



Contracting montane cloud forests: a case study of the Andean alder (*Alnus acuminata*) and associated fungi in the Yungas

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ABSTRACT

Alnus acuminata is a keystone tree species in the Yungas forests and host to a wide range of fungal symbionts. While species distribution models (SDMs) are routinely used for plants and animals to study the effects of climate change on montane forest communities, employing SDMs in fungi has been hindered by the lack of data on their geographic distribution. The well-known host specificity and common biogeographic history of *A. acuminata* and associated ectomycorrhizal (ECM) fungi provide an exceptional opportunity to model the potential habitat for this symbiotic assemblage and to predict possible climate-driven changes in the future. We (1) modeled the present and future distributions of suitable habitats for *A. acuminata*; (2) characterized fungal communities in different altitudinal zones of the Yungas using DNA metabarcoding of soil and root samples; and (3) selected fungi that were significant indicators of *Alnus*. Fungal communities were strongly structured according to altitudinal forest types and the presence of *Alnus*. Fungal indicators of *Alnus*, particularly ECM and root endophytic fungi, were also detected in *Alnus* roots. Current and future (year 2050) habitat models developed for *A. acuminata* predict a 25–50 percent decrease in suitable area and an upslope shift of the suitable habitat by ca. 184–380 m, depending on the climate change scenario. Although *A. acuminata* is considered to be an effective disperser, recent studies suggest that Andean grasslands are remarkably resistant to forest invasion, and future range contraction for *A. acuminata* may be even more pronounced than predicted by our models.

Abstract in Spanish is available with online material.

Key words: Andes; Argentina; Bolivia; DNA metabarcoding; ITS rDNA; montane cloud forests; mycorrhizal; species distribution modeling

TROPICAL AND SUBTROPICAL MONTANE CLOUD FORESTS are unique and fragile ecosystems characterized by high humidity and frequent cloud cover (even in the dry seasons) and high endemism and biodiversity (Luna-Vega *et al.* 2001, Toledo-Aceves *et al.* 2011). Andean cloud forests in particular are considered among the most biologically diverse ecosystems in the world (Gentry 1992). These forests provide crucial ecosystem services, maintaining nutrient and hydrological cycles vital for human settlements and agriculture (Brown & Kappelle 2001, Toledo-Aceves *et al.* 2011). Cloud forests are severely threatened by anthropogenic and climatic disturbances, *e.g.*, deforestations, fires, and climate change (Still *et al.* 1999, Brown *et al.* 2002, Peh *et al.* 2011). Rising temperatures may be altering the climate of tropical mountains, resulting in a shifting cloud base that threatens the long-term survival of cloud forests (Still *et al.* 1999). Therefore, tropical montane cloud forests are among the most vulnerable

terrestrial ecosystems to climate change (Foster 2001, Pacheco *et al.* 2010). Despite their biological and ecological importance, cloud forests are among the least studied terrestrial ecosystems (Stadtmüller 1987, Aubad *et al.* 2008), and most knowledge focuses on animals and plants (*e.g.*, Churchill *et al.* 1995, Gentry 1995, Brehm *et al.* 2005), while the diversity and spatial distribution of other organismal groups are still poorly known.

Here, we focused on the Andean alder (*Alnus acuminata* Kunth) and associated fungi in the Tucuman-Bolivian montane forests (hereafter, Yungas) situated on the eastern slopes of the Andes. The Yungas stretch from Tarija and Chuquisaca departments in southern Bolivia to Catamarca province in Northwestern Argentina (approx. 18–28°S) and are floristically distinct from the tropical northern Andean forests (Brown *et al.* 2001). Together with the adjacent seasonally dry piedmont forests, the Yungas constitute the southern limit of the Amazonian biogeographic domain (Cabrera 1976, Prado 2000). The vegetation in the region of interest can be divided into three major forest types: the piedmont forest (400–700 m asl), the montane forest

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(700–1500 m asl), and the montane cloud forest (1500–3000 m asl) (Grau & Brown 2000, Prado 2000, Brown *et al.* 2001).

Alnus acuminata is a defining species of the montane cloud forest zone, where it often forms monodominant forests (Brown *et al.* 2001), particularly as an early colonizer following disturbance, *e.g.*, landslides (Blodgett 1998). This species is important for agroforestry, land reclamation, watershed protection, and erosion control due to its rapid growth in disturbed habitats (National Academy of Sciences 1984). *Alnus acuminata* is among the very few tree species capable of associating with ectomycorrhizal (ECM), arbuscular mycorrhizal (AM), and root endophytic fungi simultaneously, as well as nitrogen-fixing bacteria (Cervantes & Rodríguez-Barrueco 1992, Becerra *et al.* 2009). While ECM fungi in general tend to have rather broad host ranges, often spanning plant families or even phyla, alder-associated ECM basidiomycetes represent a well-known exception, as they are generally host specific to the genus *Alnus* at a global scale (Molina 1981, Tedersoo *et al.* 2009, Kennedy & Hill 2010, Kennedy *et al.* 2011). Because *A. acuminata* is the only member of its genus, and of the family Betulaceae, in South America (Milliken 2010), alder-specific ECM fungi only occur with *A. acuminata* in the Andes. Previous data from Mexico and Argentina indicate that ECM fungi associated with *A. acuminata*, including several *Alnicola*, *Alpova*, *Cortinarius*, *Inocybe*, and *Lactarius* species, are generally conspecific in these two extremes of the geographic distribution of Andean alder (Becerra *et al.* 2002, 2005a, b, Kennedy *et al.* 2011, Geml *et al.* 2014b, Nouhra *et al.* 2015), but there are no available data from the areas in between. *Alnus acuminata* first reached northern South America *ca.* 1 million years ago (Hooghiemstra 1989) and likely colonized the cloud forests of the tropical and subtropical Andes by co-migrating from Central America with associated ECM fungi. By contrast, AM fungi, *i.e.*, the phylum Glomeromycota, are symbionts of a wide range of other plants (Becerra & Cabello 2008) and are ubiquitous in most terrestrial ecosystems (Davison *et al.* 2015). The host and habitat specificity of other fungi (*e.g.*, root endophytes) in the Yungas is virtually unknown.

The climatic niche of *A. acuminata* is, by definition, the montane cloud forest zone. Within that potential climatic niche, the actual spatial distribution of *A. acuminata* is mainly shaped by successional dynamics, as detailed above. The specificity and common biogeographic history of *A. acuminata* and associated ECM fungi provide an exceptional opportunity to model the potential habitat for the members of this symbiotic assemblage and to predict possible climate-driven changes in the future.

Species distribution models (SDMs) are increasingly used to estimate distributions of species in ecology and conservation studies. SDMs predict the potential spatial distribution of species by linking species occurrences with ecological factors that shape their distribution (Guisan & Thuiller 2005). SDMs generally rely on occurrence data that represent the known distribution of the species in the geographic region of interest. Unfortunately, fungi in general do not fit these criteria very well. Given that an estimated >95 percent of fungi are still unknown (Blackwell 2011) and that even for the known species, information on their

geographical distribution is very scarce, it has been impossible to use SDMs to estimate distribution ranges of fungi, except for some well-known pathogens of commercial importance (Lorestani *et al.* 2013). The lack of occurrence data is particularly true for undersampled and presumably highly diverse Neotropical areas, such as the Andean Yungas. To overcome the limitation posed by the lack of fungal spatial data, we estimated the present and future (2050) distribution ranges of *A. acuminata* in the Yungas and characterized the alder-associated fungal community to predict possible climate-induced shifts in their potential habitat and spatial patterns.

METHODS

SPECIES DISTRIBUTION MODELING OF *ALNUS ACUMINATA*.—We obtained species presence records of *A. acuminata* from the Global Biodiversity Information Facility (GBIF 2014) for both the Argentinian and Bolivian parts of the Yungas. We selected species presences with at least 4 pixels (around 3.6 km) distance among each other to eliminate spatial autocorrelation (Tovar *et al.* 2013). To eliminate records that likely represented cultivated specimens or erroneous coordinates, we only included presence data between 1200 and 3000 m asl, as this interval represents the greatest natural elevation range of *A. acuminata* (Résico & Burghi 2003). In total, 60 geo-referenced records of *A. acuminata* served as input for distribution modeling (Table S1).

We compiled one dataset of current environmental conditions, consisting of ten environmental variables that were used to construct the SDMs and that had Pearson correlation values of $|r| < 0.70$ (Dormann *et al.* 2013). The selected environmental variables included four bioclimatic variables (mean annual temperature, isothermality, temperature annual range, precipitation of wettest quarter) obtained from Worldclim (Hijmans *et al.* 2005), three topographic layers (slope, aspect southness and eastness) derived from a digital elevation model (Jarvis *et al.* 2008), and three layers of topsoil properties (organic carbon, pH, and bulk density) obtained from Harmonized World Soil Database (Nachtergaele *et al.* 2009) (Table S2). All environmental variables had a resolution of approximately 1 km² at the Equator. For the future projection of the *Alnus* SDM, we obtained the same bioclimatic variables for future conditions for the year 2050 from Worldclim. We selected the projections of global climate model CCSM4 for the four representative concentration pathways (RCPs) as described by Moss *et al.* (2008). These pathways consist of RCP 2.6 (represents low impact climate change), RCP 4.6 and 6.0 (represent intermediate climate change), and 8.5 (high impact climate change) (Rogelj *et al.* 2012). All environmental variables were prepared using ArcGIS 10.0 (ESRI 2011).

We used the biomod2 package v. 3.1-64 (Thuiller *et al.* 2014) for R (R core team 2015) to model the distribution of *A. acuminata* in the Yungas. We used an ensemble modeling approach constructed with five different algorithms to obtain the final SDM to offer more robust predictions for the potential distributions of species than could be obtained from single algorithm models (Araújo & New 2007). The algorithms used were as follows:

Generalized Linear Models (GLM; McCullagh & Nelder 1989), Generalized Additive Models (GAM; Hastie & Tibshirani 1990), Generalized Boosted Models (GBM; Ridgeway 1999), Random Forest (RF; Breiman 2001), and Maxent (Phillips *et al.* 2006). We selected these modeling algorithms based on a recent work that identified them as rendering consistent and high-performing models for species with varied spatial distribution and number of records (Aguirre-Gutiérrez *et al.* 2013). We carried out ten model repetitions for each algorithm, leaving 25 percent of the species presences aside to validate our model by means of the area under the curve value (AUC; Hanley & McNeil 1982). Since all algorithms need absences for model fitting (except Maxent, which uses background data), we generated a random sample of pseudo-absences. Default parameters were used for modeling, except for Maxent, where we used 1000 iterations to allow more time for model convergence. The resulting 50 models obtained from the five modeling algorithms were used to construct the ensemble model. The median of the single model predictions was used as the ensemble rule. The resulting ensemble model fitted under the present conditions was then projected to the future climatic conditions based on the CCSM 4 climate model and the four RCPs.

SOIL FUNGAL MOLECULAR WORK.—We collected soil samples at 24 sites that represent the entire latitudinal extent of the Yungas in Argentina (spanning *ca.* 2/3 of the latitudinal extent of the Yungas). We sampled localities in each of the three forest types (Fig. S1), the majority of which were in The Yungas UNESCO Biosphere Reserve (Lomáscolo *et al.* 2010). Forest type, elevation, geographic coordinates, locality, and the measured soil chemical properties of these locations are shown in Table S3. More detailed descriptions of the altitudinal forest types are available from Brown *et al.* (2001) and Geml *et al.* (2014b). We characterized and compared fungal communities of all altitudinal forest types in the Yungas after re-analyzing soil DNA metabarcoding data generated by Geml *et al.* (2014b) using an updated set of bioinformatic tools.

ALNUS ROOT MOLECULAR WORK.—We collected *Alnus acuminata* root samples from five monodominant sites (BM2, BM4, BM5, BM7, and BM8) to characterize fungi living inside the roots of *Alnus*. For each site, roots of 15 alder trees located at a minimum distance of 8 m from each other were collected from *ca.* 10 × 15 × 15 cm soil cuboids sampled at 50–100 cm from the nearest alder tree. Roots were kept in 2% CTAB in the field and were later lyophilized and ground. Genomic DNA was extracted from 1 g of dry *Alnus* roots using a NucleoSpin[®] Plant II kit (Macherey-Nagel GmbH & Co., Düren, Germany), according to the manufacturer's protocol. The ITS2 region (*ca.* 250 bp) of the nuclear ribosomal DNA repeat was PCR amplified and later sequenced using Ion Torrent as described in detail in Geml *et al.* (2014a, b). ITS (including ITS1, 5.8S, and ITS2) is the universal DNA barcode marker for fungi and has been used in a wide variety of taxonomic and ecological studies (*e.g.*, Bruns *et al.* 1991, Geml *et al.* 2005, 2010, Hempel *et al.* 2007, Schoch *et al.* 2012, Bellemain *et al.* 2013).

BIOINFORMATIC WORK.—The raw sequence data (deposited in Dryad: doi: 10.5061/dryad.8fn8j) contained 7,489,045 sequences with a modal read length of 287 base pairs (bp). We removed the primers and trimmed poor quality ends based on 0.02 error probability limit in Geneious Pro 5.6.1 (BioMatters, New Zealand). Then, we filtered sequences using MOTHRUR v. 1.32.1 (Schloss *et al.* 2009) based on the following settings: no ambiguous bases (maxambig = 0), homopolymers no longer than 10 nucleotides (maxhomop = 10), and length range from 150 to 400 bp (minlength = 150; maxlength = 400), resulting in 4,760,162 quality-filtered sequences with an average read length of 272.4 ± 49.9 (mean ± SD). We grouped the remaining sequences into 9144 operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH v.8.0 (Edgar 2010), while simultaneously removing putative chimeric OTUs. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE fungal ITS sequence data base containing identified fungal sequences with assignments to Species Hypothesis groups using dynamic assignment based on periodically updated, phylogeny-based species delimitations (Kõljalg *et al.* 2013). After excluding OTUs with <70 percent similarity or <150 bp pairwise alignment length to a fungal sequence, 7634 fungal OTUs were retained, comprising 1,416,245 high-quality sequences with an average of 59,010 reads per sample.

STATISTICAL COMPARISONS OF FUNGAL COMMUNITIES.—We used PC-ORD v. 6.0 (McCune & Grace 2002) to run non-metric multidimensional scaling (NMDS) on a primary matrix of sites by soil fungal OTUs. We used presence–absence values and relative read abundance for the ordination analyses due to demonstrated uncertainties regarding the reliability of read abundance as an indicator of taxon abundance or biomass in environmental samples (Lindahl *et al.* 2013). We calculated relative read abundance using general relativization on a per-sample basis in order to moderate the influence of OTUs with high sequence counts, while maintaining some approximation of template abundance. Given the very high sequencing coverage we achieved, ‘presence’ was defined as ≥5 sequences on a per sample basis following the recommendations of Lindahl *et al.* (2013) to minimize false positives (*i.e.*, OTUs that are common in one sample but may be low-abundant contaminants in others). The resulting final matrix contained 4635 OTUs and was used as input for ordinations. We subjected the data to 500 iterations per run using the Sørensen similarity (Bray-Curtis) index and a random number to start. We selected the solution with the lowest number of dimensions when the decrease in the final stress was <5 by adding another dimension (McCune & Grace 2002). We orthogonally rotated the resulting NMDS solution to maximize correlation between elevation and the major axes. We also tested whether fungal communities were statistically different across forest types using two different methods: multiresponse permutation procedure (MRPP) and permutation-based nonparametric MANOVA (Anderson 2001). We performed Mantel tests in PC-ORD to determine if community structure is correlated with geographic (spatial)

distance and/or environmental conditions. For the latter, we standardized mean and standard deviation values of the environmental variables so that all variables contributed equally to the data. Finally, we selected fungal OTUs characteristic ('faithful' and 'differential taxa') of *Alnus*-dominated forests using indicator species analyses (Dufrene & Legendre 1997), also in PC-ORD. For fungi that were statistically significant indicators of *Alnus* forests, we assigned functional guilds to indicator OTUs based on taxonomic identities and isolation sources of the matching reference sequences in UNITE and published ecological information about the taxon in question (Kirk *et al.* 2008, Grünig *et al.* 2011, Teder-
soo & Smith 2013).

RESULTS

SPECIES DISTRIBUTION MODELING OF *ALNUS ACUMINATA*.—The ensemble model obtained an AUC of 0.994 based on the validation dataset containing 25 percent of the *A. acuminata* presence data. The coordinates of our sampling sites support this finding, as all sampled *Alnus*-dominated forest sites were situated within the area predicted as suitable by our model. The SDM suggests that the current distribution of *A. acuminata* in the Yungas comprises a total area of 40,951 km² with a mean elevation (\pm SD) of 1906 \pm 392 masl, of which 11,695 km² is inside protected areas.

For 2050, the predicted area of suitable habitat is reduced to 30,423 km² (7060 km² in protected areas) for the model RCP 2.6, 25,417 km² (6242 km²) for RCP 4.5, 26,539 km² (6517 km²) for RCP 6.0, and 21,191 km² (4866 km²) for RCP 8.5 (Table 1). All models considered, we observed losses in the lower-elevation areas of the montane cloud forest and gains at higher altitudes, resulting in increased mean elevation values for suitable habitat: 2090 \pm 367 masl for RCP 2.6, 2190 \pm 367 masl for RCP 4.5, 2164 \pm 374 masl for RCP 6.0, and 2286 \pm 374 masl for RCP 8.5 (Table 1). Furthermore, we also observed increased separation between the Argentinean and Bolivian montane cloud forest habitats, as the low- to mid-elevation eastern mountain ranges in southern Bolivia (between 19 and 21°S) are predicted to be largely unsuitable for *A. acuminata*. The modeled future distributions for low and high impact climate change scenarios (RCP 2.6 and RCP8.5, respectively) are shown in Fig. 1. The distribution maps for the two intermediate impact scenarios (RCP 4.5 and RCP 6.0) are shown in Fig. S2.

Analyses of the importance of the variables revealed mean annual temperature as the most important variable to determine the outcome of the model, with a mean annual temperature optimum for *A. acuminata* predicted to be *ca.* 15–18°C. The importance of this variable (0.487) was by far the highest of all variables. The second most important variable, soil organic

TABLE 1. Potential habitat of *Alnus acuminata* in the Tucuman-Bolivian Yungas. The climate variables were derived from the CCSM4 climate model for the four RCP pathways: RCP 2.6 (low-impact climate change), RCP 4.6 and 6.0 (intermediate climate change), and RCP 8.5 (high-impact climate change) (Moss *et al.* 2008, Rogelj *et al.* 2012). Future range size is based on 2050 projection and is divided into three categories: stable, habitat gain, and habitat loss (also indicated as percent of current range). "Habitat loss" and "stable" indicate areas within the current range that will become unsuitable or remain suitable, respectively, while "habitat gain" represents currently unsuitable areas that will become suitable in the future. Mean elevation values for the predicted suitable habitat are calculated from elevation data of all pixels in the predicted ranges.

		Habitat Loss	Stable	Habitat Gain	Total Area
Estimated distribution of <i>Alnus acuminata</i>					
Current	Area (km ²)	–	–	–	40951
	Protected (km ²)	–	–	–	11695
RCP 2.6	Area (km ²)	14891	26060	4363	30423
	% of current area	36.4	63.6	10.7	74.3
RCP 4.5	Protected (km ²)	4635	7060	747	7807
	Area (km ²)	20746	20205	5212	25417
RCP 4.5	% of current area	50.7	49.3	12.7	62.1
	Protected (km ²)	6336	5359	883	6242
RCP 6.0	Area (km ²)	19477	21474	5065	26539
	% of current area	47.6	52.4	12.4	64.8
RCP 6.0	Protected (km ²)	6032	5663	854	6517
	Area (km ²)	25577	15374	5817	21191
RCP 8.5	% of current area	62.5	37.5	14.2	51.7
	Protected (km ²)	7837	3858	1008	4866
Modeled elevation of <i>Alnus acuminata</i>					
Current	Mean \pm SD (masl)	–	–	–	1906 \pm 392
RCP 2.6	Mean \pm SD (masl)	1618 \pm 348	2025 \pm 362	2477 \pm 402	2090 \pm 367
RCP 4.5	Mean \pm SD (masl)	1667 \pm 335	2092 \pm 360	2570 \pm 391	2190 \pm 367
RCP 6.0	Mean \pm SD (masl)	1664 \pm 337	2071 \pm 367	2558 \pm 401	2164 \pm 374
RCP 8.5	Mean \pm SD (masl)	1712 \pm 335	2151 \pm 368	2643 \pm 390	2286 \pm 374

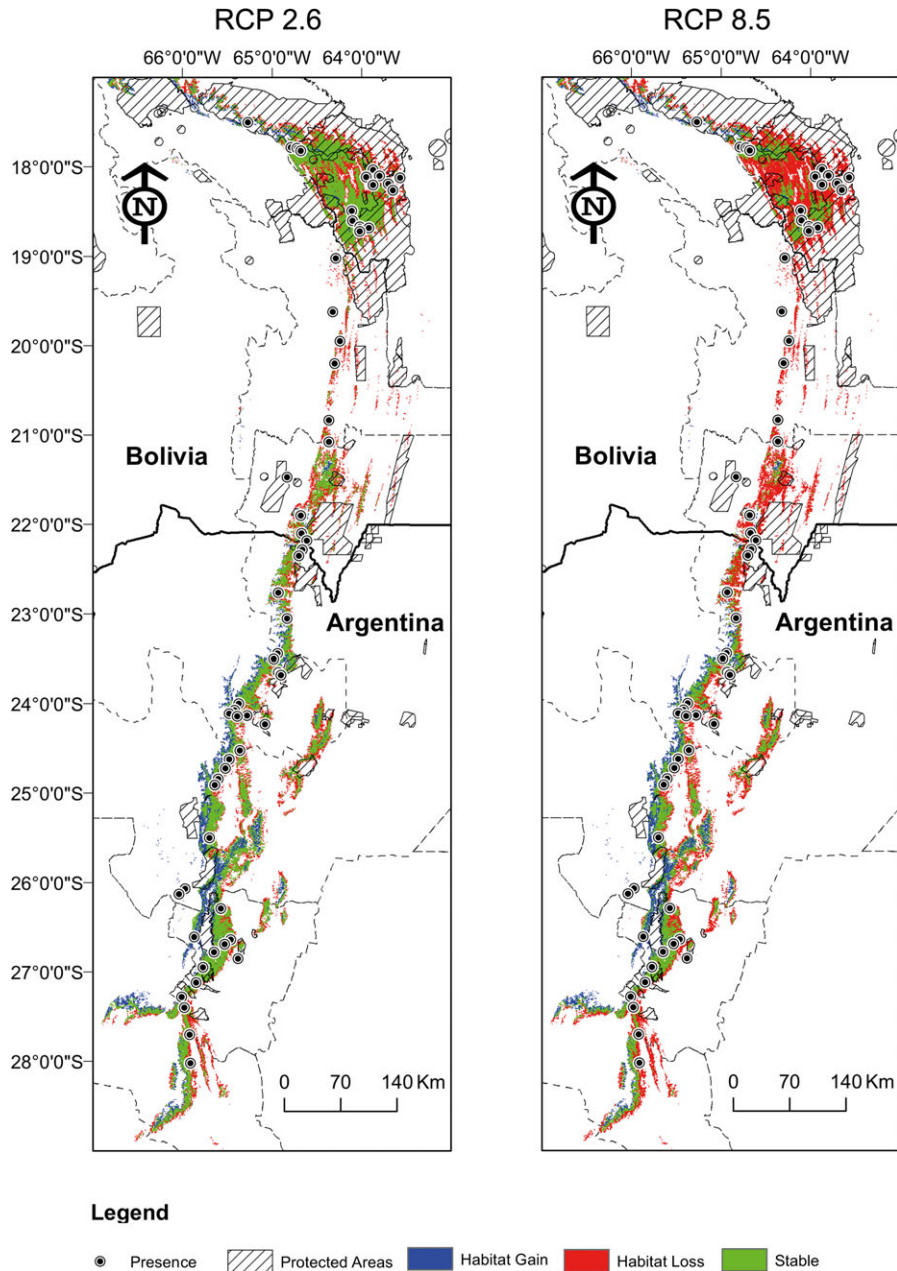


FIGURE 1. Predicted current and future (2050) distributions of *Alnus acuminata* in the Tucuman-Bolivian Yungas according to low (RCP 2.6) and high (RCP 8.5) impact climate change scenarios. Presence localities of *A. acuminata* obtained from GBIF served as input for the species distribution models. Habitat loss, habitat gains, and stable areas are indicated in red, blue, and green, respectively.

carbon, had a value of importance of 0.149, followed by ‘temperature annual range’, with a variable importance of 0.118. Other variables (in descending order of importance) were slope, aspect northness, isothermality, soil pH, precipitation of wettest quarter, aspect eastness, and soil bulk density.

FUNGAL COMMUNITIES.—NMDS analyses resulted in a 2-dimensional solution, with a final stress of 0.09189 (presence-absence) and 0.12547 (relative abundance) and final instability values

<0.00001. The coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: $r^2 = 0.769$; axis 2: $r^2 = 0.101$; total $r^2 = 0.870$; orthogonality = 96.8% (presence-absence), and axis 1: $r^2 = 0.518$, axis 2: $r^2 = 0.233$, total $r^2 = 0.751$, with orthogonality = 93.2% (relative abundance). Because the ordinations plots based on relative abundance and presence-absence were almost identical and because the final stress was lower for the latter, only the presence-absence dataset was used in

subsequent analyses. The NMDS ordinations revealed a strong structuring of soil fungal communities according to altitudinal forest types and sampling sites representing the same forest type grouped together (Fig. 2). Within the montane cloud forest, sites dominated by *Alnus* grouped together on the extreme right of the graph. The only exception was BM3, a mixed forest near the transition of montane forest into montane cloud forest co-dominated by *Juglans australis* and *A. acuminata*, which was somewhat more similar to the montane forest where *Juglans* is more prevalent. Mantel tests showed that fungal community structure was primarily explained by environmental variables ($r^2 = 0.3025$; $P = 0.00063$) and not by geographic distance ($r^2 = 0.0154$; $P > 0.05$). MRPP showed that altitudinal forest type (effect size $A = 0.07342$, probability $P = 0.00002$) as well as the presence of *Alnus* in the sampled sites ($A = 0.04179$, $P = 0.00052$) strongly correlated with fungal community composition. Similarly, permutation-based MANOVA indicated that fungal community structure differed significantly among the altitudinal zones in both the full dataset ($P = 0.0002$) and all pairwise comparisons (all $P < 0.05$). We found 160 OTUs that were significant ($P < 0.05$) indicators for *Alnus*. Of these, 57 OTUs were considered to be strong indicators ($P < 0.01$), the vast majority of which were also detected in the sampled *A. acuminata* roots, as shown in Table 2. Numerous indicators of the *Alnus*-dominated cloud forest belonged to root-associated fungal genera, such as ECM fungi (e.g., *Alnicola*, *Cortinarius*, *Inocybe*, *Lactarius*, *Tomentella*) and root endophytes (e.g., *Cadophora*, *Meliniomyces*, *Oidiodendron*, and several unidentified ascomycetes in Helotiales, Hypocreales, Venturiales, and Xylariales). In addition, there were several saprotrophic and/or pathogenic genera among the indicators, such as *Candida*, *Collophora*, *Leptosphaeria*, *Mortierella*, *Passalora*, *Parasphaeosphaeria*,

Pseudurotium, and *Umbelopsis* (Table 2). Sequences of fungal OTUs considered to be strong indicators ($P < 0.01$) of *Alnus*-dominated forests have been deposited in GenBank (KU208014-KU208070).

DISCUSSION

We modeled the present and future distribution ranges of *A. acuminata* in the Yungas to estimate climate-driven changes in potential habitat for strictly alder-associated ECM taxa and other fungi characteristic of *Alnus*-dominated forests. Our ensemble distribution model estimates that *A. acuminata* is distributed along the entire latitudinal range of the Yungas and that 28.6 percent of its range is located in protected areas. The predicted distribution largely agrees with the known distribution of *A. acuminata* with the exception that while suitable montane cloud forest habitats exist in the southern end of the predicted range, *A. acuminata* has not been observed south of 28°S. This may be due to historical reasons, although the establishment of *Alnus* there in the near future is plausible (A. Grau, pers. comm.). Given that *A. acuminata* is a defining species of the montane cloud forest zone, its distribution provides a proxy for the montane cloud forest ecosystem as a whole. In fact, SDMs generated for other cloud montane forest tree species, such as *Podocarpus parlatorei* (Quiroga *et al.* 2012), were very similar to those obtained in this study for *A. acuminata*.

All statistical analyses suggest that fungal community composition in the montane cloud forest (*i.e.*, the elevation zone representing the climatic niche of *Alnus*, regardless of its physical presence) is different from the other two forest types. Furthermore, we detected all alder-symbiotic ECM fungi and most root

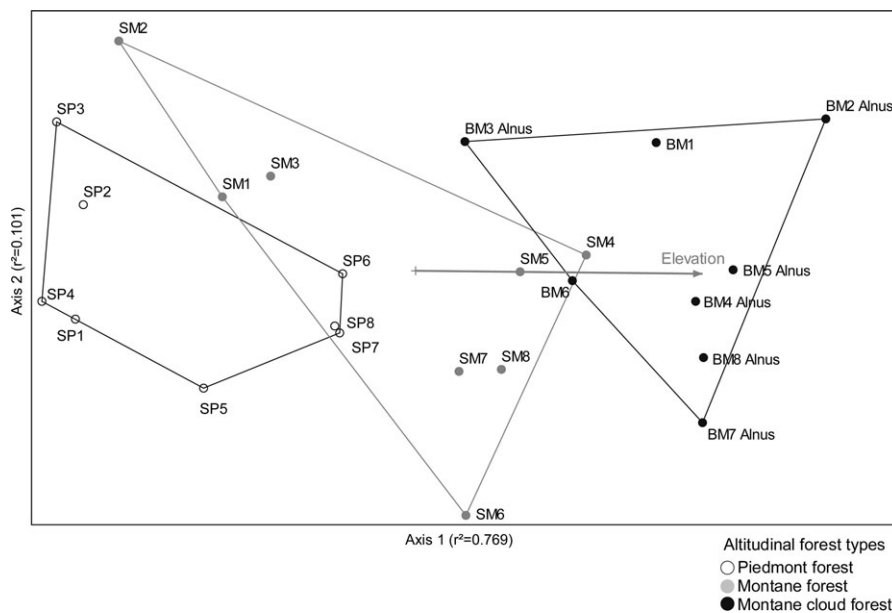


FIGURE 2. Non-metric multidimensional scaling (NMDS) ordination plot of soil fungal communities from various forest types. Labels, localities, and descriptions of the sampling sites are given in Table S1. Vectors of variables correlated with ordination axes at $|r| \geq 0.5$ are shown.

TABLE 2. Fungal OTUs considered to be strong indicators ($P < 0.01$) for *Alnus* displayed in order of decreasing significance and taxonomic identity. Accession numbers, sequence similarity (%), pairwise alignment length (bp), Species Hypothesis numbers (1.5% threshold), name, taxonomic classification, and assigned functional guild are based on the most similar sequence in the UNITE + INSD data base. OTUs in bold have also been found in *Alnus* root samples.

OTUs	P	Accession	%	bp	Species Hypothesis	Name	Phylum	Order	Functional Guild
OTU 457	0.0002	GQ996162	99.1	220	SH181150.07FU	<i>Melinionomyces</i> sp.	Ascomycota	Helotiales	ECM, DSE
OTU 3	0.0002	JN943941	99.6	289	SH218844.07FU	<i>Alnicola silvaenovae</i>	Basidiomycota	Agaricales	ECM
OTU 18	0.0002	UDB012705	99.6	263	SH188474.07FU	<i>Cortinarius</i> sp.	Basidiomycota	Agaricales	ECM
OTU 55	0.0002	UDB014077	99.6	263	SH196817.07FU	<i>Inocybe</i> sp.	Basidiomycota	Agaricales	ECM
OTU 4	0.0002	KC782511	99.6	294	SH184509.07FU	<i>Tomentella testaceogilva</i>	Basidiomycota	Thelephorales	ECM
OTU 603	0.0006	FM172802	89.1	238	SH181107.07FU	<i>Oidiendron</i> sp.	Ascomycota	Incertae sedis	ERM, DSE
OTU 93	0.0006	KT800110	98.6	255	SH211892.07FU	<i>Inocybe jacobii</i>	Basidiomycota	Agaricales	ECM
OTU 51	0.0006	JN168725	88.6	276	SH218257.07FU	<i>Inocybe</i> sp.	Basidiomycota	Agaricales	ECM
OTU 100	0.0006	HQ714728	99.7	345	SH182380.07FU	<i>Lactarius omphaliformis</i>	Basidiomycota	Russulales	ECM
OTU 604	0.0006	KF891371	99.7	366	SH182376.07FU	<i>Lactarius herrerae</i>	Basidiomycota	Russulales	ECM
OTU 11	0.0006	UDB002982	99.7	293	SH189370.07FU	<i>Tomentella ellisii</i>	Basidiomycota	Thelephorales	ECM
OTU 620	0.0006	HQ630293	96.3	162	SH185196.07FU	<i>Mortierella gemmifera</i>	Zygomycota	Mortierellales	SAP
OTU 4850	0.0008	HQ714715	99.1	290	SH218844.07FU	<i>Alnicola silvaenovae</i>	Basidiomycota	Agaricales	ECM
OTU 1495	0.0008	HQ630293	100.0	118	SH185196.07FU	<i>Mortierella gemmifera</i>	Zygomycota	Mortierellales	SAP
OTU 759	0.001	FM200596	96.7	245	SH215109.07FU	Venturiales sp.	Ascomycota	Venturiales	END
OTU 12	0.001	JF298203	99.3	293	SH184511.07FU	Thelephoraceae sp.	Basidiomycota	Thelephorales	ECM
OTU 686	0.0014	KF296911	71.2	226	SH212017.07FU	Fungi sp.	–	–	–
OTU 582	0.0014	GU174352	97.3	257	SH213267.07FU	Chaetothiriales sp.	Ascomycota	Chaetothiriales	REND
OTU 211	0.0014	KC222688	99.1	220	SH205742.07FU	Helotiales sp.	Ascomycota	Helotiales	ECM
OTU 767	0.0014	JX001638	95.0	222	SH195225.07FU	Helotiales sp.	Ascomycota	Helotiales	ECM, SAP
OTU 2279	0.0014	HQ207028	93.4	220	SH000540.07FU	Helotiales sp.	Ascomycota	Helotiales	ECM, DSE
OTU 606	0.0014	KC922122	97.2	178	SH175136.07FU	<i>Candida</i> sp.	Ascomycota	Incertae sedis	SAP
OTU 1344	0.0014	JF740217	82.2	230	SH206028.07FU	<i>Leptosphaeria errabunda</i>	Ascomycota	Pleosporales	SAP
OTU 654	0.0014	EU563602	95.4	255	SH212642.07FU	Xylariales sp.	Ascomycota	Xylariales	END
OTU 221	0.0014	UDB001040	96.8	283	SH188496.07FU	<i>Cortinarius helvelloides</i>	Basidiomycota	Agaricales	ECM
OTU 1843	0.0014	HQ604813	97.6	256	SH211892.07FU	<i>Inocybe jacobii</i>	Basidiomycota	Agaricales	ECM
OTU 71	0.0014	HE979087	99.7	295	SH177842.07FU	<i>Tomentella</i> sp.	Basidiomycota	Thelephorales	ECM
OTU 2693	0.0014	AF504842	99.0	198	SH217612.07FU	Mortierellales sp.	Zygomycota	Mortierellales	SAP
OTU 909	0.0024	FJ475780	92.2	206	SH204497.07FU	Rozellomycota sp.	Rozellomycota	Incertae sedis	–
OTU 424	0.0028	KF428228	98.2	222	SH215243.07FU	Helotiales sp.	Ascomycota	Helotiales	ECM, SAP
OTU 1296	0.003	JX243854	92.2	263	SH199052.07FU	<i>Lophiostoma</i> sp.	Ascomycota	Pleosporales	REND
OTU 550	0.004	AY699664	99.2	133	SH196475.07FU	Helotiales sp.	Ascomycota	Helotiales	ECM, SAP
OTU 3006	0.005	GQ154547	88.0	225	SH201610.07FU	<i>Collaphora rubra</i>	Ascomycota	Incertae sedis	PPATH
OTU 1069	0.0052	KF730795	96.9	223	SH215253.07FU	Helotiales sp.	Ascomycota	Helotiales	–
OTU 739	0.0054	JN943800	100.0	164	SH185972.07FU	<i>Mortierella globulifera</i>	Zygomycota	Mortierellales	SAP
OTU 539	0.006	AF250821	98.8	245	SH186980.07FU	<i>Paraphaeosphaeria pileata</i>	Ascomycota	Pleosporales	SAP
OTU 1673	0.0062	HQ207028	93.2	222	SH174913.07FU	Helotiales sp.	Ascomycota	Helotiales	REND
OTU 1348	0.0062	HQ021838	85.7	230	SH203526.07FU	Helotiales sp.	Ascomycota	Helotiales	–
OTU 103	0.0062	HM230869	98.4	122	SH194254.07FU	Helotiales sp.	Ascomycota	Helotiales	ECM, SAP
OTU 8167	0.0068	AF210691	97.1	242	SH182679.07FU	Bionectriaceae sp.	Ascomycota	Hypocreales	END
OTU 91	0.0068	FM172812	97.9	234	SH217012.07FU	<i>Oidiendron</i> sp.	Ascomycota	Incertae sedis	ERM, DSE
OTU 155	0.0072	LK052797	94.2	278	SH196094.07FU	<i>Umbelopsis</i> sp.	Zygomycota	Mucorales	SAP
OTU 366	0.0074	AY249072	96.4	222	SH196793.07FU	<i>Cadophora melinii</i>	Ascomycota	Helotiales	REND, DSE
OTU 1618	0.0074	JX076945	99.6	234	SH215929.07FU	<i>Pseudurotium</i> sp.	Ascomycota	Incertae sedis	SAP
OTU 3970	0.0078	HQ630294	99.4	334	SH090066.07FU	<i>Mortierella kublmanii</i>	Zygomycota	Mortierellales	SAP
OTU 1444	0.0082	AM999554	88.5	226	SH201665.07FU	Helotiales sp.	Ascomycota	Helotiales	–
OTU 2307	0.0082	EF152526	94.5	226	SH193938.07FU	<i>Mortierella</i> sp.	Zygomycota	Mortierellales	SAP

(continued)

Table 2 (continued)

OTUs	<i>P</i>	Accession	%	bp	Species Hypothesis	Name	Phylum	Order	Functional Guild
OTU 893	0.0082	KF359672	99.7	315	SH187861.07FU	<i>Mortierella</i> sp.	Zygomycota	Mortierellales	SAP
OTU 330	0.0086	AY699656	98.2	222	SH194252.07FU	Helotiales sp.	Ascomycota	Helotiales	ERM
OTU 528	0.009	JF519267	99.5	192	SH186050.07FU	Trechisporales sp.	Basidiomycota	Trechisporales	ECM
OTU 202	0.0092	GU214668	96.8	218	SH206832.07FU	<i>Passalora</i> sp.	Ascomycota	Capnodiales	PPATH
OTU 58	0.0092	KC222688	93.3	224	SH205742.07FU	Helotiales sp.	Ascomycota	Helotiales	REND
OTU 501	0.0092	HQ157836	93.7	222	SH181085.07FU	<i>Melinionyces</i> sp.	Ascomycota	Helotiales	ECM, DSE
OTU 2983	0.0092	JQ272370	90.1	169	SH480947.07FU	Helotiaceae sp.	Ascomycota	Helotiales	ERM, DSE
OTU 952	0.0092	FN565254	98.7	150	SH193764.07FU	Leucosporidiales sp.	Basidiomycota	Leucosporidiales	ECM, SAP
OTU 1358	0.0092	HQ271378	93.5	258	SH196145.07FU	Sebacinaceae sp.	Basidiomycota	Sebacinales	ECM
OTU 1289	0.0092	FJ475780	97.1	205	SH204497y.07FU	Rozellomycota sp.	Rozellomycota	Incertae sedis	–

Abbreviations for functional guilds: DSE, dark septate endophyte; ECM, ectomycorrhizal; END, endophyte (aboveground); ERM, ericoid mycorrhizal; PPATH, plant pathogen; REND, root endophyte (non-DSE); SAP, saprotroph.

endophytic fungi and other indicator OTUs for *Alnus* in the sampled roots, providing empirical proof that they are indeed associated with *A. acuminata* (Table 2). Among these, ECM fungi were generally represented by OTUs that were highly similar to known alder-symbiotic ECM fungi from the Northern Hemisphere, as expected based on the biogeography of *A. acuminata* (Hooghiemstra 1989, Kennedy *et al.* 2011). Even though our soil sampling is geographically biased, as it represents only the southern two-third of the Yungas, this limitation likely is of minor importance, given the migration history of alder-associated fungi. The high degree of host specificity exhibited by *Alnus*-symbiotic ECM fungi suggests that our approach of indirect estimation of potential habitat distribution based on host occurrence data is likely most reliable in the case of these ECM fungi that are restricted to *Alnus*-dominated forests. Interestingly, unlike ECM fungi, root endophytic and saprotrophic indicator fungi appeared to be more indicative of the elevation zone in general, as many of them were also found at montane cloud forest sites where alder was absent. Even for indicator root endophytic, plant pathogenic, and saprotrophic fungi that are not directly or strictly associated with *Alnus*, but nevertheless are restricted to the montane cloud forest zone, the distribution of *Alnus* provides a reasonable proxy in terms of suitable habitat.

The future (2050) distribution of *A. acuminata* is predicted to retract substantially based on SDMs generated from all four RCP climate change scenarios. According to the high impact climate change scenario (RCP 8.5), the suitable habitat for *A. acuminata* in 2050 may cover only 50 percent of the currently suitable area, <23 percent of which may fall under protection. Even the low impact climate change scenario (RCP 2.6) predicts a more than 25 percent decrease in suitable habitat, with 25.6 percent within currently protected areas. Based on our model and the variable importance, we infer that temperature increase will be a defining factor for the distribution and survival of this species in the Yungas. *Alnus* mostly occurs in regions with a mean annual temperature of 16–18°C (Fournier 2002), which was also confirmed by

the model (15–18°C). Further increase in temperature will probably affect the survival of *Alnus* in areas that currently are marginally suitable for this species. The predicted losses are at lower elevations, where it is expected that the temperature increase (and coupled changes in cloud cover that are more difficult to model) will make these areas unsuitable for *A. acuminata*. The predicted gains are at higher elevations, and all models predict an upslope shift of suitable habitat, with mean elevation increases ranging from ca. 184 m (RCP 2.6) to 380 m (RCP 8.5). This concurs with distribution trends estimated for the entire montane cloud forest and for some species, e.g., *Duranta serratifolia*, *Ilex argentina*, *Myrcianthes pseudomato*, *Prunus tucumanensis*, *Zanthoxylum coca*, and *Viburnum seemenii* (Pacheco 2015). Interestingly, a similar loss of habitat has also been estimated for the piedmont forest in Argentina, which may contract to less than half of its current area as a response to climate change, although there were some differences in response among the six tree species studied (Pacheco *et al.* 2010). In other Neotropical montane forests, shrinking habitats have been modeled for various high elevation tree species as well. For example, various species of *Quercus* and *Pinus* in Mexico and *Polylepis* in Peru have been shown to have retracting distributions (Gómez-Mendoza & Arriaga 2007, Zutta *et al.* 2012).

On a geographic scale, most habitat gain is predicted in the southern part of the Yungas, while habitat loss will likely be more pronounced in the northern (more tropical) half, particularly in the eastern low- to mid-elevation mountain ranges of southern Bolivia between 19 and 21°S, where the habitat is already only marginally suited for *A. acuminata* (Fig. 1). Further habitat loss in this region might have implications for the biological connectivity of the southern (largely Argentinian) and northern montane cloud forests.

Based on its capabilities to disperse and establish as an early-successional colonizer, we expect that *A. acuminata* has greater potential to track its climatic niche than many other trees that are restricted to old growth forests and are often poorer dispersers. For example, Blodgett (1998) reported that in Bolivia, *A. acuminata* generally colonizes newly available habitats (e.g.,

following landslides) in <3 yr following disturbance. Similarly, the dispersal of associated fungi, particularly ECM fungi, will unlikely be a limiting factor at such short distances (Perry *et al.* 1990, Geml *et al.* 2012, Pickles *et al.* 2012). *Alnus acuminata* is among the very few tree species that has been capable of migrating, together with associated ECM fungi, from Central America to Argentina along the Andes. Based on pollen records, this species first appeared in Colombia ca. 1 million years ago (Hooghiemstra 1989) and therefore likely migrated to the southern end of its present range (ca. 4000 km distance) at a minimum rate of 4 m/yr. This rate exceeds the mean migration rate of 2.5 m/yr estimated for tropical Andean tree species (Feeley & Silman 2010), suggesting that *A. acuminata* is a relatively effective disperser.

On the other hand, complex biotic and abiotic factors will likely influence the establishment of *Alnus* following seed dispersal. For example, species interactions and anthropogenic barriers (*e.g.*, land use) that may influence the distribution of *Alnus* are not taken into account by current models. The potential effects of these on predicted habitat gain may be particularly important in high elevation regions where *A. acuminata* forests border pastures near montane settlements. In Argentina, Carilla and Ricardo Grau (2010) analyzed a 150-yr chronology of tree regeneration patterns into degraded grasslands in the montane cloud forest zone. They found that despite decreasing grazing intensity and increasing rainfall, forests are not recovering into grasslands due to the increasing frequency of fires, which likely affect tree seedling mortality and/or edaphic factors and microclimate (Carilla & Ricardo Grau 2010). Similarly, the treeline ecotone between cloud forest and grassland in the Peruvian Andes is remarkably resistant to forest invasion, resulting in a more or less stationary treeline or ‘grass ceiling’ effect (Rehm & Feeley 2015). Therefore, future range contraction for *A. acuminata* may be even more pronounced than the decrease in the climatically suitable area predicted by our analyses.

Here, we demonstrated the potential of coupling large-scale fungal community assessment using DNA metabarcoding with species distribution models developed using occurrence localities of a keystone tree species to obtain initial estimates of the potential distribution of suitable habitats for co-occurring fungi. By predicting present and future distributions of potential habitats for an ecologically important tree species and its fungal associates in combination with assessment of fungal diversity in their habitat, we provide crucial data to better understand environmental factors influencing biological communities in the Yungas and to facilitate their conservation.

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DATA AVAILABILITY

Data deposited in the Dryad Repository: <http://doi:10.5061/dryad.8fn8j>.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

TABLE S1. *Species presence records of Alnus acuminata obtained from GBIF.*

TABLE S2. *Environmental variables used to fit the SDM ensemble model for present and the four RCPs.*

TABLE S3. *Sampling sites of Yungas forests included in this study with code, vegetation type, elevation, geographic coordinates, locality, and some of measured soil chemical properties.*

FIGURE S1. A map of Northwestern Argentina, showing the geographical distribution of the Andean Yungas forests with the sampling locations indicated.

FIGURE S2. Predicted current and future distributions of *Alnus acuminata* in the Tucuman-Bolivian Yungas according to two intermediate impact climate change scenarios.

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