms installation

Plate 15. (continues on next page)

D. Microcosms removal, leaf cleaning

E. Leaf drying and weighting

Plate 15. For the experiments we used senescent leaves. (A) Collection consisted on tree shaking (where fallen leaves were trapped in white sheets) or using a pruner with extension tubes. (B) Collected leaves were then discriminated in laboratory. Those presenting necrotic areas, evident signs of physical damage, galls or any type of infection, and those being too young (generally bright green colored, “clean” and firmed at contact) were discarded. Chosen leaves were dried and weighted. (C) Microcosms were randomly installed in groups of six mesh-bags on the floor of the plot covering ~4300 m^2 area. Species letters nomination is only for illustrating randomness in the experimental design. (D) Mesh bags were removed and brought to laboratory for cleaning the remaining plant material, which was finally (E) dried and weighted. ©Photos by Rafael E. Cárdenas, except when otherwise indicated.

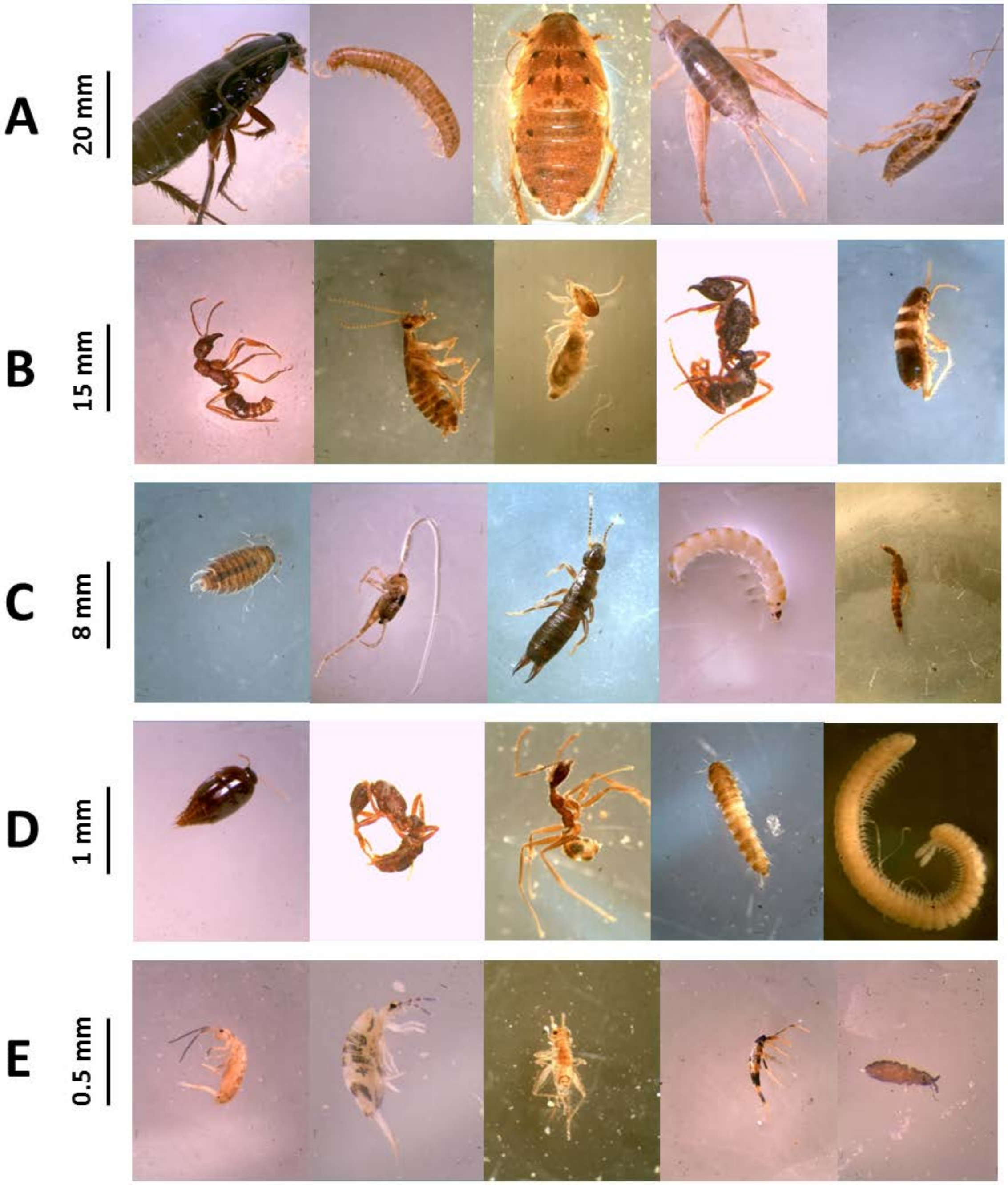

A
20 mm
B
15 mm
C
8 mm
D
1 mm
E
0.5 mm

Plate 16. Example of soil arthropod morphospecies found in both Pitfall and Winkler samples used for soil fauna collection. Invertebrates were identified and measured at 0.01 mm precision. Collection courtesy of David A. Donoso. From left to right (A): Blatellidae nymph (cockroach, m.sp. 7), Polydesmidae (millipedes, m.sp. 2), Blatellidae nymph (cockroach, m.sp. 1), Gryllidae (m.sp. 10), Blatellidae nymph (cockroach, m.sp. 2); (B): *Ectatomma* (ant, m.sp. 1), Blattidae nymph (cockroach, m.sp. 4), Isoptera (termite, m.sp. 1), *Gnamptogenys* (ant, m.sp.2), Blattidae nymph (cockroach, m.sp. 3); (C): Isopoda (m.sp. 1), Gryllidae (m.sp. 9), Labiidae (dermapterous, m.sp. 1), Coleoptera larva (m.sp. 3), Staphylinidae (Oxytelinae, m.sp. 10); (D): Staphylinidae (Tachyporinae, m.sp. 3), *Gnamptogenys* (ant, m.sp.1), *Pheidole* (ant, m.sp. 1), Staphylinidae (larva, m.sp. 2), Chordeumidae (millipedes, m.sp. 1); (E): Entomobryidae (Collembola, m.sp. 19), Entomobryidae (Collembola, m.sp. 17), Gryllidae (m.sp. 2), Entomobryidae (Collembola, m.sp. 10), Hypogastruridae-Neanuridae (Collembola, m. sp. 4). ©Photos by Adriana Argoti, Mario Herrera and Rafael E. Cárdenas.

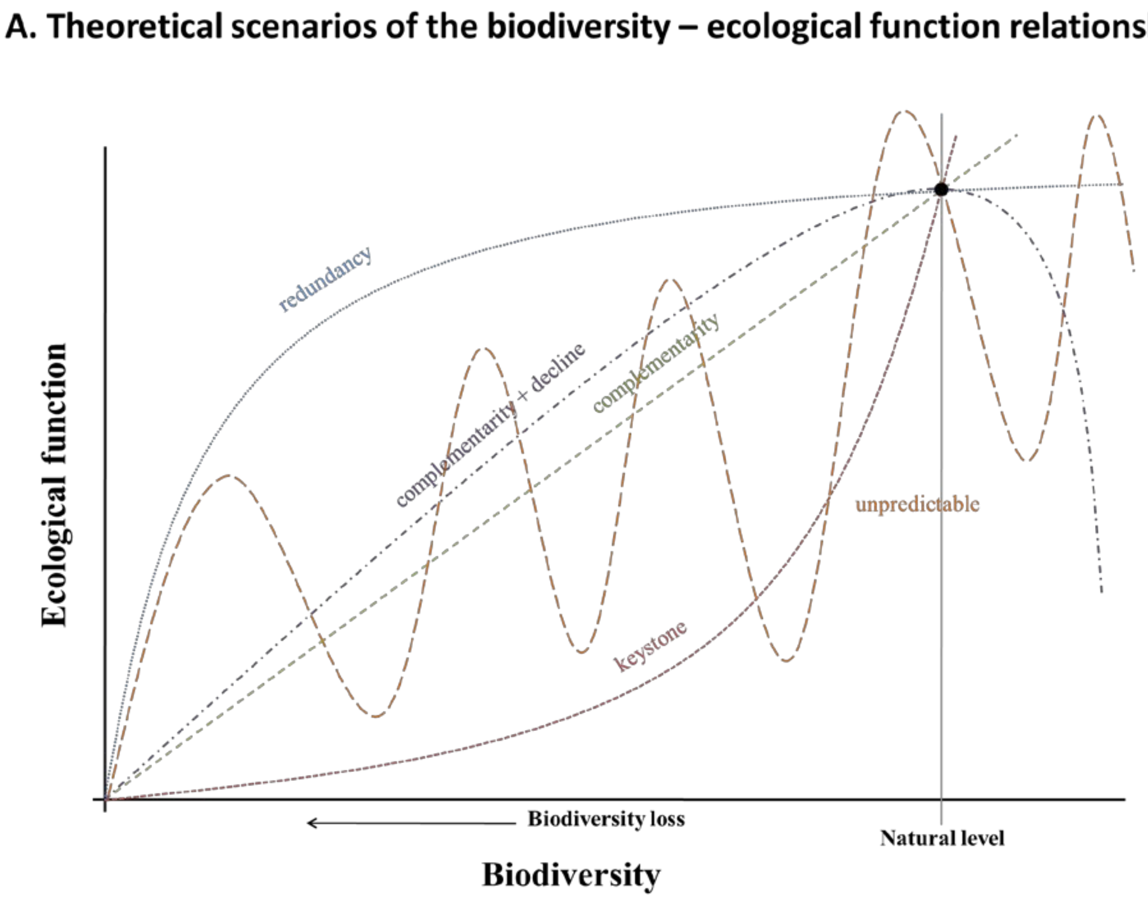


Plate 17. (continues on next page)

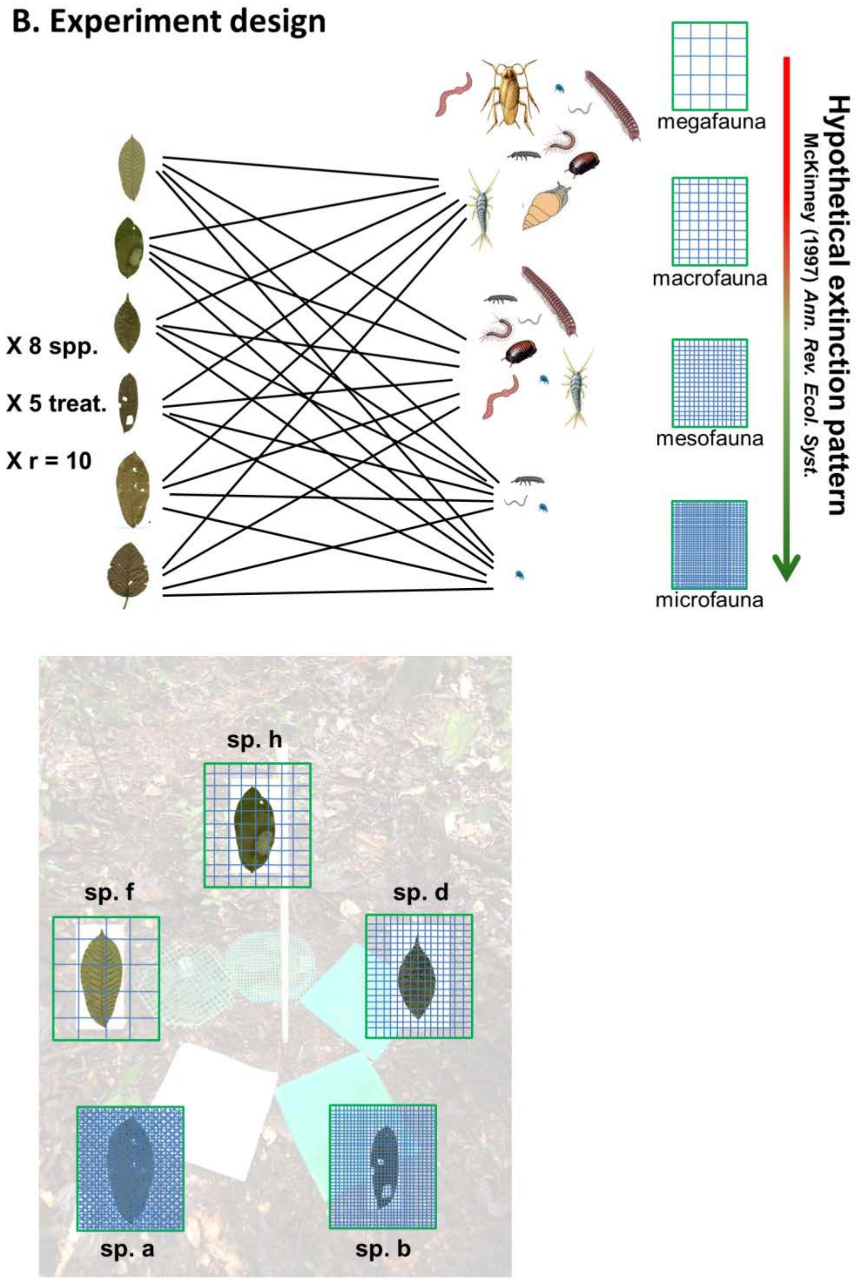


Plate 17. For this experiment we used the same leaf collection and sample processing methodologies as described in Plate 15. (A) Biodiversity-ecological function relationships may be revealed when excluding from ecological processes (Figure modified from Naeem *et al*. 2009). (B) Hypothetical extinction scenario (McKinney 1997) suggests that bigger invertebrates are more (and first) susceptible to extinction as consequence of current climate change (image above). To simulate such a scenario (image below), leaves of eight common tree species were packed in five plastic mesh bags with different holes size, randomly installed on the forest floor in an area that covered 4000 m^2 and finally quantified its mass loss after 104 days of decomposition process. Species letters nomination is only for illustrating randomness in the experimental design.

—CHAPTER 1—

Plant traits predicting herbivory in a highly diverse Neotropical rain forest

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Running headline: Plant traits affecting herbivory

Summary

1. One key issue of plant-herbivore interaction research is to elucidate which plant traits contribute to explain observed differences in herbivory damage among plant species and individuals. Chemical, physical and ecological traits of plants have been recognized as relevant factors driving herbivory damages yet their relative importance is still subject to debate, particularly in species-rich systems such as tropical rainforests.

2. To address this issue, we quantified over one year leaf herbivory damages obtained, in 53 common tree species of the Yasuní forest dynamic plot (YFDP) in the Ecuadorian Amazon. Performing our study in YFDP allowed us to obtain long-term data on several aspects of tree ecology potentially affecting herbivory such as tree growth or spatial distribution. We then selected the 28 most leaf-productive tree species for which we measured 7 chemical, 7 physical and 4 ecological traits. Using a combination of multivariate analyses and generalized linear models, we assessed trade-offs between physical and chemical traits and the relative effect of all these traits on leaf herbivory damage.

3. Herbivory damage was highly variable among and within the 28 common species, with a mean value of 13.4 % (2.5–29.5 %). We found no significant trade-off between physical and chemical defences for the 28 studied tree species. Overall, shearing resistance, ash content, shearing resistance × C:N ratio, and leaf size were, in order of importance, the best predictors of herbivory damage. Surprisingly, secondary metabolites such as condensed tannins or latex did not significantly correlate with herbivore damage. Also we found no relationships between herbivory damage and tree growth rates and density. However, we found barely significant effect of tree clustering and strong effect of tree leaf production on herbivory damage.

4. *Synthesis*. In the western Amazon, leaves are defended against herbivores using a combination of physical (toughness), chemical (toughness-related elements) and phenological (tree leaf replacement and potentially conspecifics tree spatial clustering) characteristics that do not necessarily present trade-offs amongst each other. Conventional strategies such as condensed tannins or latex do not seem to be strongly involved as a defence against herbivores.

Keywords: Amazonia, Ecuador, forest dynamics plot, leaf-litter production, plant-herbivore interactions, Resource Availability Hypothesis, Yasuní National Park.

Introduction

Plant-herbivore interactions have been a topic of intense research over the past 40 years with significant contributions from empirical and experimental studies, theory, and meta-analyses (Price *et al.* 1980; Ohgushi 2005; Endara & Coley 2011; Johnson *et al.* 2012). To date, differences in herbivore damage among plant species and conspecific individuals are thought to be driven by two main factors: defence traits and phenological strategy. First, a number of studies have shown that the extent of herbivore damage is mainly driven by plant physical and chemical traits (Coley & Barone 1996; Loranger *et al.* 2013). While high nitrogen content and SLA (specific leaf area) index can make plants more palatable to herbivores (Mattson 1980; Choong *et al.* 1992; Hanley *et al.* 2007), the presence of secondary metabolites and structural traits is assumed to increase plant resistance to herbivory (Agrawal 2007; Hanley *et al.* 2007). The assumption that defences are costly (Bazzaz *et al.* 1987) led to explain the distribution of defences among plant parts (e.g. optimal defence theory, Rhoades & Cates 1976) and among individuals and species with varying access to resources (e.g. growth-differentiation balance hypothesis, Herms & Mattson 1992) or with different life-history characteristics (such as growth rates, Coley 1988). Similarly, within a plant, defences have been predicted to trade-off against one another because a finite pool of resources is being divided between different types of defence (Read *et al.* 2009). These ideas have been used to suggest that there will be trade-offs between physical and chemical defences (Twigg & Socha 1996; Read *et al.* 2009).

Second, phenological aspects of plants such as some leaf production patterns (e.g. synchronous flushing, leaf expansion rate) have shown to be correlated to the amounts or rates of herbivory (Aide, 1988, 1992, 1993; Coley & Barone 1996). For example, leaves produced during the dry season (when herbivores are less abundant), in synchronous flushes (as a strategy to satiate herbivores) or presenting rapid leaf expansion (minimizing vulnerability interval) receive significantly less damage from herbivores (Aide 1988; Coley & Barone 1996). Other aspects such as the abundance and spatial distribution of plants might also affect the risk of discovery by herbivores (Coley 1983), with consequences for survival (Maron & Crone 2006). Herbivore pressure is predicted to increase with conspecific plant density (Janzen 1974; Sullivan 2003) while spatial differences in resource availability may also affect plant susceptibility to herbivore damage (Fine, Mesones & Coley 2004). In low-resource environments, the impact of herbivory will be especially large because of the cost of replacing lost tissue, and *vice versa* (Lamarre *et al.* 2012).

Studies on the interactions between plant defences and herbivore damage take on particular significance in tropical forests, as herbivores are thought to play an important role in maintaining high plant species diversity in these systems (Janzen 1970; Connell 1971; Fine, Mesones & Coley 2004). However there are relatively few community-wide studies examining the relative importance of species-specific (intrinsic) and ecological traits of trees on herbivory damage in tropical forests (Bardgett & Wardle 2010; Agrawal 2011; Cárdenas & Dangles 2012). This is partly due to the difficulty of obtaining reliable and exhaustive data on tree dynamics and spatial distribution in high diversity communities containing hundreds of species. In this context, forest dynamics plots such as the Yasuní 50 ha plot in Ecuador (Valencia *et al.* 2004b) offer a unique, yet surprisingly poorly considered, opportunity to test the factors driving herbivory damage pattern in tropical forests.

Observational studies have the advantage of providing natural conditions and fully intact assemblages of animals and plants with natural interactions that are not reproducible in short-term experiments.

Here we examine the relative importance of intrinsic plant traits (e.g. leaf quality) and ecological factors (e.g. tree growth rate, spatial distribution) in driving variation in leaf herbivore damage in a species-rich tree community of the Ecuadorian Amazon. We first quantified variability in leaf herbivory damage for the 75 most abundant tree species in our study plot over the course of one year. We then examined the effects of several key leaf functional traits on herbivory damage and tested whether there is evidence of a trade-off between physical and chemical defences among species (Moles *et al.* 2013). Finally, we used the ecological census data of the forest dynamics plot to test the following hypotheses: (1) fast-growing tree species have lower amounts of constitutive defences and therefore higher herbivory damages than slow-growing species (from the Resource Availability Hypothesis, Coley, Bryant & Chapin 1985); (2) species with higher leaf production support lower herbivory damages than low leaf-productive species; (3) spatially clustered-species have higher herbivory damage than evenly-distributed species; and (4) tree surrounded by a higher number of conspecifics exhibit higher herbivory damages than trees isolated from their conspecifics. The two latter hypotheses were based on Janzen-Connell effect (Janzen 1970; Connell 1971) which states that if adult trees serve as reservoirs for natural enemies, conspecifics of the surroundings may increase such effect (i.e. attracting herbivores) thus presenting higher levels of herbivory damage.

Material and methods

Study site

The Yasuní National Park (YNP) and the adjacent Waorani Indigenous territory cover 1.6 million ha of forest (1.8 times the Yellowstone National Park in the USA) and form the largest protected area in Amazonian Ecuador (~17.7 % of the Ecuadorian Territory; Valencia *et al.* 2004a), harboring the world's most diverse tropical forests (Bass *et al.* 2010). YNP is an evergreen lowland wet forest ranging in altitude from 200 m to 300 m above sea level. It has a 15–30 m canopy with some emergent trees reaching 50 m (Dangles, Carpio & Woodward 2012). Rainfall and temperature are aseasonal with a mean annual rainfall of 2826 mm (none of the 12 calendar months averaging < 100 mm) and a mean monthly temperature ranging from 22 to 32°C (min: 16.9; max: 38.9°C) (see Valencia *et al.* 2004a for more details; data obtained from YRS meteorological station, http://www.yasuni.ec).

The study plot was located in the vicinity of Yasuní Research Station of the *Pontificia Universidad Católica del Ecuador* (YRS-PUCE; 76°24´1.8´´W; 00°40´16.7´´S). As part of a global network of permanent forest dynamics plots the YRS-PUCE, in collaboration with the Smithsonian Tropical Research Institute and Aarhus University, created in 1995 the Yasuní 50-ha plot (500 × 1000 m; 76° 24´ W; 00°41´ S; see http://www.puce.edu.ec/portal/content/Dinámica del Bosque Yasuní). Its primary objective is to describe the long-term demography of thousands of plant species and explain their dynamics with ecological theories (e.g. Kraft, Valencia & Ackerly 2008; Romero-Saltos 2011). To achieve this goal, all trees with ≥1 cm DBH (diameter at breast height) have been tagged, mapped and identified at species level (Valencia *et al.* 2004b), indispensable when searching for correlations between species identity and functional traits at the community level. The census methodology is described in detail in Condit (1998). The plot lies at 230 m above sea level, and contains three large ridges and intervening valleys (Appendix S1) that include small streams and a small swamp. There is a 33.5 m difference between altitude extreme points in the plot which presents an average slope of 13 % (Valencia *et al.* 2004a).

Leaf litter collection

In order to test the herbivory damage in the study plot leaf litter was collected from 100 litter fall traps (mesh of 0.71 m^2 fixed on 0.8m high plastic tubes) located in a Western 650 × 400 m sub-plot (see Appendix S1) and censured monthly between February 2011 and January 2012 (except May 2011). Collected leaves of the 75 most abundant freestanding woody plants tree species of the plot (accounting for >36 % of the total number of individuals) were identified in the field with the expertise of a para-taxonomist botanist. The identity of each species was confirmed using voucher material deposited at the YRS-PUCE. Tree commonness was assessed using the 2009 complete census of the 50 ha plot (Valencia, unpublished data).

Leaf herbivory damage quantification

Of the 75 most abundant tree species in the plot, leaves from 53 species were recovered in the litter fall traps. For these 53 species, collected leaves were pressed, dried at 60°C for 48 h, weighted and scanned at 2300 × 3300 pixels resolution for further herbivory damage quantification. As we collected more than 30 leaves for only 28 tree species we did not consider the 25 remaining species for subsequent analyses. We defined herbivory damage as the proportion of eaten area over the complete leaf lifespan. It represents the cumulative percentage of leaf area lost (Schuldt *et al.* 2012) including herbivory events from previous seasons (see Brenes-Arguedas, Coley & Kursar 2008). The few leaves totally eaten (i.e. with only the petiole remaining) were not taken into account for herbivory damage estimations. Our herbivory damage metrics therefore mainly refers to the action of leaf chewers (e.g. Orthopterans, Lepidopteran larvae, Coleopterans like Chrysomelids, Molluscs). Piercing/sucking damages (e.g. Cicadellids, Curculionids, Cercopids) were not taken into account as it was impossible to measure their damage impact visually. Also, leaves presenting mines, galls, scraped/scratched/ripped leaf surfaces, and necrotic areas, which represented < 20% of the total leaf collection (RC personal observation), were not evaluated.

Eaten area was quantified using ImageJ (http://rsb.info.nih.gov/ij/; Abràmoff, Magalhães & Ram 2004). For this, leaf images were cleaned (i.e. erase shadows, fill scratches, eliminate the petiole) and binary-transformed. We then quantified total and damaged areas, and then calculated the proportion herbivory damage (eaten area divided by total area). In the case of leaf-margin damage we cloned the missing edge from the opposite side of the same leaf or from similar size-shape leaves to infer the initial total area in Photoshop CS (Adobe Systems Incorporated, San José, California, USA).

Sampling for leaf trait measurements

Foliar material was collected from randomly selected individual trees located nearby YRS-PUCE trails, rejecting individuals that showed heavy impact of herbivores, or that lacked sufficient recently produced, fully expanded and hardened leaves (Cornelissen *et al.* 2003). We targeted outer canopy green-leaves from adult trees in the 28–276 mm diameter at breast height size class (Valencia *et al.* 2004b) that were readily accessed from the ground. We chose green-leaves as most of the herbivory damage occurs on young/expanding leaves (Coley & Barone 1996). In total, 40 to 60 leaves were sampled from 5-6 individuals from the 28 tree species. Leaves designated for chemical analyses (10–25 depending on its size and tangible biomass) were dried at 45°C for 48–96 hours, separated into five groups, homogenized in a coffee grinder and kept in dry conditions until analyses.

Leaf traits measurements

We selected a range of vegetative functional traits, both physical and chemical, that have been shown to be correlated with herbivory or anti-herbivory properties (Cornelissen *et al.* 2003; Hanley *et al.* 2007; Kurokawa & Nakashizuka 2008; Carmona, Lajeunesse & Johnson 2011; Moles *et al.* 2013). We first characterized the “structural” and “indirect defences” of each species (Hanley *et al.* 2007; Carmona, Lajeunesse & Johnson 2011; Kessler & Heil 2011) by determining the presence/absence of hairs/scales on the leaves and the presence/absence of extra-floral nectaries/glands on the twigs. Physical traits – or resistance to physical damage traits (Carmona, Lajeunesse & Johnson 2011) – corresponded to leaf thickness, and necessary force for punching, shearing and tearing (Onoda *et al.* 2011). Tests were performed over 28–30 leaves *per* species, except for tearing tests where only 15–18 leaves were used for three species (*Inga auristellae* Harms, *Eugenia florida* DC., *Eugenia minicomun* [*nomen nudum*]). Thickness was measured avoiding primary and secondary veins using an analog 0–25 mm micrometer caliper at 0.005 mm precision (Amico Corporation, Ontario, Canada). For punching, shearing and tearing quantification, we used a digital dual-range force sensor (Vernier Software & Technology, 2010, Beaverton, Oregon, USA) fixed to a handmade steel instrument that allowed to perform standard movements for all the three tests. Forces were measured in Newtons (N) at 0.01 or 0.05 N precision for resistances ± 10 N or ± 50 N, respectively. For the punching test we screwed on the force sensor a 1.68 mm diameter Aluminum flat-ended rivet (area: 2.22 mm^2) and measured the maximum force *per* unit of fracture (N_{max}/mm^2) to perpendicularly punch the leaf avoiding primary and secondary veins (Onoda *et al.* 2011). For the shearing test we screwed on the force sensor a peg-like folded steel sheet that supported a super thin Gillette blade (Gillette®, Procter & Gamble Co., Brazil). The leaf was fixed between two under pressure plaques leaving a space for shearing. Blades were replaced every 28–30 measurements (i.e. one blade per species) to avoid the damage of the sharp edge. Measures were recorded as the force *per* unit of time (s × N; the area under the curve) and normalized to force *per* unit of fracture (s × N/mm; Onoda *et al.* 2011). For tearing tests we cut a leaf segment from the central part of the leaf, in parallel to its main axis and avoiding the midrib. Length and width were measured (mm) using a digital caliper (Fowler Tools of Canada, Ontario, Canada) and the leaf strip was fixed between two under-pressure plaques –one fixed, the other mobile– leaving a space for tearing. Force Sensor was attached to an arm-like that performed a horizontal movement. A rubber bumper directly screwed to the sensor pushed the mobile plaque until the leaf strip was ripped. As tearing tests measured the maximum force to tear a leaf strip, we normalized the data *per* unit of fracture (N_{max}/mm; Onoda *et al.* 2011). Finally, leaf size (cm^2) and specific leaf area (SLA, defined as the ratio of fresh leaf area in cm^2 to dry weight) of the 28 tree species were taken from Kraft, Valencia & Ackerly (2008) and Kraft & Ackerly (2010, and unpublished data).

For the 28 tree species, we quantified the following chemical traits: nitrogen, carbon, lignin, cellulose, ash, condensed tannins, and the presence/absence of latex. Nitrogen and carbon values were taken from Kraft & Ackerly (2010 and unpublished data). All other traits tests were measured at Colorado State University (Fort Collins, Colorado, USA) Soil, Water and Plant Testing Laboratory (http://www.soiltestinglab.colostate.edu/). Lignin and cellulose contents followed the gravimetric determination of acid detergent fiber (ADF) and acid detergent lignin (ADL) methodology (Möller *et al.* 2009). Ash content (a measure of leaf toughness, see below) corresponded to the leaf mass remaining after combustion of ADL samples at 550°C for two hours (Möller *et al.* 2009). Condensed tannins were measured using the Butanol-HCl method and expressed as leucocyanidin equivalent (% DM) following Porter, Hrstich & Chan (1986).

Statistical analyses

Plant trait relationships

We first explored the relationships among all plant traits simultaneously using a categorical principal component analysis (CATPCA; Gifi 1991) in SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA). CATPCA allows the analysis of categorical and numerical variables, and the existence of a nonlinear relationship between traits (Ellis *et al.* 2006; Costantini, Linting & Porzio 2009). Binary traits were treated as ordinal, while continuous traits were treated as numerical.

We then tested for potential trade-offs between chemical and physical defence trait groups by plotting the first axes eigenvalues of the CATPCA (considered as indexes of overall physical and chemical defences, see Moles *et al.* 2013). Extra-floral nectaries/glands and hairs/scales traits were included in the model as physical factors (Moles *et al.* 2013), while latex was included as a chemical factor (Gershenzon *et al.* 1999; Agrawal & Konno 2009). A simple linear fit was used to characterize the general form of the relationship between both defences groups.

Herbivory damage predictors

A Generalized Linear Model (GLM) analysis was used to test the effects of plant traits on herbivory damage. As the inclusion of the 16 measured plant traits (and their interactions) would have resulted in an over-parameterization on the model we decided to perform the analyses using only traits that were the best linear predictors of herbivory damages. We selected six plant traits (thickness, leaf size, shearing, C:N, cellulose and ash contents) which showed the best linear regression with leaf damage ($P \leq 0.14$ while $P > 0.32$ for all other plant traits). A parallel stepwise multiple regression analyses including all plant traits confirmed that these six traits were the best predictors of herbivory damage. In order to test for potential multicollinearity among selected plant traits, we built a pairwise regression matrix, which showed that none of the predictors included in the GLM were auto correlated ($r < 0.60$). Plant trait significance was identified using the corrected Akaike's Information Criterion (AICc) which is recommended for small sample sizes (Hurvich & Tsai 1989). Following Vuong (1989), the statistical significance of each factor was assessed using likelihood ratio test (LRT) based on comparison of deviances under full and reduced models. Contributions of more than two predictor interactions were not taken into account for interpretations. All analyses were performed using R software (R Development core team 2013).

Herbivory damage vs. tree growth rates, leaf production and spatial distribution

We considered three factors related to the concentration of leaf resources for herbivores. First, we evaluated the relationship between species inherent growth rates averages with leaf physico-chemical eigenvalues obtained from PCA analysis (axes 1 and 2) and herbivory damage data. Growth data rates were obtained measuring the diameter of the trunk of every tree ≥1 cm d.b.h. during a period of seven years (1995 and 2002; see Valencia *et al.* 2004a). Second, we confronted herbivory damage to the leaf production (measured as the number of leaves counted in the litter fall traps divided by tree abundance) of each of the 28 selected tree species. Third, we explored the relationship between leaf damage and tree clustering in the forest plot. We analysed the degree of tree clustering using the Ripley's K statistics (Ripley 1981) and the associated $L(r)$ function (normalized with the complete spatial randomness function). For each tree species, a tree clustering index (TCI) was defined as the area under the curve of the $L(r)$ function. Aggregation is indicated when $TCI > 0$, whereas $TCI < 1$ indicates spacing at some scale, and $TCI = 0$ a random distribution. Fourth, we assessed the potential local effect of tree clustering on herbivory damage by testing for a correlation between herbivory damage of a focal tree species near a focal litter fall trap and the density nearby conspecifics. This analysis could only be performed for tree species *Eschweilera coriacea* (DC.) S.A. Mori, for which we collected a total of 1503 leaves in 53 litter fall traps (traps with < 3 leaves were excluded from the analysis). Using *E. coriacea* tree locations acquired during the 2009 census, we were able to count the number of *E. coriacea* trees with in a 20 m radius around each of 100 traps. We then compared herbivory damage (averaged using all leaves in each trap) with tree densities around the trap. All spatial distribution analyses were performed using the SpatStat package in R (R Development Core Team 2013).

Results

Patterns of leaf litter fall and herbivory damage

Over 11 months, a total of 6802 leaves from 53 of the 75 most common tree species in the plot were collected from the 100 litter fall traps (Fig. 1). This means that leaves of some 22 common species in the plot were never collected in our leaf-litter survey. We found no significant relationship between tree abundance in the plot and leaf-litter abundance in the traps (Spearman correlation, R = 0.01; d.f. = 51; P = 0.488). For example, the second most abundant tree species in the plot, *Brownea grandiceps* Jacq. (n = 2573), contributed with only 1.06 % of the total number of fallen leaves. Contrastingly, *E. coriacea* (n = 1530), ranked in the 10th position of tree abundance in the plot represented 24.08 % of the total fallen leaves, the highest leaf-litter contribution in the plot. Temporal trends over one year of litter fall for the 28 species are presented in Appendix S2.

For the 28 most common tree species, mean herbivory damage was of 13.4% ± 5.9 (min: 2.49 %; max: 29.46 %).There was a high herbivory damage variability among and between tree species (Fig. 2). Average standard deviation of the full dataset was 15.64 % (min: 10.72; max: 22.69). *Otoba glycycarpa* (Ducke) W. Rodrigues & T.S. Jaramillo, *Matisia malacocalyx* (A. Robyns & S. Nilsson) W.S. Alverson, *Matisia bracteolosa* Ducke, and *Rinorea apiculata* Hekking showed the highest proportions of herbivory damage, > 20 % on average. On the opposite end, *Inga 6cuadra* (*nomen nudum*), *E. minicomun* and *Sorocea steinbachii* C.C. Berg presented the lowest proportions of herbivory damage, < 5 % on average. For most species (82.1 %), we found that herbivory damage occurred mostly (> 50 %) on the leaf edge rather than on the lamina (Fig. 2). *Brownea lore* (*nomen nudum*) was the species that presented the highest proportion of herbivory damage on the edges (85.1 %), while *S. steinbachii* presented the highest herbivory damage on the lamina (71.6 %).

Relationships among plant traits

The categorical PCA used to investigate multivariate relationships among all plant traits explained 19.59 % of the total variance on the first axis, which separated species with high values of latex, from those with high values of Carbon and lignin (Fig. 3). The second axis explained 16.46 % of the variation and separated species with high values of N from those with high values of C:N, tearing, and cellulose. Hairs/scales, thickness and condensed tannins were best explained by the third axis (11.50 %), punching, ash and shearing by the fourth axis (9.41 %), nectaries/glands by the fifth axis (8.13 %), leaf size by the sixth axis (7.32 %) and finally, SLA was best explained by the seventh axis (6.45 %).

We found no significant relationship between indexes of overall physical and chemical traits ($R^2 = 0.066$, $P = 0.188$), despite a general tendency of negative linear regression (Fig. 4; slope = -0.306). This means that species with high physical defences may also present high chemical defences (e.g. *B. lore* and *E. minicomun*), and vice-versa (e.g. *Guarea sylvatica* C. DC. and *Neea comun* [*nomen nudum*]).

Plant traits affecting herbivory damage

GLM analyses showed significant relationships between herbivory damage and several physico-chemical traits and their interactions (Table 1). When considering factors without interactions, shearing, ash, and to a lesser extent leaf size, best explained differences in herbivory damage (Table 1, Fig. 5). Contrastingly, thickness, C:N and cellulose were not significant predictors of herbivory damage ($P = 0.33$; $P = 0.19$; $P = 0.21$ respectively) but showed a general positive relationship (Fig. 5B,E,F). The same GLM model including interaction showed that the terms shearing × ash, leaf size × ash, and shearing × C:N were significant predictors of herbivory damage (Table 1).

Herbivory damage vs. tree growth rates, leaf production and spatial distribution

Species growth rates showed no significant relationships neither with physico-chemical defences (eigenvalues from axes 1 and 2) nor with herbivory damage (Fig. 6 A-B, linear regressions $P > 0.05$ in all cases). For example, higher physico-chemical defended species (*G. sylvatica*, *N. comun* and *I. 6cuadra*) presented the lowest growth rates but not necessarily the lowest herbivory damage proportions. On the opposite side, lower physico-chemical defended species *I. auristellae* coincided with higher growth rates only, while *B. lore* and *Eschweilera giga* (R. Knuth) J.F. Macbr. presented higher herbivory damage proportions only.

The relationship between herbivory damage and leaf production resulted in a marginally not significant fit to a logarithmic regression (Fig. 7A; $P = 0.073$). However, the relationship became highly significant when the fit excluded one outlier species *O. glycycarpa* ($P = 0.001$; $y = 7.32 - 2.96\ln x$), suggesting that species with low leaf production may be more susceptible to herbivory than species with mid/high production. This was confirmed when comparing the five more productive species in terms of the number of leaves *per* tree (*E. coriacea*, *E. minicomun*, *I. 6cuadra*, *Protium sagotianum* Marchand, *Pseudolmedia laevis* [Ruiz & Pav.] J.F. Macbr.) vs. the less productive species (*B. grandiceps*, *E. giga*, *Perebea xanthochyma* H. Karst., *R. apiculata*, *R. viridifolia*), against herbivory damage. We found the first group in average was 15 times less damaged comparing to the second group (Table S1).

Herbivory damage was not significantly correlated with the spatial distribution of tree species ($P = 0.816$, Fig. 7B). Here again, the relationship was strongly affected by one species, *R. apiculata* as, when it was removed from the fit, a significant logarithmic regression could be adjusted to the data ($P = 0.031$; $y = 12.37 – 0.92\ln x$). This suggests that species with very low clustering indexes tend to be more susceptible to herbivory damage than clustered species. Finally, we found that tree density of *E. coriacea* at a given location in the plot did not affect local herbivory damage ($P = 0.191$, Fig. 7C).

Discussion

Leaf herbivory patterns in a species-rich tropical forest

Data on leaf damage levels in rain forests are not common, and very few of them have involved the Amazonian region. The overall range of herbivory damage for the 28 common species in our study was of 13.4 % (2.49–29.46 %), within the range of those observed in previous studies. Landsberg & Ohmart (1989)'s review suggests that defoliation levels of tropical rain forests are between 5 and 15 %. Lowman (1984) found an average of 14.6 % of damage level in Australian subtropical rainforest canopies while Sterck, van der Meer & Bongers (1992) found that overall damage was slightly > 5 % (0.8–12.8 % in range) in French Guiana. More recently, Brenes-Arguedas, Coley & Kursar (2008) found ranges between 25 % and 40 % (32 % in average) for *Inga* species in Panamá (BCI) and Ecuador (Yasuní). Note however that our survey methodology did not take into account the leaves that were completely eaten (nor galls or mining damaging), underestimating the real herbivory damage occurring in Yasuní (Lowman 1984). Factors such as local environment, microhabitat (i.e. canopy, understorey, and gaps), temporality (i.e. dry vs. wet season) and the species of plants involved, may be explaining result variability worldwide (Landsberg & Ohmart 1989; Coley & Barone 1996).

Overall, we found a high variance in leaf herbivory damages at both inter– and intraspecific levels, which coincides with other studies in both tropical and subtropical ecosystems (Kurokawa & Nakashizuka 2008; Schuldt *et al.* 2012). Coley (1983) found that intra-specific variance in leaf damage was rather similar among tree species showing different life histories, defensive characteristics, and levels of leaf damage. Our results fully support these findings, and further revealed that herbivory damage was also highly variable in space and time (results not shown), without any obvious patterns.

Physico-chemical plant traits influencing leaf herbivory

Of the 14 plant traits we analysed, three physical and three chemical traits (but no secondary metabolites) were strongly correlated to herbivory damage. GLM analysis showed that shearing resistance, ash content and leaf size were the best factors explaining herbivory damage. Positive correlation with leaf size supports the idea that larger leaves may attract more herbivores (Garibaldi, Kitzberger, Ruggiero 2011). Our results reveal that very small leaves present very low herbivory proportions as well and this could be explained, at least in part, through biomechanical constraints as very small or highly divided and dissected leaves may reduce feeding efficiency (Brown, Lawton & Grubb 1991). Interestingly, *E. minicomun* and *I. auristellae,* both possessing the smallest leaves of the whole data set, also present nectaries/glands as indirect defences while *Pourouma bicolor* Mart., *Protium nodulosum* Swart and *M. malacocalyx* possessing the largest leaves lack this kind of indirect defences. The presence of extra-floral nectaries has proven to be an effective defence that are used by hymenopterans (ants particularly) that might be contributing to protect the plants against herbivory damage (Rosumek *et al.* 2009; Kessler & Heil 2011).

The force to shear (a measure of toughness) showed a significant (negative) relationship with herbivory damage, confirming that toughness is an efficient anti-herbivore defence (Lowell *et* *al.* 1991; Choong *et al.* 1992; Coley & Barone 1996). This result makes sense biologically if we consider that most herbivory damages in the tropics are caused by invertebrates (~75 %, Coley & Barone 1996) comparing to mammals (< 20 %, Coley & Barone 1996) and that invertebrate radulas and mandibles act mainly as shears. Shearing × C:N interaction was significantly associated to herbivory damage, although C:N did not explain herbivory damage variation by itself (see Schuldt *et al.* 2012 for similar results) and simple linear regression between C:N and herbivory damage showed a c.a. neutral correlation. We found shearing and C:N were positively correlated (linear regression equation: $y = 659.71x + 14.71$; $R = 0.48$; F = 7.94; $P < 0.01$; results not shown). This result agrees with other studies that have found different measures of leaf toughness were positively correlated with C:N (e.g. Agrawal & Fishbein 2006; Paul *et al.* 2011) and confirms that the multiple carbon-based structural compounds are contributing factors improving leaf resistance to mechanical damage (Coley & Barone 1996; Schuldt *et al.* 2012).

Ash content is a measure of defences such as Calcium oxalates and silica-based phytoliths (the latter helps increasing toughness of plant tissues, Massey, Ennos & Hartley 2007; Moles *et al.* 2013), two components that strongly reduce herbivory (Korth *et al.* 2006; Massey, Ennos & Hartley 2006). Our results corroborate these findings showing a negative relationship between herbivory damage and ash content. Furthermore, GLM analysis showed a significant interaction between ash and leaf size and between ash and shearing suggesting a generalized physico-chemical defence strategy in our studied plant community respecting these functional traits. This suggests that from the physiological perspective, these strategies (syndromes) are compatible, not mutually exclusive, and are consequences of particular habitat selection pressures (Kursar & Coley 2003; Agrawal 2007).

Surprisingly, we found no support that secondary metabolites may represent anti-herbivores chemical defences. Considering condensed tannins our results coincide with other large-scale analyses that found no- or weak relationships between these phenolic compounds and herbivory damage (Coley 1983; Kurokawa & Nakashizuka, 2008; Carmona, Lajeunesse & Johnson 2011). On the other hand, in spite that the frequent occurrence of laticiferous plants in tropical America (Amazon: 20–35 % *sensu* Lewinsohn 1991) compared to the rest of the world (8.9 % *sensu* Farrell, Dussourd, Mitter 1991) shall be consistent with the defensive roles of latex against herbivorous insects (Konno 2011), our results did not support latex as a plant defence compound. Jones, Firn & Malcolm (1991) explicitly argue that plants actually contain –and retain– a very high diversity of mostly inactive secondary compounds because they increase the probability of producing new active compounds. Secondary metabolites might then have secondary role in defending plants from herbivores, second to morphology and physical resistance traits (Carmona *et al.* 2011; Schuldt *et al.* 2012).

About 30 % of the studied species showed either high physical and high chemical defences (*G. sylvatica*, *N. comun*, *I. 6cuadra* and *Inga capitata* Desv.) and low physical and low chemical defences (*B. lore*, *E. minicomun*, *E. giga* and *I. auristellae*). When these species were excluded, a simple negative linear regression fitted significantly (P = 0.02, results not shown) suggesting that in the rainforest, most species may invest in a balanced set of physical and chemical defences. Our results, presenting single and interacting non-traditional physical and chemical traits as deterrents of herbivores, agree with a pluralistic approach in the study of plant defences that considers a wider spectrum of traits and their correlations (Agrawal 2007). Further studies following this approach would be necessary for unravelling the holistic strategies of plant defences against herbivores in highly diverse neotropical rainforests.

Effect of tree growth and spatial distribution on herbivory damages

In a recent revision, Endara & Coley (2011) evaluated the RAH (Coley, Bryant & Chapin 1985) that in part expounds that, "for fast-growing species the optimal defence level is low, even though this leads to high rates of herbivory, and for slow-growing species, the optimal defence level is high, even though this cost further reduces the realized growth rate" (Endara & Coley 2011). Our results however did not support these predictions as species with higher growth rates did not present necessarily lower levels of defences (with few particular exceptions, see p. 111) or higher levels of herbivory damage. More detailed analyses where we distinguished physical from chemical defences, as well as some of its interactions (e.g. leaf size:ash and shearing:CN ratio, see Table 1), did not support the predictions neither (results not shown). Other factors not measured in our study, such as root-feeding herbivory, may be contributing on the control of plants defence/growth strategies, as different kinds of herbivory (e.g. piercing) have different effects on the response of the plant and its consequent fitness (Strauss & Agrawal 1999).

A novel finding of our analysis was that less leaf productive trees were much more susceptible to suffer biomass loss by the action of herbivores comparing to more productive ones. Our results strongly suggest that high values of leaf replacement may help plant species to deter herbivores more efficiently. This might not agree with Lamarre *et al.* (2012) who found that herbivory rates did not correlate with leaf production rate. Aide (1993) meanwhile, showed that species producing leaves more or less continuously suffered lower rates of herbivore damage, presumably by using chemical defences. Unfortunately, we are not currently able to identify which of the species present continuous or synchronous leafing, at what season were produced and what is its life-span (see Coley & Barone 1996; Kurokawa & Nakashisuka 2008 for these traits implications on herbivory rates).

Tree clustering (when excluding *R. apiculata*), but not tree density showed significant correlation with herbivory damage. The less the species were clustered, the more they were damaged by herbivores. In two experimental studies Fine, Mesones & Coley (2004), and Fine *et al.* (2006) showed that species grew best on their home soil type (habitat specialization) in the presence of herbivores. Soil properties and microtopography have been shown to shape species distributions in Yasuní (Valencia *et al.* 2004a; Endara & Jaramillo 2010). Clustering may thus be a measure of habitat specialization (Svenning 1999) and implies specialized species may inhabit only very specific microenvironments, which are closer to its optimal. Following Janzen (1974), clustered species adapted to resource-rich soils may tolerate better herbivory and invest less in defence, and vice-versa, species adapted to resource-poor soils may not tolerate herbivory very well and may invest more in defence. According to this, our five more clustered species (*R. apiculata*, *B. lore*, *Acidoton nicaraguensis* [Hemsl.] G.L. Webster, *Rinorea viridifolia* Rusby and *E. minicomun*) showed medium-to-high physico-chemical defence investment and a medium effectiveness to deter herbivores.

The defensive strategy chosen by any species may thus depend on the particular herbivory pressure each species is submitted to and the microenvironment where it grows (Janzen 1974; Fine *et al.* 2006). This confirms that the underlying factors and mechanisms behind defence syndromes and its continuums is a complex mosaic of action/reaction between plants and herbivores, especially in extreme biodiverse ecosystems such as tropical rain forests where there is an enormous variation of herbivory kinds and consequently plant responses.

Acknowledgements

We thank the staff of the Yasuní Research Station, in particular the administrative personnel, David Lasso, Pablo Jarrín, Carlos Padilla and Karla Rivadeneira for constant support during the stays at the Station. Milton Zambrano and Pablo Alvia provide invaluable expertise on plant taxonomy in the field. This study would have been logistically impossible to carry out without them. We are grateful to Pablo Alvia, Anelio Loor and Gabriela Torrens for their help in plant measurements. Jim Self provided guidance and full support for leaf analyses at Soil, Water & Plant Testing Laboratory at CSU. We are grateful to the School of Biological Sciences of the *Pontificia Universidad Católica del Ecuador* (PUCE), in particular to its dean Hugo Navarrete for constant support. The PUCE (project codes H13164 and J13041) and the French Institute for Research and Development (IRD) supported this study through research grants. This study was endorsed by the *Ministerio de Ambiente del Ecuador* permits MAE: 09-FLO-MA-DPO-PNY and 06-2011-FAU-DPAP-MA.

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Tables

Table 1. Results of the generalized linear model (GLM) analysis of six leaf plant traits (thickness, leaf size, shearing, C:N, cellulose and ash contents, see Material and Methods) on herbivory damage. AICc is the corrected Akaike's Information Criterion for the initial model after removal of the "effect" term. ΔAICc corresponds to the difference between the AICc of the initial model and that of the reduced model. Likelihood-ratio test (LRT) and associated *P*-values test the hypothesis that the suppression of the 'effect' term provides no better fit than the initial model. Only significant results of the GLM analysis are shown.

Effect	Terms included in the initial model	AIC_c	Δ AIC_c	LRT	*P*-value
Leaf size	Thickness, Leaf Size, Shearing, C:N, Cellulose, Ash	160.22	3.12	4.092	0.04
Shearing	"	102.78	60.57	8.957	<0.001
Ash	"	130.28	33.06	7.964	0.005
Shearing × ash	Thickness, Leaf Size, Shearing, C:N, Cellulose, Ash + interactions	169.42	6.12	5.197	0.023
Leaf size × ash	"	172.42	3.12	4.095	0.043
Shearing × C:N	"	168.26	7.28	5.482	0.019

Figure legends

Fig. 1. Spatial distribution of trees and leaf litter input in soil. Y_1: Leaf litter abundance in traps; Y_2: Tree abundance in in study area; Shapiro-Wilk normality test showed no-normality distribution for both data set ($W_{trees} = 0.804$ and $P < 0.0001$; $W_{leaves} = 0.514$ and $P < 0.0001$). Tree abundance and leaf-litter abundance in the traps were not correlated (Spearman correlation coefficient $R = 0.01$; d.f. = 51; $P = 0.933$). Dashed-grey line corresponds to 30 leaves criterion used to discriminate species for analyses.

The following correspond to morphospecies names (*nomen nudum*: waiting for a formal description): *Brownea lore*, *Eugenia minicomun*, *Inga 6cuadra*, *Mabea superbrondu*, *Miconia purpono*, *Miconia tipica*, *Neea comun*, *Siparuna bigll3i* and *Piper obchic*.

Fig. 2. On the left, herbivory damage (%) on fully expanded green-leaves from adult trees of 28 common species in Yasuní National Park. On the right, herbivory damage (%) differentiated by margin (grey) vs. lamina (black). Error bars and empty circles correspond to standard deviations and outliers respectively.

Fig. 3. Herbivory *vs*. indirect, mechanical and resin-type defences traits of the 28 more common spp in Yasuní National Park (fully expanded green-leaves). First and second axes explained by 19.59 % and 16.46 % respectively.

Fig. 4. Relationship of physical (X) and chemical defences (Y) from CATPCA eigenvalues representing the factor loadings for traits on the first two axes. The solid line represents the linear tendency of the relationship (linear regression: $R^2 = 0.257$, F = 1.825, $P = 0.188$). Upper and lower dash-lines represent 5 % and 95 % confidence intervals.

Fig. 5. Herbivory *vs*. physical and chemical traits of 28 common species in Yasuní National Park (fully expanded green-leaves). The solid lines represent the tendency of the relationship. Upper and lower dash-lines represent 5% and 95% confidence intervals. R^2 and P values correspond to regression fit and GLM results respectively.

Fig. 6. Relationship of species growth rates with physico-chemical eigenvalues (A) and herbivory damage proportions (B). In (A), black dots represent axis 1 eigenvalues, and grey dots represent axis 2 eigenvalues. Simple linear regressions are denoted ($P > 0.05$ in all cases).

Fig. 7. Relationship of herbivory damage (%) with (A) leaf production/tree and (B) tree clustering (from 28 common species in the study area. (C) Herbivory damage (%) and *Eschweilera coriacea* tree density (number of trees in a 20 m circle radius around the litter fall traps) relationship. Empty circles in grey correspond to excluded data from the regressions that led to logarithmic adjustments with P values < 0.05 in both (A) and (B).

Figures

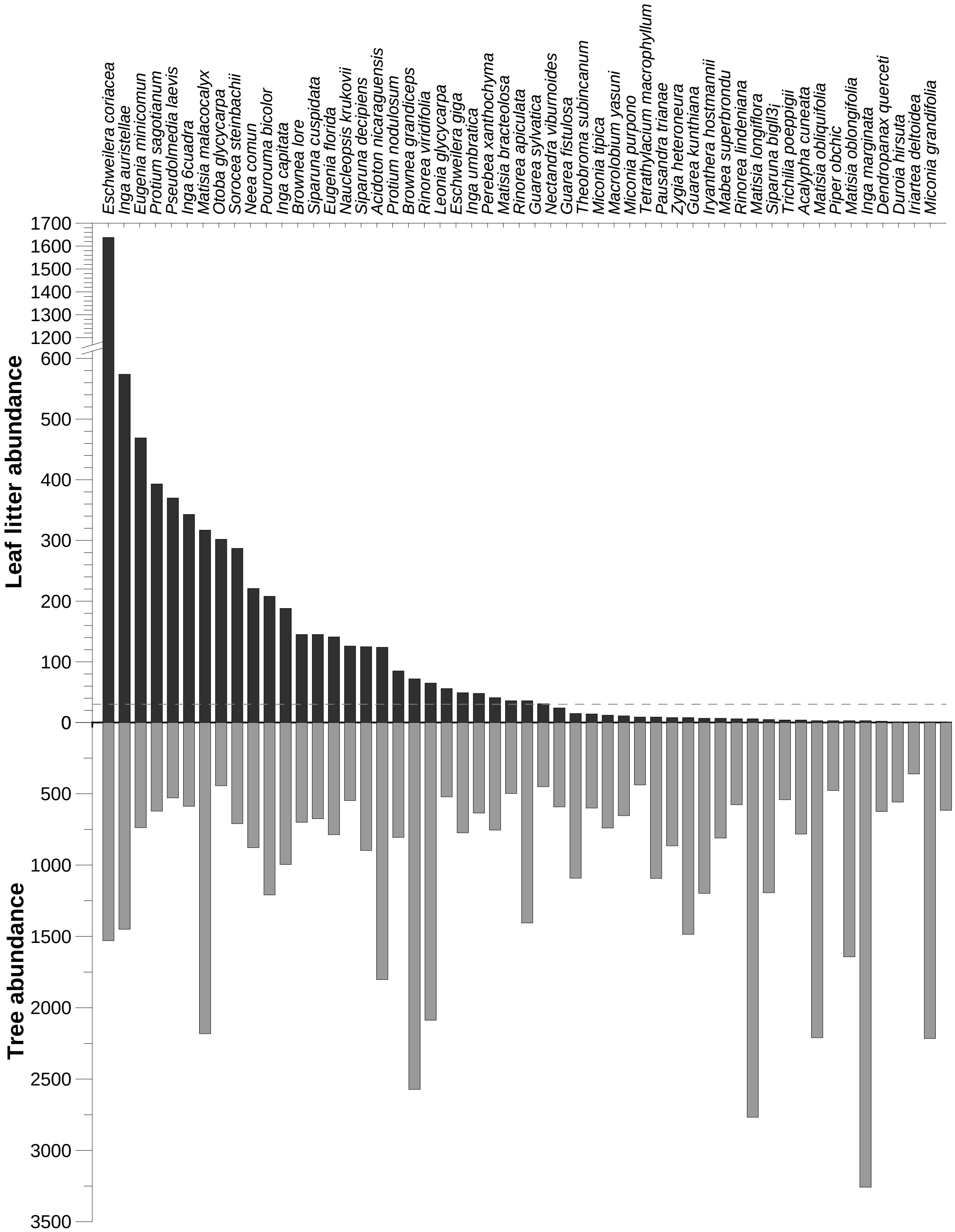
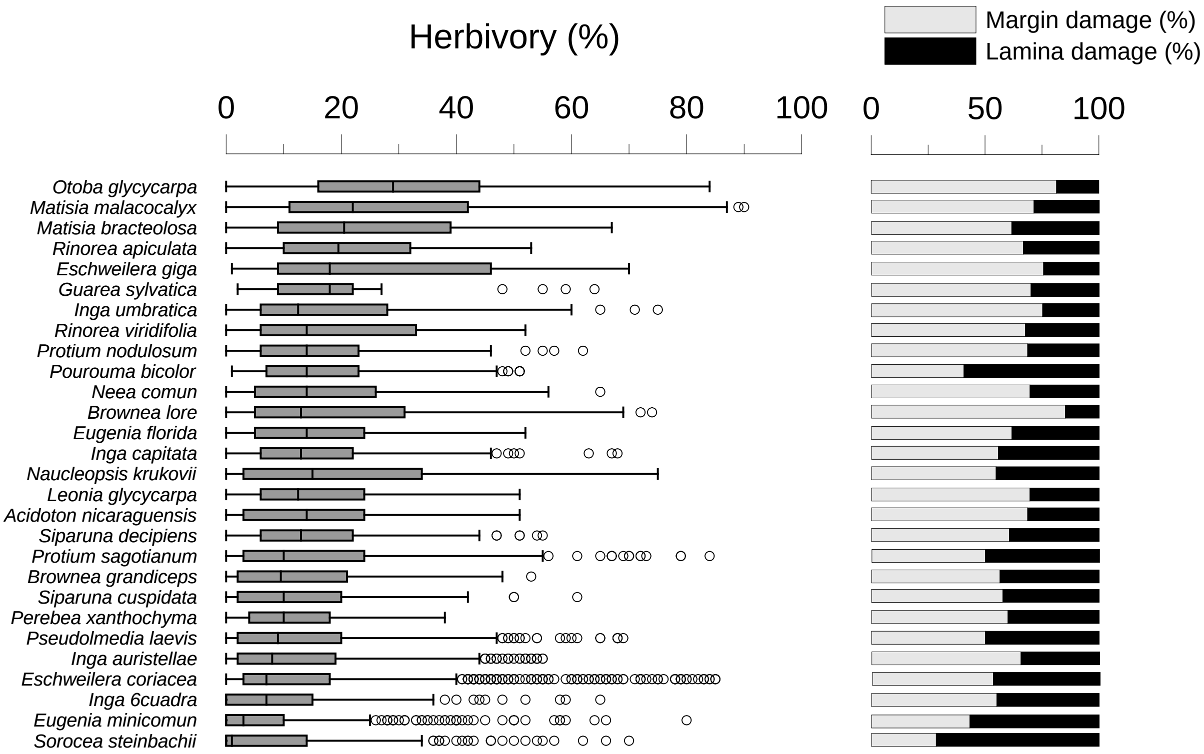


Fig. 1. Cárdenas *et al.*



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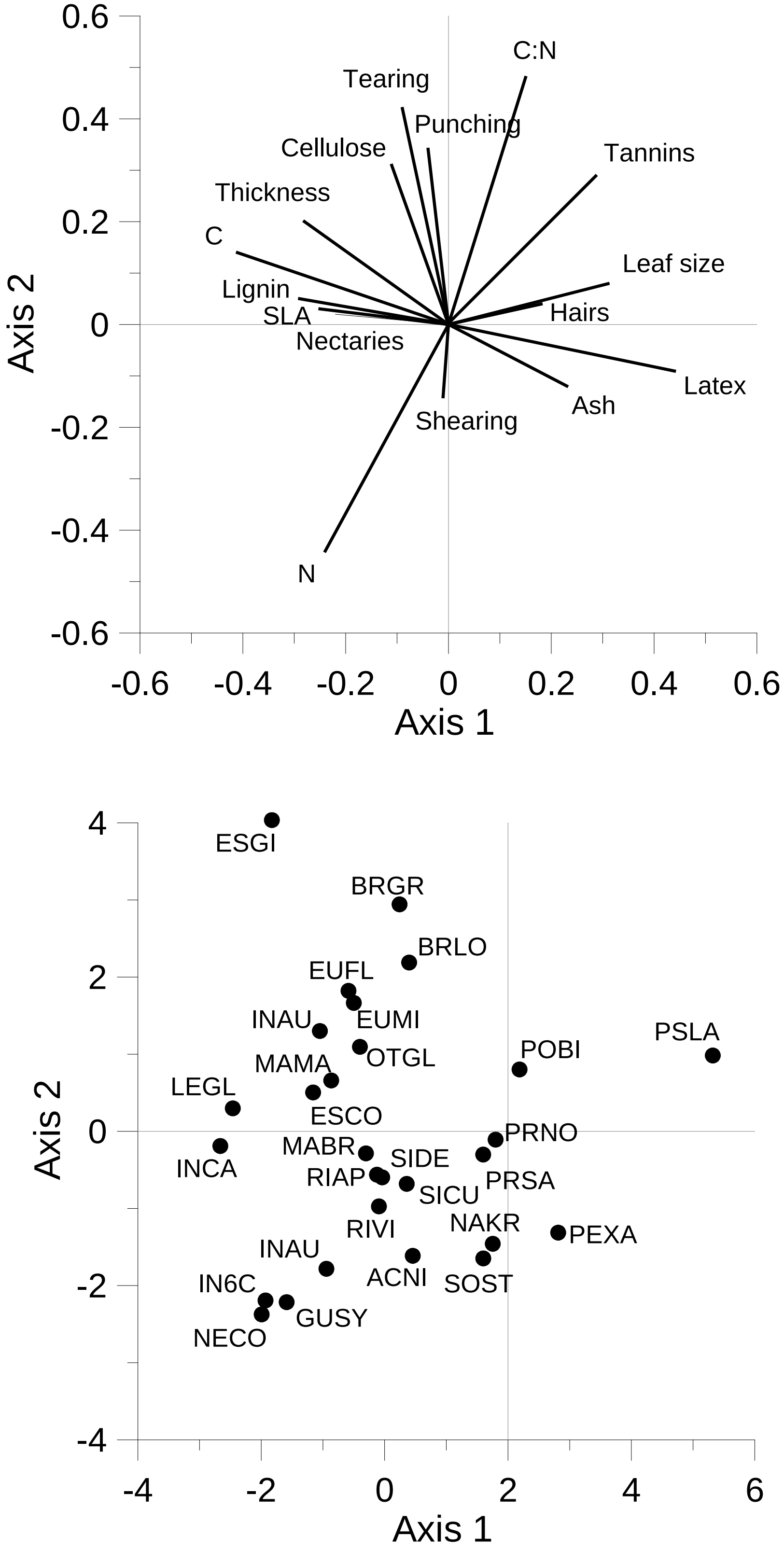

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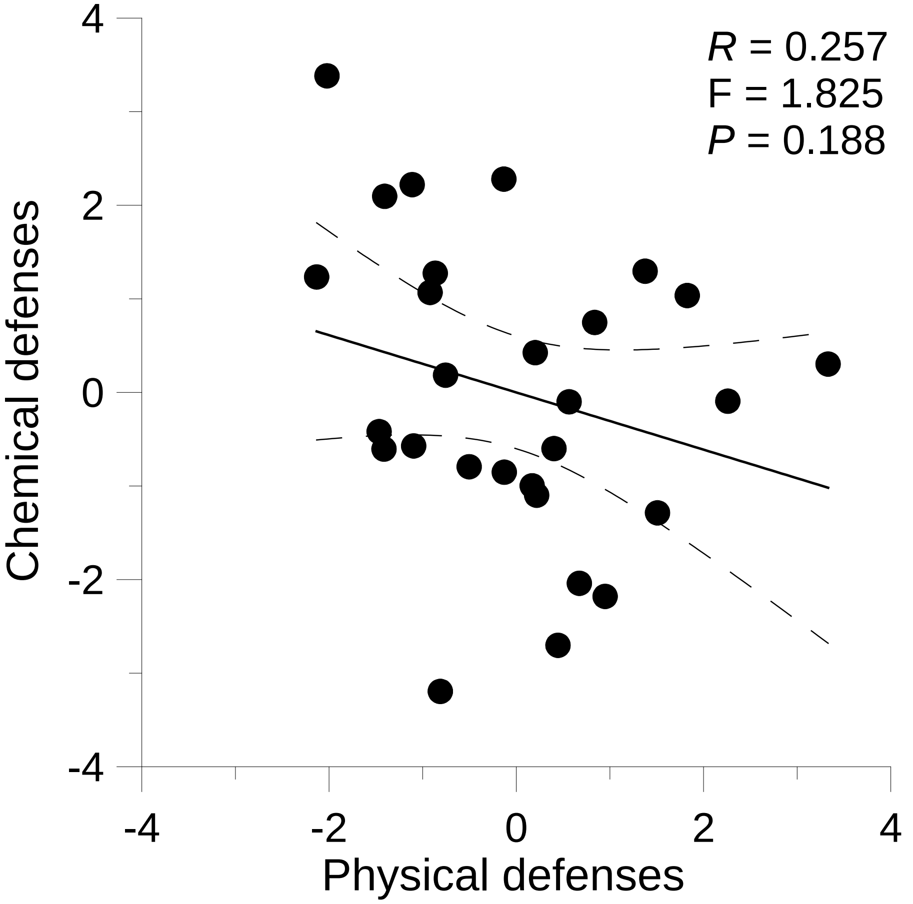

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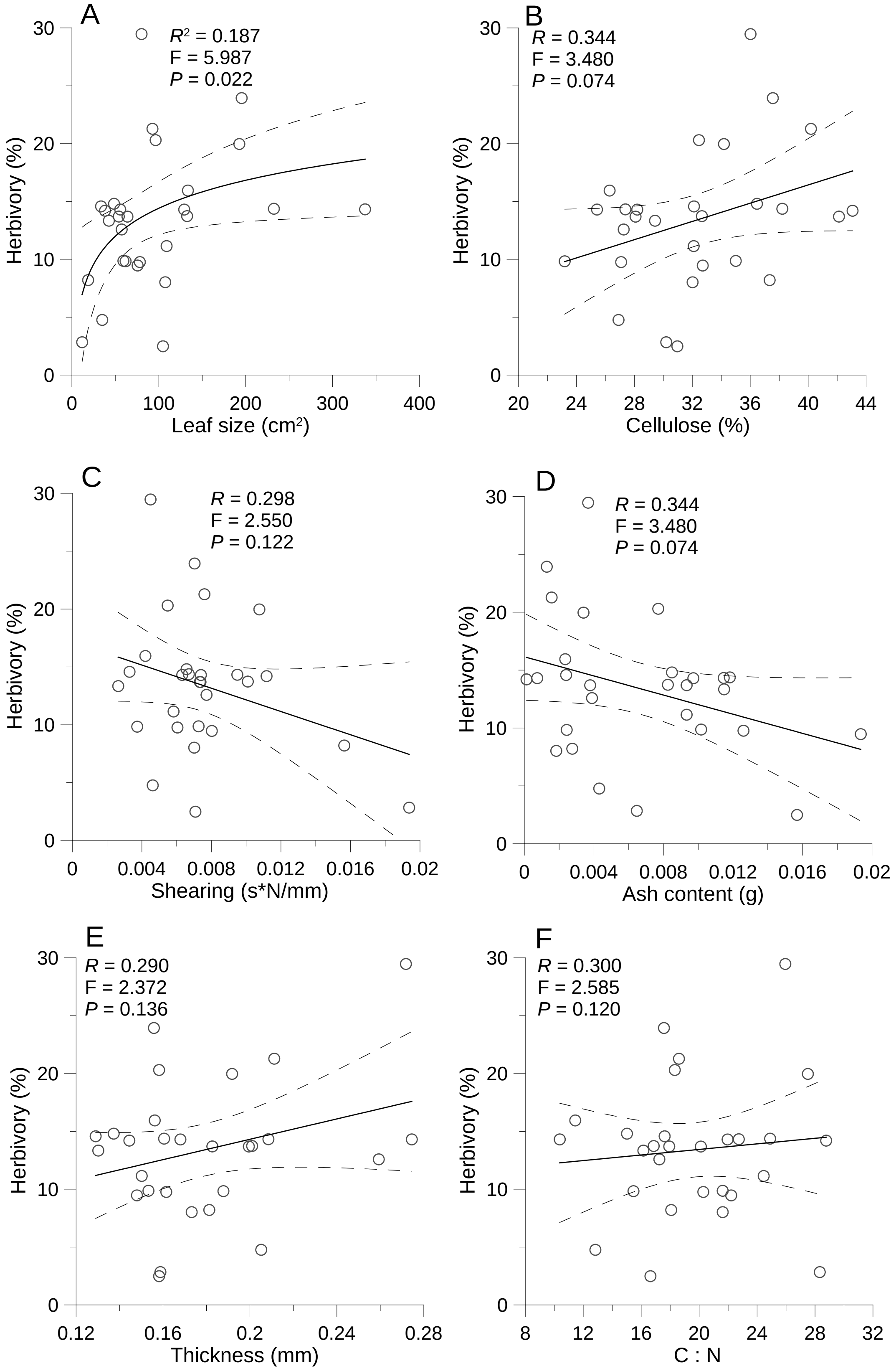

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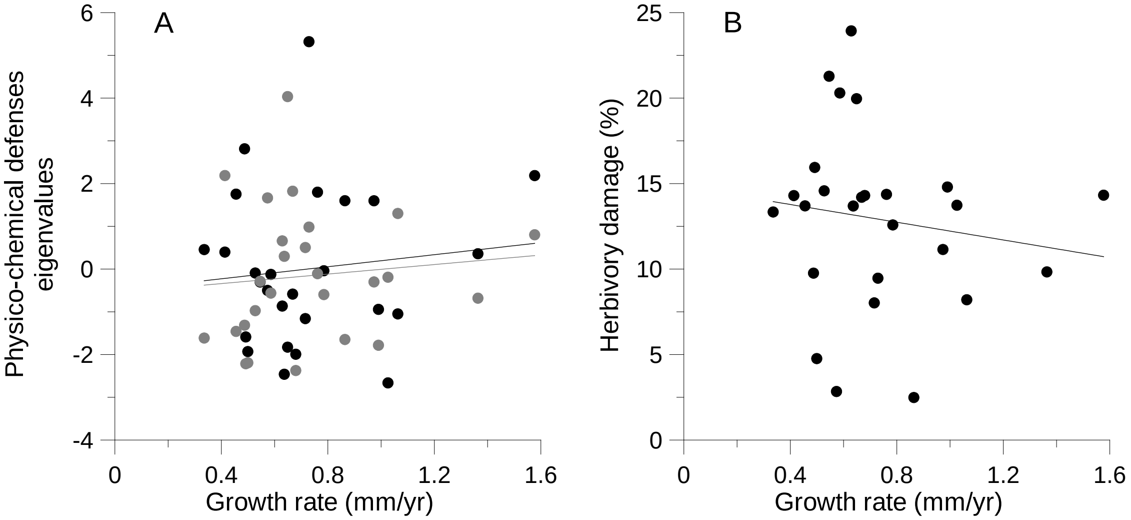


Fig. 6. Cárdenas *et al.*

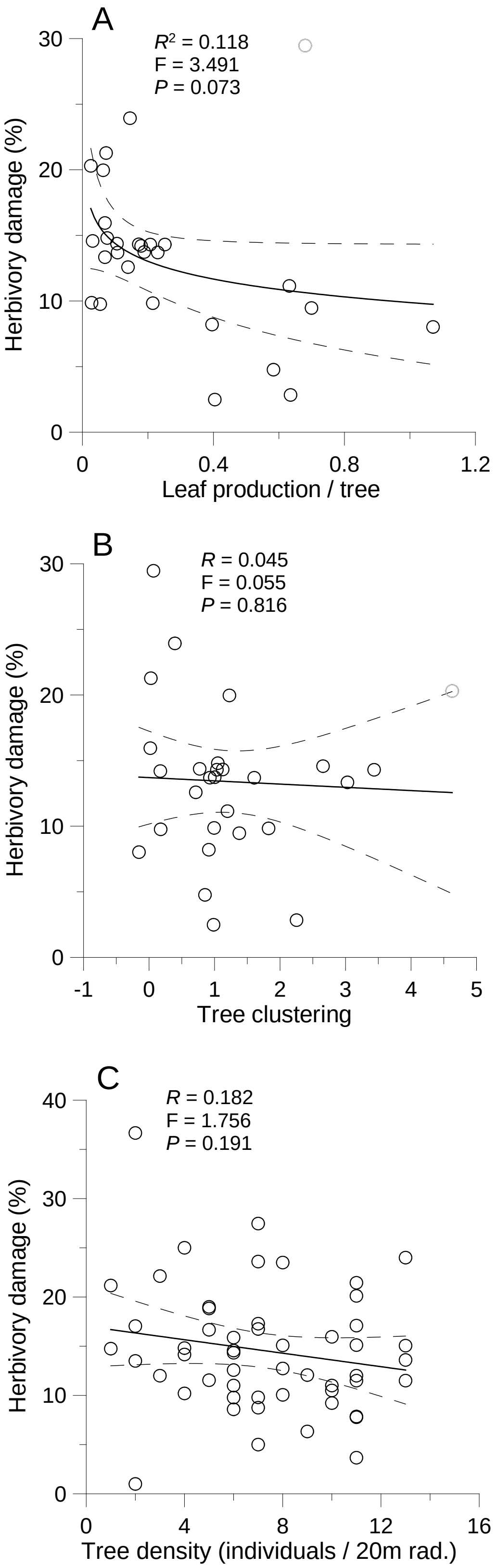
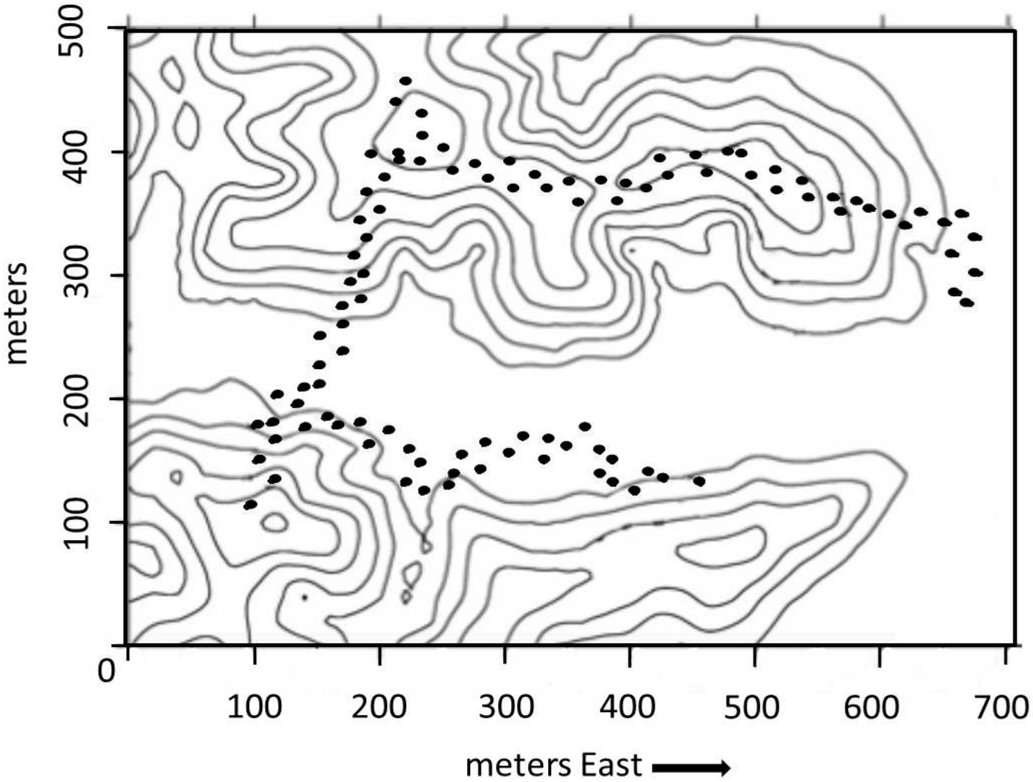


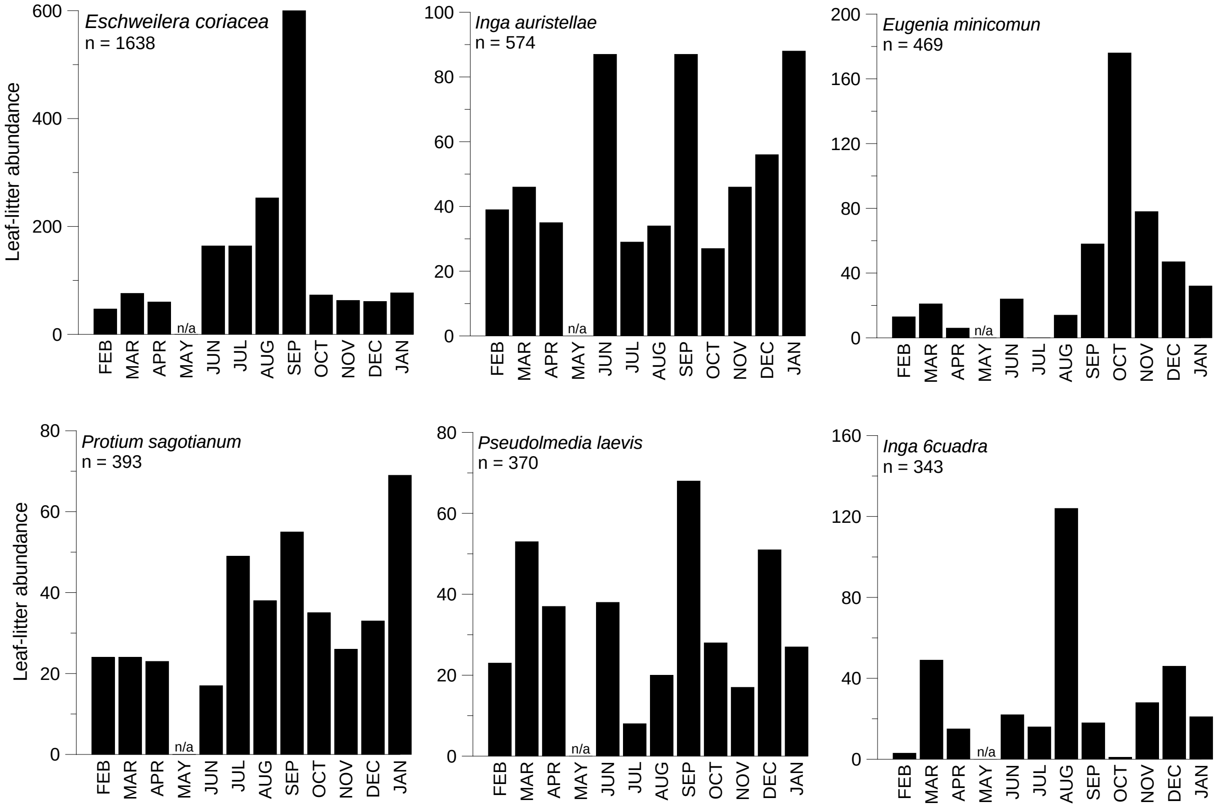
Fig. 7. Cárdenas *et al.*

Supporting information

Appendix S1. Topographic map of the YRS-PUCE sub-plot and the location of the 100 litter fall traps used for this assessment (coordinates X: 50–700; Y: 100–500). Lines represent 4m contour interval. Image modified from Metz *et al.* (2010).



Appendix S2. Specific leaf-litter proportion *per* month for one-year census for those species represented by ≥ 100 leaves in litter traps. Some tree species showed a one-year seasonality of leaf-litter production. Some species like *Brownea lore*, *Eugenia florida* and *Neea comun* resulted particularly conspicuous in this aspect as 78.62 %, 75.18 % and 51.13 % of its fallen leaves were respectively recorded in one survey date. For other species like *Eugenia minicomun* and *Eschweilera coriacea*, 54.16 % and 52.08 % of its fallen leaves were respectively recorded in two consecutive survey dates. *Sorocea steinbachii* on the other hand, presented 68.64 % of its fallen leaves in six dates (two sets of three consecutive survey dates) suggesting a bimodal distribution of leaf abscission.



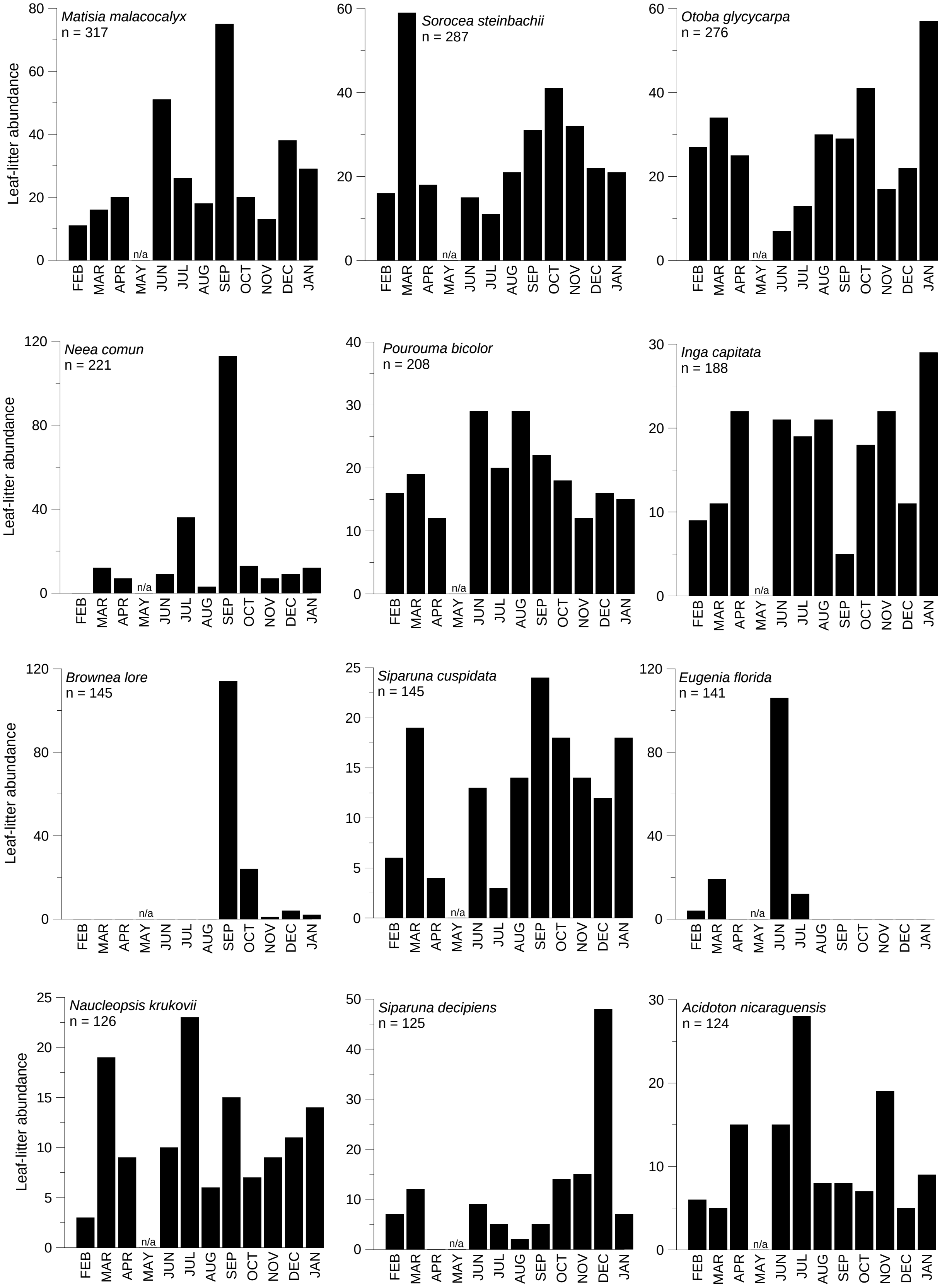

Leaf-litter abundance
Matisia malacocalyx
n = 317
0
20
40
60
80
Sorocea steinbachii
n = 287
0
20
40
60
Otoba glycycarpa
n = 276
0
20
40
60
Neea comun
n = 221
0
40
80
120
Pourouma bicolor
n = 208
0
10
20
30
40
Inga capitata
n = 188
0
10
20
30
Brownea lore
n = 145
0
40
80
120
Siparuna cuspidata
n = 145
0
5
10
15
20
25
Eugenia florida
n = 141
0
40
80
120
Naucleopsis krukovii
n = 126
0
5
10
15
20
25
Siparuna decipiens
n = 125
0
10
20
30
40
50
Acidoton nicaraguensis
n = 124
0
10
20
30
n/a
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN

Table S1. Table showing the leaf abundance in litter fall traps (LA), the number of trees (N, in the 25+ ha study plot = tree abundance), the herbivory average per species (H), the leaf production of the species (LP = LA:N), the relationship between leaf abundance and herbivory (LA:H), and the relationship between leaf production and herbivory (LP:H) of the 28 most leaf productive tree species in the Yasuní National Park (Amazonia, Ecuador) study plot.

	LA	**N**	**H (%)**	**LP**	**LA:H**	**LP:H**
Acidoton nicaraguensis	124	1803	13.339	0.069	9.296	0.005
Brownea grandiceps	72	2573	9.865	0.028	7.299	0.003
Brownea lore	145	700	14.299	0.207	10.141	0.014
Eschweilera coriacea	1638	1530	8.021	1.071	204.206	0.133
Eschweilera giga	49	774	19.966	0.063	2.454	0.003
Eugenia florida	141	787	14.200	0.179	9.930	0.013
Eugenia minicomun	469	738	2.838	0.636	165.267	0.224
Guarea sylvatica	31	451	15.944	0.069	1.944	0.004
Inga 6cuadra	343	588	4.764	0.583	72.005	0.122
Inga auristellae	574	1450	8.205	0.396	69.961	0.048
Inga capitata	188	996	13.733	0.189	13.689	0.014
Inga umbratica	48	635	14.800	0.076	3.243	0.005
Leonia glycycarpa	56	522	13.690	0.107	4.091	0.008
Matisia bracteolosa	36	498	21.278	0.072	1.692	0.003
Matisia malacocalyx	317	2182	23.933	0.145	13.245	0.006
Naucleopsis krukovii	126	548	13.697	0.230	9.199	0.017
Neea comun	221	878	14.304	0.252	15.451	0.018
Otoba glycycarpa	302	444	29.464	0.680	10.250	0.023
Perebea xanthochyma	41	755	9.762	0.054	4.200	0.006
Pourouma bicolor	208	1209	14.321	0.172	14.524	0.012
Protium nodulosum	85	806	14.370	0.105	5.915	0.007
Protium sagotianum	393	622	11.145	0.632	35.262	0.057
Pseudolmedia laevis	370	529	9.466	0.699	39.086	0.074
Rinorea apiculata	36	1406	20.300	0.026	1.773	0.001
Rinorea viridifolia	65	2088	14.576	0.031	4.459	0.002
Siparuna cuspidata	145	675	9.833	0.215	14.746	0.022
Siparuna decipiens	125	898	12.582	0.139	9.935	0.011
Sorocea steinbachii	287	710	2.486	0.404	115.435	0.163

—CHAPTER 2—

Ecol Res (2012) 27: 975–981
DOI 10.1007/s11284-012-0979-8

ORIGINAL ARTICLE

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Do canopy herbivores mechanically facilitate subsequent litter decomposition in soil? A pilot study from a Neotropical cloud forest

Received: 11 November 2011 / Accepted: 23 July 2012 / Published online: 14 August 2012

Abstract There is increasing evidence that the above- and belowground components of ecosystems influence one another, thereby controlling key processes such as organic matter decomposition. The aim of this study was to test the hypothesis that leaf herbivory in forest canopies could facilitate subsequent leaf litter decomposition in soils, through changes in leaf quality (i.e., litter palatability) or geometric form (i.e., increased availability of leaf edges made by herbivore damages). In a 9-month field experiment in an Ecuadorian tropical cloud forest, we compared the decomposition rates of entire leaves (EL) and 15 %-damaged leaves (DL) of *Ficus cuatrecasana* showing similar initial leaf chemistry. We found that DL decomposed significantly faster than EL in early stages of decomposition (i.e., between 0 and 38 days). A parallel experiment using cellulose discs on which we simulated different degrees of damages revealed, however, that geometry per se (i.e., increased edge availability) did not influence decomposition rates. We discuss these contrasting results and propose that higher edge availability in damaged leaves may promote the access of microbes and/or macro-detritivores to leaf tissues thereby enhancing the initial stages of leaf decomposition.

Keywords Detritivores · Herbivory · Mechanical facilitation · Above belowground interaction

Introduction

It is estimated that, worldwide, 90 % of total plant biomass is not consumed by herbivores and enters the soil system directly as dead organic matter (DOM) (Gessner et al. 2010). Decomposition of DOM relies on several factors, such as climate, the physical and chemical properties of plant litter, and the sequential action of soil invertebrates, fungi and bacteria (Chapin et al. 2002). Several studies have shown that herbivores can play a significant role in litter decomposition by affecting the activity of soil decomposers and detritivores through modification of their biomass distribution (Mulder et al. 2008), and of organic matter input quality and quantity (van Dam and Heil 2011; Wardle et al. 2004), thus affecting nutrient availability and plant productivity (reviewed by Vitousek and Sanford 1986; Hunter 2001; Cebrián and Lartigue 2004). Positive effects of herbivory could be related to a high consumption of net primary production, high return of labile fecal material to soil, and an improvement of litter quality through reduced leaf content of phenolics, lignin and structural carbohydrates (Chapman et al. 2006; Wardle et al. 2004). While herbivores have been shown to increase litter decomposition rates in particular ecosystems such as grasslands, coniferous forests, and semi-arid woodlands (Chapman et al. 2006; Wardle et al. 2004) herbivory-litter decomposition relationships in the tropics remain controversial, with several studies suggesting a weak association between both processes (Didham 1998; Kurokawa and Nakashizuka 2008).

While most attention has focused on the effects of herbivores on resource quality, the importance of their physical modification (fragmentation that modifies organic matter geometric form) for subsequent processing remains poorly studied.

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Plant decomposition is generally speeded up by any activity that grinds up and fragments vegetal tissues (Chapin et al. 2002). This process could be referred as to "mechanical facilitation" and fits into the concept proposed initially by Heard (1994): the "processing chain ecology". Heard (1994) argued that resource transformation rate can be regulated by a consumption chain of species adapted to the specific conditions of that resource. For example, resource consumption by species B may depend on pre-treatment of the resource by species A. In such a system, consumers specialize on resources in each condition (e.g., herbivory), influencing the rate at which the resource is transformed between conditions (i.e., to litter). Microcosm studies have shown the synergistic action of different detritivores taxa in leaf litter decomposition (Zimmer et al. 2005; De Oliveira et al. 2010). Other studies have proposed the existence of mechanical facilitation among a variety of consumers, such as stream detritivores that fragment whole leaves and leave behind smaller particles to be exploited by others (Daugherty and Juliano 2002; Flecker 1996; Jonsson et al. 2002), or tuber moth larvae that make holes on the tuber and facilitate the entrance of other burrowers (Dangles et al. 2009). Also, Muller et al. (2002) suggested that feeding Scolytid beetles facilitates fungal colonization in decaying wood. To our knowledge, no analogous studies have been performed using canopy herbivores and soil decomposers as study models in order to test whether the areas opened as a result of the chewing action of herbivores would facilitate litter palatability, accessibility and consumption by decomposers and detritivores.

Fragmentation creates fresh surfaces that increase the proportion of vegetation mass accessible to attack by micro- and/or macro-organisms (Chapin et al. 2002). Accessibility is enhanced greatly by the removal of protective barriers such as cuticle and lignin cell walls, and by increasing the ratio of litter surface area to mass (Chapin et al. 2002). A great variety of leaf damage is caused by insect herbivores due to their different modes of feeding (Mithöfer et al. 2005). In most cases, mechanical damaging by insects (e.g., through holes or scars) removes amounts of plant material and creates new foliar edges (Hargrove and Crossley 1988; R.E.C and O.D., personal observation). These edges may promote the entrance of plant pathogens, such as fungi (Hargrove and Crossley 1988; Moran 2005), which themselves increase the nutritional value of leaf litter and palatability for detritivores (Graça 2001). Leaf edges may also facilitate the action of other macro-decomposers that seem to prefer feeding on leaf edges (e.g., Jonsson et al. 2002).

In this study, by comparing the decomposition rate of entire and damaged leaves, we tested the hypothesis that canopy herbivores facilitate the action of soil detritivores and decomposers via mechanical damage to leaves. We then tested for the role of initial leaf chemical quality versus geometric form in explaining the observed differences in decomposition rates in entire leaves (EL) versus damaged leaves (DL) using (1) initial leaf chemistry analyses and (2) perimeter/area ratio manipulated cellulose discs. Cellulose disc manipulation was necessary to test whether higher edge availability in damaged leaves can promote the access of microbes and/or macro-detritivores to leaf tissues thereby enhancing leaf decomposition, while eliminating variations resulting from litter quality (Yin et al. 1989).

Methods

Study site

The study was conducted in a tropical cloud forest on the western Andean slopes of Ecuador, at Otongachi Reserve (00°18′60″S, 78°56′53″W, 950 m a.s.l.). The reserve represents a 150 ha patch of primary–secondary forest surrounded by pastures and cattle farms. Seasons in this region can be well separated into a dry (June–November: 65 ± 16 mm per month) and a rainy (December–May: 315 ± 90 mm per month) season (see Appendix 1). The average annual precipitation is about 2,300 mm and the mean temperature ranges between 16.0 and 25.0 °C (see Appendix 1). Soil at the study site had an average slope > 70 %, a root depth (i.e., depth to bedrock) > 100 cm, and a "moderately" thick soil texture (sandy loam, silt loam, sensu AEE 2000). Soil analyses performed at the Center for Environmental and Chemical Services of the Pontifical Catholic University of Ecuador (PUCE) (using ten 1 kg samples collected randomly in the study area) revealed a soil electric conductivity of 42.3 ± 25.7 µS/cm, a moisture of 28.2 ± 5.3 %, a pH of 6.7 ± 0.4, and a C/N ratio of 10.57.

Decomposition of entire versus damaged leaves

In May 2008, 4,000 leaves were picked-out randomly from a single tree as our specific aim was to test for the mechanical effect of herbivory per se on decomposition. The species *Ficus cuatrecasana* (Dugand) was chosen for our study because of its broad distribution in Ecuador and the Neotropics across a wide altitudinal range (Jørgensen and León-Yánez 1999) and because of the palatability of its leaves for mammals (Castellanos et al. 2005; Giraldo et al. 2007) and insects (R.E.C and O.D., personal observation) in Andean forests. In order to avoid the effect of potential confounding factors (such as age or position in the tree) on leaf chemistry, only green leaves (i.e., neither emergent nor senescent) were picked-out randomly from a single 10-m-high *F. cuatrecasana* tree. Moreover, all leaves were "shade leaves" as the study tree was located entirely in the forest understorey.

Green leaves were mixed and sorted into "entire" (EL, showing no damage by herbivores) and "damaged" (DL, eaten to some extent by herbivores) (EL:DL in the tree = 0.67). From the damaged leaves pool, we randomly selected 500 damaged leaves, scanned them (HP Scanjet 4070, Hewlett-Packard, Los Angeles, CA), and quantified eaten areas using imaging software (Scion Image 4.0.2., Frederick, MD). The median damaged surface was 15.2 ± 5 % per leaf (Fig. 1), which corresponded roughly to damaged areas measured on fallen *F. cuatrecasana* leaves collected previously in 1 m^2 litter traps (18.9 %) in the study area. Two groups of leaves, entire (non-eaten) and damaged (15.2 ± 5 % eaten) were sorted, air-dried to constant weight, and weighed into 7.0 ± 0.1 g portions using an analytical balance (FA2104N, Ningbo Utech International, Ningbo, China). This value was chosen based on data obtained from a litter fall census realized during our experiment, and was sufficient to ensure that a minimum leaf mass would remain at the end of our study period. Using 1 m^2 litter traps, we indeed measured a mean litter fall input of 292 g m^{-2} day^{-1} of *F. cuatrecasana* leaves (dry weight) at the beginning of the experiment, which corresponds to 6.57 g/0.15 m^{-2} day^{-1}. This estimation did not change significantly over time as we found an annual litter input of 5.34 g 0.15 m^{-2} day^{-1} over the whole study period.

The leaves were remoistened to make them pliant, and enclosed in 15 × 15 cm (0.15 m^2) plastic mesh bags. As mentioned above, leaf edge availability may enhance leaf decomposition in soils either through an increased colonization of microbes, the facilitated action of detritivores, or both. In order to assess whether leaf damage would preferentially benefit microbes, micro- or macro-decomposers, we performed our leaf litter decomposition experiment using different types of mesh bags: coarse-mesh (CM, 10 mm mesh size) and fine-mesh (FM, 0.5 mm mesh size). While the fine-mesh bags excluded macro-detritivores, coarse-mesh bags did not. In total, 240 bags (60 replicates for each of the four treatments) were prepared and placed for decomposition in 24 different sites selected randomly in an area ~2,000 m^2 around the *F. cuatrecasana* tree. Bags were covered with a handful (~75 g) of forest mixed litter and 10 bags of each treatment were removed every 40 days, from June 2008 to March 2009. In the laboratory, leaves from each litter bag were cleaned gently to remove soil particles, adhering debris, and invertebrates, then dried (60 °C, 48 h) and weighed.

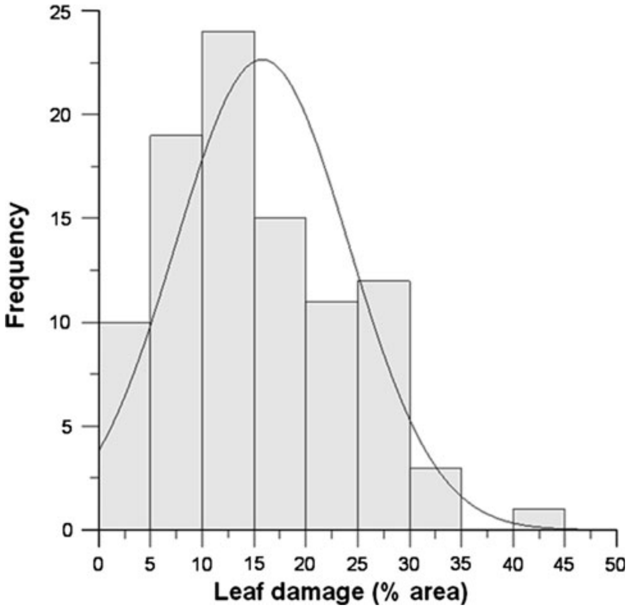

Fig. 1 *Histogram* of the leaf damage area of 500 scanned damaged-leaves from a *Ficus cuatrecasana* tree. The *distribution curve* shows damaged area median (=15.2 %)

Leaf chemistry analyses

To assess the potential role of leaf chemistry in observed difference (due to herbivory on the tree) between EL and DL decomposition rates, a subsample of both types of leaves ($n = 5$) was used to analyze basic litter components at the beginning of the experiment (0 days of decomposition). Percentages of critical elements, C, N, P and K were measured following Kaspari et al. (2008) and were performed at the Oklahoma State Soil, Water and Forage Analytical Laboratory (OSU 2009, available at http://www.soiltesting.okstate.edu/). N percentage was estimated from crude protein based on a nitrogen-to-protein conversion factor of 4.4 (Milton and Dintzis 1981).

Perimeter:area ratio manipulation with cellulose discs

We tested the specific effect of edge availability on litter decomposition by manipulating the perimeter:area ratio of cellulose discs. Standard cellulose filter discs (Fisher Scientific, Pittsburg, PA; 28.3 cm-perimeter) were cut using sterilized scissors to simulate a gradient of edge availability corresponding to three different values of perimeter:area ratio (the total damaged area remained constantly equal to 15 %): (1) a ratio of 1.1 obtained by cutting one hole of 3.6-cm diameter, (2) a ratio of 1.6 obtained by cutting two opposite holes of 2.6-cm diameter, and (3) a ratio of 2.2 obtained by cutting four opposite holes of 1.8-cm diameter (see drawings in Fig. 4). Thirty replications of each treatment were decomposed in CM bags for 58 days in the same field area between November 2008 and January 2009. Mass loss was measured as described in the leaf decomposition experiment.

Statistical analyses

We tested for differences between treatments (EL vs. DL) using a one-sample *t* test of DL:EL decomposition ratios (based on mass loss %) versus a theoretical mean of 1 in both types of mesh bags for all dates. In this test, no significance meant that DL and EL decomposed at equivalent rates, so that their ratio = 1 (Carta et al.

2004). Additionally, the decomposition rate coefficient k was calculated for each litterbag using a negative exponential decay model ($M_t = M_0 \exp^{-kt}$, where M_0 is initial litter mass and M_t is mass remaining at time t), which reflects the most commonly observed functional response for leaf decomposition (Graça et al. 2005). An analysis of covariance (ANCOVA) was used to test for differences in leaf-litter decomposition rates between CM and FM bags (as independent categorical variable) and between EL and DL (the covariate) over time. We used one-way ANOVA to test for differences among both treatments. For the cellulose disc experiment, we used the Anderson–Darling P value (Stephens 1974) (±95 % confidence intervals) to determine whether the DL:EL discs fitted into a log-normal distribution (as expected for random decomposition). The Anderson–Darling is a goodness-of-fit statistic to test the hypothesis that a random sample $X_1,\ldots, X_m$, with empirical distribution $F_m(x)$, comes from a continuous population with empirical distribution function $F(x)$ where $F(x) = F_0(x)$ (normal distribution) (Stephens 1974; Scholz and Stephens 1986). All the analyses were performed using Minitab 15.1 (Minitab, State College, PA).

Results

Over time, the decomposition rates (k) of leaf litter in CM bags reached 0.0091 ± 0.0015 day^{-1} for EL versus 0.0090 ± 0.0016 day^{-1} for DL. In FM bags, the k values were between 0.0037 ± 0.0005 and 0.0042 ± 0.0013 for EL and DL, respectively. Differences between CM and FM treatments were highly significant (ANCOVA, $P < 0.0001$), with leaf litter mass decomposing ~50 % slower in FM bags than in CM bags (Fig. 2). When considering the whole study period, we found no significant differences in decomposition rates between EL versus DL within mesh size treatments (ANCOVA, $P_{FM} > 0.05$; $P_{CM} > 0.05$). However, date by date analysis showed that DL from the CM bags treatments decomposed at a significantly faster rate than EL ($\bar{x}_{CM} = 1.31$; $\bar{x}_{FM} = 1.03$) in the early stages of decomposition (0–38 days; $t = 2.42$, $P < 0.05$; Fig. 3). Overall, 8.9 % of leaf biomass was processed more rapidly during the first month of decomposition. There was also a close to significance (although not significant) trend of faster decomposition of DL in the period of day 38–82 ($t = 1.54$, $P < 0.08$). After 82 days, we likewise found no significant difference in the decomposition rates between EL and DL ($P > 0.05$). No differences in decomposition rates were found among dates for the FM bag treatments ($P > 0.05$; Fig. 3).

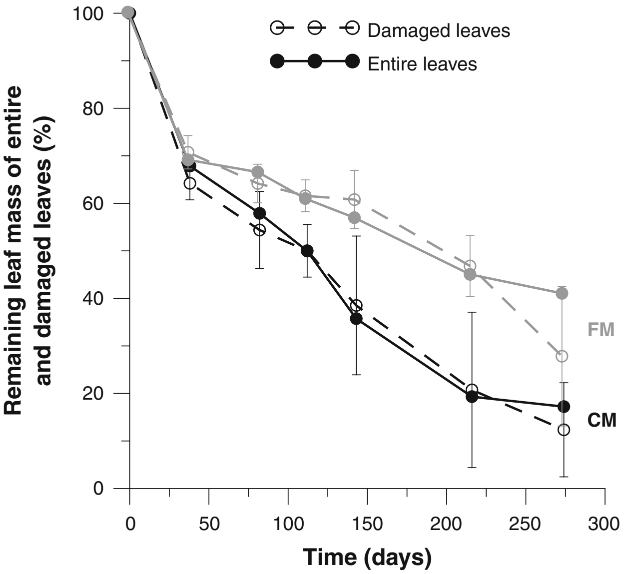

Fig. 2 Decomposition rates (mean values of ten replicates ± SD) of entire and damaged leaves in coarse (10 mm) and fine mesh (0.5 mm) leaf-bag treatments over the study period. *Open circles* Damaged leaves (DL), *filled circles* entire leaves (EL)

Overall, we found no differences in the initial leaf quality between EL and DL except with K, which was lower in DL than in EL (t test, $P < 0.05$; Table 1).

With regards to the cellulose disc experiment, no significant differences were found in decomposition rates among the three perimeter:area ratio treatments (one hole, $P = 0.165$; two holes, $P = 0.068$; four holes, $P = 0.104$; Anderson-Darling test, Fig. 4a–c). Comparison of the decomposition rate between leaves and cellulose discs at 58 days (based on decomposition k rate model) revealed that EL decomposed 16.55 % faster than entire discs, and DL decomposed 17.95 ± 3.7 % faster than herbivore-simulated cellulose discs.

Discussion

Our results support previous experiments that showed the importance of a sequence order of consumers on preconditioned resource utilization (Heard 1994), but from a new perspective of consumption sequence, from herbivores to detritivore facilitation. Jonsson et al. (2002) and Dangles et al. (2009) evidenced facilitation between consumers that resulted in resource exploitation that was 1.8- to 4-fold more efficient. This suggests that any variation in the sequence of detritivore activity would alter not only the first stages, but the whole decomposition process. This was also supported by the fact that, in the absence of macro-detritivores (i.e., in FM bags), EL and DL always decomposed at similar rates.

At the beginning of the decomposition process (i.e., 0–38 days) DL decomposed significantly faster than EL in CM bags, but not in FM bags, suggesting that leaf edges may facilitate the breakdown action of soil macro-detritivores. As a potential mechanism, higher availability of edges may have promoted the colonization of microbes (e.g., Hargrove and Crossley 1988; Moran 2005) thereby increasing the nutritional value of detritus and their palatability for detritivores (Graça 2001). Leaf edges may have also facilitated the action of some groups of macro-detritivores that seemed to prefer

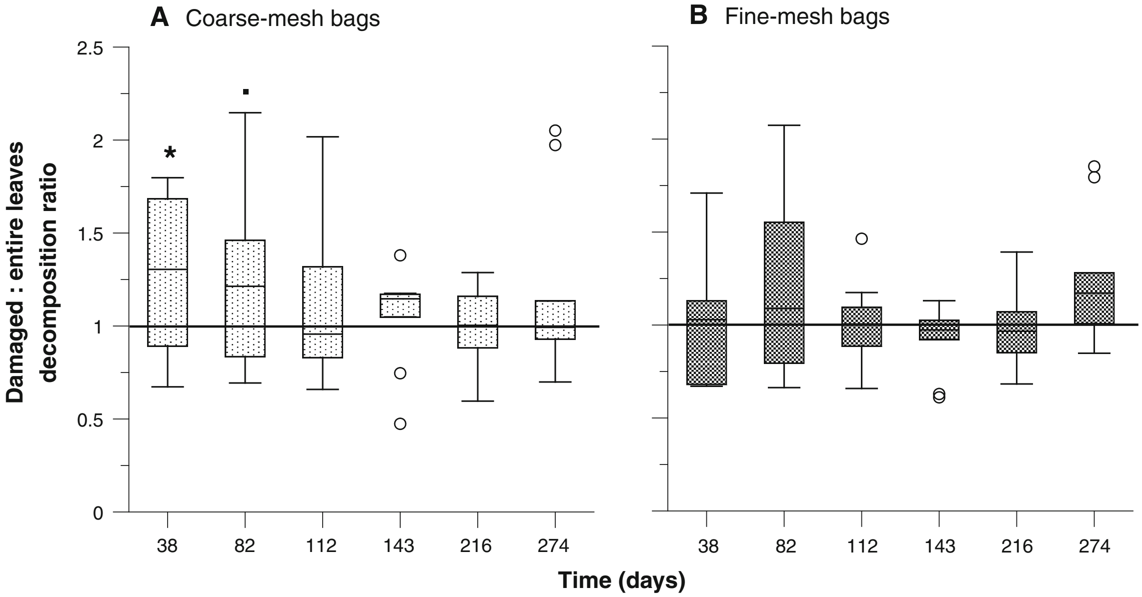

Fig. 3 Damaged:entire decomposition ratio *t* test analysis against a theoretical mean of 1. Outliers are represented by *empty circles*. *Asterisk* Significant at $P < 0.05$; *black dot* near-significance for $P = 0.08$; *no symbols above boxes* no statistical difference. **a** Coarse mesh (CM) bags, **b** fine mesh (FM) bags

Table 1 Initial litter chemistry analyses for green fallen damaged and entire leaves at 0 days of decomposition

	Leaves collected in litter traps (0 days of decomposition)	
	Damaged	Entire
% Crude protein	7.8^a	6.7^a
% N	1.77^a	1.52^a
% C	44.6^a	44.1^a
% P	0.07^a	0.06^a
C:N	25.2^a	29.01^a
N:P	25.32^a	25.38^a
% K	0.73^b	1.06^a

Values in rows followed by the same letter were not significantly different (*t* test, $P \geq 0.05$). Entire leaves collected in litter traps presented 68.9 % more K rate than damaged leaves (*t* test, $P < 0.05$)

feeding on leaf edges rather than eating leaves from top to bottom (Jonsson et al. 2002, R.E.C. and O.D, personal observation). The similarity of decomposition rates between EL and DL in fine mesh bags (FM) confirms that the positive effect of higher leaf edge availability on the decomposition process necessarily involves the presence and action of macro-detritivores. Further studies on the evolution of leaf litter quality over decomposition and on the feeding habits of the soil macro-detritivore community would be needed to better understand the mechanisms involved. Hättenschwiler et al. (2008), for example, compared the CNP concentrations of green versus senescent leaves of 45 spp. of Amazonian trees. They found little variation in C concentration between both types; however, N and P concentrations differed significantly. Averaged across all species, N and P concentrations were 30 and 65 % lower, respectively in senescent leaves compared to green ones. This implies demanding components such as N or P are first reabsorbed by plants before abscission. Once on the ground, these scarce components are exploited rapidly by micro-decomposers by lowering their C-use efficiency as suggested by Manzoni et al. (2008) for N. This would imply that, during the decomposition process, the C:N or C:P ratios gradually increase until the whole organic matter is finally mineralized. In this context, we assume microbial colonization might be more intense in the early stages of decomposition because of the higher concentration of soluble organic matter. Besides, macro-detritivores may prefer litter that is in early stages of decomposition both because of the presence of microbes and because of the higher concentrations of labile and essential substances.

Our hypothesis that leaf herbivory in forest canopies could facilitate subsequent leaf litter decomposition by soil macro-detritivores through changes in leaf litter palatability (e.g., via microbial colonization) was not supported when considering the entire study period. Moreover, modifications in the geometric form per se as an effect of changes in the perimeter-to-size ratio did not have an effect on cellulose disc decomposition. Three reasons, not mutually exclusive, may explain our results. First, the filter papers, being a uniform substance, may be poorly attractive to detritivores (and microbial decomposers) in comparison to DL because of the absence of exposed suitable cell layers and fresh surfaces for decomposing colonizers and nutrients (Chapin et al. 2002; Muller et al. 2002). Second, mechanical facilitation through increased leaf edge availability may be less important in terrestrial than aquatic systems where both

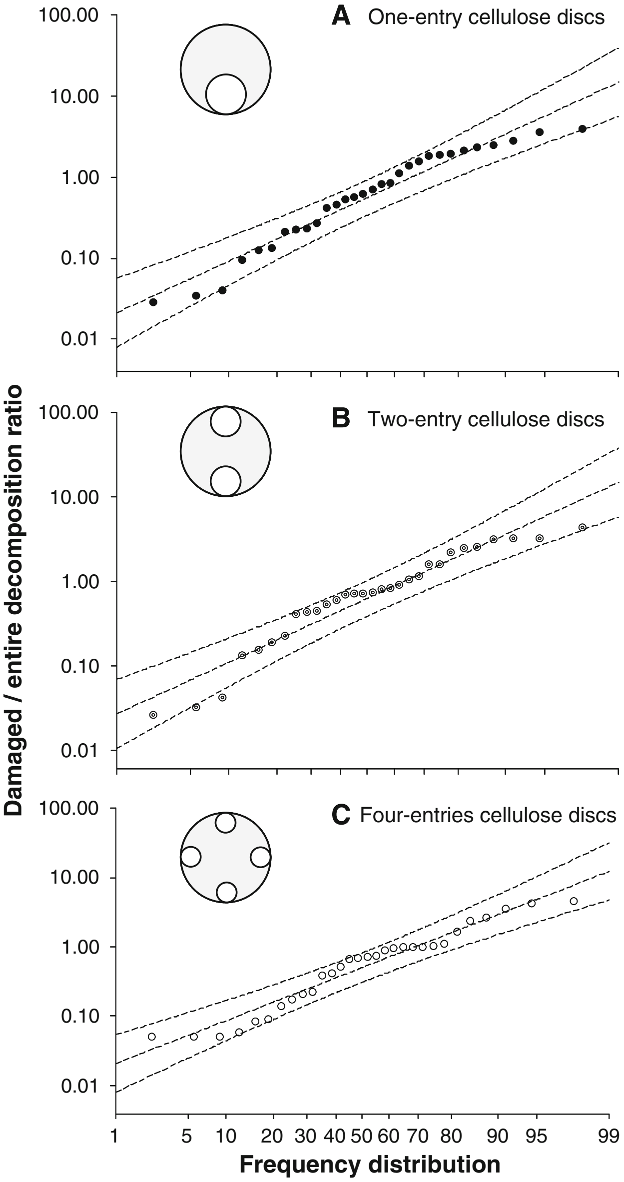

Fig. 4 Decomposition ratio of "damaged" and entire filter paper discs tested against a log-normal distribution model ($n = 30$ for all cases). *Lower* and *upper dashed lines* represent ±95 % confidence intervals. **a** One hole (11.31 cm perimeter), $P = 0.165$. **b** Two holes (7.99 cm perimeter each), $P = 0.068$. **c** Four holes (5.66 cm perimeter each), $P = 0.104$

leaf litter material and detritivores are found in patches (see Dangles 2002) thereby promoting niche segregation at the leaf level among detritivore species (Jonsson et al. 2002). Third, decomposition rates may also depend on litter quality parameters, such as secondary compounds induced by herbivory, that were not measured during our experiment (Kurokawa and Nakashizuka 2008). Although not surprising, the fact that both entire and damaged leaf treatments decomposed faster than entire and simulated damaged cellulose discs after 58 days (results not shown), supports the first and third of these explanations.

In conclusion, canopy herbivores seem to mechanically facilitate soil detritivore action at least at the beginning of the decomposition process. Because our results cannot be generalized to the whole litter plant community, further studies could obtain a more general relationship by using a range of tree species that differ in the physical (Kurokawa and Nakashizuka 2008) and chemical (Hättenschwiler et al. 2008; Kagata and Ohgushi 2011) properties of their leaves. In tropical rainforests, leaf herbivory ranges between 25 % and 40 % (32 % in average, Brenes-Arguedas et al. 2008; 68 % occurring in young leaves, Coley and Barone 1996). It is therefore likely that soil litter has a higher proportion of damaged leaves in tropical forests than in temperate ones (where herbivory rates range between 22 % and 26 %, Lowman 1984). The potential role of canopy herbivores in modifying leaf litter quality and geometric form may therefore be crucial to better understand leaf litter decomposition patterns and mechanisms in tropical forest ecosystems.

Acknowledgments We are grateful to Verónica Mesías, Rodrigo Espinosa, Patricio Andino, Mónica Páez, Mario Herrera and Belén Proaño for their technical assistance, and David Donoso for leaf nutrient quantification. We thank Álvaro Pérez for helping us in *Ficus* identification. The School of Biological Sciences of the PUCE provided financial support to R.E.C. We warmly thank Sylvain Coq, Clifford Keil, Tanya Handa and three anonymous reviewers for providing valuable comments on previous versions of the manuscript.

Appendix 1

See Fig. 5.

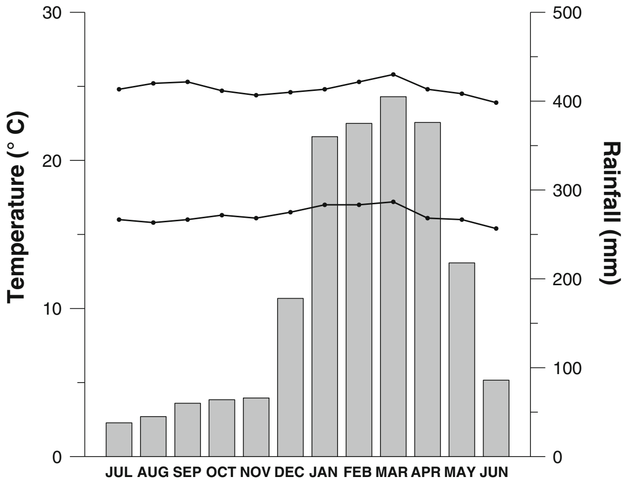

Fig. 5 Monthly means of temperature range (*solid lines*) and rainfall (*bars*) at the Otongachi reserve based on 30s WORLDCLIM 1.4 shapefile at http://www.diva-gis.org/ (Hijmans et al. 2005)

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—CHAPTER 3—

Weak association between leaf herbivory and decomposability at both inter and intra-specific levels in a tropical rainforest tree community

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Summary:

- Terrestrial ecosystems consist of both above- and belowground subsystems whose feedbacks play a crucial role in regulating ecosystem processes. One key question in this topic has led to investigate how plant responses to foliar herbivory (e.g. plant defenses) influence subsequent leaf litter decomposability in soil. While several studies have examined the association between leaf herbivory and decomposability at the inter-specific level, none have documented how intra-specific variations in leaf herbivory may influence decomposability.
- Using 17 neotropical tree species, we experimentally addressed this issue by assessing whether leaves with different levels of herbivory damage in the canopy differ in their subsequent decomposition rates in soil and correlating to potential leaf traits affecting both processes.
- We found that leaf herbivory does not influence its subsequent decomposition in the Amazonian forests. Moreover, while herbivory was mainly affected by physico-chemical plant traits such as toughness and carbon-based structural compounds, decomposition was regulated by complex recalcitrant molecules such as condensed tannins and lignin.
- The analyses based on the variability in herbivory at inter- and intra-specific level failed to explain any strong association between leaf herbivory and litter decomposability. We concluded that herbivory and decomposition are two unrelated processes controlled by different intrinsic leaf traits.

Key words: above- belowground interaction, canopy, detritivores, decomposers, Ecuador, folivory, soil, Yasuní National Park

Introduction

Biotic interactions at the interface between above- and belowground compartments play a fundamental role in regulating the structure and functioning of terrestrial ecosystem processes such as nutrient cycling (Bardgett & Wardle, 2010). At the heart of this interface, the decomposition of dead organic matter (OM) is a key process that results on factors acting both aboveground (e.g. the quality of decaying litter, Kaspari *et al*., 2008; Hättenschwiler *et al*., 2011) and belowground (e.g. the sequential action and diversity of soil decomposers, Pramanik *et al*., 2001; Gessner *et al*., 2010). Several studies have shown that herbivores can play a significant role in leaf litter decomposition by affecting the activity of soil decomposers and detritivores through modification of (i) energy fluxes and hence soil fauna biomass distribution (Mulder *et al*., 2008), and (ii) of OM input quality and quantity (van Dam & Heil, 2011; Wardle *et al*., 2004), with consequences on nutrient availability and plant productivity (reviewed by Vitousek & Sanford, 1986; Hunter, 2001; Cebrián & Lartigue, 2004). However, different ecosystems differ in their response to herbivory. While herbivores have shown to increase litter decomposition rates in particular ecosystems such as grasslands, coniferous forests, and semi-arid woodlands (Chapman *et al*., 2003, 2006; Wardle *et al*., 2002, 2004), herbivory-litter decomposition relationships in the tropics remain controversial, with several studies suggesting a weak association between both processes (Didham, 1998; Kurokawa & Nakashizuka, 2008; Cárdenas & Dangles, 2012). A potential reason for such discrepancy may rely on the fact that the high levels of herbivore diversity and herbivory pressure in the tropical rain forests (Novotny *et al*., 2006; Salazar & Marquis, 2012) has led to large variations in tree strategies for escaping herbivory such as phenological defenses (growth rates, leaf production, synchrony flushing; Aide 1988; Coley 1988; Aide 1993; Cárdenas *et al*., [Chapter 1]), indirect defenses (extra-floral nectaries/glands on the twigs; Kessler & Heil, 2011), physical defenses (leaf toughness, presence of hairs, spines; Lowell *et al*., 1991; Choong *et al*., 1992; Hanley *et al*., 2007) and chemical defenses (Coley & Barone, 1996; Fine *et al*., 2013).

Studies have typically analyzed the herbivory-decomposition relationship through an inter-specific perspective (i.e. comparing averaged trait values among species). However, intra-specific variability in leaf herbivory is known to be high in forest ecosystems (Coley, 1983a; Brenes-Arguedas *et al*., 2008), reaching up to 100-fold in terms of the percentage of damaged area in tropical forests (Lowman, 1984; Cárdenas *et al*., [Chapter 1]). Factors such as local environment, microhabitat (i.e. canopy, understorey, gaps, soil properties; all influencing phenotypic variation in defense, Coley & Barone, 1996), temporality (i.e. dry vs. wet season; Lowman, 1984; Aide 1988), and tree spatial distribution (Coley, 1983b), may be contributing factors explaining such variability (Landsberg & Ohmart 1989; Coley & Barone 1996). Therefore, it is possible that the high intra-specific difference in leaf quality in the canopy may result in significant difference in decomposability of the leaf litter in soils. Conceptually, the action of canopy herbivores may affect subsequent decomposition by detritivores, a sequential process which could be understood within the ‘‘processing chain ecology’’ framework proposed by Heard (1994). This author argued that resource transformation rate can be regulated by a consumption chain of species adapted to the specific conditions of that resource. For example, resource consumption by species B may depend on pre-treatment of the resource by species A. In such a system, consumers specialize on resources in each condition (e.g. herbivory), influencing the rate at which the resource is transformed between conditions (i.e. to litter).

While association between herbivory and decomposability has focused on inter-specific comparisons, no studies have assessed the potential association between the intra-specific variability in herbivory and decomposability. In an attempt to contribute filling this gap, our study examined the association between herbivory and decomposition processes in a Neotropical rainforest using leaf litter from 17 tree species. We first assessed, at the inter-specific level, whether there exist or not any significant relationship between leaf herbivory and litter decomposability and examined whether leaf traits controlling herbivory were similar to those controlling decomposition. Second, we quantified intra-specific variability in both leaf herbivory damage and litter decomposition for these 17 species. We then experimentally assessed whether the intra-specific variability in herbivory damage would affect litter decomposability by responding the following questions. Does the action of canopy herbivores over the leaf lifetime affect the quality of falling leaf litter? Do leaves (mechanically) damaged by canopy herbivores decompose at a different rate, and are controlled by different traits, than those that are not damaged?

Materials and Methods

Study site

The Yasuní National Park (YNP) and the adjacent Waorani Indigenous territory cover 1.6 million ha of forest (1.8 times the Yellowstone National Park in the USA) and form the largest protected area in Amazonian Ecuador (~17.7% of the Ecuadorian territory; Valencia *et al.*, 2004) harboring the world's most diverse tropical forests (Bass *et al.*, 2010). YNP is an evergreen lowland wet forest ranging in altitude from 200 m to 300 m above sea level. It has a 15–30 m canopy with some emergent trees reaching 50 m (Dangles *et al.*, 2012). Rainfall and temperature are aseasonal with a mean annual rainfall of 2826 mm (none of the 12 calendar months averaging < 100 mm) and a mean monthly temperature ranging from 22 to 32°C (min: 16.9; max: 38.9°C) (data obtained from YRS meteorological station, http://www.yasuni.ec; see Valencia *et al.*, 2004 for more details). The study area was located in the vicinity of Yasuní Research Station of the *Pontificia Universidad Católica del Ecuador* (00°40'16.7" S, 07°24'1.8"W) in an extent of ~4300 m^2 forest floor plot composed by slope- and valley-type habitats (see Valencia *et al*., 2004 for a detailed description of micro-habitats).

Herbivory data

Except for *Duroia hirsuta*, leaf damage data of the 17 species were obtained from Cárdenas *et al*., (see Chapter 1) which briefly consisted on quantifying the proportion of eaten area of the pressed, dried and scanned leaves using ImageJ open source image processor (http://rsb.info.nih.gov/ij/; Abràmoff *et al.* 2004). For this, leaf images were previously cleaned (i.e. erase shadows, fill scratches, eliminate the petiole) and binary-transformed. Leaves totally eaten were not taken into account for herbivory damage estimations. *Duroia hirsuta* herbivory damage data were taken from Jaramillo (2012) which were measured with similar methods as described by Cárdenas *et al*. (Chapter 1). *D. hirsuta* leaves were collected in the same study area as ours.

Leaf litter collection

A potential approach for understanding herbivory-decomposition relationships is to compare the decomposability of senescent leaves showing various degrees of herbivory damage (Cárdenas & Dangles, 2012). This method integrates the accumulated amount of herbivory damage over the course of leaf lifetime as a function of the variation of leaf physical and chemical properties (e.g., toughness or chemical composition). It has also the advantage to measure herbivory damage on leaves that will be found on the forest floor (as opposed to methods based on herbivory rate quantification at different phenological stages, e.g., Kurokawa & Nakashizuka, 2008).

In April 2011, senescent leaves were collected from young to sub-adults trees of 17 common angiosperm species belonging to 11 families (most aboveground production involves angiosperm trees, Kurokawa & Nakashizuka, 2008): *Matisia malacocalyx* (Bombacaceae), *Mabea superbrondu*

(Euphorbiaceae), *Inga capitata* (Fabaceae), *Macrolobium yasuni* (Fabaceae), *Nectandra viburnoides* (Lauraceae), *Miconia purpono* (Melastomataceae), *Siparuna cuspidata* (Monimiaceae), *Siparuna decipiens* (Monimiaceae), *Naucleopsis krukovii* (Moraceae), *Pseudolmedia laevis* (Moraceae), *Sorocea steinbachii* (Moraceae), *Iryanthera hostmannii* (Myristicaceae), *Neea comun* (Nyctaginaceae), *Duroia hirsuta* (Rubiaceae), *Leonia glycycarpa* (Violaceae), *Rinorea lindeniana* (Violaceae), *Rinorea viridifolia* (Violaceae). Collection consisted on 5–20 tree-shaking repetitions from the trunk or branches. Leaves fell on white sheets (cotton 1.5 m × 3.5 m) that were tied at 1m height from the floor. Leaves that were too young (typically presenting bright green colors), too old (i.e. rotten or presenting large amounts of necrosis), or presenting evident fungi infection or insects galleries were discarded (*c.a.* 5–20% of the total leaves collected *per* species). Once collected, leaves were stored in an acclimatized room for no more than 24 h and dried at 40°C for up to 72 h in cotton fabric bags (depending on the leaf dryness rate; no more than 30 leaves *per* bag).

Decomposition experiments

Our experiment consisted in testing the decomposition rate of entire, damaged and artificially punched leaves of the 17 mentioned tree species. Punched leaves were supposed to simulate the physical effect of herbivory damage (increased leaf-edge accessibility for consumers) while keeping the quality of entire leaves thereby sorting out potential mechanical and chemical effects of canopy herbivores (see Cárdenas & Dangles, 2012 for further details). Collected leaves were visually divided into ‘entire’ leaves (those presenting 0–5% of herbivory damage) and ‘damaged’ leaves (those presenting 30–50% of herbivory damage). These classes were chosen based on previous experiments carried out in tropical forests (e.g. Lowman, 1984; Landsberg & Ohmart, 1989; Sterck *et al*., 1992; Brenes-Arguedas *et al*., 2008; Cárdenas *et al*., [Chapter 1]) and after accounting for the community leaf damage proportions (i.e. leaf scanning, see *Hervivory data* section above). Leaf punching was performed using a 14 mm diameter iron tube to make standard holes for comparison with entire and damaged leaves. Since leaf size differed within and among species, the number of holes *per* leaf necessary to reach a damage of about 30–50% also differed.

For each treatment, about 2.91 g ± 1.57 (min.: 0.59 g; max.:8.91 g) of leaves without *peciolum* (weighted using a portable balance FA2104N, Ningbo Utech International, Ningbo, China) were placed in 20-cm diameter plastic mesh bags. The bottom mesh had squared holes of 100 mm^2 that allowed the access/exit of micro, meso and macrofauna while preventing the significant loss of coarse non-consumed litter material. Top-size mesh had squared holes of 900 mm^2 to allow the free access/exit of micro, meso, macro and megafauna (see Swift *et al*., 1979 for detritivores size classification). In total we followed the decomposition process of leaves placed into 510 mesh bags (17 species × 3 treatments × 10 replicates). Mesh bags were divided into 85 lots (3 treatments × 2 species) and placed on *terra firme* soils, in the same area where leaves had been collected. The minimal distance between each lot was 5 m. After 100 days, all litter bags were collected for analyses. In the laboratory, leaves were gently cleaned to remove soil particles, adhering debris, and invertebrates, then dried at 40°C for up to 96 h, and weighed.

Leaf-litter trait analyses

For the 17 studied tree species, a subset of 10–30 entire and damaged leaves *per* species were separated from the rest of collected leaves and randomly sorted into 5 groups for subsequent leaf quality analyses. All leaves was kept at -20°C after collection, dried at 40°C for up to 72 h, homogenized in a coffee grinder and kept in dry conditions until analyses.

We selected a range of 10 vegetative functional traits, both physical and chemical, that have been shown to be correlated with decomposition (Cornelissen *et al*., 2003; Kurokawa & Nakashizuka, 2008; Hättenschwiler *et al*., 2011). Thickness was measured avoiding primary and secondary veins using an analog 0–25 mm micrometer caliper at 0.005 mm precision (Amico Corporation, Ontario, Canada). Leaf size (cm^2) and specific leaf area (SLA, defined as the ratio of fresh leaf area in cm^2 to dry weight) were taken from Kraft & Ackerly (2010). Chemical traits were measured at Colorado State University (Fort Collins, Colorado, USA) Soil, Water and Plant Testing Laboratory (http://www.soiltestinglab.colostate.edu/). N and C were measured in a CN analyzer that used an infrared detection for carbon and thermal conductivity detection for nitrogen system (Leco® TruSpec Micro CN analyzer, St. Joseph, Minnesota, USA). Lignin and cellulose content determination followed the gravimetric determination of acid detergent fiber (ADF) and acid detergent lignin (ADL) methodology (Möller *et al*., 2009). Ash content (considered as a measure of defenses such as silica-based phytoliths and calcium oxalates, Moles *et al*., 2013) corresponded to the leaf mass remaining after combustion of ADL samples at 550°C for two hours (Möller *et al*., 2009). Condensed tannins were measured using the Butanol-HCl method and expressed as leucocyanidin equivalent (% DM) following Porter *et al*., (1986). Finally, micronutrients content were estimated using the ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) methodology (Boumans, 1987).

Data analyses

All the analyses were performed in Past software v.2.17 (Hammer *et al*., 2001) unless otherwise indicated. The relationship between leaf herbivory damage (defined as the percentage of damage at leaf abscission) and decomposability (mass loss percentage of the dry matter) was first evaluated by correlation and simple linear regression models. The significance of the relationship was assessed with a non-parametric Spearman's correlation analysis, and ANOVA test, the latter using Table Curve 2D software v.5.01 respectively. Intra-specific variability of both processes was analyzed through the coefficient of variation (CV), and both distributions were compared using Kolmogorov-

Smirnov goodness of fit test (herbivory data did not adjust to normality: Shapiro-Wilk test, $P_{\text{herbivory}} < 0.05$).

For exploring the potential effect of herbivory on falling leaf litter quality, the relationship in leaf trait values between senescent (both damaged and entire) and green leaves of 10 of the 17 tree species were fitted to linear regressions whose slopes were compared using an analysis of covariance (ANCOVA, see Gotelli & Ellison, 2004). We assumed significant differences between slopes for $4.10 < F < -4.10$ (i.e. $P < 0.05$).

The effect of mechanical damage on leaves was assessed by comparing the percentage of mass loss of entire vs. damaged leaves, and entire vs. punched leaves, against a 1:1 relationship. Differences between slopes were evaluated using an ANCOVA as described above.

Whether leaf traits were related to herbivory and decomposability of entire and damaged leaves (separated or pooled) was measured using multiple correlation analyses (Kurokawa & Nakashizuka, 2008; Coq *et al.*, 2010; Kurokawa *et al.*, 2010). Decomposition k rates (constant that characterizes the decomposition rate based on an exponentially litter matter mass loss) was calculated following Levins (1968) as,

$$k = -\left(\frac{\ln\left(\frac{L_t}{L_0}\right)}{t}\right)$$

where L_t is the litter mass at time t, and L_0 is the litter mass at time 0.

Because of logistical reasons shearing and shearing × CT correlations with decomposition were measured using 10 of the 17 species. For both, decomposition and herbivory, correlations were calculated using data from green leaves as measures of toughness have shown to do not differ significantly between green leaves and leaf litter (Kurokawa & Nakashizuka, 2008).

Finally, the relationship between leaf decomposition and trait values for the 17 tree species were evaluated by simple linear and three nonlinear regression analyses (log, power and hyperbolic) that had been previously used in the literature (see Melillo *et al*., 1982; Prescott 2010). The significance of the relationship was assessed with an ANOVA test using Table Curve 2D software v.5.01.

Results

Inter-specific variability in leaf herbivory and decomposability

We found no significant relationships (neither linear nor curvilinear) between herbivory damage and decomposability for the 17 studied tree species (Fig. 1, simple linear regression model; R^2 = 0.09; F = 1.489; P = 0.241; Spearman rank test, $P > 0.05$). The best model (Power function) indicated only a weak negative trend between both sets of values ($y = 89.96x^{-0.17}$; P = 0.097). We found all types of associations between herbivory and decomposability among the 17 species: low herbivory/high decomposability (e.g. *S. steinbachii*, *M. superbrondu*, *S. cuspidata*), high herbivory/low decomposability (e.g. *I. hostmannii*, *N. viburnoides*) high herbivory/high decomposability (e.g. *M. malacocalyx*, *M. purpono*), and low herbivory/low decomposability (*D. hirsuta*, *P. laevis*).

Among the 16 tested factors (including interactions), the best predictors of decomposition rates for the pool of both entire and damaged leaves were lignin, condensed tannins, lignin:N, CT:N and lignin×CT (Table 1). Simple linear and non-linear best regression models of all the interacting plant traits with significant correlations in Table 1 were fitted and plotted in Fig. 2 where lignin×CT fitted to logarithmic model (R^2 = 0.43, F = 11.322, P = 0.004), lignin:N to power model (R^2 = 0.41, F = 10.563, P = 0.005), and CT:N to linear models (R^2 = 0.38, F = 9.004, P = 0.009). Thickness, SLA, and carbon showed to be barely significantly correlated to decomposition rates ($P_{\text{thick.}}$ = 0.097; P_{SLA} = 0.081; P_{C} = 0.066). We finally found leaf size, cellulose and ash resulted barely significantly correlated to herbivory ($P_{\text{leaf size}}$ = 0.095; $P_{\text{cellul.}}$ = 0.073; P_{ash} = 0.074).

Intra-specific variability in leaf herbivory and decomposability

Intra-specific variability in both herbivory damage and decomposition (% mass loss) were high. Herbivory damage was more variable than decomposability, except for three species: *M. superbrondu*, *R. lindeniana* and *S. cuspidata*. In all, standard deviations of herbivory were 1.59 times higher than those of decomposition (Fig. 3). Both, herbivory and decomposition presented high values of CV (in average 95.47% and 16.32%, respectively). CV distributions resulted significantly different (Kolmogorov-Smirnov goodness of fit $P < 0.001$, Fig. 4). CV herbivory showed a skewness of $g_1 = 1.42$ (i.e. right skewed), and decomposition a skewness of $g_1 = -0.31$ (i.e. left skewed). Compared to a normal distribution, CV Kurtosis showed that herbivory CV distribution is more expanded to the tails ($g_2 = 1.79$) compared to decomposition's which is more centered ($g_2 = -0.15$).

Herbivory effect on falling leaf litter quality

In order to assess the potential effects of canopy herbivores on leaf quality, we compared the relationship between green and senescent leaves quality metrics in 17 species, for both damaged and entire leaves (Fig. 5). First, ANCOVA showed significant differences among entire and herbivory damaged senescent leaves for lignin and ash contents ($F_{\text{lignin}} = 12.09$; $F_{\text{ash}} = -4.46$). Both damaged and entire leaves were more lignified when still green comparing to senescent, and herbivory damaged leaves were more lignified than entire leaves. The opposite occurred for the ash content, where entire leaves presented a higher proportion of ash compared to damaged leaves (and to green leaves). Second, the ANCOVA between entire and damaged leaves showed significant differences from the 1:1 relationship line for six of the parameters: thickness ($F_{\text{entire}} = 6.26$; $F_{\text{damaged}} = 6.09$), C:N ($F_{\text{damaged}} = -4.52$), lignin ($F_{\text{entire}} = 10.64$) and ash ($F_{\text{entire}} = -15.79$; $F_{\text{damaged}} = 16.96$).

Decomposition of entire and damaged leaves

Our results showed that entire, damaged and punched leaves did not decompose at significantly different rates (Fig. 6 and Table S1). ANCOVA showed $F < 4.10$ (i.e. $P > 0.05$) for all kinds of comparisons: entire/damaged vs. entire/punched; entire/damaged vs. 1:1; and entire/punched vs. 1:1. Entire and damaged leaf treatments decomposition were significantly negative correlated to condensed tannins, condensed tannins:N ratio, and lignin × condensed tannins interaction. Damaged leaves decomposition alone was additionally correlated to lignin and lignin:N ratio (Table 2).

Discussion

Association between herbivory and decomposability at the inter-specific level

At an inter-specifc level, tree species presenting higher levels of herbivory damage did not necessarily present higher levels of decomposability and *vice versa*. We only found a barely significant negative herbivory- decomposability relationship ($P = 0.097$) in agreement with Kurokawa & Nakashizuka (2008) who reported a weak association between the rates of both processes. Except for some particular species, these results suggest that "better defended" leaves (or less susceptible to herbivory damage) may not turn into necessarily less decomposable litter.

In agreement with Kurokawa & Nakashizuka (2008) findings, our results showed that factors controlling decomposition are not the same controlling herbivory. This confirms that in the tropics canopy and soil are two independent subsystems that are regulated by different components and mechanisms (Kurokawa & Nakashizuka 2008). In particular, we found that condensed tannins and lignin correlated negatively with leaf litter mass loss in agreement with other studies in tropical ecosystems (Kurokawa & Nakashizuka, 2008; Coq *et al.*, 2010; Hättenschwiler & Bracht Jørgensen, 2010). Large and complex molecules are indeed more difficult to digest and therefore mostly processed first extracellularly by fungi and bacteria exoenzymes (Chapin *et al*., 2002). Moreover, difficulty on decomposing these elements may rely on the irregularity of lignin structure and the toxicity of condensed tannins (Chapin *et al*., 2002). Also, the 'lignin:N' and 'CT:N' ratios showed a significant negative correlation with decomposition rates. The former ratio has often been identified as a good predictor of decomposition in a wide range of terrestrial ecosystems (Melillo *et al*., 1982; Taylor *et al*., 1989; Moore *et al*., 1999; Kurokawa & Nakashizuka, 2008; Wieder *et al*., 2009), although Hättenschwiler *et al*., (2011) found no significant effect of this ratio on decomposition in the Guyana forest.

Leaf size, cellulose and ash were barely significantly correlated to herbivory. Positive correlation with leaf size supports the idea that larger leaves may attract more herbivores (Garibaldi *et al*., 2011), a characteristic that may not necessarily produce the same effect on the detritivore community (Weerakkody & Parkinson, 2006; but c.f. Bärlocher & Schweizer, 1983). Ash-related elements have demonstrated to be efficient in defending plants from the action of herbivores (Hanley *et al*., 2007; Cooke & Leishman, 2012; Cárdenas *et al*., Chapter 1). Ash content is a measure of defenses such as Calcium oxalates and silica-based phytoliths (the latter helps increasing toughness of plant tissues, Massey *et al*., 2007; Moles *et al*., 2013), two components that strongly reduce herbivory (Korth *et al*., 2006; Massey *et al*., 2006). Cellulose is a macromolecule hard to degrade (Abril & Bucher, 2002) where animals gut symbiotic bacteria and flagellates, and specialized detritivores such as termites are the main responsible of its metabolization (Wenzel *et al*., 2002; Tokuda &Watanabe, 2007).

Overall, our findings support the idea that, at the inter-specific level, litter decomposability cannot be reliably predicted by leaf herbivory in the tropics, perhaps because plant diversity results in diverse plant–herbivore interactions and of particular (micro-) habitat selection pressures (Kursar & Coley, 2003; Agrawal, 2007). In contrast to other types of ecosystems (see Introduction), herbivores may not necessarily generate positive feedback for carbon and nutrient cycling in diverse tropical forests.

Intra-specific variability in herbivory and decomposition

Intra-specific herbivory damage in tree canopies resulted significantly more variable than decomposition in soils. This could be explained by the fact that the ‘green’ pool is highly heterogeneous in terms of intrinsic quality (e.g., differences in plant defenses) between sun and shade leaves or young, mature and senescent leaves (Coley & Barone, 1996; Dominy *et al*., 2003; Boege & Marquis, 2005). Additionally, trees may be responding to local herbivory pressures like herbivore clustering or outbreaks events (Miler & Straile, 2010; Salazar & Marquis, 2012). Also it remains unclear whether tropical plants invest in non-volatile defenses (such as food supply limitation, nutrient value reduction, physical structures disruption, and/or herbivore chemical pathways inhibition) by local or systemic induction, and at which level (leaflet, leaf, branch, or the whole tree) after a sporadic herbivore attack in the wild (Miler & Straile, 2010; Warman *et al*., 2011). Growing evidence suggests that the defense response can be limited to the site of attack (local induction), or can be expressed in remote, undamaged plant parts (systemic induction), ranging from structural defenses to toxic chemical compounds (Bezemer & van Dam, 2005). Besides, chemical plant defenses could finally be non-uniformly distributed at the leaf level (Shroff *et al*., 2008). Finally, Freschet *et al*., (2013) found high plasticity in resource acquisition in response to local environmental stress suggesting differences in plant nutrition, which may be influencing herbivores leaf consumption.

Contrastingly, the ‘brown’ pool may be surrounded by more homogenous conditions, with soil communities (invertebrates detritivores, bacteria, fungi and endomycorrhizae) adapted to efficiently transform all type of dead OM (Lavelle 2002; Madritch & Lindroth 2011). In a macroecological analysis, Makkonen *et al*. (2012) indeed showed that decomposer communities present little specialization and high metabolic flexibility in processing all type of plant litter material.

Herbivory effect on falling leaf-litter quality

Before abscission, when leaves enter the senescent state, plants resorb nutrients through the phloem to other plant tissues (Aerts, 1996; Hättenschwiler *et al*., 2008). Nutrient resorption is crucial for plant survival (Freschet *et al*., 2010), and it may be influenced by so many factors that no single environmental control has been identified (Chapin *et al*., 2002). Herbivory that represents twice the loss of N and P comparing to leaf renewal, has been recognized as one of those factors triggering nutrient resorption as a plant anti-herbivore response to defoliation (Tuomi *et al*., 1984).

Our results suggest that the action of canopy herbivores had significant consequences on the lignin and ash contents of leaves. In particular, lignin content was better conserved in damaged leaves, and ash-related elements in entire leaves, probably as an intrinsic arrangement of plants to defend them against herbivores action. Tuomi *et al*. (1984) suggested that the excess of carbon that cannot be resorbed to growth was diverted to the production of plant secondary metabolites. Lignin and other structural carbohydrates (and ash-related elements as described above) provide the supporting skeleton of the leaf that has the particularity of reducing leaf digestibility (Bazzaz *et al*., 1987). Coley (1983b) and Pérez-Harguindeguy *et al*. (2003) showed that lignin concentrations had the largest effect on the herbivore damage since leaf consumption should be directly linked to leaf nutritional quality. Although most previous studies documenting the importance of lignin have worked with woody plant species from tropical rainforests results have not been consistent (Coley, 1983; Poorter *et al*., 2004; Kurokawa & Nakashizuka, 2008; Kurokawa *et al*., 2010).

Real-time effect of herbivores on plant defense strategies (hormone-regulated) has been proven for herbaceous and woody plants in temperate ecosystems (Agrawal *et al*., 2012; Giron *et* *al*., 2013). Evidence suggests that in natural conditions such responses are relaxed after 3–4 years in woody plants (Tuomi *et al*., 1984), suggesting an "after-herbivory effect" in the leaf quality. That is, leaf physico-chemical traits found in our study may be probably representing past herbivory events that conditioned the whole pool of leaves of a tree. This could potentially explain the intra-specific variability in herbivory damage (i.e. related to individual past events), and would confirm that lignin and ash-related elements are effectively and immediately conserved in damaged leaves as a local herbivore deterring strategy.

Canopy leaf herbivores do not mechanically facilitate subsequent litter decomposition in soil

In agreement with a previous pilot study (Cárdenas & Dangles, 2012), our results did not support the hypothesis that leaf herbivory by canopy herbivores could facilitate subsequent leaf litter decomposition by soil detritivores and decomposers. Entire, damaged and punched leaf treatments did not differ in their decomposition rates, in spite that fragmentation (e.g. carried out by herbivores) creates fresh surfaces that increase the proportion of vegetation mass accessible to attack by micro- and/or macro-organisms (Chapin *et al*., 2002). Cárdenas & Dangles (2012) found that herbivores may facilitate soil detritivore action at the beginning of the decomposition process only (i.e. the first 38 days), suggesting that decomposition rates of the two leaf types may depend on litter quality parameters, such as secondary compounds induced by herbivores activity. This hypothesis was not supported in the present study in spite of significant differences in initial lignin and ash contents found between senescent 'entire'-punched and damaged leaves (Fig. 3). As a matter of fact we actually found that leaf traits affecting both entire and damaged leaves were practically the same and at comparable statistical power. Potential increased availability of leaf edges made by herbivore damage seems to not represent a mechanical facilitation for detritivores and decomposers consumption in the long term (i.e. after > 30 days of decomposition process). This suggests that leaf litter chemical traits are the main controlling factors of decomposition regardless of the shape of the plant material. The potential role of herbivores as ecosystem engineers mechanically facilitating the decomposition process by soil fauna may be currently rejected in the tropics.

Conclusion

Our finding on a wide range of tree species strongly supports that the consumption of leaf material is governed by different processes in tree canopy and soils of neotropical forests. While physical traits may provide an effective barrier against herbivore consumption, chemical traits seem to be the main factors controlling leaf litter decomposability in soils. Leaf litter with low levels of lignin and CT may be nutritionally much more attractive for the decomposer soil community compared to the inverse content levels of these elements. Our study also suggests weak associations between the activities of canopy herbivores and that of soil decomposers. Unlike in other systems, there is no evidence of a processing chain (sensu Heard, 1994) between herbivores and decomposers and the high variability in herbivory damages found at the canopy level, at both inter-specific and intra-specific levels, might be masking potential direct consequences for the subsequent decomposition of leaf litter in soils. Mega-biodiverse ecosystems such as the tropical rain forests, where there is an enormous variation of herbivory kinds and consequently plant responses (i.e. defensive strategies), may explain the extremely high variability in the herbivory ranges at inter and intra-specific levels. Such a complex mosaic of action/reaction between plants and herbivores may be at the base of the inconsistencies of the actual relationship between leaf herbivory and litter decomposability found in the literature. Future studies should focus on analyzing this relationship from the perspective of the different herbivore-defense strategies in the plant communities.

Acknowledgements

We are grateful to Liliana Jaramillo and Selene Baez for providing herbivory damage data for *D. hirsuta*. We thank the staff of the Yasuní Research Station, in particular the administrative personnel, David Lasso, Pablo Jarrín, Carlos Padilla and Karla Rivadeneira for constant support during the stays at the Station. We are grateful to Pablo Alvia who provided invaluable expertise on plant taxonomy in the field. Patricio Andino, Rodrigo Espinosa, Mayra Buenaño, Fernanda Samaniego and Pamela Arias helped in field work and laboratory. This study would have been logistically impossible to carry out without them. Jim Self provided guidance and full support for leaf analyses at Soil, Water & Plant Testing Laboratory at CSU. We are grateful to the School of Biological Sciences of the *Pontificia Universidad Católica del Ecuador* (PUCE), in particular to its dean Hugo Navarrete for constant support. The PUCE (project codes H13164 and J13041) and the French Institute for Research and Development (IRD) supported this study through research grants. This study was endorsed by the *Ministerio de Ambiente del Ecuador* permits MAE: 09-FLO-MA-DPO-PNY and 06-2011-FAU-DPAP-MA.

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Tables

Table 1. Pearson correlation coefficients of the decomposition rates (entire and damaged senescent leaves averaged) and herbivory, with plant functional traits.

Variable	**Decomposition rate *k*(a^{-1}) (*s* = 17)**	**Herbivory (%) (*s* = 28)**
Thickness	**-0.415·**	0.289
SLA	**0.435·**	0.180
Leaf size	-0.115	**0.322·**
Shearing	-0.269	-0.299
Nitrogen (N)	0.164	-0.050
Carbon (C)	**-0.456·**	0.300
Lignin	**-0.540***	0.190
Cellulose	0.056	**-0.344·**
Ash	0.011	**-0.344·**
CT	**-0.622****	-0.067
C:N	-0.395	0.098
Shearing × CT	-0.424	-0.199
Lignin:N	**-0.519***	0.236
Cellulose:N	-0.118	0.197
CT:N	**-0.612****	0.013
Lignin×CT	**-0.598***	0.008

Significant correlations are indicated in bold. Herbivory correlations were measured from Cárdenas *et al*., (Chapter 1, unpublished data). Shearing and Shearing × CT correlations were measured using *s* = 10 species. Because toughness measures did not differ significantly among green leaves and leaf litter (Kurokawa & Nakashizuka, 2008), shearing leaf resistance data for both decomposition and herbivory correlations were taken from green leaves. · $P < 0.1$; *$P < 0.05$; **$P < 0.01$.

Table 2. Pearson correlation coefficients of the decomposition rates of entire and damaged senescent leaves with plant functional traits.

Variable	Decomposition rate k(a^{-1})	
	Entire	Damaged
Thickness	-0.394	**-0.441·**
Nitrogen (N)	0.107	0.218
Carbon (C)	**-0.465·**	**-0.430·**
C:N	-0.328	**-0.450·**
CT	**-0.622****	**-0.599***
Lignin	**-0.432·**	**-0.647****
Cellulose	0.079	-0.001
Ash	-0.026	0.074
Lignin:N	**-0.423·**	**-0.609****
Cellulose:N	-0.004	-0.211
CT:N	**-0.595***	**-0.614***
Lignin×CT	**-0.526***	**-0.646****

Significant correlations are indicated in bold. (s = 17 and n = 8–10 for all treatments).

· $P < 0.1$; *$P < 0.05$; **$P < 0.01$.

Figure legends

Fig.1. Herbivory damage (species average) and decomposition (entire and damaged leaves averaged) relationship for 17 tree species in the Yasuní National Park. Neither linear nor non-linear tendency regressions gave significant fits (simple linear regression model $P = 0.241$; $F = 1.489$; $R^2 = 0.09$; Spearman rank test, $P > 0.05$). DUHI: *Duroia hirsuta*; INCA: *Inga capitata*; *Iryanthera hostmannii*; LEGL: *Leonia glycycarpa*; MAMA: *Matisia malacocalyx*; MASU: *Mabea superbrondu*; MAYA: *Macrolobium yasuni*; MIPU: *Miconia purpono*; NAKR: *Naucleopsis krukovii*; NECO: *Neea comun*; NEVI: *Nectandra viridifolia*; PSLA: *Pseudolmedia laevis*; RILI: *Rinorea lindeniana*; RIVI: *Rinorea viridifolia*; SICU: *Siparuna cuspidata*; SIDE: *Siparuna decipiens*; SOST: *Sorocea steinbachii*.

Fig. 2. Decomposition rates of all pooled leaves as a function of interacting chemical plant traits parameters. Values of R, *F* and *P* are given for log, power or hyperbolic regressions (equations described). Dashed lines correspond to ±95% confidence intervals.

Fig. 3. A comparison of the leaves hebivory damage and leaf litter decomposition proportions of 17 tree species in Yasuní National Park. Decomposition corresponds to the average of entire and damaged leaves. *D. hirsuta* herbivory damage data were taken from Jaramillo (2012). Error bars are standard deviations.

Fig. 4. A comparison of coefficients of variation of herbivory damage and decomposition, and its frequencies, among the studied plant species. Dark-grey and light-grey bars correspond to decomposition and herbivory data respectively.

Fig. 5. Leaf traits relationships between green and senescent leaves of 10 species. Full grey circles correspond to entire senescent leaves and empty black triangles to damaged senescent leaves (dam.). Grey solid and black dashed-point lines represent simple linear regressions of entire and damaged leaves respectively. Light grey dashed diagonal line represents 1:1 relationship. *P* values of simple linear regressions are given. *R* and *F* statistics are presented in Table S2.

Fig. 6. Decomposition of damaged and punched leaves compared to entire leaves. Light grey dashed diagonal line represents 1:1 relationship. Error bars are standard deviations.

Figures

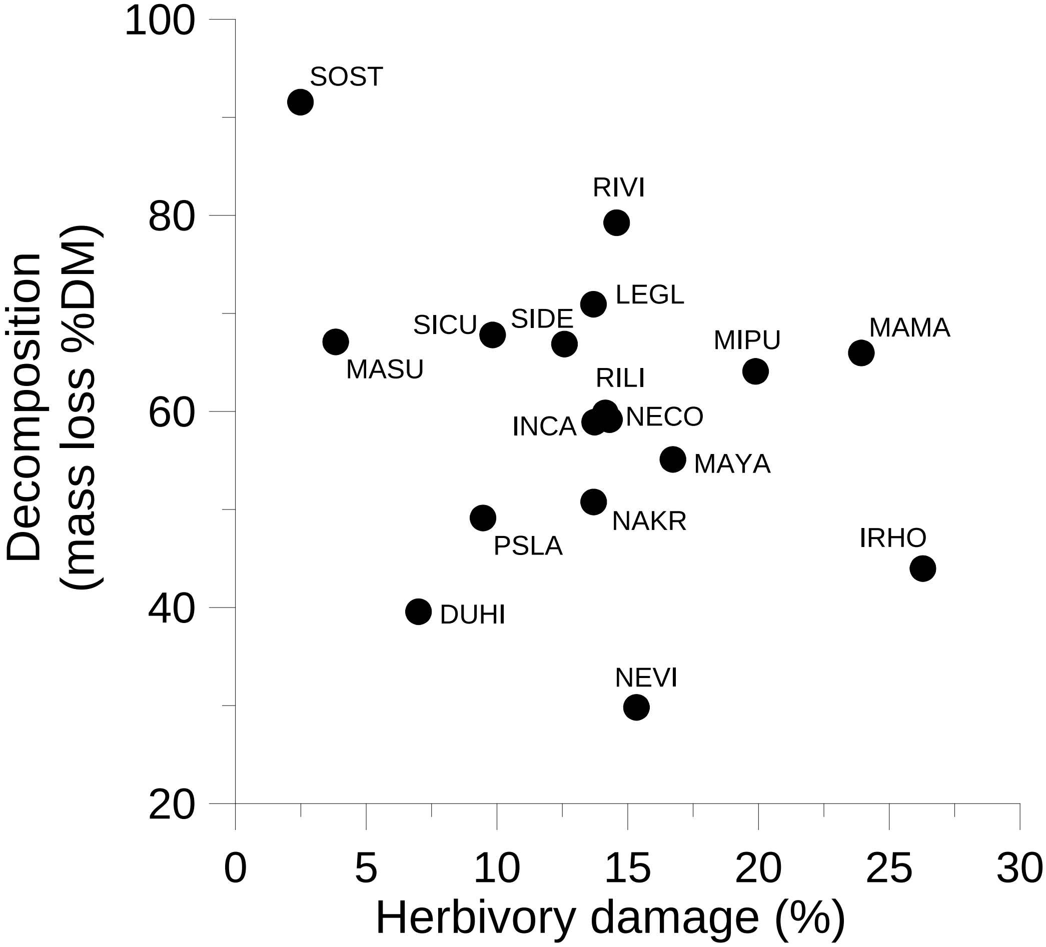

Fig. 1. Cárdenas *et al*.

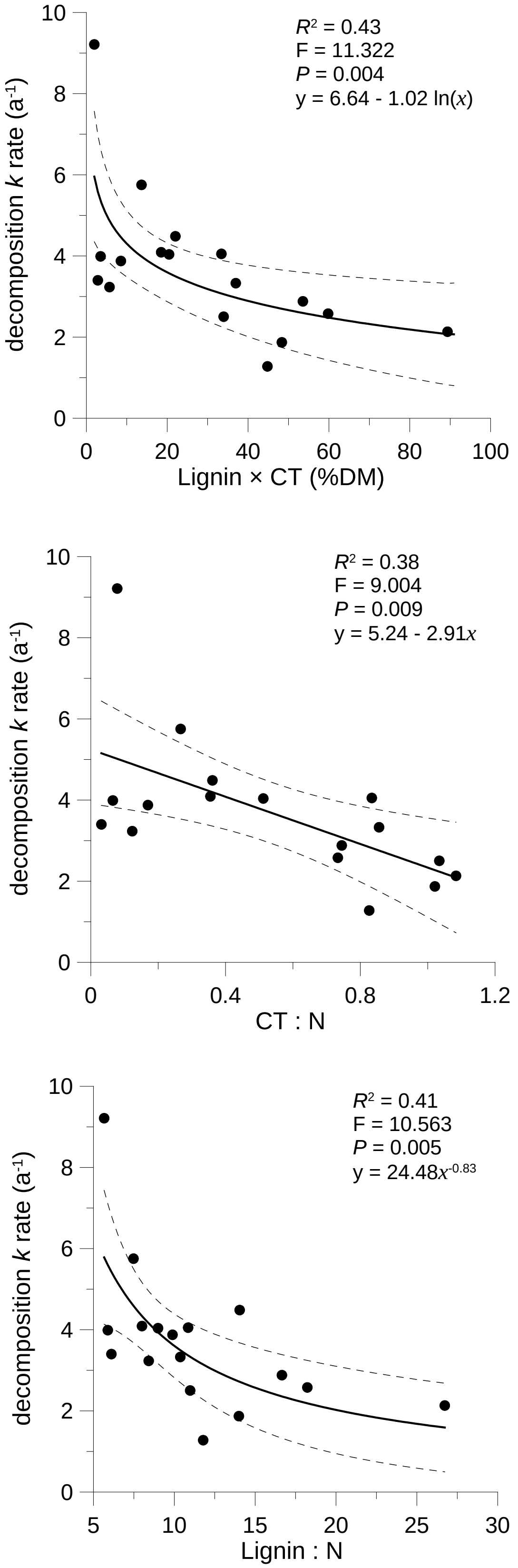

Fig. 2. Cárdenas *et al*.

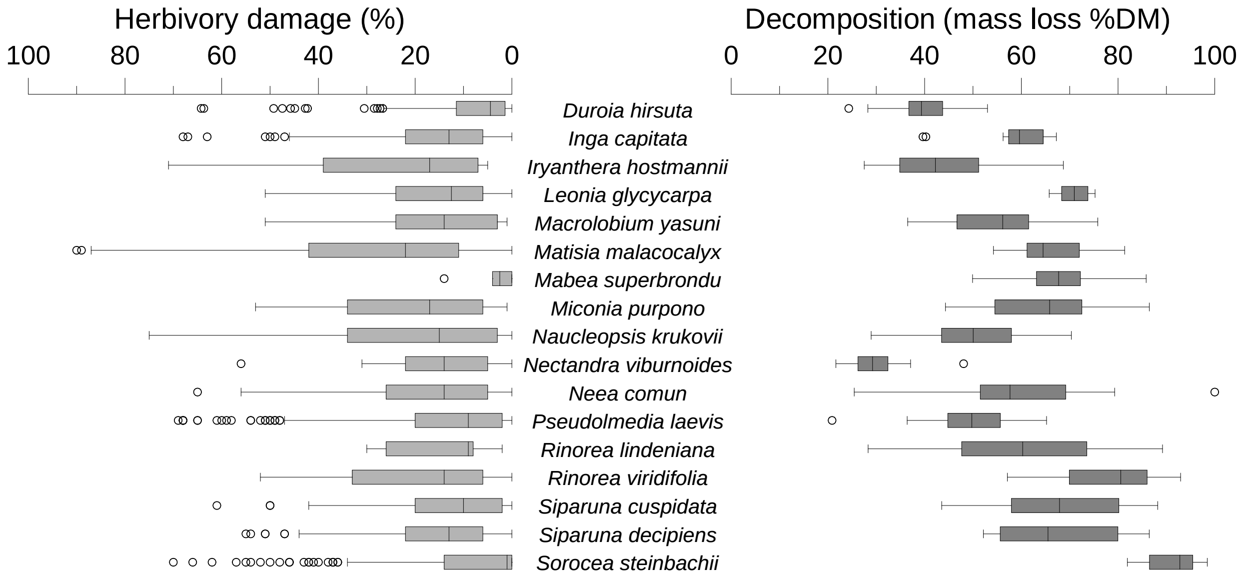

Fig. 3. Cárdenas *et al*.

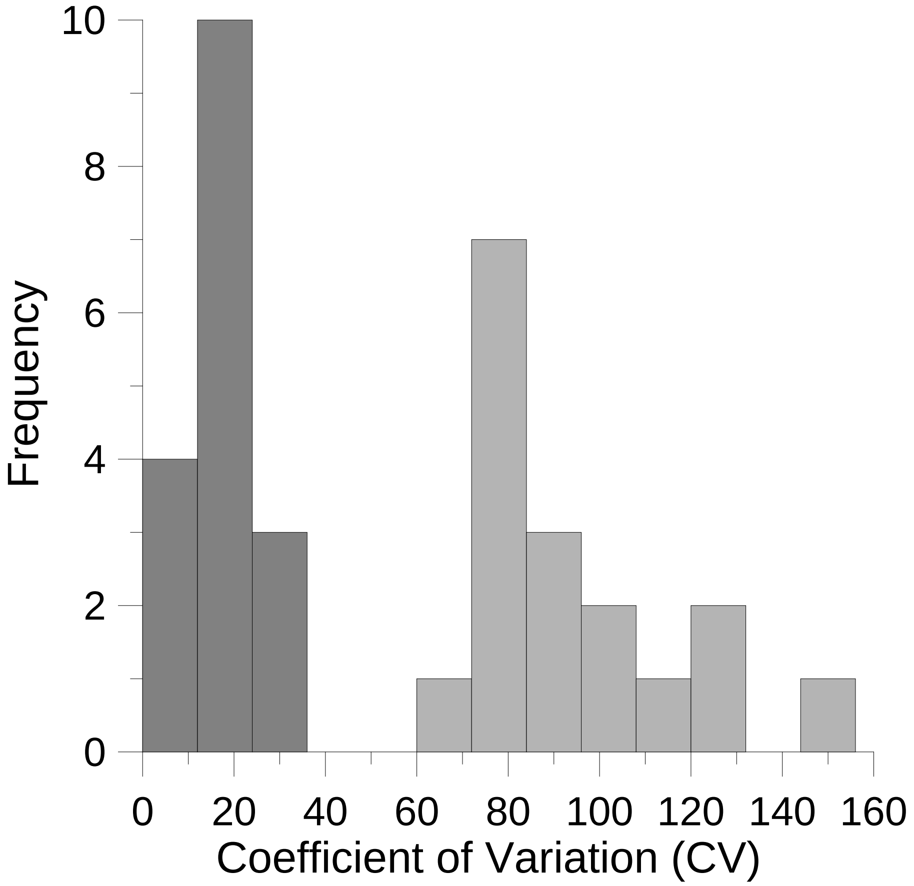

Fig. 4. Cárdenas *et al*.

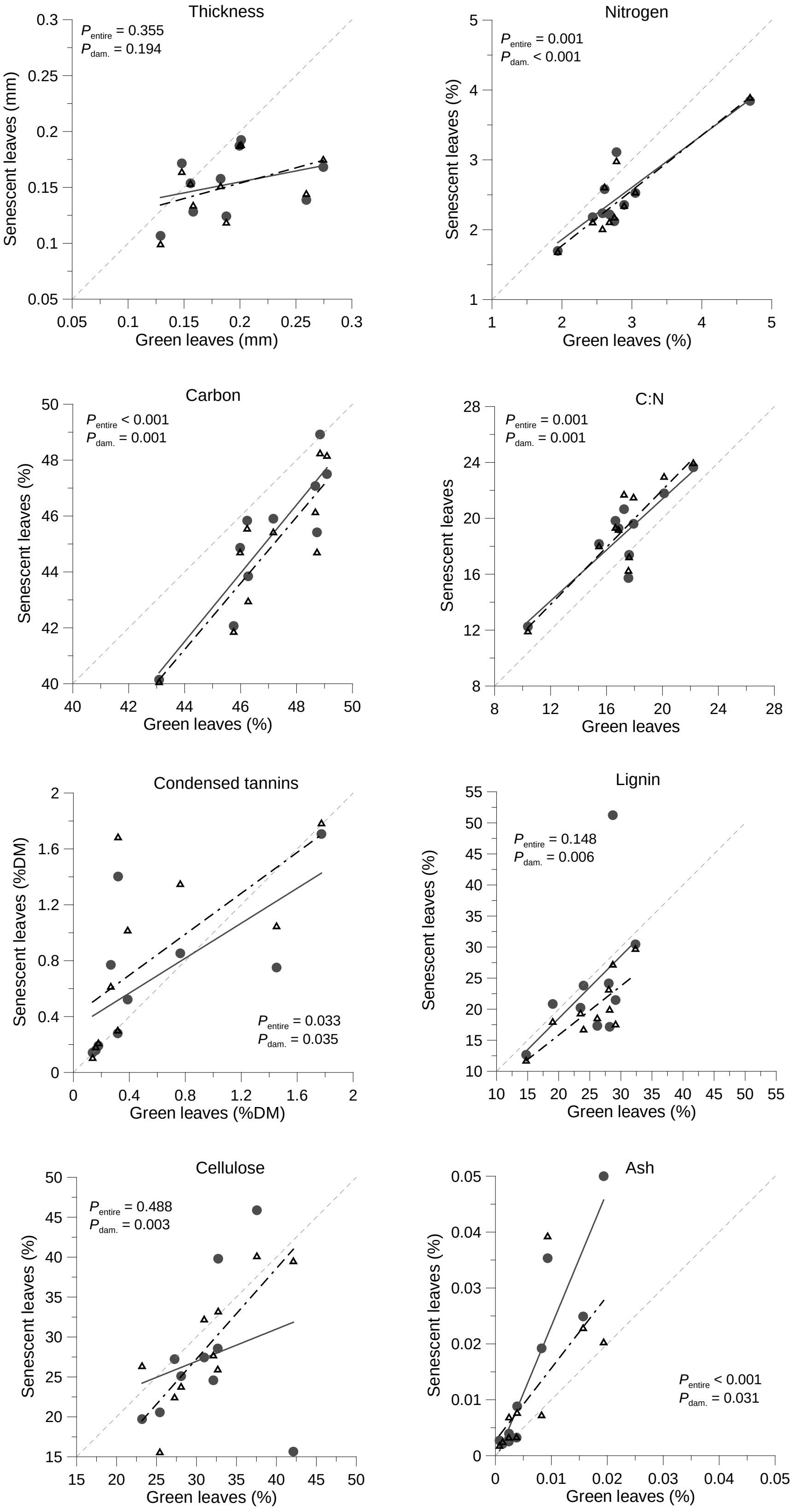

Fig. 5. Cárdenas *et al*.

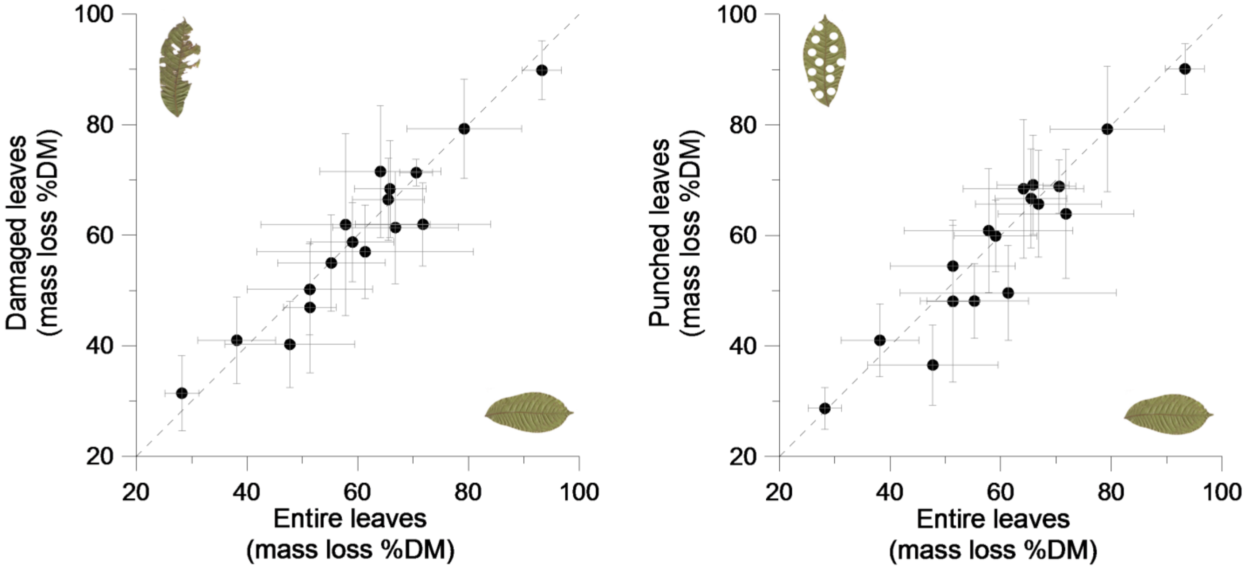

Fig. 6. Cárdenas *et al*.

Supporting information

Table S1. Annual decomposition *k* rates, mass loss during the 100 days of experimentation and calculated annual mass loss for entire and damaged (Dam.) leaves. Kolmogorov-Smirnov test was used for comparing the annual *k* rates between the three treatments (data not adjusted to normality). t-test was used for comparing both mass loss results (data adjusted to normality). No significant differences were found between the three treatments for any of the decomposition parameters ($P > 0.05$).

species	***k* rates (a^{-1})**			**Mass loss$_{100}$ (%DM)**			**Mass loss(a^{-1}) (%DM)**		
	Entire	Punched	Dam.	Entire	Punched	Dam.	Entire	Punched	Dam.
Duroia hirsuta	1.83	1.86	1.91	38.16	41.02	40.99	83.95	84.48	85.22
Inga capitata	3.24	3.30	3.22	59.07	59.91	58.75	96.10	96.30	96.01
Iryanthera hostmannii	2.35	1.68	1.91	47.72	36.56	40.23	90.51	81.32	85.18
Leonia glycycarpa	4.44	4.13	4.53	70.56	68.85	71.30	98.82	98.38	98.92
Matisia malacocalyx	3.92	3.89	4.06	65.50	66.68	66.44	98.02	97.95	98.27
Mabea superbrondu	3.90	4.38	4.20	65.84	69.16	68.35	97.98	98.75	98.50
Macrolobium yasuni	2.89	2.41	2.87	55.24	48.13	54.97	94.45	91.01	94.33
Miconia purpono	4.19	4.09	3.56	66.81	65.69	61.37	98.49	98.33	97.15
Naucleopsis krukovii	2.64	2.84	2.51	51.35	54.45	50.18	92.90	94.15	91.87
Neea comun	3.68	2.47	3.12	61.34	49.59	56.99	97.49	91.58	95.56
Nectandra viridifolia	1.21	1.23	1.34	28.22	28.70	31.40	70.30	70.87	73.86
Pseudolmedia laevis	2.62	2.36	2.38	51.36	48.10	46.91	92.76	90.58	90.74
Rinorea lindeniana	3.22	3.36	3.44	57.80	60.86	61.91	96.00	96.54	96.79
Rinorea viridifolia	5.71	5.72	5.79	79.24	79.24	79.25	99.67	99.67	99.69
Siparuna cuspidata	3.77	4.38	4.41	64.10	68.45	71.51	97.70	98.75	98.78
Siparuna decipiens	4.55	3.79	3.52	71.79	63.91	61.96	98.95	97.75	97.05
Sorocea steinbachii	10.02	8.62	8.41	93.28	90.14	89.83	100.00	99.98	99.98

Table S2. ANOVA test of the simple linear regression models of the relationship between senescent entire and damaged leaves with plant leaf traits. (C:N = carbon:nitrogen ratio; CT = condensed tannins).

Leaf trait	**entire leaves**			**damaged leaves**		
	R	***F***	***P***	***R***	***F***	***P***
thickness	0.327	0.961	0.355	0.448	2.009	0.194
nitrogen	0.888	29.696	< 0.001	0.906	36.748	< 0.001
carbon	0.894	31.718	< 0.001	0.870	24.896	0.001
C:N	0.871	25.208	0.001	0.875	26.253	< 0.001
CT	0.674	6.654	0.033	0.666	6.393	0.035
lignin	0.492	2.561	0.148	0.791	13.334	0.006
cellulose	0.249	0.529	0.488	0.836	18.539	0.003
ash	0.920	44.384	< 0.001	0.678	6.802	0.031

—CHAPTER 4—

Structure and function of soil detritivore communities in a lowland tropical rainforest

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Running headline: Soil invertebrate biodiversity and decomposition

Abstract

During the last 20 years, the accelerated rates of global biodiversity loss have intensified the need of understanding the role of organisms on the functioning of ecosystems and the services they provide. Decomposition of dead organic matter (OM) is a key ecosystem function that ensures soil formation, nutrient availability, and carbon sequestration. However, how biodiversity loss in natural communities of soil detritivores may affect OM decomposition process remains poorly understood. In order to explore the effect of biodiversity on the decomposition process in a tropical rain forest, we (1) provide a detailed description of soil fauna community structure and function in the study area, and (2) explore the functional role of this community by setting an exclusion experiment where we manipulated the accessibility of soil detritivore size-classes to eight types of plant leaf litter resources. Our results revealed ants and collembolans (both leaf litter transformers) were the most abundant soil taxa, and that gradual decreases in the number of species and functional groups significantly reduced the decomposition rates of two of the eight leaf litter treatments. When considering the pooled data, we found a barely significant ($P = 0.058$) positive linear relationship between detritivore size community and the percentage of leaf litter mass loss. Our results suggest that the different detritivore size-classes have a complementary effect on the decomposition process in this ecosystem. We concluded the extinction of larger invertebrates may not necessarily represent a challenge for the decomposition process in Yasuní, but this must not imply they are not essential for the correct functioning of this tropical ecosystem.

Key words: belowground, detritivores, decomposers, Ecuador, extinction order, invertebrates, Yasuní National Park

Introduction

Compelling evidence shows that biodiversity loss disrupts the functioning of ecosystems directly undermining ecosystem services, and ultimately affecting human well-being (Cardinale *et al*. 2012; Díaz *et al*. 2006). Central to this issue, biodiversity and ecosystem functioning (BEF) research seeks to determine how species diversity is related to the magnitude and stability of ecosystem processes (Griffin *et al*. 2009). Three tenets of BEF are well understood. Biodiversity is known to improve productivity (Tilman *et al*. 1996), stability (Tilman *et al*. 2006) and to enhance the magnitude of a variety of ecosystem processes (Balvanera *et al*. 2006; Hooper *et al*. 2005). However, biodiversity effects on ecosystem functioning may vary among ecosystem types and study groups, and depends on the trophic relationships therein involved (Schmid *et al*. 2009).

One key ecosystem function is decomposition of dead organic matter (OM) (categorize as a supporting service in the Millenium Ecosystem Assessment 2005) that ensures soil formation, nutrient availability for plants and carbon sequestration (Chapin *et al*. 2002). It relies on several factors, such as climate (Wall *et al*. 2008), the physical and chemical properties of dead OM (Hättenschwiler *et al*. 2011; Kaspari *et al*. 2008), the sequential action of soil invertebrates, fungi and bacteria (Pramanik *et al*. 2001) and biodiversity of both dead OM (e.g. plant litter) and soil consumers (Dangles *et al*. 2012; Gessner *et al*. 2010; Hättenschwiler & Gasser 2005).

Much of BEF research has largely focused in the role played by invertebrate fauna (detritivores) on the decomposition of leaf litter in both stream and terrestrial habitats (Gessner *et al*. 2010). Detritivore biodiversity has proven to be critical to the biogeochemical and ecological functioning of terrestrial ecosystems having consequences in fertility, plant growth, environmental structure and carbon storage (Brussaard *et al*. 1998). However, we know little on how detritivore biodiversity loss may affect leaf litter decomposition and other ecosystem processes (Wall *et al*. 2010). Especially in real field conditions and in the tropics where studies manipulating the effects of detritivore diversity on leaf litter decomposition are virtually non-existent (Schmid *et al*. 2009) in spite that macro- and meso-detritivore fauna play a crucial role in fragmenting dead organic matter (Swift *et al*. 1979). For example, in an Ecuadorian cloud forest, Cárdenas & Dangles (2012) found a decline of 50% on leaf litter decomposition rates when preventing the access of both macro and meso-detritivore fauna using mesh-bags exclusion experiments. Likewise, Coq *et al*. (2010) found a decline of 17.4% and Yang & Chen (2009) of 40% in leaf litter decomposition rates in French Guiana and in tropical China respectively when preventing the access of macro-detritivores. Furthermore, factors such as land use, nitrogen enrichment, acidification and climate change have been reported to alter soil and streams detritivore diversity (Gessner *et al*. 2010). Moreover, the loss of key species and trophic groups, such as symbionts, predators, shredders, fungivores, root feeders and bioturbators, may have far-reaching and unpredictable consequences for ecosystem functioning (Wall *et al*. 2010).

Experiments that manipulate the detritivore diversity are of extremely importance for understanding the consequences of their potential exctinction in decomposition process. However, one specific problem in soil biology, and especially in the tropics, is to deal with diverse and complex groups such as soil fauna. Especially in these mega diverse systems, where most species (> 80% of all invertebrates of tropical forests) have yet to be described by science, and almost nothing is known of the remainder’s ecology (Primack & Corlett 2005; Wall *et al*. 2010). Ecosystem processes are a product of multiple biological and environmental variables (Petchey *et al*. 1999), reason why more realistic experiments, ideally in natural conditions, are encouraged (e.g. O’Connor & Crowe 2005). Biodiversity that may be coarsely defined as the variety of life includes variation among genes, species and functional traits (Cardinale *et al*. 2012). In this context, one potential approach to biodiversity is to use body size (equivalent to body mass) as a key functional trait of species (Reiss *et al*. 2011). Body size may reflect the mass-dependent metabolic needs of an individual or a species community thus predicting the impact on a given ecosystem function when their natural abundances are submitted to drastical changes (Reiss *et al*. 2009). This is of extremely importance when one considers that large species are especially vulnerable to various types of environmental perturbations, including climate change (McKinney 1997; Sheridan & Bickford 2011), habitat fragmentation (Klein 1989) or land use (McCracken & Bignal 1998). Moreover, recent research has proven that smaller species are not simply miniature copies of larger ones (due in part to mass-specific metabolic constraints, Reiss *et al*. 2011) suggesting that many animal size-classes are needed to maintain ecosystem functioning (Dangles *et al*. 2012).

Invertebrate soil bio- and functional-diversity is practically unexplored in the Amazonian tropical ecosystems (Moreira *et al*. 2008; Primack & Corlett 2005). In order to evaluate a realistic effect of biodiversity loss (i.e. body-size and feeding habits functional traits) on the leaf litter decomposition process we found indispensable to first present a detailed description of the soil fauna diversity of Yasuní National Park (Amazonian tropical rain forest, Ecuador). Then, to reveal whether large, medium and small species are complementary in terms of efficiency on exploiting a particular resource, we set an exclusion experiment where we manipulated the accessibility of soil detritivore size-classes to eight types of plant leaf litter resources (analogous to a removal experiment, see Díaz *et al*. 2003).

Material and methods

Study site

The Yasuní National Park (YNP) and the adjacent Waorani Indigenous territory cover 1.6 million ha of forest and form the largest protected area in Amazonian Ecuador (~17.7% of the Ecuadorian Territory; Valencia *et al*. 2004) harboring the world's most diverse tropical forests (Bass *et al*. 2010). YNP is an evergreen lowland wet forest ranging in altitude from 200 m to 300 m above sea level. It has a 15–30 m canopy with some emergent trees reaching 50 m (Dangles *et al*. 2012). Rainfall and temperature are aseasonal with a mean annual rainfall of 2826 mm (none of the 12 calendar months averaging < 100 mm) and a mean monthly temperature ranging from 22 to 32°C (min: 16.9; max: 38.9°C) (see Valencia *et al*. 2004 for details; data obtained from Yasuní Research Station (YRS) meteorological station, http://www.yasuni.ec). The study area was located in the vicinity of YRS of the *Pontificia Universidad Católica del Ecuador* in an extent of ~4000m^2 in the forest floor in 'ridge-slope' -type microhabitats (see Valencia *et al*. 2004 for a detailed description of microhabitats designation).

Soil detritivore biodiversity survey

We sampled detritivore communities potentially involved in the fragmentation of leaf litter material from the humic leaf litter layer using two commonly used and complementary sampling methods: pitfall traps and Winkler extraction. For the first method, we set a nested rectangular grid of six different spatial scales (smallest scale: 10 × 5 m; largest scale: 1000 × 500m) across the forest floor. At each of these scales we sampled four plots (one in each corner of each scale) for a total of 24 plots (Appendix 1). Each plot consisted of one pitfall trap which remained open for 24 hours. Pitfall traps consisted of plastic cups of 5 cm in diameter and 10 cm in depth and were buried to soil level. A total of 20 Winkler extractions (from 1 m^2 of soil leaf litter) were performed in a 200 m transect separated by 10 m between each other following ALL-protocol (Ants of the Leaf Litter, see Agosti & Alonso 2000 for details).

Species identification and functional group allocation

For identification, specimens were examined under the stereoscope at 0.68X–50X (Leica M275, Leica Microsystems AG, Wetzlar, Germany), separated to the finest taxonomic group as possible using specialized literature, and counted (Appendix 2). When a morphospecies was recognized for the first time, a lateral, dorsal and ventral image was taken using an adaptable digital camera (Future Optics Sci. & Tech. Co., Ltd, 1.3 MP, MEM1300 model, Hangzhou, China). This image served for comparisons every time any similar specimen appeared in the collection. Larvae of holometabolous insects could not be associated to any adult species, so they were classified into different morphospecies. In the case of hemimetabolous, when nymphs showed structural differences (but not color differences) to any adult morphospecies, they were assumed as new ones. When available, long, width and height were measured for up to 10 specimens of the same morphospecies for having more accurate morphometric dimensions of morphospecies. Finally, one or more functional group categories were assigned to each morphospecies based on Moreira *et al*. (2008) classification: herbivores, ecosystem engineers (Boze *et al*. 2012; Jones 2012; Jones *et al*. 1994; Lavelle *et al*. 1997), litter transformers, decomposers, predators, microregulators and soil-borne pests and diseases (primary producers, microsymbionts and prokaryotic transformers categories were not part of our collection target). Feeding habits were determined using specialized literature and internet resources (e.g. Brandão *et al*. 2012; Gillot 2005; Triplehorn & Jonson 2005; http://soilbugs.massey.ac.nz/index.php; http://www.collembola.org/). Scolytines (Coleoptera) were considered ecosystem engineers because of their digging holes behavior that physically changes its surrounding environment allowing the access of subsequent decomposers (Muller *et al*. 2002). Although Acari taxonomic order represents an important group in the soil food web, we were unable to discriminate specimens at morpho-species level and accurately assign them to any of the many functional groups they could possibly belong. Appendix 3 shows however independent analyses of this rich and complex group.

Leaf litter collection

Leaves were collected from sub-adult trees of eight common angiosperm species (most aboveground production involves angiosperm trees, Kurokawa & Nakashizuka 2008) belonging to eight different families: *Matisia malacocalyx* (Bombacaceae), *Inga capitata* (Fabaceae), *Nectandra viridifolia* (Lauraceae), *Miconia purpono* (Melastomataceae), *Siparuna decipiens* (Monimiaceae), *Pseudolmedia laevis* (Moraceae), *Neea comun* (Nyctaginaceae), *Leonia glycycarpa* (Violaceae). These species and families were chosen in order to represent a wide range of intrinsic chemical and physical traits. Collection consisted on 5–20 shaking repetitions of 2 to 7 trees from the trunk or branches. Leaves fell on white fabric sheets (cotton, 1.5m × 3.5m) that were tied at 1 m height from the floor. Leaves characteristically young (i.e. presenting bright green or reddish colors and/or soft lamina) too old (i.e. rotten or presenting large amounts of necrosis), or presenting evident fungi infection or insects galleries, were discarded. For the experiment we collected only senescent leaves with herbivory damaged < 30% of the leaf area.

Soil detritivore exclusion and decomposition experiments

To assess the functional importance of soil detritivore community, we designed an exclusion experiment in *terra firme* using 20 cm diameter plastic and polyester fabric leaf mesh-bags with five different mesh sizes that allowed the access/exit of different detritivores/decomposers size groups (see Swift *et al*. 1979 for soil microflora and fauna size classification): 268.8 mm^2 (15.2 × 17.7 mm; micro, meso, macro and megafauna), 118 mm^2 (10 × 11.8 mm; micro, meso and macrofauna), 16.1 mm^2 (3.2 × 5.1 mm; micro, meso and macrofauna), 2.7 mm^2 (1.1 × 2.5 mm; micro and mesofauna) and <0.01 mm^2 (~0.1 × 0.1 mm; microfauna). *Peciolum* of every leaf was removed and leaves were placed to dry at 40°C for 48–72 hours in cotton fabric bags (containing no more than 10 leaves *per* bag) and weighed to 0.001 precision (FA2104N, Ningbo Utech

International, Ningbo, China). The leaves were remoistened using rain water to make them pliant, and enclosed the mesh-bags.

The experiment consisted on testing the leaf decomposition rate of the eight mentioned species in the same period of time and area, but in different mesh-bags types. In total we analyzed the decomposition process of 8 (species) × 5 (treatments) × 10 (replications) × 5 (leaves *per* species, or 2–4 depending on the leaf size), that was 400 leaf litter mesh-bags and up to 2000 leaves placed randomly in the study area. Mesh-bags were set in groups of five treatments with species previously shuffled, in a 50 × 80 m grid-type plot (Appendix 1). After 104 days of decomposition process (mass loss of ~58% in average for the same eight species after 103 days, see Chapter 2) mesh-bags were collected for analyses. In the laboratory, leaves from each litter bag were gently cleaned to remove soil particles, adhering debris, and invertebrates, then dried at 40°C for 2–4 days, and weighed (FA2104N, Ningbo Utech International, Ningbo, China).

Data analyses

Sampling efficiency evaluation

Rarefaction ‘sample-based’ accumulation curves (Sanders 1968) were analyzed in order to assess whether pitfall traps and winkler extractions reached an asymptote in terms of species richness and functional groups. For this we used Past v.2.17 software (Hammer *et al*. 2001) that implements the analytical solution known as "Mao tau" following Colwell *et al*. (2004) where standard errors are transformed in ±95% confidence intervals. Sample-based rarefaction curves implicitly reflect empirical levels of within-species aggregation of individuals by considering only incidence, thus providing a realistic estimate of the number of species to be found in sets of real-world samples (Gotelli & Colwell 2001).

Describing the soil fauna community

General description

We first described the community structure of Yasuní soil fauna (Appendix 2) using rank plots (Magurran 2004) at order and morpho-species levels in relation to their abundance and mass-volume measures (volume corresponded to body height × width × long). Then, following Preston's (1948) boundaries of octaves as a measure of commonness degree, we classified the number of species in relation to its abundance in eight categorical ranges, and finally fitted data to log-normal distribution using Table Curve 2D software v.5.01. Distribution of the mass volume and body width morphometrics of the soil community were finally plotted in relation to specimen frequency.

Soil functional groups cluster analysis

Groups of organisms in the invertebrate community overlap in terms of feeding habits and body sizes, hence in their functional role and impact extent in soil ecological processes. A cluster analysis was performed to statistically classify the different functional groups found in the forest floor. For this, we used morphospecies body length, width and height, and 0-1 binary data of the seven functional group categories from Moreira *et al*. (2008). Ten groups of species were defined using Gower distance (minimum spanning tree) and Ward linkage (minimum variance) as statistical methods. We chose Gower's distance as it allows mixed scale types of data (quantitative, interval, nominal or ordinal, ratios and/or missing values) and has proven to consistently provide the best results (Mouchet *et al*. 2008). Ward method was chosen as, comparing to average linkage, it produced the more clearly defined clusters (Pla *et al*. 2012). Cluster analysis was performed using InfoStat software with default program data standardization (Di Rienzo *et al*. 2012). Finally, we manually separated one additional group belonging to the 'omnivores' cluster that was specifically represented by omnivore ants because of their particular impact on ecosystem processes (Blüthgen *et al*. 2003). Cutting at a distance of ~5.48 did not affect the other 10 clusters. Bio-volume distribution of the clusterized functional groups was finally plotted to better appreciate the spreading in body size of the invertebrate community.

Spatial heterogeinity analyses

In order to assess whether soil fauna diversity was aggregated or not, we compared both individual- and sample-based rarefaction curves by plotting them together. Gotelli & Colwell (2001) explain when the sample-based curve lies below the individual-based curve one can assume aggregation of species.

Considering soil detritivore fauna only (i.e. litter transformers, see below), we compared every collection unit content (pitfall trap or Winkler extraction site) to one another using similarity and distance indexes (Hammer *et al*. 2001) to evaluate whether there soil litter transformer communities were patchy-distributed or not . Sørensen similarity coefficient was used for species-area analysis using 0-1 binary data of presence-absence in the traps. Bray-Curtis similarity index was used for abundance– and biomass-area relationships using real data. Finally, functional groups distribution was analyzed using Hamming distance index with categorical data.

Size-dependent litter decomposition

Body width is probably the most important morphometric parameter that firstly discriminates the access to the leaf litter resource through the mesh treatments (width:height average ratio of the litter transformers community = 1.24). In a first step we described the distribution of this parameter in relation to the number of species, its abundance and community biomass. Then, we assess the biomass (N × mm^3) of litter transformers that potentially had access to the different mesh treatments.

Our mass loss (%) per mesh size (mm) data were fitted to power or linear regression models (Cardinale *et al*. 2006) for every species (individually and pooled) and mesh-size treatments. We plotted the power or linear regression that better fitted to the data in terms of P values. Regressions were performed using Table Curve 2D software v.5.01. k-rates (a^{-1}) were compared among treatments using Kruskal-Wallis test with Mann-Whitney pairwise comparisons (Bonferroni corrected) using Past software v.2.17 (Hammer *et al*. 2001).

Results

Soil invertebrate community structure

In the soil detritivore community, hymenopterans (predominantly ants), collembolans (mostly Hypogastruridae/Neanuridae and Entomobryidae) and coleopterans (largely characterized by Staphylininae subfamily and other Curculionidae) were the most abundant groups represented by 1095, 880 and 678 individuals, respectively. Lepidopterans (larvae), neuropterans (Myrmeleontidae) and diplurans were on the other hand the less frequent groups of invertebrates collected (Figure 1A, Appendix 2). At the morphospecies resolution, the species rank abundance (SRA) plot showed the classical distribution of natural (pristine) environments with very few common species, some moderately common, and a great majority rare Figure 1B). When SRA was plotted in a $\log_2$ scale, data fitted significantly to a lognormal distribution ($P = 0.002$; $R^2 = 0.97$, Figure 1C).

The species rank of the invertebrates mass volume, showed a ‘hollow curve’ distribution (log normal fit: $P < 0.001$, curve no shown) indicating that invertebrate community was composed of relatively few large species and many small ones (Figure 2A). In terms of mass volume and body width abundance frequency, distributions resulted slightly left-skewed bell-shaped (Figure 2B and

2C) although were not normally distributed (Shapiro-Wilk normality tests: $W_{volume} = 0.969$, $P < 0.001$; $W_{width} = 0.963$, $P < 0.001$).

Sampling efficiency

Rarefaction curves showed that none of the collection methodologies (either Pitfall or Winkler) collected a sufficient number of species to reach an asymptote (Figure 3A). Overall, Winkler collections were significantly more efficient in terms of the number of species collected per number of samples in a smaller area. At 20 samples stop vertical line, curves and their ± 95% confidence intervals did not overlap (see Figure 3A). However, both methodologies were complementary concerning the identity of the invertebrates collected. For example Pitfall traps were highly represented by four morphospecies: *Gnamptogenys* sp.2, Scolytinae sp.1, *Polydesmida* sp.2 and Entomobryidae sp.5. Winkler extractions on the other hand were highly represented by Pseudoscorpionida sp.1, Proteininae sp.2, Cecidomyiidae sp.3 and *Hylomyrma* sp.1 (results not shown). Both methodologies needed a relatively small number of samples (~7) to characterize the total number of functional groups in a relatively small sampling area (i.e. Winkler extractions were performed in a 200 m transect) (Figure 3B).

Soil invertebrate functional groups characterization

Cluster analysis discriminated four main functional group classes in the invertebrate community (cophenetic correlation = 0.546): litter transformers (LT), omnivores (O), predators (P) and pest-diseases-predators-parasites (Pd), divided in 11 sub-classes (Table 1 and Figure 4). From these, 'litter transformers and microregulators' (LT4) were the most speciose and abundant represented by 63 species and 920 individuals (14.8% and 27.2% respectively), followed by 'general predators' (P1) with 54 species (12.7%) and omnivores 'ants' (O2) with 464 individuals (13.7%) (Table 1).

‘Litter transformers and ecosystem engineers’ (LT2) were those with the higher total biomass with 1275.12 mm^3 (42.7%) followed by ‘litter transformers’ (LT1) with 593.39 mm^3 (19.9%) (Table 1). Mass volume data showed a rough sized-distribution of different functional groups where Pd classes were frequently small-sized, O and P classes medium/large-sized, and LT classes occupied the whole size spectrum from the smaller to the larger specimens (Figure 5). LT2 and LT1 were the biggest classes in terms of volumetric mass with an average of 667.4 mm^3 and 384.7 mm^3 respectively (no $\log_{10}$ transformation values, Figure 5).

Soil invertebrate spatial heterogeinity

Comparisons of samples- and individuals-based rarefaction curves of both Pitfall and Winkler extractions showed species in the forest appeared to be aggregated at smaller sampling spatial scales (i.e. Winkler, Figure 6A) although this pattern did not persist at larger sampling spatial scales (i.e. Pitfall, Figure 6B).

Similarity and distance indexes showed that the invertebrate community was heterogeneous in terms of species diversity, its abundance, the functional groups and biomass, and that such heterogeinity was independent of the spatial scale (Figure 7). That is, the same levels of heterogeinity may be found in small as well as in large sampling areas. We must emphasize that community biomass distribution was the most variable of all the parameters analyzed (Figure 7D).

Leaf litter transformer communities

Metrics description and accessibility to mesh-bag treatments

Concerning the ‘litter transformers’ functional communities (LT1–LT4), we found that the great majority of them were ‘thin’ belonging to the first octave of the whole body width ranges (Figures

8A–B). As biomass was highly related to individual body width results showed a strong positive association between both parameters (Figures 8C). Moreover, invertebrates between 12000-16000 µm body width (the last two octave ranges) presented almost identical biomass measures (Figures 8C). Figure 9 showed that different classes of biomass LT had different accessibility to resources through the five mesh treatments, except for T4 and T5 that were potentially accessed by virtualy the same LT community (Table 2). T1 accessibility was of 14.8% and 5.16% of the total LT species and abundance respectively, represented by only LT4 class (mostly collembolans, Table 2). T2–T5 were potentially accessed by all functional groups and by > 80% and 94% of species and specimens respectively (see Table 2 for details).

Detritivore body mass and leaf litter decomposition

Power regressions were significant for *Miconia purpono* ($P < 0.05$, $R^2 = 0.98$) and *Nectandra viburnoides* ($P < 0.05$, $R^2 = 0.98$), and barely significant for the pool of species computed together ($P = 0.058$, $R^2 = 0.75$) (Figure 10). All the species, except *Neea comun*, *Pseudolmedia laevis* and *Siparuna decipiens* showed a positive relationship between more LT accessibility and percentage of mass loss. This tendency persisted for the pool of species as well (Figure 10, Table 3). Mass loss variability showed to be independent on the mesh treatment, except for *M. purpono* (and in smaller proportion *P. laevis*) where the biggest the hole size, the biggest the mass loss variation. Finally, we found biomass decompositon were not significantly different between mesh treatments (Appendix 4, pooled data).

Discussion

The soil invertebrate community

Soil organisms are essential for the functioning of terrestrial ecosystems (e.g., nutrient cycling, soil formation, and soil aeration), however just as in the oceans, most of it is yet to be discovered about their exact functional role and importance of species diversity in tropical soils (Wall *et al*. 2010). Our results showed a predominant abundance of hymenopterans (mainly ants), collembolans and coleopterans (mostly bark beetles and weevils). These three groups are heterogeneous in terms of feeding habits and may occupy a wide range of niches in the forest food web. Ants represent one of the most animal diverse and ecologically dominant groups, and are extremely important in terms of biomass and relative local abundance (Wilson & Hölldobler 2005). Nutritional biology of ants could be wide-ranging including predators, leaf cutters, fungus growers, sap feeders, pollinivorous, saprophytes and generalists (Brandão *et al*. 2012). Recent studies suggest that niche diversity drives ants' specialization and supports high species diversity in the neotropics (Ryder Wilkie *et al*. 2010; Vasconselos & Vilhena 2006). Comparing to ants, collembolans belong to less diverse niches and includes saprophagous, fungivorous –including some spore feeders–, phytophagous –including pollen feeders–, and very rarely predators (Gillot 2005). However, they also represent one of the most abundant terrestrial arthropods globally, and despite their relatively low biomass, they are extremely important in influencing the structure of soils (Hopkin 1997). Their main contributions consist on the regulation of fungal populations and the enhancement of mycorrhizal functioning improving plant growth (Gange 2000; Hopkin 1997). Bark beetles are principally woodborers where they typically construct tunnels facilitating fungal colonization in decaying wood (Muller *et al*. 2002), subsequent bacterial access (de Boer *et al*. 2005), and further OM decomposition. Weevils on the other hand are mostly phytophagous (sap suckers) at both above- and belowground (i.e. leaves and roots), and their diet may also include fresh and decaying fruits as well as seeds

(Triplehorn & Jonson 2005). These three abundant groups belong to the meso- and macrofauna classification of Swift *et al*. (1979) who fragment (ecosystem engineering) and ingest litter coated with microbial biomass producing large amounts of fecal material which is more favorable for decomposition (Lavelle *et al*. 1997; Hopkin 1997).

Further in the analysis of diversity, our ranked abundance plots fitted significantly to a lognormal distribution, agreeing with other large-scale invertebrates samplings in the neotropics, indicating a few very abundant species and many rare species (Longino *et al*. 2002; Ryder Wilkie *et al*. 2010). Unlike other popular models such as the geometric model that predicts extremely uneven abundances, or the broken stick that predicts extremely even abundances, lognormal is intermediate (McGill *et al*. 2007). It is supposed to describe the distribution patterns of natural communities in pristine ecosystems (could be considered as a biological model) based on the assumption that abundance of species in an ecological community are proportional to the resources they allocate (i.e. niche apportionment; Magurran 2004). Although discussions on the use of species abundance distribution models are still a hot debate (there have been described dozens of them), many are intended to provide significant insights into basic and applied ecological science (McGill *et al*. 2007).

With respect to the distribution of community body-sizes, our mass volume species rank fitted significantly to a log-normal distribution revealing that soil community size structure at species level was composed of few voluminous species and many small ones. Litter transformers community followed the same tendency. Moreover, mass volume and body width abundance frequency distributions resulted slightly left-skewed bell-shaped (but not normally distributed). This is, peak species richness occurred at the intermediate body size that led to the maximum number of individuals, coinciding with other large-scale samplings (e.g. Jonsson *et al*. 2005; Siemann *et al*. 1999). These distributions may respond to ecological and evolutionary features where community size patterns correlate to the number of taxonomical groups. Siemann *et al*. (1996) found similar biovolume distributions patterns among the most common orders of insects, though they found significant differences (more than 100-fold) between their medians. They suggest that shared physiologies and/or morphologies of a monophyletic group limit related organisms to similar sizes and conclude that there may be a general rule, independent of body size, for the relations among interspecific resource division, abundance and diversity. This contradicts in part however the idea that biomass (and/or total volume) of different species in a community is likely to be a better measure of the apportionment of available resources than relative abundance (Mouillot *et al*. 2003; Poulin 1998). Relationships between species diversity, body sizes and abundances are still not well understood, and future studies must consider individuals (instead of communities) for revealing distribution patterns driving mechanisms (White *et al*. 2007).

Revealing the structure and distribution of soil invertebrate communities

Winkler and pitfall collections did not reach an asymptote, they differed in terms of efficiency (i.e. number of species per sample unit), were complementary concerning the species identity composition, and characterized all the functional groups in a relatively small sampling effort. That is, a more intensive sampling (i.e. time; see Krell *et al*. 2005) is necessary for revaling the real soil food web biodiversity richness in Yasuní. Although both methodologies are actually no directly comparable among them in this study, Winkler extraction seemed to be more efficient for capturing more species in a smaller area, disagreeing with other tropical studies that found pitfall traps were ideal in terms of abundance of the most representative taxa (e.g. Sabu & Shiju 2010). Complementary results of both collection methodologies may be explained by their own particularities. Pitfall traps target invertebrate taxa that are nocturnally active on the soil surface, while Winkler extractions are suitable for capturing leaf litter-inhabiting and rapidly mobile invertebrates (particularly ants and beetles) (Agosti & Alonso 2000; Moreira *et al*. 2008; Sabu & Shiju 2010). Functional groups rapid assessment intuitively suggests that the Amazonian forest floor is upholstered of all kinds of taxa representing multiple behaviors, strategies and feeding habits, suggesting a high rate of redundancy *per* unit of area. In this context, aggregation tests (i.e. similarity and distance indexes, and individuals/samples rarefaction plots) showed an 'even' heterogeinity of soil fauna at all the levels (species, abundance, functionality and biomass) especially at larger areas (see Figure 6), reinforcing the idea of redundant taxa and functionality at all spatial scales. The relationship between species and functional diversity remains poorly understood for most of the ecosystems around the world (Micheli & Halpern 2005). Some studies however, in other highly diverse (aquatic) environments, have shown low levels of redundancy (Bellwood *et al*. 2003) or high levels of functional redundancy but low of functional diversity (Strauß *et al*. 2010) which has direct implications on the impact of biodiversity loss in the ecological resilience of ecosystems (Peterson *et al*. 1998; Reich *et al*. 2012). Finally, it remains to test whether redundancy in Yasuní tropical forest is expected to happen at a small temporal and spatial scale only as stable coexistence might to be incompatible with functional redundancy as suggested by Loreau (2004).

Soil invertebrate functional groups

In spite of the wide range of body sizes between and within functional groups originally assigned, and the overlap of feeding habits among them, cluster analysis showed average cophenetic correlation, meaning that clustering result was reasonably representative of the pairwise differences. It was noteworthy that collembolans (and gryllids; LT4) were statistically considered as an "outgroup" in the dendrogram. This could be biologically interpreted as their ecological role and potential impact (i.e. relative to their feeding habits and biomass) are well defined in the food web.

Seastedt (1984) explained that a reasonable generalization about the dominant trophic function of microarthropods (acari and collembolans) is the predominance of fungal-feeders (mycophages). Collembolans mouth parts are capable to fragment organic matter (e.g. leaf litter) while feeding on the microflora adhering to this detritus and increasing leaf litter decomposition rates by 23% in average (based on numerous studies, Seastedt 1984). Remaining LT groups were spreadly distributed in the tree, and closely related to other groups such as omnivores (e.g. LT3 and O3). This might be an effect and a consequence of overlapping feeding habits and the body size range of LTs as was detailed in Figure 5. Predators (P1 and P2) were totally grouped in a separated branch of the tree illustrating their particular ecological position in the soil food web. More importantly, the cluster analysis kept ants (P2) separated from other predators (e.g. arachnids) highlighting its physiological and morphological characteristics that might delineate their particular role and position in this ecosystem (Brandão *et al*. 2012; Philpott *et al*. 2010). In the core of the tree we found omnivore groups (O1 and O2 grouped on one side), and pest-diseases-predators-parasites (Pd1 and Pd2, separated by their body size, Table 1) in a central place (closely related to LT3 and O3). Their central position in the dendrogram coincided with their overlapping feeding habits and body dimensions (Figure 5).

Exclusion experiment

Considering the litter transformer community accessibility to the different mesh treatments, we found that T1 filtered most of the soil fauna in terms of biomass, species, abundance and functional groups. Although the remaining treatments (T2–T5) allowed comminuting by most of the LTs community it was noteworthy that all the treatments, except for T4 and T5, were complementary in terms of biomass that is, resource consumption potential. Body size is one of the primary determinants of metabolism and, therefore, resource use (Brown *et al*. 2004). Biggest individuals need and consume more resources, and the relationship between size and abundance (i.e. biomass accessibility to resources in our experimental set) may also reveal how resources are partitioned in ecological systems (White *et al*. 2007). Our experiment pointed out figuring how resources were exploited following gradual extinction of larger soil detritivores in a natural scenario. Results showed a barely significant complementarity (i.e. linear fit) of the different body size classes of detritivores (for the pool data set averaged), suggesting that species size classes contributed similarly to the functioning of the decomposition process at the leaf litter layer of the forest floor in Yasuní (Naeem *et al*. 2009). However this was not the common pattern for most of plant species used in this experiment. Of the eight plant species, only two presented a significant (positive) regression fit: *M. purpono* and *N. viburnoides*. With our experimental design, we are not able to assess whether these two species were the only exploited by the large-size detritivore community. Moreover, Table 3 showed averaged *k*-rates (a^{-1}) were not significant between mesh-groups, that is as a whole, decomposition rates were independent on the size and biomass of the soil fauna that had access to that resource, suggesting a functional redundancy in the LT community not significantly affecting the time of the decomposition process. This would not to be contradicting the regression fit of the pooled-averaged data on mass loss that predicts a potential complementary function of the LT community instead of redundancy (Figure 10) because these data fitted also to power ($y = ax^b$; $R^2 = 0.66$, $P = 0.096$) and log functions ($y = a + b\ln x$; $R^2 = 0.65$, $P = 0.099$) with a similar fidelity as for the linear regression fit (i.e. $0.05 < P < 0.1$, results not shown). Figure 5 showed LT categories were represented in the entire size-class continuum. That is, there were litter transfomers that could enter into all the mesh treatments (see Table 2), and of these, the tiny Collembola that were particularly abundant (Figure 1) and may have had a significant impact on leaf litter decomposition rates (Seastedt 1984). We conclude, the extinction of larger invertebrates may thus not necessarily represent a challenge for the decomposition process in Yasuní, and conversely, species loss may potentially generate an increment on the diversity and abundance of the smaller as shown by Kunte (2008) after removal of dominant butterfly species in a tropical rain forest. Our results may not imply however that larger invertebrates are not essential for the correct functioning of this tropical ecosystem. Detritivores role in the forests go far beyond their feeding habits as they are prey, and many are essential for soil aeration or act as pollinators (Triplehorn & Jonson 2005) hence, the impact of their potential extinction in the ecosystem functioning remains highly unpredictable because changes in the food web with successive extinctions make it difficult to predict which species will show compensation in the future (Ives & Cardinale 2004). A more extensive experimentation, using more plant species (i.e. that represents a wider spectrum of the forest litter quality mosaic, see Chapter 2 and complementary use of resources concept in Vos *et al*. 2013), may reveal more realistic patterns of leaf litter decomposition as a function of a gradual loss of body-size biodiversity of soil detritivores in Yasuní.

Additional remarks

Results of this experiment lead to many questions when considering non-significant regression relationships between gradual loss of detritivore size categories and leaf litter decomposition: for example we asked ourseleves (1) whether there would be an exclusion competition between small, medium and large-size detritivores for the rest of the plant species leaf litter. The term 'complementarity' suggests non-competitive interspecific interactions with potential positive effect on ecosystem process, but it actually also incorporates all non-additive effects arising from multi-species interactions including interspecific interference competition (Basset & Rossi 1990; Fox 2005; McKie *et al*. 2008). That is, smaller and/or medium-size detritivores might probably not enter into competition in the presence of large-size detritivores. (2) Ecosystem engineering usually occurs after the fragmentation work of big detritivores that facilitate the ingestion/colonization of OM particles by the smaller ones (including micro-flora), but not the inverse (Jonsson *et al*. 2002; Lavelle *et al*. 1997). Future research should consider if larger detritivores may prefer fresh leaf litter for one or more plant species (i.e. no pre-treated by smaller ones, or already invaded by fungi and bacteria), which would also explain in part the broadest variation of OM mass loss of *M. purpono* or *P. laevis* in T4 and T5 mesh treatments. This would make sense if we consider that smaller detritivores are more abundant than larger ones, and that early accessibility to “fresh” resources might be hazardous.

Acknowledgements

We thank the staff of the Yasuní Research Station, in particular the administrative personnel, David Lasso, Carlos Padilla, Miguel Rodriguez and Karla Rivadeneira for constant support during the stays at the Station. Pablo Alvia, for sharing his invaluable plant taxonomic expertise, and help in field and laboratory work. This experiment would have been logistically impossible to perform without him. Jim Self provided guidance and full support for leaf analyses at Soil, Water & Plant Testing Laboratory at CSU. We are grateful to the School of Biological Sciences of the *Pontificia Universidad Católica del Ecuador* (PUCE), in particular to its dean Hugo Navarrete for constant support. The PUCE (project codes I13045 and J13041) and the French Institute for Research and Development (IRD) supported this study through research grants. This study was endorsed by the *Ministerio de Ambiente del Ecuador* permit Flora No. 0001 MAE-DPO-PNY (2012).

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Tables

Table 1. Functional group (FG) categories and sub-categories showing the number of species (S), abundance (N) and biomass (B).

Category 1	FG	Category 2	S	N	B
litter transformers	**LT1**	litter transformers	29	170	593.39
	LT2	litter transformers and ecosystem engineers	47	401	1275.12
	LT3	litter transformers, predators and herbivores	46	213	63.52
	LT4	litter transformers and microregulators	63	920	177.91
	total		**185**	**1704**	**2109.94**
omnivores	**O1**	general	43	186	21.18
	O2	ants	17	464	254.97
	O3	omnivores and microregulators	43	248	12.99
	total		**103**	**898**	**289.14**
predators	**P1**	general predators	54	321	30.40
	P2	ants	36	340	382.90
	total		**90**	**661**	**413.30**
pest, diseases, predators and parasites	**Pd1**	larger organisms	21	63	173.69
	Pd2	smaller organisms	26	54	1.74
	total		**47**	**117**	**175.43**

Table 2. Detail of the total biomass (B), species number (S), abundance (N), litter transformers functional groups (FG) and representative groups potentially involved on the decomposition process of leaf litter resources within the five different mesh-bag treatments. (d = diagonal) (+ = additional litter transformers groups).

	T1 **(~0.1 × 0.1mm)** **(d = 0.14mm)**	**T2** **(1.1 × 2.5mm)** **(d = 2.73mm)**	**T3** **(3.2 × 5.1mm)** **(d = 6.02mm)**	**T4** **(10 × 11.8mm)** **(d = 15.47mm)**	**T5** **(15.2 × 17.7mm)** **(d = 23.33mm)**
B (N×mm^3)	0.002%	4.7%	13.6%	78.8%	“100%”
S	28	167	180	189	189
N	5.16%	94.8%	98.8%	99.9%	“100%”
FG	LT4	all	all	all	all
Representative groups	mostly collembolans	+ isopterans and smaller isopods	+ larger isopods and gryllids	+ large blattids and diplopods	+ larger blattids and diplopods

Table 3. Decomposition *k*-rates (a^{-1}) (averages) of the eight and pooled plant species in relation to the five mesh-bag treatments. No differences of *k*-rates were found among treatments.

MAMA: *Matisia malacocalyx* (Bombacaceae); INCA: *Inga capitata* (Fabaceae); NEVI: *Nectandra viridifolia* (Lauraceae); MIPU: *Miconia purpono* (Melastomataceae); SIDE: *Siparuna decipiens* (Monimiaceae); PSLA: *Pseudolmedia laevis* (Moraceae); NECO: *Neea comun* (Nyctaginaceae); LEGL: *Leonia glycycarpa* (Violaceae).

variable	**T1**	**T2**	**T3**	**T4**	**T5**
			***k*-rates (a^{-1})**		
INCA	2.384	2.519	2.633	2.327	2.533
LEGL	2.587	2.800	3.278	3.214	3.217
MAMA	2.514	1.900	2.600	2.533	3.093
MIPU	1.319	1.394	1.694	2.133	2.344
NECO	2.625	2.583	2.546	2.545	2.467
NEVI	0.986	1.001	1.005	1.060	1.074
PSLA	1.959	1.822	2.107	1.834	1.870
SIDE	2.674	2.659	2.689	2.501	2.624
Pool	1.978	1.947	2.211	2.175	2.196

Figure legends

Figure 1. Rank abundance plots showing (A) the relative distribution of the number of species for major soil animal groups, (B) the variation in the relative abundance of soil animal species ordered from most to least abundant, and (C) the Preston's plot of the number of species *per* $\log_2$ abundance ranges. For detailed information on Acari biodiversity description, refer to Appendix 3. On (C), $\log_2$ series followed Preston (1948) ranges: 2^0 (1–2), 2^1 (2–4), 2^2 (4–8), 2^3 (8–16), 2^4 (16–32), 2^5 (32–64), 2^6 (64–128), 2^7 (128–256).

Figure 2. Distributions of the log mass volume and width of soil fauna in relation to species rank (A) and frequency (B and C). P, F and R^2 values in (A) correspond to the lognormal regression (curve fit not shown). In (B) and (C) frequencies correspond to mass volume and width averages of the species.

Figure 3. Rarefaction curves (simple-based) showing Winkler extraction and Pitfall traps collections in relation to the number of species in (A) and number of functional groups in (B). Vertical grey line indicates the number of samples where both methodologies are comparable. Smooth full or dashed lines correspond to ±95% confidence intervals.

Figure 4. Cluster analysis showing functional grouping of soil fauna communities. The Ward clustering method with Gower distance was used. Group abbreviations: litter transformers (LT), omnivores (O), predators (P) and pest-diseases-predators-parasites (Pd).

Figure 5. Box-plot showing the distribution of the mass volume of the eleven categories of functional groups. The black line inside the box represent the median and empty circles are outliers.

Figure 6. Sample- (black) and individual-based (grey) rarefaction curves of both collecting methodologies Winkler extraction (A) and Pitfall traps (B). Smooth full or dashed lines correspond to ±95% confidence intervals.

Figure 7. Plots showing the effect of the geographical distance on the similarity of (A) community species, (B) abundance, (C) functional groups and (D) biomass between Winkler extraction sites (grey dots) and Pitfall traps (black dots). Plot (C) corresponds to a distance index where values close to 1 must be interpreted as dissimilar.

Figure 8. Histograms showing the frequency distribution of litter transformers width in relation to (A) species, (B) abundance and (C) biomass.

Figure 9. Total biomass of litter transformers soil fauna having potential access to the five different mesh-bags treatments.

Figure 10. Percentage of mass loss of the leaf litter material of the eight plant species in relation to the five mesh-bag treatments (mesh size corresponds to the measure of the diagonal of the hole). Dashed lines correspond to non-significant power or linear regression fits, and where plotted only for illustrating the general tendency of the data. Full lines (and ± 95% CI) correspond to significant (and barely significant for the ‘pooled’ data) regression fits. Pooled regression was performed based on averaged data and error bars represent its standard deviations.

Figures

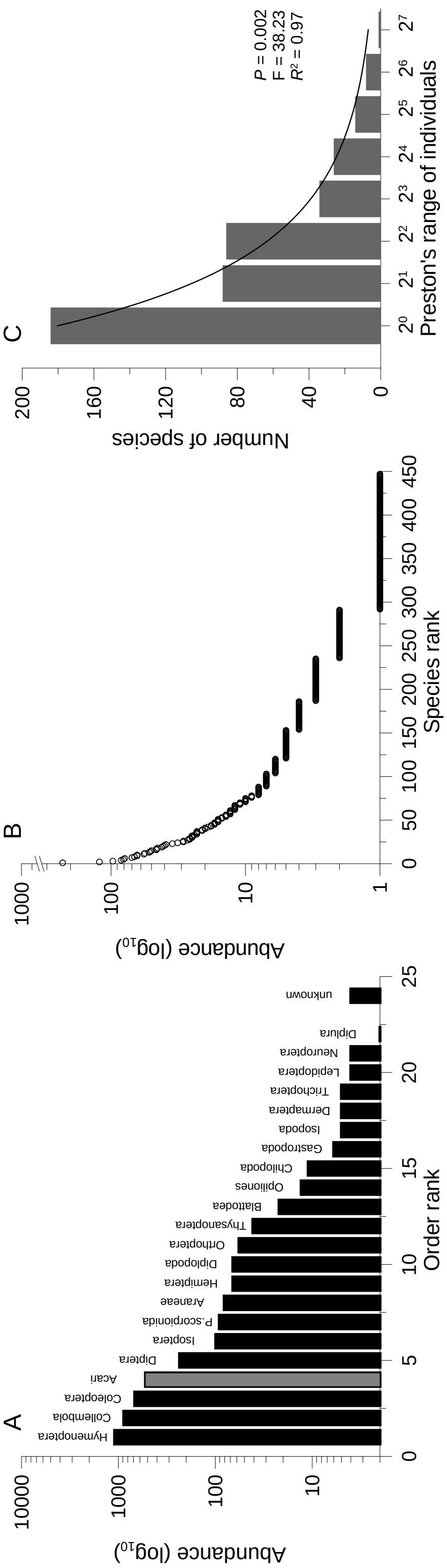


Figure 1. Cárdenas *et al*.

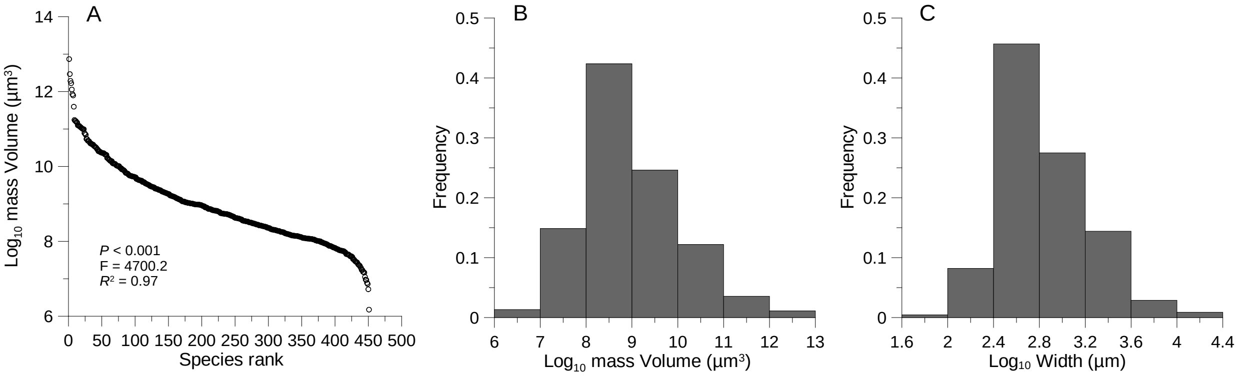
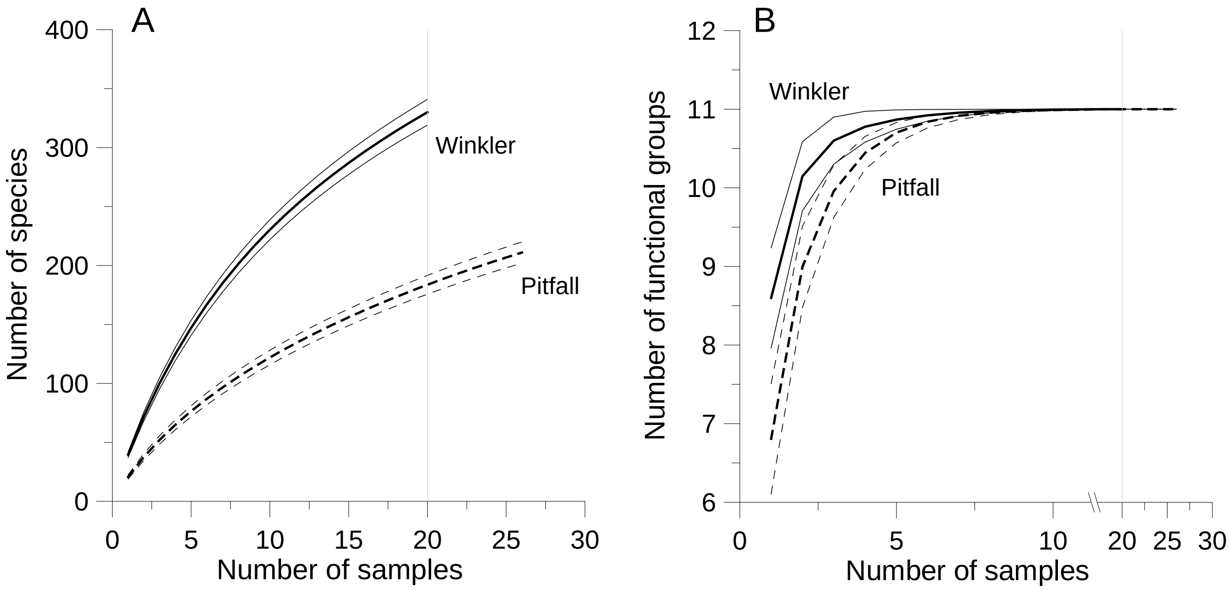


Figure 2. Cárdenas *et al*.



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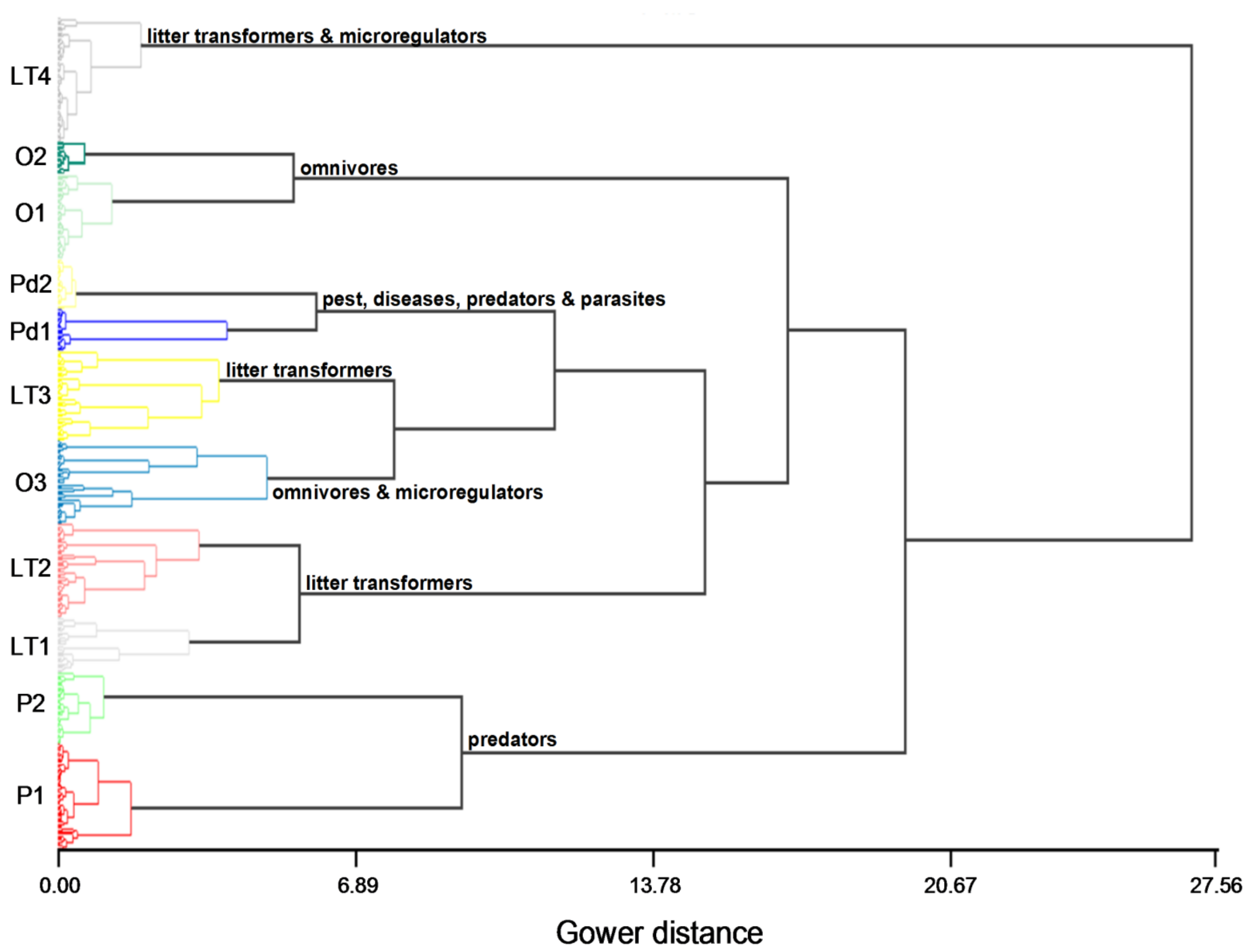

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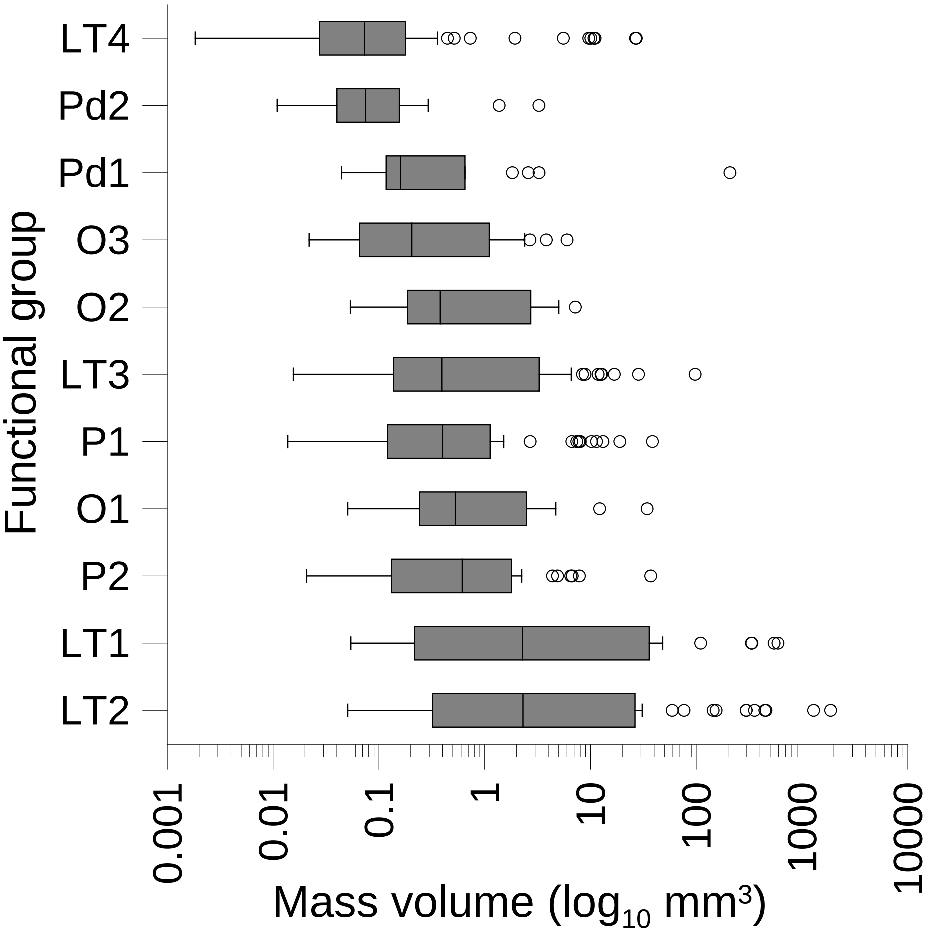

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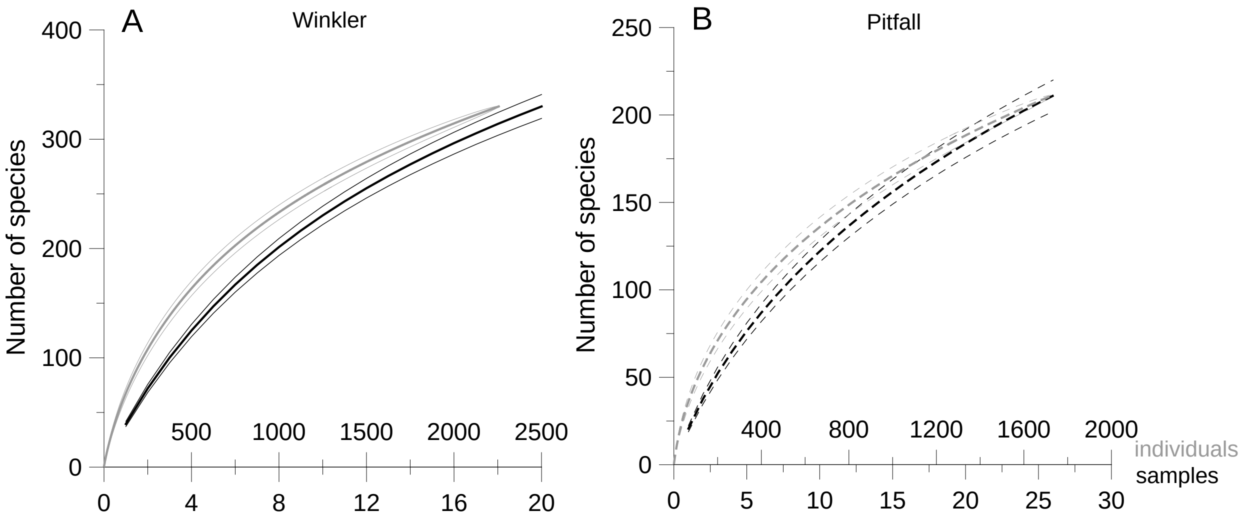

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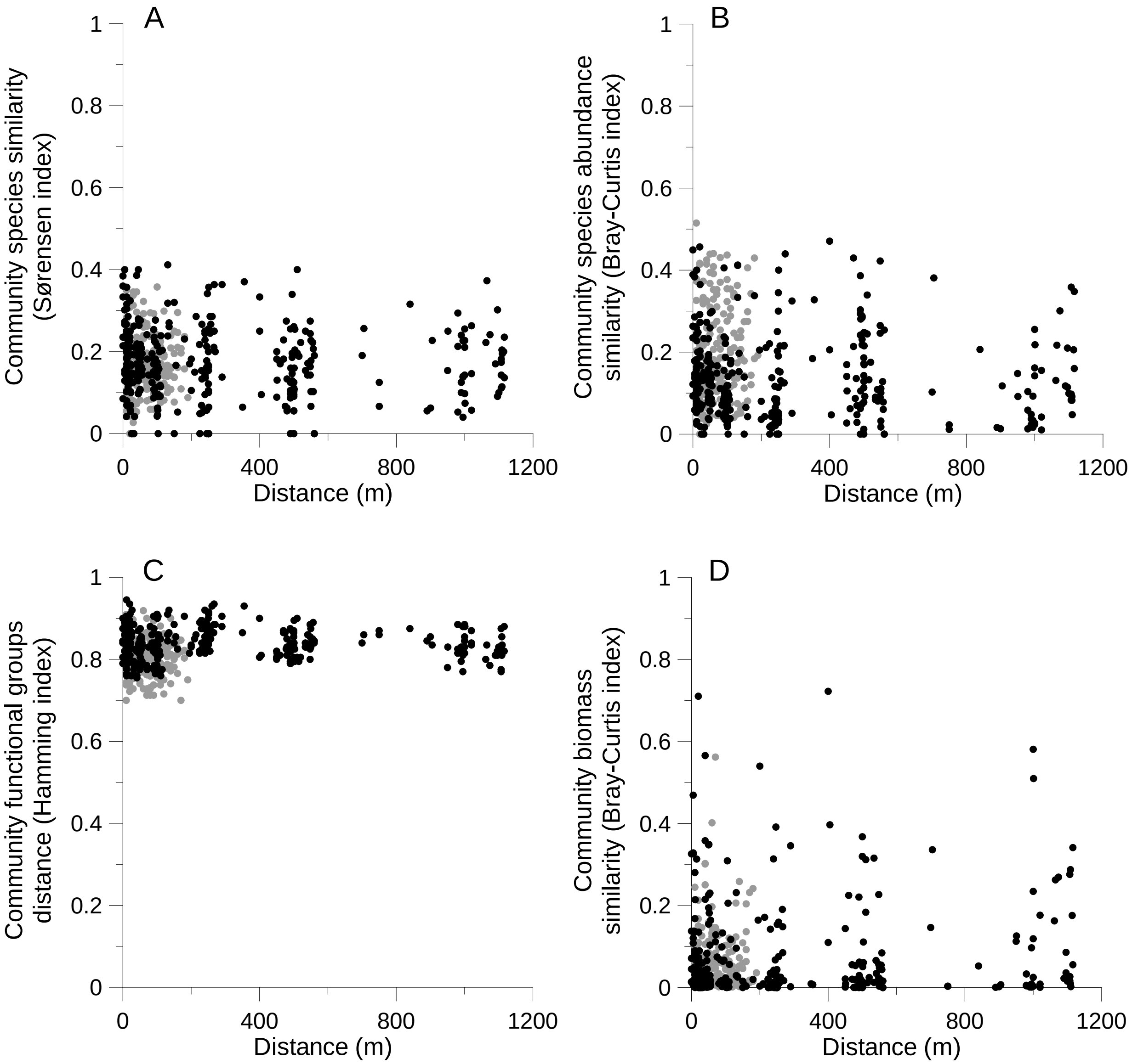
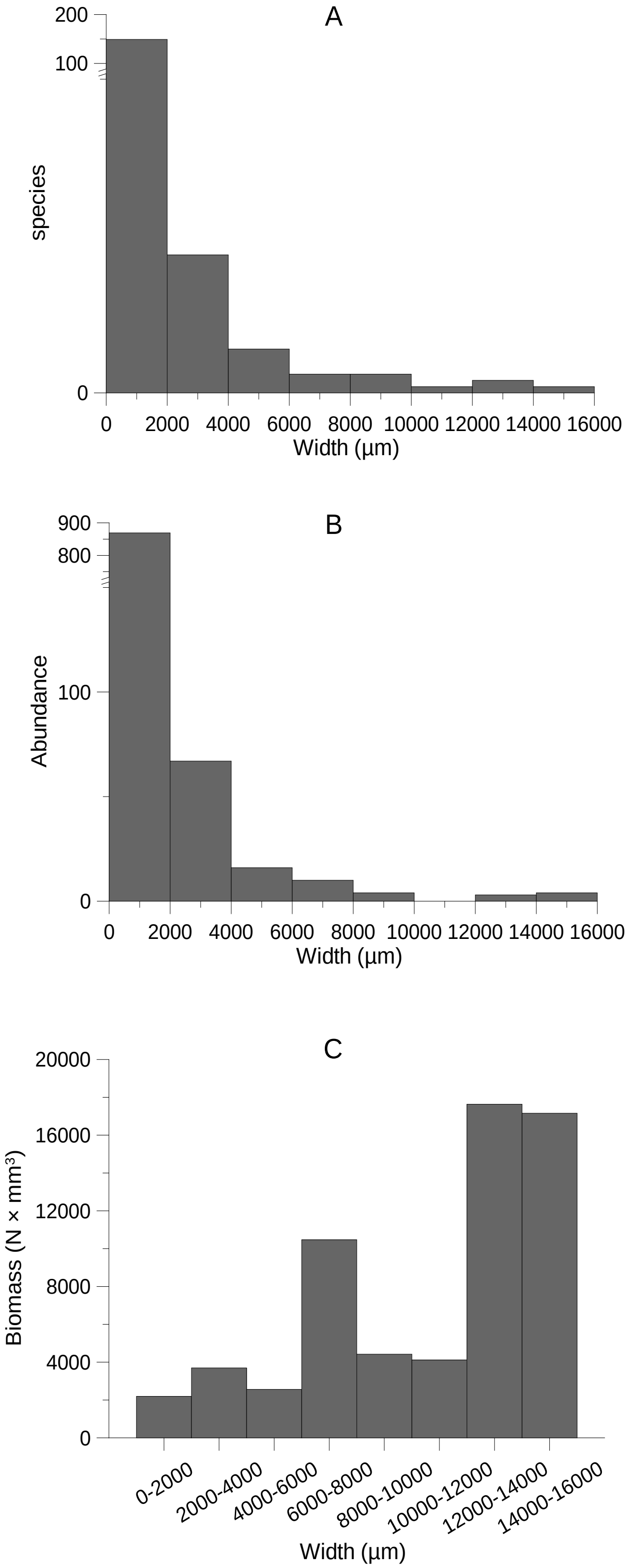


Figure 7. Cárdenas *et al*.



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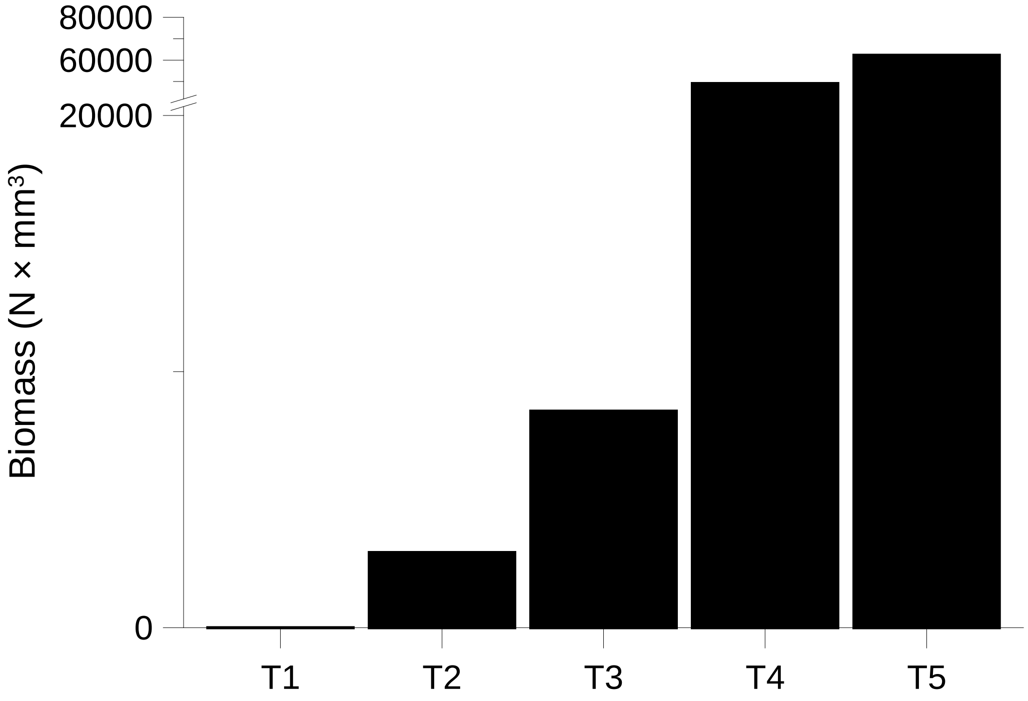


Figure 9. Cárdenas *et al*.

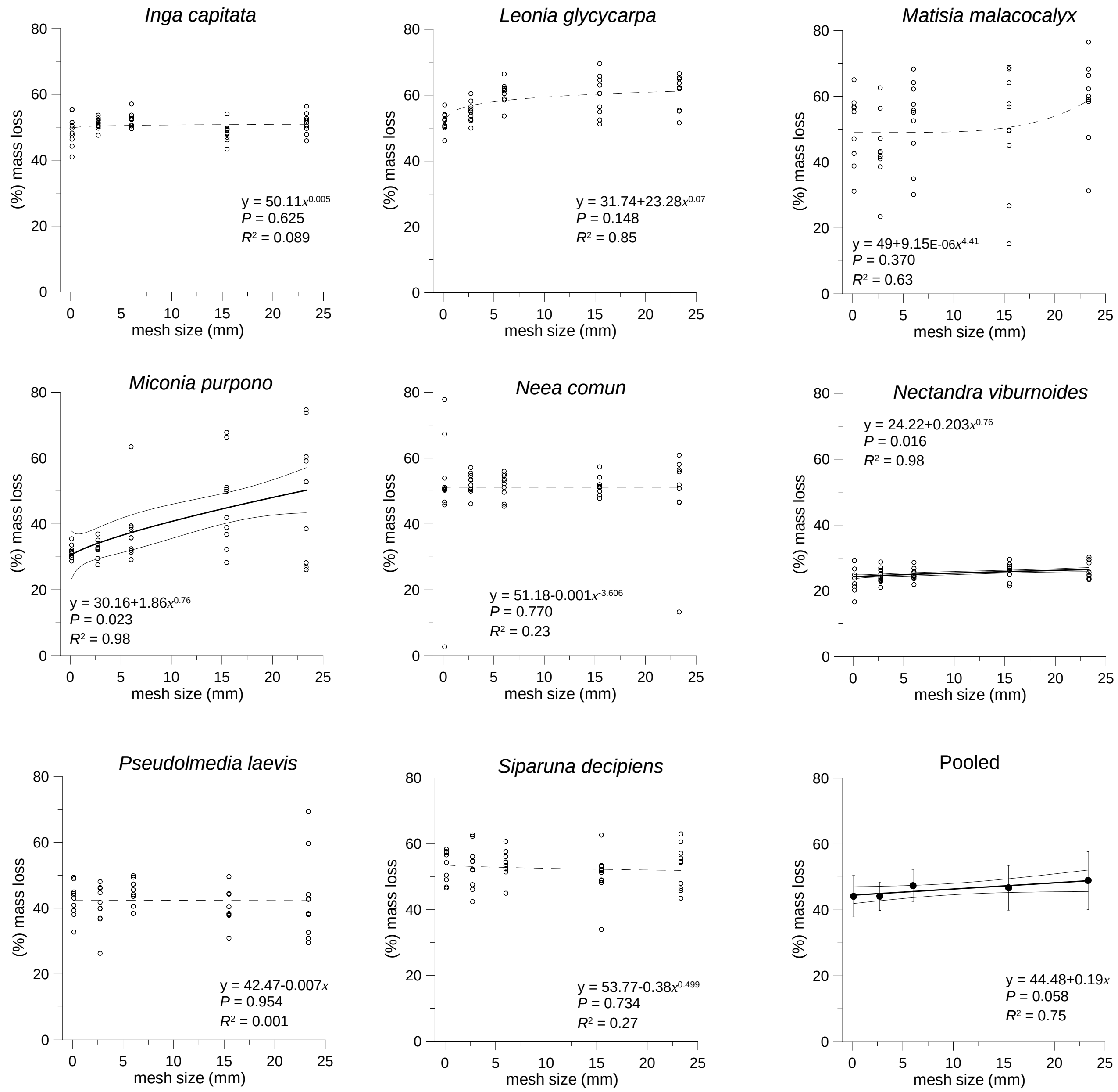
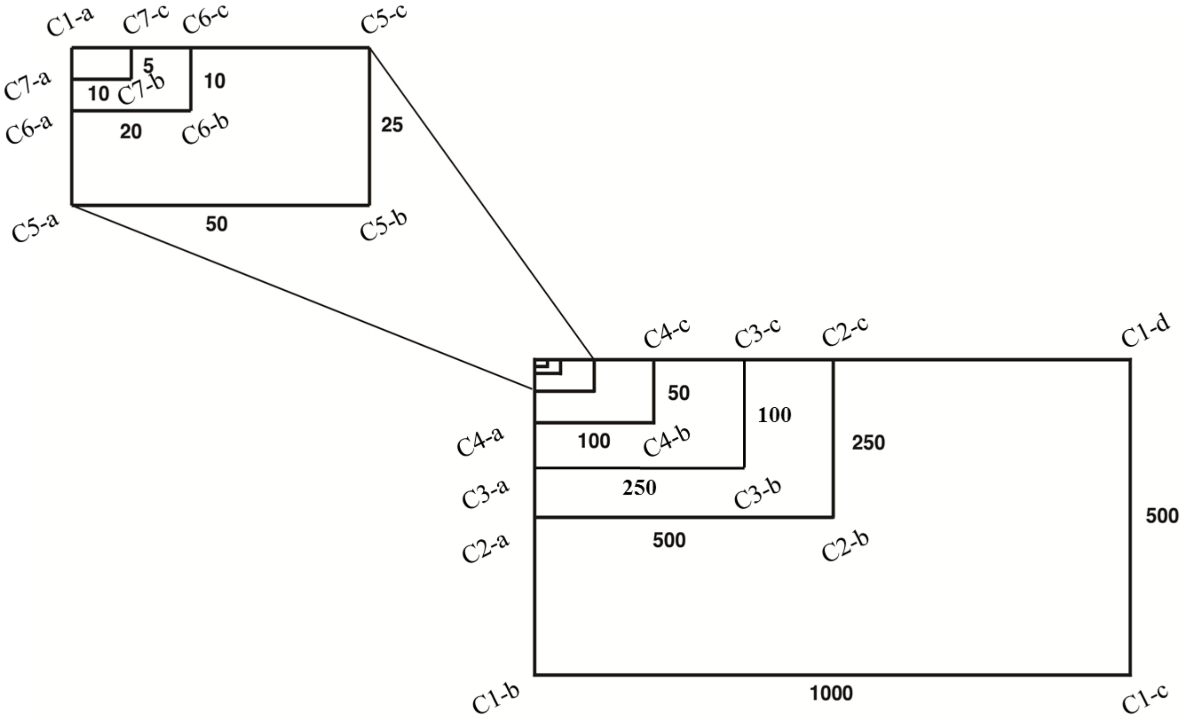


Figure 10. Cárdenas *et al*.

Supporting information

Appendix 1. Sampling design of the pitfall trapping. Line numbers correspond to distances (m). Corner codes refer to the quadrat number and position, where C means quadrat, the following number gives a reference of quadrat size where 1 is the biggest and 7 the smaller, and the lower case letter corresponds to each of the corners.



Appendix 2. Complete list of all the morphospecies collected in Yasuní National Park.

Phylum	Class	Order	Family/Subfamily/Genus	species	Abundance
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp4	229
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Camponotus	Camponotus sp1	122
Arthropoda	Insecta	Coleoptera	Curculionidae/Scolytinae	Scolytinae sp1	97
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Solenopsis	Solenopsis sp1	84
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp5	81
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Camponotus	Camponotus sp3	79
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp7	70
Arthropoda	Insecta	Collembola	Isotomidae/Oncopoduridae	Isotomidae/Oncopoduridae sp3	67
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp1	64
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Strumigenys	Strumigenys sp2	64
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Hypoponera	Hypoponera sp1	57
Arthropoda	Insecta	Isoptera		Isoptera sp3	56
Arthropoda	Insecta	Diptera	Cecydomyiidae	Cecidomyiidae sp2	52
Arthropoda	Arachnida	Pseudoscporpionida		Pseudoscorpionida sp1	51
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Wasmannia	Wasmannia sp1	48
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp2	46
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Strumigenys	Strumigenys sp1	46
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp1	45
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp5	42
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp8	41
Arthropoda	Arachnida	Pseudoscporpionida		Pseudoscorpionida sp2	40
Arthropoda	Insecta	Diptera	Drosophilidae	Drosophilidae sp2	39
Arthropoda	Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp3	34
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp1	32
Arthropoda	Myriapoda	Diplopoda		Diplopoda sp2	29
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp6	28
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp3	27
Arthropoda	Insecta	Coleoptera	Curculionidae/Scolytinae	Scolytinae sp2	26
Arthropoda	Insecta	Coleoptera	Staphylinidae/Aleocharinae	Aleocharinae sp2	26
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Hylomyrma	Hylomyrma sp1	26
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp2	25
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp1	25

Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp4	24
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp6	23
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp6	23
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp2	23
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Hypoponera	Hypoponera sp2	23
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae?	Myrmicinae? sp1	22
Arthropoda	Insecta	Coleoptera	Ptiliidae	Ptiliidae sp1	21
Arthropoda	Insecta	Collembola	Isotomidae/Oncopoduridae	Isotomidae/Oncopoduridae sp2	21
Arthropoda	Insecta	Coleoptera	Curculionidae/Scolytinae	Scolytinae sp4	20
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp5	20
Arthropoda	Insecta	Isoptera		Isoptera sp4	19
Arthropoda	Insecta	Coleoptera	Ptiliidae	Ptiliidae sp4	18
Arthropoda	Insecta	Diptera	larva	Diptera larva sp1	18
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp11	17
Arthropoda	Insecta	Hymenoptera	Formicidae/Ectatomminae/Gnamptogenys	Gnamptogenys sp1	17
Arthropoda	Insecta	Hymenoptera	larva	Hymenoptera larva sp1	17
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp7	16
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp5	16
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp7	16
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Solenopsis	Solenopsis sp2	16
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp3	15
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp4	15
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp3	14
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Cyphomyrmex	Cyphomyrmex sp1	14
Arthropoda	Insecta	Coleoptera	Ptiliidae	Ptiliidae sp5	13
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp1	13
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp6	13
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp17	13
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Camponotus	Camponotus sp2	13
Arthropoda	Insecta	Coleoptera	Staphylinidae/Aleocharinae	Aleocharinae sp1	12
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp6	12
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp14	12
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp5	12
Arthropoda	Insecta	Isoptera		Isoptera sp5	12
Arthropoda	Insecta	Coleoptera	Phalacridae	Phalacridae sp1	11
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Acropyga	Acropyga sp1	11

Arthropoda	Myriapoda	Diplopoda	larva	Diplopoda larva sp1	11
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp19	10
Arthropoda	Insecta	Diptera	Phoridae	Phoridae sp3	10
Arthropoda	Insecta	Hemiptera	Pleidae	Pleidae sp1	10
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Nylanderia	Nylanderia sp2	10
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp3	10
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp4	9
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp7	9
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Sericomyrmex	Sericomyrmex sp1	9
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp6	8
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp8	8
Arthropoda	Insecta	Collembola	Isotomidae/Oncopoduridae	Isotomidae/Oncopoduridae sp1	8
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp2	8
Arthropoda	Insecta	Hymenoptera	Formicidae/Ectatomminae/Ectatomma	Ectatomma sp1	8
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Crematogaster	Crematogaster sp1	8
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Wasmannia	Wasmannia sp2	8
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Odontomachus	Odontomachus sp1	8
Arthropoda	Insecta	Isoptera		Isoptera sp1	8
Arthropoda	Arachnida	Araneae		Araneae sp10	7
Arthropoda	Arachnida	Araneae		Araneae sp11	7
Arthropoda	Arachnida	Araneae		Araneae sp7	7
Arthropoda	Insecta	Coleoptera	Curculionidae/Dorytosomimus	Dorytosomimus sp1	7
Arthropoda	Insecta	Coleoptera	Curculionidae/Scolytinae	Scolytinae sp6	7
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp16	7
Arthropoda	Insecta	Coleoptera	Ptiliidae	Ptiliidae sp2	7
Arthropoda	Insecta	Coleoptera	Staphylinidae/larva	Staphylinidae/larva sp2	7
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae_Aleocharinae	Oxytelinae/Aleocharinae sp1	7
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp4	7
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp1	7
Arthropoda	Insecta	Diptera	Mycetophilidae	Mycetophilidae sp2	7
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Apterostigma	Apterostigma sp1	7
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole?	Pheidole? sp2	7
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Rogeria	Rogeria sp1	7
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp4	7
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp2	7
Arthropoda	Arachnida	Araneae	Philodromidae	Philodromidae sp1	6

Arthropoda	Arachnida	Araneae		Araneae sp15	6
Arthropoda	Arachnida	Opiliones		Opiliones sp2	6
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp5	6
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp7	6
Arthropoda	Insecta	Coleoptera	Staphylinidae/Tachyporinae	Tachyporinae sp1	6
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp9	6
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp1	6
Arthropoda	Insecta	Diptera	larva	Diptera larva sp2	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Megalomyrmex	Megalomyrmex sp1	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Myrmicocrypta	Myrmicocrypta sp1	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Octostruma	Octostruma sp1	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Solenopsis	Solenopsis sp4	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Strumigenys	Strumigenys sp3	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Pachycondyla	Pachycondyla sp1	6
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Pachycondyla	Pachycondyla sp2	6
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp2	6
Arthropoda	Myriapoda	Chilopoda		Chilopoda sp2	6
Arthropoda	Arachnida	Araneae		Araneae sp13	5
Arthropoda	Crustacea	Isopoda		Isopoda sp1	5
Arthropoda	Insecta	Coleoptera	Mycetophagidae	Mycetophagidae sp1	5
Arthropoda	Insecta	Coleoptera	Phalacridae	Phalacridae sp3	5
Arthropoda	Insecta	Coleoptera	Ptiliidae	Ptiliidae sp3	5
Arthropoda	Insecta	Coleoptera	Scaphidiidae	Scaphidiidae sp1	5
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp7	5
Arthropoda	Insecta	Coleoptera	Staphylinidae/Aleocharinae	Aleocharinae sp3	5
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp10	5
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp5	5
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp9	5
Arthropoda	Insecta	Coleoptera	Tenebrionidae/Lagriinae/Anaedus	Anaedus sp1	5
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp3	5
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp1	5
Arthropoda	Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp5	5
Arthropoda	Insecta	Diptera	Cecydomyiidae	Cecidomyiidae sp1	5
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogonidae sp1	5
Arthropoda	Insecta	Diptera	larva	Diptera larva sp3	5
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp4	5

Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp7	5
Arthropoda	Insecta	Hymenoptera	Formicidae/Ectatomminae/Gnamptogenys	Gnamptogenys sp2	5
Arthropoda	Insecta	Hymenoptera	Formicidae/Ectatomminae/Gnamptogenys	Gnamptogenys sp3	5
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Basiceros	Basiceros sp2	5
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Carebara	Carebara sp1	5
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Megalomyrmex	Megalomyrmex sp2	5
Arthropoda	Insecta	Hymenoptera	Scelionidae	Scelionidae sp1	5
Arthropoda	Insecta	Orthoptera	Gryllacrididae	Gryllacrididae sp2	5
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp1	5
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp11	5
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp4	5
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp7	5
Arthropoda	Myriapoda	Diplopoda		Diplopoda sp1	5
Arthropoda	Arachnida	Araneae		Araneae sp1	4
Arthropoda	Arachnida	Araneae		Araneae sp12	4
Arthropoda	Arachnida	Araneae		Araneae sp5	4
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp5	4
Arthropoda	Insecta	Coleoptera	Curculionidae/Scolytinae	Scolytinae sp3	4
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp9	4
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp5	4
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp3	4
Arthropoda	Insecta	Coleoptera	Staphylinidae/Tachyporinae	Tachyporinae sp2	4
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp10	4
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp8	4
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp9	4
Arthropoda	Insecta	Diptera	Phoridae	Phoridae sp4	4
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp1	4
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp5	4
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp9	4
Arthropoda	Insecta	Hemiptera	Tingidae	Tingidae sp1	4
Arthropoda	Insecta	Hymenoptera	Eulophidae	Eulophidae sp1	4
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pyramica (=Strumigenys)	Pyramica (=Strumigenys) sp2	4
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Strumigenys	Strumigenys sp4	4
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Trachymyrmex	Trachymyrmex sp1	4
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Trachymyrmex	Trachymyrmex sp2	4
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Discothyrea	Discothyrea sp1	4

Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Hypoponera	Hypoponera sp3	4
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp1	4
Arthropoda	Insecta	Hymenoptera	Sapygidae	Sapygidae sp1	4
Arthropoda	Insecta	Orthoptera	Gryllacrididae	Gryllacrididae sp1	4
Arthropoda	Myriapoda	Diplopoda	Polydesmida	Polydesmida sp1	4
Arthropoda	Myriapoda	Diplopoda	Polydesmidae	Polydesmidae sp3	4
Mollusca	Gastropoda			Gastropoda sp2	4
Arthropoda	Arachnida	Araneae		Araneae sp19	3
Arthropoda	Arachnida	Araneae		Araneae sp20	3
Arthropoda	Arachnida	Araneae		Araneae sp22	3
Arthropoda	Arachnida	Araneae		Araneae sp9	3
Arthropoda	Arachnida	Opiliones		Opiliones sp1	3
Arthropoda	Crustacea	Isopoda		Isopoda sp2	3
Arthropoda	Crustacea	Isopoda		Isopoda sp3	3
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp1	3
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp3	3
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp7	3
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp8	3
Arthropoda	Insecta	Blattodea	Blattidae	Blattidae sp2	3
Arthropoda	Insecta	Blattodea	Blattidae	Periplaneta americana sp1	3
Arthropoda	Insecta	Coleoptera	Curculionidae/Molytinae	Molytinae sp2	3
Arthropoda	Insecta	Coleoptera	Curculionidae/Scolytinae	Scolytinae sp5	3
Arthropoda	Insecta	Coleoptera	Endomychidae	Endomychidae sp1	3
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp11	3
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp6	3
Arthropoda	Insecta	Coleoptera	Mycetophagidae	Mycetophagidae sp2	3
Arthropoda	Insecta	Coleoptera	Scarabaeidae/larva	Scarabaeidae/larva sp1	3
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp4	3
Arthropoda	Insecta	Coleoptera	Staphylinidae/larva	Staphylinidae/larva sp1	3
Arthropoda	Insecta	Coleoptera	Staphylinidae/larva	Staphylinidae/larva sp4	3
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp7	3
Arthropoda	Insecta	Coleoptera	Staphylinidae/Tachyporinae	Tachyporinae sp3	3
Arthropoda	Insecta	Collembola	Cyphoderidae/Oncopoduridae	Cyphoderidae/Oncopoduridae sp1	3
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp10	3
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp15	3
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp20	3

Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp3	3
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp4	3
Arthropoda	Insecta	Dermaptera	ninfa	Dermaptera ninfa sp1	3
Arthropoda	Insecta	Diptera	Culicidae	Culicidae sp1	3
Arthropoda	Insecta	Diptera	Drosophilidae	Drosophilidae sp1	3
Arthropoda	Insecta	Diptera	Mycetophilidae	Mycetophilidae sp1	3
Arthropoda	Insecta	Diptera	Mycetophilidae	Mycetophilidae sp3	3
Arthropoda	Insecta	Diptera	Phoridae	Phoridae sp1	3
Arthropoda	Insecta	Diptera	Simuliidae	Simuliidae sp1	3
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp8	3
Arthropoda	Insecta	Hemiptera	Pleidae/ninfa	Pleidae/ninfa sp1	3
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Nylanderia	Nylanderia sp1	3
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp6	3
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Anochetus	Anochetus sp1	3
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp6	3
Arthropoda	Insecta	Hymenoptera	Sapygidae	Sapygidae sp4	3
Arthropoda	Insecta	Isoptera		Isoptera sp2	3
Arthropoda	Insecta	Neuroptera	Myrmeleontidae	Myrmeleontidae sp1	3
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp8	3
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp9	3
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp3	3
Arthropoda	Myriapoda	Diplopoda	Chelodesmidae	Diplopoda sp1	3
Arthropoda	Myriapoda	Diplopoda	Chelodesmidae	Diplopoda sp2	3
Arthropoda	Arachnida	Araneae		Araneae sp6	2
Arthropoda	Arachnida	Opiliones		Opiliones sp3	2
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp2	2
Arthropoda	Insecta	Blattodea	Blattidae	Blattidae sp1	2
Arthropoda	Insecta	Coleoptera	Carabidae	Carabidae sp1	2
Arthropoda	Insecta	Coleoptera	Curculionidae/Dryophthorinae/Rhynchophorini/Metamasius	Metamasius sp1	2
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp3	2
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Aphodinae	Aphodinae sp1	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp11	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp12	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp2	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp4	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp5	2

Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae_Aleocharinae	Oxytelinae/Aleocharinae sp2	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Pselaphinae	Pselaphinae sp2	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp7	2
Arthropoda	Insecta	Coleoptera	Staphylinidae/Tachyporinae	Tachyporinae sp4	2
Arthropoda	Insecta	Coleoptera	Trogossitidae	Trogossitidae sp1	2
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp10	2
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp12	2
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp16	2
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp2	2
Arthropoda	Insecta	Collembola	Hypogastruridae/Neanuridae	Neanuridae sp3	2
Arthropoda	Insecta	Diplura		Diplura	2
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogonidae sp2	2
Arthropoda	Insecta	Diptera	Ceratopogonidae/larva	Ceratopogonidae/larva sp1	2
Arthropoda	Insecta	Diptera	Drosophilidae	Drosophilidae sp3	2
Arthropoda	Insecta	Diptera	Drosophilidae	Drosophilidae sp4	2
Arthropoda	Insecta	Diptera	Sciaridae	Sciaridae sp1	2
Arthropoda	Insecta	Hemiptera	Anthocoridae	Anthocoridae sp1	2
Arthropoda	Insecta	Hymenoptera	Chalcididae	Chalcididae sp1	2
Arthropoda	Insecta	Hymenoptera	Cynipidae	Cynipidae sp1	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Amblyoponinae/Stigmatomma	Stigmatomma sp1	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Carebara	Carebara sp2	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Crematogaster	Crematogaster sp2	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Myrmicocrypta	Myrmicocrypta sp3	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp7	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pyramica (=Strumigenys)	Pyramica (=Strumigenys) sp3	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Sericomyrmex	Sericomyrmex sp2	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Trachymyrmex	Trachymyrmex sp3	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Tranopelta	Tranopelta sp1	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Hypoponera	Hypoponera sp4	2
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Pachycondyla	Pachycondyla sp3	2
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp5	2
Arthropoda	Insecta	Hymenoptera	Sapygidae	Sapygidae sp3	2
Arthropoda	Insecta	Hymenoptera	Scelionidae	Scelionidae sp2	2
Arthropoda	Insecta	Hymenoptera	Trichogrammatidae	Trichogrammatidae sp2	2
Arthropoda	Insecta	Lepidoptera	larva	Lepidoptera larva sp1	2
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp10	2

Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp6	2
Arthropoda	Insecta	Orthoptera	Tetrigidae	Tetrigidae sp1	2
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp2	2
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp8	2
Arthropoda	Myriapoda	Diplopoda	larva	Diplopoda larva sp2	2
Arthropoda	Myriapoda	Diplopoda	Polydesmida	Polydesmida sp2	2
Arthropoda	Myriapoda	Diplopoda	Polydesmidae	Polydesmidae sp1	2
Arthropoda	Myriapoda	Diplopoda		Diplopoda sp4	2
Arthropoda	Myriapoda	Diplopoda		Diplopoda sp5	2
Mollusca	Gastropoda			Gastropoda sp1	2
Arthropoda	Insecta	Hemiptera	Alydidae/ninfa	Alydidae/ninfa sp1	1
Arthropoda	Arachnida	Araneae	Cyrtaucheniidae	Cyrtaucheniidae sp1	1
Arthropoda	Arachnida	Araneae	Dipluridae	Dipluridae sp1	1
Arthropoda	Arachnida	Araneae		Araneae sp14	1
Arthropoda	Arachnida	Araneae		Araneae sp16	1
Arthropoda	Arachnida	Araneae		Araneae sp17	1
Arthropoda	Arachnida	Araneae		Araneae sp18	1
Arthropoda	Arachnida	Araneae		Araneae sp21	1
Arthropoda	Arachnida	Araneae		Araneae sp23	1
Arthropoda	Arachnida	Araneae		Araneae sp24	1
Arthropoda	Arachnida	Araneae		Araneae sp25	1
Arthropoda	Arachnida	Araneae		Araneae sp26	1
Arthropoda	Arachnida	Araneae		Araneae sp27	1
Arthropoda	Arachnida	Araneae		Araneae sp28	1
Arthropoda	Arachnida	Araneae		Araneae sp29	1
Arthropoda	Arachnida	Araneae		Araneae sp3	1
Arthropoda	Arachnida	Araneae		Araneae sp4	1
Arthropoda	Arachnida	Araneae		Araneae sp8	1
Arthropoda	Arachnida	Opiliones		Opiliones sp4	1
Arthropoda	Arachnida	Opiliones		Opiliones sp5	1
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp4	1
Arthropoda	Insecta	Blattodea	Blattellidae	Blattellidae sp6	1
Arthropoda	Insecta	Blattodea	Blattidae	Blattidae sp3	1
Arthropoda	Insecta	Blattodea	Blattidae	Blattidae sp4	1
Arthropoda	Insecta	Coleoptera	Biphyllidae	Biphyllidae sp1	1
Arthropoda	Insecta	Coleoptera	Curculionidae/Conoderinae/Eulechriops	Eulechriops sp3	1

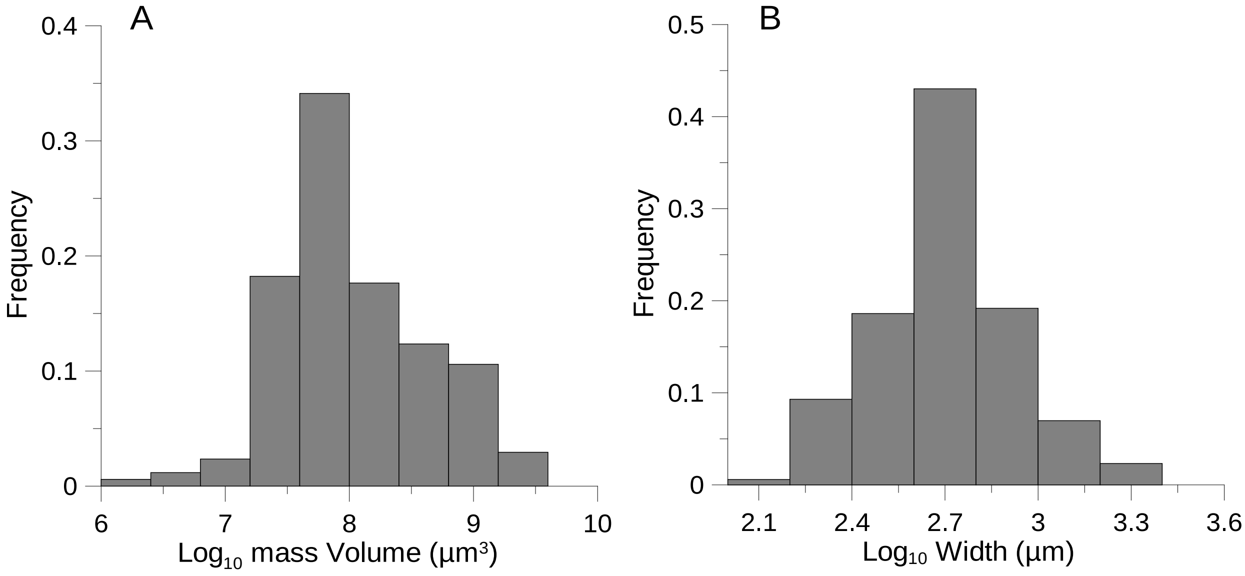
Arthropoda	Insecta	Coleoptera	Elateridae/larva	Elateridae/larva sp1	1
Arthropoda	Insecta	Coleoptera	Histeridae/Euspilotus	Euspilotus sp1	1
Arthropoda	Insecta	Coleoptera	Histeridae/Omalodes	Omalodes sp1	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp1	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp13	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp14	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp15	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp17	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp18	1
Arthropoda	Insecta	Coleoptera	larva	Coleoptera larva sp8	1
Arthropoda	Insecta	Coleoptera	Phalacridae	Phalacridae sp2	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp1	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp2	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp3	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp6	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp8	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae	Scarabaeinae sp9	1
Arthropoda	Insecta	Coleoptera	Scarabaeidae/Scarabaeinae/Eurysternus?	Eurysternus sp1	1
Arthropoda	Insecta	Coleoptera	Staphylinidae	Staphylinidae sp4	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Aleocharinae	Aleocharinae sp4	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/larva	Staphylinidae/larva sp3	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp10	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Oxytelinae	Oxytelinae sp9	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Proteininae	Proteininae sp1	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Proteininae	Proteininae sp2	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp2	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp4	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae	Staphylininae sp8	1
Arthropoda	Insecta	Coleoptera	Staphylinidae/Staphylininae_Oxytelinae	Staphylininae_Oxytelinae sp1	1
Arthropoda	Insecta	Collembola	Actaletidae	Actaletidae sp2	1
Arthropoda	Insecta	Collembola	Actaletidae	Actaletidae sp2	1
Arthropoda	Insecta	Collembola	Arrhopalitidae	Arrhopalitidae sp8	1
Arthropoda	Insecta	Collembola	Arrhopalitidae?/Sminthuridae?	Arrhopalitidae?/Sminthuridae? Sp1	1
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp13	1
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp18	1
Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp21	1

Arthropoda	Insecta	Collembola	Entomobryidae	Entomobryidae sp9	1
Arthropoda	Insecta	Collembola	Sminthuridae	Sminthuridae sp1	1
Arthropoda	Insecta	Collembola	Sminthuridae	Sminthuridae sp2	1
Arthropoda	Insecta	Dermaptera	Labiidae	Labiidae sp1	1
Arthropoda	Insecta	Dermaptera	Labiidae	Labiidae sp2	1
Arthropoda	Insecta	Diplura?		Diplura? sp1	1
Arthropoda	Insecta	Diptera	Cecidomyiidae	Cecidomyiidae sp4	1
Arthropoda	Insecta	Diptera	Ceratopogonidae/larva	Ceratopogonidae/larva sp2	1
Arthropoda	Insecta	Diptera	larva	Diptera larva sp4	1
Arthropoda	Insecta	Diptera	larva	Diptera larva sp5	1
Arthropoda	Insecta	Diptera	larva	Diptera larva sp6	1
Arthropoda	Insecta	Diptera	larva	Diptera larva sp7	1
Arthropoda	Insecta	Diptera	larva	Diptera larva sp8	1
Arthropoda	Insecta	Diptera	larva	Diptera larva sp9	1
Arthropoda	Insecta	Diptera	Mycetophilidae	Mycetophilidae sp4	1
Arthropoda	Insecta	Diptera	Phoridae	Phoridae sp2	1
Arthropoda	Insecta	Diptera	Phoridae	Phoridae sp5	1
Arthropoda	Insecta	Diptera	Phoridae	Phoridae sp6	1
Arthropoda	Insecta	Diptera	Pipunculidae	Pipunculidae sp1	1
Arthropoda	Insecta	Diptera	Scatopsidae	Scatopsidae sp1	1
Arthropoda	Insecta	Diptera	Simuliidae	Simuliidae sp2	1
Arthropoda	Insecta	Hemiptera	Anthocoridae	Anthocoridae sp2	1
Arthropoda	Insecta	Hemiptera	Aradidae	Aradidae sp1	1
Arthropoda	Insecta	Hemiptera	Ceratocombidae	Ceratocombidae sp1	1
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp3	1
Arthropoda	Insecta	Hemiptera	Cicadellidae	Cicadellidae sp6	1
Arthropoda	Insecta	Hemiptera	Coreidae	Coreidae sp1	1
Arthropoda	Insecta	Hemiptera	Cydnidae	Cydnidae sp2	1
Arthropoda	Insecta	Hemiptera	Cydnidae_ninfa	Cydnidae_ninfa sp1	1
Arthropoda	Insecta	Hemiptera	ninfa	Hemiptera ninfa sp1	1
Arthropoda	Insecta	Hemiptera	Pentatomidae	Pentatomidae sp1	1
Arthropoda	Insecta	Hemiptera	Reduviidae	Reduviidae sp1	1
Arthropoda	Insecta	Hemiptera	Reduviidae	Reduviidae sp2	1
Arthropoda	Insecta	Hemiptera	Reduviidae	Reduviidae sp3	1
Arthropoda	Insecta	Hymenoptera	Braconidae	Braconidae sp1	1
Arthropoda	Insecta	Hymenoptera	Braconidae	Braconidae sp2	1

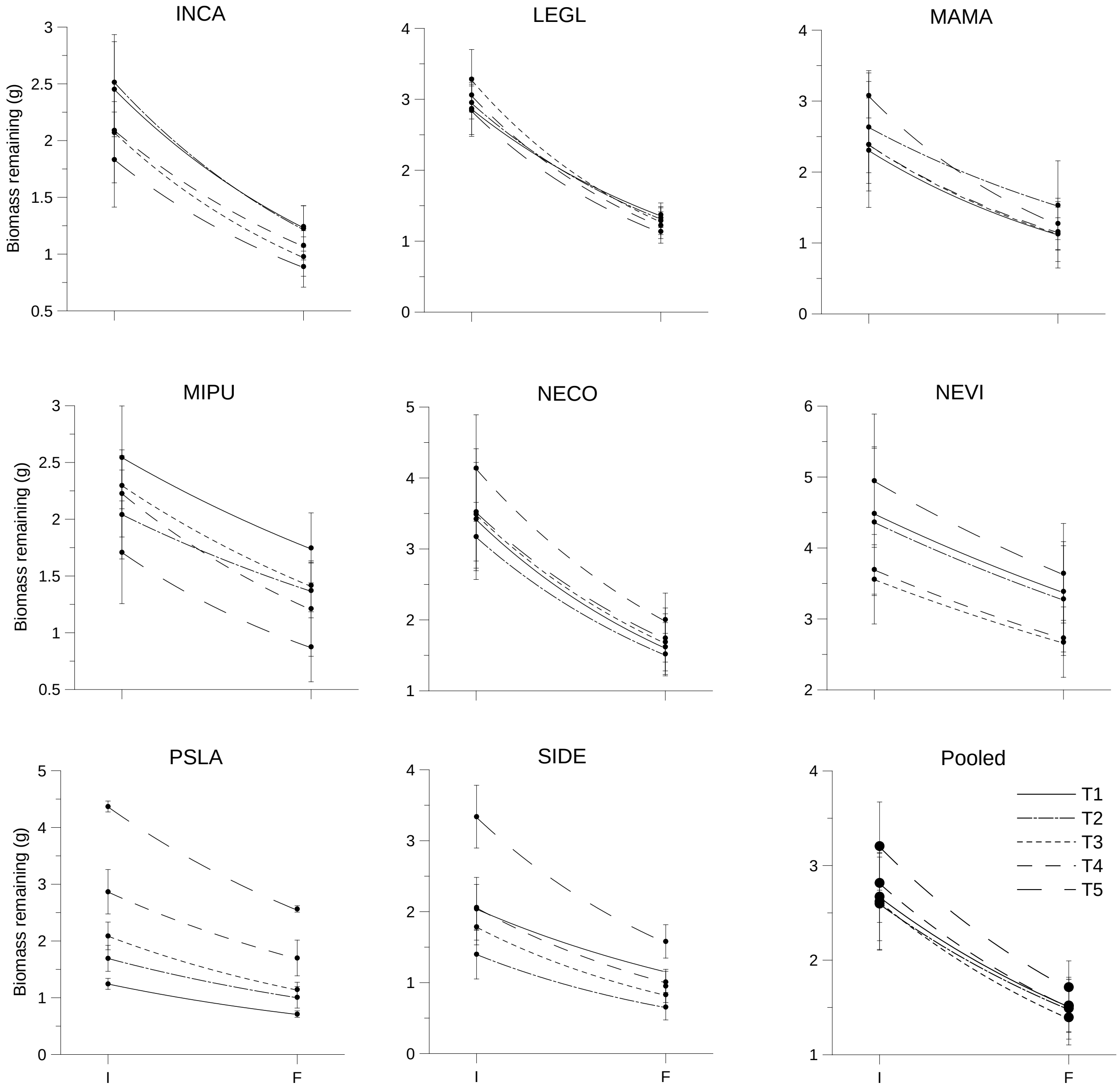
Arthropoda	Insecta	Hymenoptera	Encyrtidae	Encyrtidae sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Cerapachyinae/Cerapachys	Cerapachys sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Dolichoderinae/Azteca	Azteca sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Ectatomminae/Typhlomyrmex	Typhlomyrmex sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Brachymyrmex	Brachymyrmex sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Formicinae/Paratrechina	Paratrechina sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Basiceros	Basiceros sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Hylomyrma	Hylomyrma sp2	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp8	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pheidole	Pheidole sp9	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Pyramica (=Strumigenys)	Pyramica (=Strumigenys) sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Rogeria	Rogeria sp2	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Rogeria	Rogeria sp3	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Rogeria	Rogeria sp4	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Solenopsis	Solenopsis sp3	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Myrmicinae/Trachymyrmex	Trachymyrmex sp4	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Leptogenys	Leptogenys sp1	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Ponerinae/Odontomachus	Odontomachus sp2	1
Arthropoda	Insecta	Hymenoptera	Formicidae/Pseudomyrmecinae/Pseudomyrmex	Pseudomyrmex sp1	1
Arthropoda	Insecta	Hymenoptera	Ichneumonidae	Ichneumonidae msp1	1
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp3	1
Arthropoda	Insecta	Hymenoptera	Mymaridae	Mymaridae sp7	1
Arthropoda	Insecta	Hymenoptera	Pteromalidae	Pteromalidae sp1	1
Arthropoda	Insecta	Hymenoptera	Sapygidae	Sapygidae sp2	1
Arthropoda	Insecta	Hymenoptera	Sapygidae	Sapygidae sp5	1
Arthropoda	Insecta	Hymenoptera	Scelionidae	Scelionidae sp3	1
Arthropoda	Insecta	Hymenoptera	Signiphoridae	Signiphoridae sp1	1
Arthropoda	Insecta	Hymenoptera	Trichogrammatidae	Trichogrammatidae sp1	1
Arthropoda	Insecta	Hymenoptera	Trichogrammatidae	Trichogrammatidae sp3	1
Arthropoda	Insecta	Hymenoptera	Trichogrammatidae	Trichogrammatidae sp4	1
Arthropoda	Insecta	Hymenoptera	Trichogrammatidae	Trichogrammatidae sp5	1
Arthropoda	Insecta	Hymenoptera		Hymenoptera sp1	1
Arthropoda	Insecta	Isoptera		Isoptera sp6	1
Arthropoda	Insecta	Lepidoptera	larva	Lepidoptera larva sp2	1
Arthropoda	Insecta	Lepidoptera	larva	Lepidoptera larva sp3	1
Arthropoda	Insecta	Lepidoptera	larva	Trichoptera larva sp2	1

Arthropoda	Insecta	Neuroptera	Myrmeleontidae/larva	Myrmeleontidae larva sp1	1
Arthropoda	Insecta	Orthoptera	Acridoidea	Acridoidea sp1	1
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp5	1
Arthropoda	Insecta	Orthoptera	Gryllidae	Gryllidae sp7	1
Arthropoda	Insecta	Orthoptera	Tettigoniidae	Tettigoniidae sp1	1
Arthropoda	Insecta	Protura?		Protura? sp1	1
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp4	1
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp5	1
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp6	1
Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Phlaeothripidae sp9	1
Arthropoda	Insecta	Trichoptera	larva	Trichoptera larva sp1	1
Arthropoda	Insecta	Trichoptera	larva	Trichoptera larva sp2	1
Arthropoda	Insecta	Trichoptera	larva	Trichoptera larva sp4	1
Arthropoda	Insecta	Trichoptera	larva	Trichoptera larva sp5	1
Arthropoda	Insecta	Trichoptera		Trichoptera sp3	1
Arthropoda	Insecta			Insecta sp1	1
Arthropoda	Myriapoda	Chilopoda	Geophilomorpha	Geophilomorpha sp1	1
Arthropoda	Myriapoda	Chilopoda	larva	Chilopoda larva sp1	1
Arthropoda	Myriapoda	Chilopoda	Scolopendromorpha/Scolopendridae	Scolopendridae sp1	1
Arthropoda	Myriapoda	Chilopoda		Chilopoda sp1	1
Arthropoda	Myriapoda	Chilopoda		Chilopoda sp3	1
Arthropoda	Myriapoda	Chordeumida		Chordeumida sp1	1
Arthropoda	Myriapoda	Diplopoda	larva	Diplopoda larva sp3	1
Arthropoda	Myriapoda	Diplopoda	Polydesmidae	Polydesmidae sp2	1
Arthropoda	Myriapoda?			Myriapoda? sp1	1

Appendix 3. Acari distribution of frequencies of mass volume (A) and body width (B). Distributions resulted bell-shaped and only body-width distribution was Normal (Shapiro-Wilk normality tests: $W_{volume} = 0.975$, $P < 0.01$; $W_{width} = 0.986$, $P = 0.09$).



Appendix 4. Leaf litter biomass remaining of the eight plant species after 104 days of decomposition process in Yasuní National Park for the five mesh-bag treatments. No differences were found within species including the pooled data. Error bars correspond to standard deviations values. I = initial. F = final of the decomposition experiment.



—DISCUSSION—

Studies synthesizing the biotic and abiotic factors affecting herbivory and decomposition that additionally seek for relationships among both processes are central for a better understanding of ecosystems nutrient cycling (Bardgett & Wardle 2010). In this Discussion part we first summarize our more significant results considering some topics not examined in the chapters. Then, we analyze the future perspectives of insect-plant interaction ecological research in Ecuador.

Canopy herbivory and soil decomposition in the tropics: two independent processes governed by different factors

Since Grime *et al*. (1996)'s seminal study of a causal connection between anti-herbivore defense and leaf decomposition rate in a large-scale experiment in the temperate region, many additional works have been carried out in the last two decades. Both positive and negative effects of herbivores on subsequent leaf litter decomposition have been documented, suggesting associations between both subsystems are context-dependent (Bardgett & Wardle 2010) and may be affected by climate change (Rasmann *et al*. 2013).

Compared to those performed in temperate biomes, tropical studies assessing the herbivory-decomposition relationship are very scarce. This thesis project has focused on trying to elucidate, in a tropical lowland rainforest, which biotic and/or ecological factors were controlling herbivory and decomposition, and whether both processes were linked or not. Coinciding with another large scale tropical study (e.g. Kurokawa & Nakashizuka 2008) we found that leaf herbivory and subsequent litter decomposition processes were not associated. That is, resources in both canopy and soil strata were exploited according to its intrinsic characteristics (i.e. quality at the species and individual level) and consumers capabilities (i.e. specialists, generalists). No 'processing chain ecology' (Heard 1994) was found between both resources consumption, confirming that detritivores in soil did not depend on leaf pre-treatment by herbivores in the canopy. Although one can roughly synthesize that 'palatability' is the main controlling factor acting on leaf herbivory and litter decomposability, this term differs totally from an herbivore or a detritivore perspective. In the following we present a synthesis of plant traits controlling both herbivory and detritivory in the study area. Each bullet number in the text refers to Figure D1.

Herbivory. – **(1) Herbivory is controlled by specific leaf physical and tree traits.** Leaf 'toughness' is considered as an efficient strategy for deterring herbivores: the more a leaf is tough, the more is compounded with carbon-based structural elements, and the more difficult for herbivores to fragment and digest with consequences at the herbivore physiological level –e.g. lengthen insect developmental times– (Coley & Barone 1996). In the literature one finds 'toughness' has been measured in several ways, but the force to punch appears to be the most widely used method (Sanson *et al*. 2001). However, a single test method may show a single angle of the relation between structural plant defense and herbivore damage. Thus additional toughness tests like the force to shear and the force to tear are suitable in herbivory studies (Sanson *et al*. 2001; Onoda *et al*. 2011). Our results showed the force to shear (but not the force to punch or the force to tear), and its interaction with ash and C:N had a significant negative effect on herbivory. These results are new in the literature and may contribute to route future research on this topic, especially when seeking to understand the mechanisms behind the patterns.

Other factors such as leaf size and the tree leaf production (or leaf replacement) were two characteristics closely related to the amount of damage by herbivory. The biggest a leaf is, the most attractive and accessible to herbivores (Garibaldi *et al*. 2011). Smaller or highly divided leaves or leaflets are mechanically less accessible (especially to medium–large size invertebrates because lamina may fail on supporting their weight) reducing the foraging efficiency of herbivores and making effective feeding more difficult (Brown *et al*. 1991). Less productive trees resulted much more susceptible to suffer biomass loss by the action of herbivores comparing to more productive ones. Our results suggest that high values of leaf replacement may help plant species to deter herbivores more efficiently, in agreement with Aide (1993) who showed that species producing leaves more or less continuously suffered lower rates of herbivore damage, presumably by using chemical defenses. However this latter statement is to be confirmed in the Amazonian forests, because leaf replacement and chemical defenses are energetically costly which may be confronting plants to trade-off between the energy invested in growing or in defense. Plants must grow fast enough to compete, while maintaining the defenses necessary to survive in the presence of pathogens and herbivores (Herms & Mattson 1992).

(2) Herbivory affects leaf litter quality. Our results showed herbivory affects the quality of leaves in respect to the lignin and ash content. We found entire senescent leaves were less lignified and contained less ash-related products (associated to leaf structural resistance) comparing to damaged senescent leaves. Following Agrawal *et al*. (2012) our results suggest these chemical alterations were potentially a consequence of plant strategies to deter herbivores where lignin and ash-related elements were effectively and immediately conserved (i.e. not resorbed) in damaged leaves as a local anti-herbivore strategy.

Decomposition. – **(3) Leaf litter fall biomass is independent to tree size and tree abundance.** Our findings showed that leaf litter fall is a property intrinsic of a plant species. Our results showed it is independent on the growth form of the trees (i.e. tall tree, mid-sized tree, treelet) and its abundance. Leaf litter patterns are probably directly responding to biotic and abiotic factors such as herbivory pressure and defenses (i.e. amount of leaf production and leaf replacement rates as herbivore deterring strategy), soil properties or climate.

(4) Leaf litter quality differences between entire and damaged leaves are not important for decomposition rates. Once fallen on soils, small physico-chemical differences found between entire and herbivory-damaged leaves were not sufficient to affect decomposition patterns. From a detritivore perspective, both entire and damaged leaf litter are pretty much the same type of resources, except for the first month of decomposition process where higher edge availability in damaged leaves seem to promote the access of microbes and/or macro-detritivores to leaf tissues (Cárdenas & Dangles 2012).

(5) Decomposition is mainly controlled by leaf litter chemical traits. Irrespective of the physical state of fallen leaf litter (i.e. entire or damaged), we found decomposition was regulated by intrinsic chemical elements such as complex recalcitrant molecules like lignin and condensed tannins (CT) (and interactions, lignin:N, CT:N, and lignin × CT). Lignin, and more specifically, lignin:N ratio have been concurrently negative correlated to decomposition rates (Melillo *et al*. 1982; Taylor *et al*. 1989; Moore *et al*. 1999; Kurokawa & Nakashizuka 2008; Wieder *et al*. 2009), although Hättenschwiler *et al*. (2011) found no significant effect of this ratio on decomposition in the Guyana forest. We argue that differences between both Amazonian results might rely mainly on differences on experimental designs: the use of different plant species with particular traits (i.e. litter types used), and the participation of different decomposers/detritivores communities with particular nutrient necessities and degrading capacities. Our study considered the consuming contribution of micro- to megafauna, while Hättenschwiler *et al*. (2011) mesh bags (of 0.068mm) allowed the consuming contribution of microfauna only, thus preventing the activity of a range of macrodetritivore species that would probably bring to light the negative effect of lignin:N ratio.

The negative role of CT on decomposition has been widely documented in the literature (e.g. Hättenschwiler *et al*. 2005; Kurokawa & Nakashizuka 2008; Coq *et al*. 2010), and our results in this respect are in total agreement. However the ‘chemical ecology’ of these complex molecules, i.e. the mechanisms behind their toxicity and its impact on the ecosystems, are not yet well understood. Salminen & Karonen (2011) started by challenging the classical definition that considered tannins as “water-soluble phenolic compounds able to bind and precipitate proteins and other macromolecules within aqueous solutions.” Such a definition suggested that the nutritive content of an herbivore diet could be severely affected if tannins bound and precipitated all the proteins and micronutrients. For a long time, tannin anti-herbivore activity was thought to derive from their protein precipitation capacity that rendered plant tissues non-nutritious and unpalatable for herbivores. Recent evidence suggests however that tannin activity cannot be explained quite as simply, as tannin oxidation should also be taken into account as a defense mechanism for plants. CT belong to the proanthocyanidins group that present the most active protein precipitants, but are the least oxidatively active at the same time. Future studies considering tannins as anti-herbivore compounds, must consider other class of hydrolysable tannins such as the ellagitannins (which are highly oxidatively active, Salminen & Karonen 2011). Other methodologies such as the polyethylene glycol (PEG)-binding capacity provide a straightforward quantitative index that address the importance of the degree to which tannins bind to plant proteins by considering the activity of many groups of tannins at the same time (Moles *et al*. 2013).

For technical reasons, the role of micronutrients was not assessed in our analyses (see Introduction, p. 52 of this manuscript). However, some large minute data we could obtain suggest that they may have a role in driving decomposition. As shown by previous authors (e.g. Berg 2000), our analyses found that Manganese (Mn), Copper (Cu) and the Mn:Cu ratio were constantly good predictors of litter decomposition (results not shown in the main paper). Mn content has direct implications for lignin degradation as it is essential for the production and activity of Mn-peroxidase, a lignin-degrading enzyme (Pérez & Jeffries 1992) and is involved in the regulation of other lignolytic enzymes, including laccase (Archibald & Roy 1992) and lignin peroxidase (Pérez & Jeffries 1992). Contrastingly, Cu is considered an inhibitor of soil microbial respiration (Doelman & Haanstra 1984). High concentrations of this element in leaves make them poorly palatable for detritivores. Our findings strongly supports that tropical leaf litter showing a combination of high levels of Mn and low levels of Cu may be highly suitable for the decomposer soil community (Figure D2). The role of other microelements in the decomposition process has been scarcely evaluated and still poorly undertood (Kaspari *et al*. 2008). Calcium (Ca), Copper (Cu), Manganese (Mn), Sodium (Na), and Phosphorous (P) seemed to play an important role in the decomposition process (results not shown). P has shown to increase by 49% and 30% cellulose and leaf-litter decomposition (Kaspari *et al*. 2008) confirming its position as a rate-limiting nutrient in the synthesis of cellulolytic enzymes (Sterner & Elser 2002). Ca is essential for calcareous detritivores nutrition like gastropods, isopods, diplopods, annelids and oribatid mites (its exoskeleton containing up to 18% of Ca) (Pramanik *et al*. 2001; Schamp *et al*. 2010). Na is essential to the metabolism of plant consumers, both decomposers and herbivores, as it maintains homeostasis, and gradients of cell solutes concentration and membranes voltage (Dudley *et al*. 2012). Kaspari *et al*. (2009) showed that adding NaCl solution to the leaf litter in an Amazonian rainforest, enhanced litter mass loss by 41% in no more than 20 days. The role of micronutrients on decomposition is still poorly understood, more detailed analyses are necessary to understand its role and its relationship with detritivore and decomposer communities physiological needs.

The role of detritivore functional diversity in the decomposition process: do size matters?.– **(6) Detritivore diversity has complementary functionality in Yasuní.** In the tropical rainforests, the soil fauna makes an important contribution on the ecosystem nutrient cycling by controlling litter decomposition (Swift *et al*. 1979 and Lamina 3; Hättenschwiler *et al*. 2011). Our study described for the first time the functional diversity of soil detritivores in the Yasuní forest, and documented the implications of their size gradual loss in the process of decomposition to make qualitative predictions about the functional consequences of such an extinction scenario (Gross &

Cardinale 2005). Our results showed a predominant abundance of hymenopterans (mainly ants), collembolans and coleopterans (mostly bark beetles and weevils) representing a wide range of feeding habits. Functional groups assessment suggested that the Amazonian forest floor is upholstered of all kinds of taxa representing multiple behaviors and physiological strategies within the trophic chain, suggesting a high rate of redundancy per unit of area. These results contrasted to other highly diverse (aquatic) environments, which have shown low levels of redundancy (Bellwood *et al*. 2003) or high levels of functional redundancy but low of functional diversity (Strauß *et al*. 2010). As a whole, soil community size structure was composed of few voluminous species and many small ones (a pattern that was kept when analyzing litter transformers community distribution alone). Finally, we found a barely significant positive linear relationship between detritivore size community and the percentage of leaf litter mass loss suggesting that the different detritivore size-classes have a complementary effect on the decomposition process in this ecosystem. We concluded the extinction of larger invertebrates may not necessarily represent a challenge for the decomposition process in Yasuní.

(7) Weak association between herbivory and decomposability. Overall, when considering our findings and taking account results published in specialized literature, we can truthfully confirm and conclude that in the tropics canopy and soil are independent subsystems where leaf herbivory and leaf litter decomposition processes are weakly associated. One novel approach to reveal any relationship between them was to analyze data counting on their differences at inter- and intra-specific levels. Tropical forests must be seen as a mega-diverse mosaic of strategies and adaptations where species inner variability is at the base of its complexity. Plant diversity have resulted in diverse plant–herbivore interactions and of particular (micro-) habitat selection pressures (Kursar & Coley 2003; Agrawal 2007) with consequences over the entire ecosystem. At both, inter- and intra-specific levels, greatest variability occurred within the herbivory process when comparing to decomposition. This might be a consequence of the higher levels of specialization occurring in herbivores comparing to detritivores (Makkonen *et al*. 2012). Finally, analyses based on inter- and intra-specific variability in herbivory and decomposition failed to explain any relationship between both processes confirming they are independent between one another and respond to different factors typical to each subsystem (i.e. canopy and soil).

Perspectives

Further new and long-term ecological research in the tropics, but more importantly, generated by local researchers from the tropics, is of extreme relevance due to the current degraded environmental state of the planet. For example, understanding the responses of tropical ecosystems to changes in environmental conditions is crucial for predicting global carbon cycling (Zuidema *et al*. 2013). According to this, dynamic plots such as the 50-ha in Yasuní in the Ecuadorian Amazon offer a great opportunity for ecologists to carry out studies that understand connections and mechanisms of various ecosystem processes (Condit 1995; Valencia *et al*. 2004b). However, while extensive studies have been performed on plant ecology relatively few studies have addressed the role of insects on the maintenance of ecosystem processes in such biodiverse forests. In the continuity of this thesis project, there is some basic information that it is imperative to fulfill as it may improve the scope of future research experiments.

Invertebrate monitoring

We have a very poor knowledge of invertebrate identity and role in the tropical forests and this issue needs to be urgently considered. The use of barcoding to accurately identify the total species number of canopy and especially soil detritivore fauna will certainly open the black box of the real impact of ecosystem functioning in extinction scenarios of both groups of organisms (see Wall *et* *al*. 2010). This of course needs to be complemented with physiological studies (using stable isotopes analyses for example, e.g. Davidson *et al*. 2003) that describe the role, trophic-chain position and real biomass-consumption relationship of each of the different groups of animals (at the taxonomic finest resolution). Long-term annual monitoring of herbivore and detritivore invertebrate populations, and tree leaf production patterns in Yasuní dynamic plot should be the next step. A more accurate functional diversity of invertebrates could be suitable for having more realistic results when for example matching it with leaf area seasonal swings satellite data (see Myeni *et al*. 2007), herbivory/decomposition annual monitoring values and leaf production patterns. With such information we would better understand the impact of climate, leaf size and leaf production seasonality in herbivore outbreaks and in plant physiological defense adaptations, and whether this has consequences on decomposition rates.

Going deeper into our knowledge on plant-invertebrate interactions in the tropics

Combining plant and invertebrate ecological studies in forest dynamic plots future research should consider new approaches in order to reach a thorough panorama of the invertebrate-plant interactions involved in nutrient cycling. For example new studies should focus at both ‘micro’ (leaf, branch, individual, local site habitat) and ‘macro’ (species, communities, ecosystems) plant ecological levels. Because of the magnitude of local factors influencing plant and invertebrates biology, micro-ecological level analyses in the future need more controlled conditions considering the physiology of both groups of organisms. Experiments *in situ* green houses for example, with control over the accessibility of herbivores and/or detritivores with plants growing in the same soil conditions may help to better understand the impact of both groups of invertebrates on the physiological responses of plants (i.e. growth and defense). At macro-ecological level new analyses must dig deeper based on the results already obtained and accessible as published data. For example, the anti-herbivore defensive strategy chosen by any plant species may depend on the particular herbivory pressure each species is submitted to and the microenvironment where it grows (e.g. plants growing in gaps) (Fine *et al*. 2006; Janzen 1974). Thus future experiments should consider this kind of information to reduce the number of contributing factors acting over herbivory process and plant defense strategies and test those not yet evaluated.

Forest structure and dynamics effects on herbivory and decomposition

As soil properties and microtopography have been shown to shape species distributions in Yasuní (Endara & Jaramillo 2010; Valencia *et al*. 2004a) another line of research should aim to identify whether highly clustered species in the plot (e.g. *Rinorea* spp., *Brownea lore*) grow in rich or poor environments, present fast or low growing rates, and whether these characteristics are correlated to defense strategies and herbivory (Coley *et al*. 1985; Fine *et al*. 2004). The ‘home field advantage’ hypothesis (HFA, Gholz *et al*. 2000) could be tested in different scenarios (clustered vs. unclustered species in rich and poor environments for example) and this would allow assessing whether in such local conditions there is a significant relationship between herbivory, plant defense strategies and decomposition. The HFA hypothesis predicts that litter may decompose faster in an area dominated by the plant species from which it derived (i.e. at home) than in an area dominated by another plant species (i.e. away) (Gholz *et al*. 2000) because of local detritivores and decomposers more efficiency (specialization) to degrade ‘home’ litter. Although HFA has been proven to explain part of the decomposition variability in meta-analyses (Gholz *et al*. 2000; Ayres *et al*. 2009), intensive experiments have not been conducted in the tropics yet. However experiments in specific soil micro environments such as those generated by leaf litter fall of clustered plant species have not been performed and may show unsuspected results in the sense that evidence suggest no association between soil fauna and tree species in a tropical rainforest (Donoso *et al*. 2010), suggesting poor probabilities of an important role of HFA in the decomposition process in this type of ecosystems.

In the context of phenological aspects of plants, and its relation to leaf herbivory, defenses and litter decomposition, Kurokawa & Nakashizuka (2008) found that ‘continuous’ leaf emergence defense strategy (and not ‘synchronous’ leafing strategy) was associated to a positive relationship between herbivory and decomposition (refer to Kursar & Coley 2003 for a full diagnosis on defense syndromes). This is interesting in the sense that continuous-leafing species present the higher levels of chemical defenses (Kursar & Coley 2003), and in the tropics, chemical defenses seem not to be as effective as physical defenses for deterring herbivores (Cárdenas *et al*. Chapter 1; Kurokawa & Nakashizuka 2008). Further analyses for example should elucidate which intrinsic functional traits (e.g. all kinds of plant defenses) are mechanistically explaining the role of continuous-leafing on the subsequent decomposition process. These kinds of analyses require meticulous long-term monitoring of leaf production to determine which species have chosen which strategy.

Establishing ‘the role of invertebrates in the ecosystem functioning’ research in Ecuador

For establishing such a new research discipline it is absolutely necessary to rely on solid and long-term interdisciplinary collaborations among researchers and institutions globally, but at Ecuadorian and Latin American levels principally. For example, invertebrate Museums in Latin America are complementary in respect of the taxonomic-diversity knowledge (i.e. groups of organisms described/identified by taxonomic authorities may differ among the Museums). This is of extreme relevance if one considers that several species could be distributed in geographically remote countries and that reliable taxonomic information is at the base of B-EF research. Moreover, many ecosystems are shared between countries. For example the Amazonian region is shared by 9 countries and the Andean region by 7 which mean that results of a research in one country may be replicable in another one (in the same type of ecosystem) and may be useful for studying large-scale ecological patterns. Parallel to this, chemical and molecular analyses need top laboratories managed by qualified experts in each area to properly face problems during the different steps until protocols are established. In Ecuador, as well as in the rest of Latin America, these kinds of laboratories are still scarce and dispersed reason why inter-institutional collaborations may facilitate analyses in terms of paperwork and costs. In this context, it would be of extremely importance to build-up a new team of ecologists and equipped laboratory in Ecuador for the study of the role of invertebrates on the ecosystem functioning. Investigations should focus on the role of ecosystem engineers in the tropical ecosystems, the importance of biodiversity in the ecosystem functioning and its impact on ecosystem services, whose results are useful to the society and help in reaching the so-yearned *Sumak-Kawsay* world (‘living well’, an autochthonous notion of human wellbeing based on the Andean *Kichwas* cosmovision) (Ecuador Constitution 2008; Radcliffe 2012).

Figures

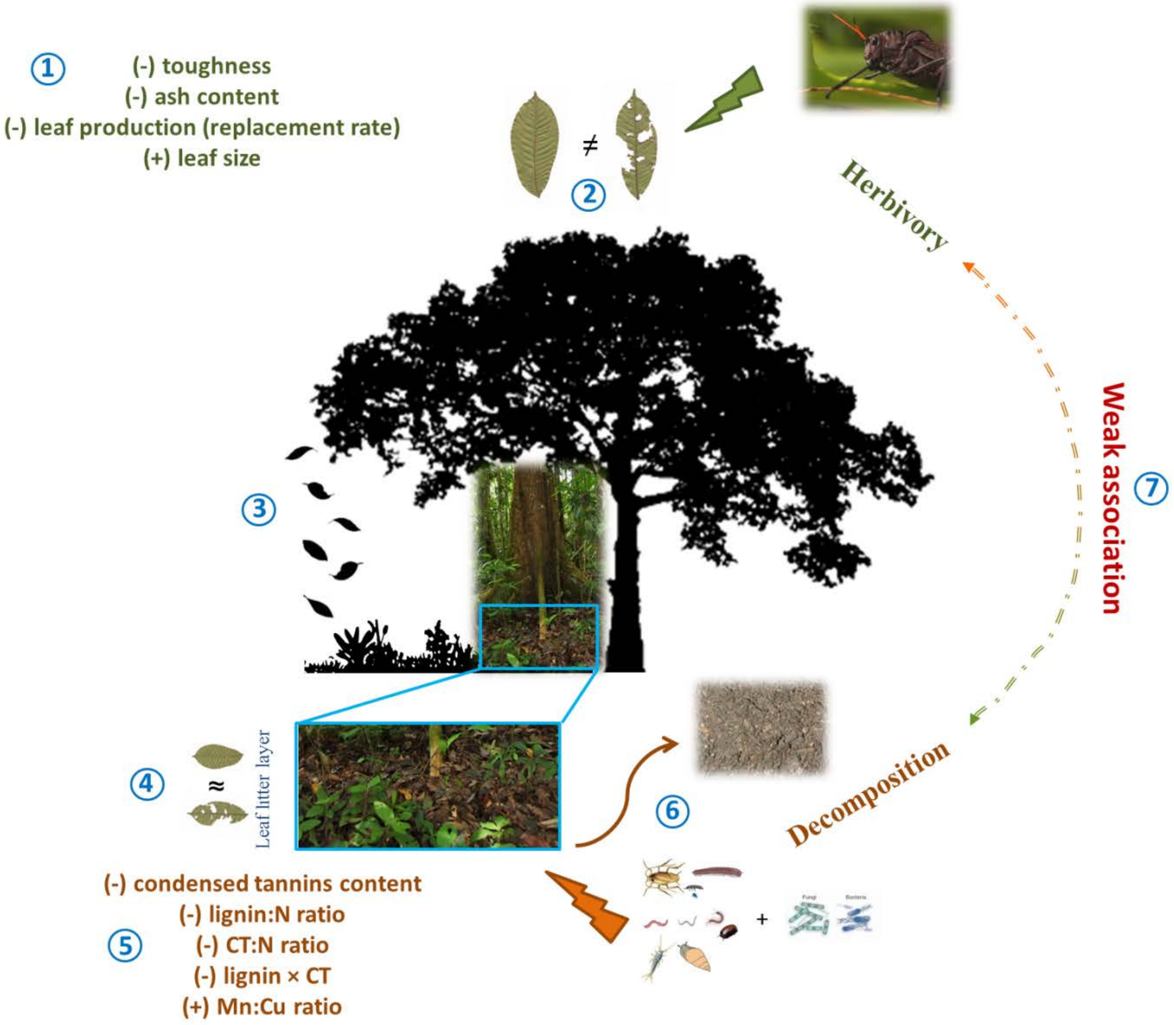


Figure D1. Diagram of the most important leaf/litter traits controlling herbivory and decomposition in the Yasuní tropical rainforest. Bullet points are explained in the main text.

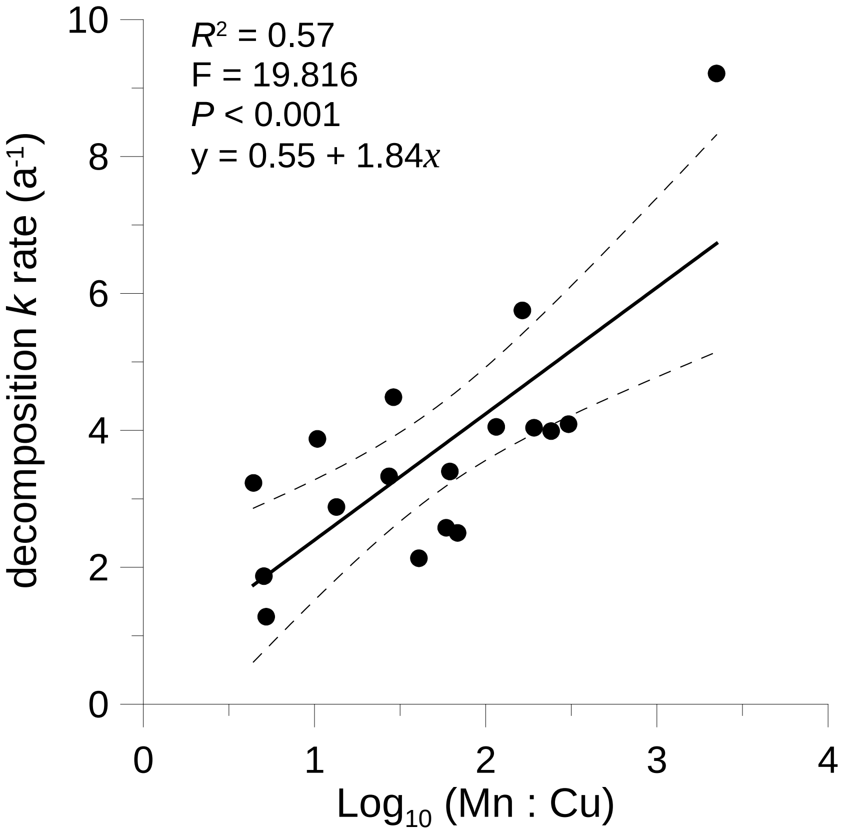


Figure D2. Decomposition rates of all pooled leaves as a function of manganese:copper ratio. Values of R, F and *P* are given for log, power or hyperbolic regressions (equations described). Dashed lines correspond to ±95% confidence intervals.

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