**Title.** Land-use history shapes tree aboveground biomass patterns in tropical montane cloud forest landscapes

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**Keywords**

**Abstract**

**Introduction**

How does small-scale farming shapes regional patterns of AGB in tropical montane cloud forest across environmental gradients?

1. How much AGB TMCFs hold and where is it allocated?
2. How does land-use shape AGB spatial patterns along an environmental gradient?
3. What is the relationship between AGB and tree diversity in TMCF?

Acronyms: TMCF, FI

**Methods**

*Study Area*

We delimited a study area following a TMCF regionalization conducted by Toledo-Aceves *et al.* in 2011 based on geomorphology, forest cover, watershed margins, rivers, and cultural differences (such as presence of indigenous groups). We focused on the Northern Mountains of Oaxaca (NMO), a region that harbors some of the most biodiverse forests in Mexico including the largest and most continuous TMCF, and where forest conservation is considered a critical priority (Toledo-Aceves et al., 2011). In the NMO, TMCF are found on hillslopes and humid ravines with frequent fog and drizzle. The mountains impose an environmental gradient that goes from warmer to cooler temperatures as elevation increases. Soils in these forests usually develop from the weathering of metamorphic rocks and volcanic outcrops, they tend to be deep, and rich in clay and organic matter (Torres, 2004). We defined the distribution of TMCF within NMO using the official map of vegetation and land-use series V published by the National Institute of Statistic and Geography (INEGI) in 2013 (CITA) (Figure 1).

*Data Collection and Processing*

We gathered and integrated information on forest structure and composition, environment, topography, and land-use, from different sources into a single dataset. The main data source for this work is the publicly available Mexican National Forest Inventory database, which contains information on forest structure and composition, as well as forest disturbance. From this database we estimated forest structural attributes, AGB, and land-use variables. To complement this data, we obtained information on mean annual precipitation and temperature from WorldClim (CITA). Lastly, we retrieved topographical information from NASA’s Shuttle Radar Topography Mission digital elevation data. A detailed description of the foregoing variables can be found below (see also Table 1 for a summary of the variables).

*Forest Inventory Sites.* FI data collection was carried out between 2009 and 2014 following a hierarchical nested sampling design with 1-ha circular sites as the main sampling unit. All sites were established 25 km apart from each other in a grid-like fashion. Within each site, four circular plots of 400 m2 were established. One in the center of the site, and the other three in a north, southeast, and southwest direction, respectively, at 45.14 m from the central plot. All trees, lianas, shrubs, palm trees and ferns within the plots with a diameter at breast height (DBH) larger than 7.5 cm were sampled for height, DBH, basal area (BA), and taxonomic identification. Information about the geographic location, vegetation type, and land ownership of each site was also documented (CONAFOR, 2018). For selecting FI sites relevant to our study, we performed a spatial intersection in QGIS 3.16 between FI sites and the NMO using FI sites’ geographic coordinates and the NMO shapefile we acquired from the National Commission of Biodiversity (CONABIO) GeoPortal (CITAS).

*Forest Structural Attributes and Aboveground Biomass Estimation.* Based on FI raw data, we derived three structural attributes at plot level that were then averaged by site: (1) stem density, *i.e.*, the number of trees per hectare; (2) basal area, defined as the sum of the cross-sectional surface area of trees per hectare; and (3) Lorey’s height, which is a measure of forest stand height weighted by its basal area.

To estimate AGB, we first calculated each individual’s AGB using 47 different allometric equations (Table 2). Whenever allometric equations were available in the scientific literature at species or genus level we would use them. Otherwise, we used the following generic allometric equation developed by Chave *et al.* (2014(2015?)) for tropical trees based on tree wood density (*ρ*), height (*H*) and DBH (*D*):

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We corrected taxonomic names collating a list of species with the Taxonomic Name Resolution Service and searched for the wood density value of each species or its closest relative in global wood density databases using the R package BIOMASS (Réjou‐Méchain, 2017).

We calculated AGB per plot adding up the biomass of each individual tree and AGB per site averaging plot’s AGB. There is always some uncertainty inherent to upscaling biomass estimates from trees to forest stands that arises from the propagation of errors in field data collection, allometric equations, wood density estimates, and forest variation. To account for this uncertainty, we estimated plot AGB standard deviation following error propagation through a Monte Carlo scheme, assuming 95% of the samples have a low diameter error and the remaining 5% a high diameter error (close to 5 cm), and a height error of 10%, as suggested in Chave et al. (2004). To estimate site’s AGB error, we assumed standard error independence and used the following equation:

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*Tree Diversity.* We calculated species richness, *i.e.*, the total number of species, Shannon (H) and Simpson (D) diversity indices with the following equations, respectively:

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Where *pi* is the proportion of species *i* and *S* is the number of species.

*Environmental and Topographic Variables*. We calculated annual precipitation extracting WorldClim bio12 values per plot using the package *raster* in R. When plot’s annual precipitation values within a single site varied, we calculated a mean annual precipitation value per site. WorldClim’s annual mean temperature (bio1) was also extracted following the same procedure. Then, we extracted slope (in degrees) and aspect values for each plot from NASA’s Shuttle Radar Topography Mission digital elevation data (~30 m resolution), using Google Earth Engine (Farr et al., 2007).

*Land-use Variables*. To quantify land-use in TMCF we used three variables: forest disturbance related to agricultural activities, forest disturbance related to cattle grazing activities, and a land-use gradient we built on information about the landscape composition of each site (*i.e.*, the proportion of different types of land cover within a site). We assessed FI sites’ forest disturbance related to agricultural and grazing activities at the time of data collection using FI’s disturbance data set, which contains information about the cause and severity of vegetation disturbance. The causes of disturbance are classified in 11 classes: fires, hurricanes, floods, roads, logging, land-use change, grazing, pests and diseases, electrical cables, mining, and urbanization. The severity of disturbance is classified in a four category nominal scale: very low, low, medium, and high severity. Both the cause and severity of disturbance where qualitatively assessed during field data collection (CONAFOR, 2018). Agriculture and grazing activities are reported within the categories of land-use change, grazing, logging, and fires with labels such as ‘clearing for growing coffee’, ‘swidden agriculture’, or ‘conversion from forest to cattle ranch’. We reviewed all recorded disturbance causes in the database, identified, and extracted data related to agricultural and cattle grazing activities. Then, we assigned each site a disturbance severity value from 0, when no disturbance was reported, to 4, indicating high severity disturbance. Whenever a site presented more than one reported disturbance related to agriculture or grazing, we averaged the disturbance severity value.

To assess the landscape composition of each site in terms of the proportion of mature forest, secondary forest, and agricultural or grazing lands present in the site, we first identified an approximate successional state of FI plots. To do so, we classified FI plots with a k-means analysis using structural attributes, including tree height, DBH, and tree density. K-means is a non-hierarchical cluster analysis where the user defines the initial number of centers. We run the analysis using two, three, four and five initial centers with 25 random sampling sets each. Then, we compared 30 indices to define the best number of clusters and chose the one that was better supported by most indices. Most of the indices suggested three clusters as the best classification. Thus, from this analysis, we obtained three structural clusters: the first one groups together plots with very low tree density, low basal area, and low tree height; the second cluster groups together forest plots with high tree density, and medium basal area and tree height; and a third one groups together plots with very high basal area and tree height, but medium tree density (Figure S1). Other studies conducted in TMCF in Oaxaca have shown that tree height, DBH, and tree density change through time after disturbance and are useful for estimating an approximate stage of forest succession in forest-agriculture mosaics (Velasco-Murguía et al., 2021). In general, TMCFs after disturbance show an increase in tree density, height, and basal area. Over time, tree height and basal area continues to increase but stem density decreases. This transition usually happens around 50 years after disturbance and differentiates young forest from mature forest (del Castillo, 2015). Because the three clusters we obtained from the non-hierarchical cluster analysis follow this general trend, we assigned approximate successional stages to each cluster as follows: we defined cluster one as very young forest, cluster two as young forest, and cluster three as mature forest. It is important to note that forest succession is a continuum and a complex process. Here, we classified forest succession in discrete categories as a methodological approach conducted for the sake of the analysis. This approach has proven to be useful for understanding forest ecosystem dynamics elsewhere (CITAS).

Once plots were classified in three successional stages, we assessed the composition of very young, young, and mature forests in each site. Interestingly, some of the sites have plots that fall across different structural classes, showing the patchiness in these forest-agriculture mosaic landscapes. To describe this patchiness, we defined a landscape composition value adding up all successional stages within a site and normalizing the value as follows:

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Where SS is the sum of the successional stage categories of the plots in a site. Successional stage categories can take the following values: very young forest = 1; young forest = 2, and mature forest = 3. Thus, considering there are four plots in each site, the minimum possible SS value is always 4, and the maximum is always 12. This way, a landscape composition value from 0 to 1 is assigned to all sites, where 0 represents sites dominated by mature forests, 1 represents sites dominated by very young forest stands, and everything in between are sites with a combination of forests at different successional stages. Thus, this variable represents a land-use gradient where sites dominated by very young forests (*i.e.*, sites closer to 1) show a greater degree of forest disturbance. It is important to note that other causes of forest disturbance unrelated to land-use can result in values closer to 1, such as pest outbreaks. However, agriculture and cattle ranching expansion have been identified as main causes of forest loss in TMCF (CITAS), and there is a correlation between our landscape composition variable and the presence of forest disturbance related to agricultural and grazing activities reported in the FI disturbance database (Figure S2). Therefore, other sources of disturbance were excluded from the analysis.

*Data Selection and Quality Control*

Because TMCF has a scattered distribution along the NMO, not all sites within this region correspond to our study system. To filter FI sites further, we used the following criteria: (1) sites should be within an elevation range between 1,000 and 2,800 m asl; (2) sites should receive at least 1,000 mm of annual precipitation; (3) sites should be described as cloud forest in the vegetation type column of the FI database; and (4) all sites must have epiphytes.

We performed data quality control homogenizing missing data values, correcting taxonomic and places names, removing diacritics, and filtering out rows with missing information. Additionally, we removed plots with many unidentified species or where most trees were dead. Because some plots are in places impossible to collect field data (such as very steep ravines), not all sites have four sampled plots. To avoid a biased sampling design, we selected only sites where four plots were sampled. After applying these filters, we ended up with a dataset of 40 sites located between 16.8938 and 18.6155 degrees N and -95.6699 and -97.0214 degrees W, within the distribution of TMCF (Figure 1).

*Statistical Analysis*

We performed statistical analysis at two sampling levels. On the one hand, we used sites (n= 40) as our main unit of analysis to assess the amount of AGB in TMCF, and its variation across space in relation to tree diversity, environmental and land-use gradients. On the other, we used plots (n= 160; a smaller sampling unit) for understanding the contribution of tree size to AGB and stem density, as well as changes in structural attributes in forest at different successional stages. First, we gained a general sense of the amount of AGB in TMCF and its variation performing basic summary statistics at site level and estimating the correlation between all variables. Then, we conducted analyses to answer our three main questions. The following sections describe these analyses further.

*AGB distribution in TMCF*. We explored the contribution of tree size to AGB and stem density following a similar approach as Cuni-Sanchez et al. (2021) and classified trees in six size classes based on their DBH: ≤10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, >50 cm. Then, we calculated the proportion of AGB and stem density per size class in each plot and performed an analysis of variance (ANOVA) to assess the difference in the contribution of tree size to AGB and stem density. Additionally, we computed a two-way ANOVA to evaluate the differences in the contribution of tree size classes within and among forests at different successional stages. Finally, we carried out four more ANOVA to test whether tree density, tree height, basal area and AGB are significantly different in forests at different successional stages.

*AGB patterns along environmental and land-use gradients*. We explored the relationships among tree biomass, diversity, environmental variables, and land-use in TMCF sites using multiple linear regression models. First, to reduce the number of variables, we computed a principal component analysis (PCA) of altitude, precipitation, temperature, and slope. Aspect is not a continuous variable and lacks variation in our dataset as most sites are facing either south or west, and thus, was excluded from the analysis. The first principal component (PC1) explained 68% of the variation and is correlated to temperature, precipitation, and altitude. Thus, PC1 represents an environmental gradient from warmer and moister sites at lower elevations to cooler and drier sites at higher elevations (Figure S3). Therefore, we selected PC1 and slope as our environmental predictors. We fitted a model with AGB as the response variable and tree diversity, environmental gradient, slope, and the three land-use variables as predictors. Because data is not normally distributed, we log-transformed AGB. Then, following an approach similar to Tredennick et al. (2021) for model exploration, we performed variable selection by comparing the full model against a series of reduced models in which each predictor is dropped in a stepwise fashion. We selected the best model comparing their adjusted R2, Mallows' Cp (CP), and Bayesian Information Criterion (BIC). To test for spatial autocorrelation, we calculated the Moran’s I of the residuals of the final model using a neighboring distance of 10, 25, and 50 km, and visualized the spatial distribution of residuals with a map.

*AGB relationship to tree diversity.* To understand the relationship between AGB and tree diversity in TMCF, we performed a series of nonparametric regression analysis. Nonparametric regressions allow to test relationships between two or more variables without assuming linearity. Given tree diversity and AGB in our dataset show a positive but week correlation, we could not assume a linear relationship. Here, we assessed the relationship between AGB and stem density with Simpson diversity index and species richness at site level fitting locally weighted regression (loess) curves. Similarly, we performed these nonparametric regression analyses at the plot level. Although we do not have specific ages after disturbance for assessing how diversity and AGB change over time, taking a space-by-time approach we evaluated these trends using the three-category successional stages we assigned to each plot.

**Results**

*AGB allocation in TMCF*

Average tree AGB in TMCF in the NMO is 137.49 ± 121.29 Mg/ha and it ranges from as low as 8.266 Mg/ha to as high as 414.524 Mg/ha, showing the wide variation of AGB existing in these landscapes (Table 3). In fact, out of the 160 plots analyzed, 30 are outside this range. Specifically, 17 plots show values below 8.26 Mg/ha, several of which were completely devoid of trees larger than 7.5 cm of DBH at the time of data collection. In contrast, 13 plots exhibit higher values than the highest averaged AGB found at site level, some of them even surpassing 500 Mg/ha. Variation in structural attributes within and among sites is also large. For instance, average stem density in all 40 sites is 641.56 but some sites have as few as 81.25 trees/ha. This variation results from the diversity in landscape composition found in these forest-agriculture mosaics and it is driven by the successional stage of each plot. In very young forests, tree density is low, and trees are short and thin. As forests develop, all these structural attributes increase. Thus, young forests show larger trees and higher stem density. In mature forests, the density of trees decreases as they become even taller and bigger. As a result, AGB in very young forests averages only 33.38 Mg/ha, in young forests this value notably increases to 151.69 Mg/ha, and in mature forests it reaches 354.86 Mg/ha (Table 4).

In these landscapes there is a large proportion of small trees (DBH < 20 cm), and trees with a DBH > 30 cm are uncommon. However, large trees contribute the most to AGB, particularly the largest ones (tree size class 6: DBH > 50 cm). Interestingly, this pattern is different in forest plots at different successional stages. Although the proportion of stems of different size classes remains somewhat constant across the three different successional stages, their contribution to AGB varies widely. In very young and mature forests, largest trees are the ones contributing the most to total AGB, but in young forest the contribution to AGB is equally distributed across all tree size classes (Figure 2).

*Patterns of Tree AGB Across Land-use and Environmental Gradients*

Tree AGB in TMCF sites are controlled mainly by land-use, and secondly by environmental factors. Here, we assessed both land-use and environmental factors with multiple linear regression models. Through a stepwise model selection process, we found the best model includes three variables: landscape composition, forest disturbance related to agriculture and slope (p < 0.05, adjusted R2= 0.811, Table 5). The second best model includes environmental gradient as a fourth predictor (Table 6).

*AGB and tree diversity*

**Discussion**

TMCF sites are found along two compounding gradients: an environmental one, going from warmer and wetter areas at lower elevations to cooler and drier ones at higher elevations; and a land-use gradient, from higher to lower forest disturbance related to agricultural and cattle gazing activities.

Spatial heterogeneity caused by small-scale forest disturbance determines AGB patterns at landscape and regional scales.

The effect of environmental variables on tree AGB and diversity is evident once land-use is taken into consideration.

**Conclusions**

**Tables**

Table 1. Variables used in this study

Table 2. Allometric equations

Table 3. Summary statistics of structural attributes, tree aboveground biomass (AGB) and diversity in Tropical Montane Cloud Forest sites (n= 40).

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| **Variable** | **Min ± S.E.** | **Max ± S.E.** | **Mean ± S.D.** |
| Stem density (tree/ha) | 81.25 ± 15.72 | 1806.25 ± 444.10 | 641.56 ± 355.23 |
| Basal area (m/ha) | 2.26 ± 0.64 | 51.08 ± 6.57 | 20.42 ± 13.48 |
| Lorey’s height (m) | 5.92 ± 0.68 | 24.91 ± 1.00 | 12.46 ± 4.63 |
| Aboveground biomass (Mg/ha) | 8.26 ± 1.02 | 414.52 ± 19.29 | 137.49 ± 121.29 |
| Species richness | 1.75 ± 0.25 | 9 ± 1 | 4.4 ± 1.63 |
| Simpson diversity index (D) | 0.19 ± 0.12 | 0.78 ± 0.05 | 0.54 ± 0.15 |
| Shannon diversity index (H) | 0.33 ± 0.21 | 1.75 ± 0.25 | 1.06 ± 0.36 |

Table 4. Structural attributes and tree aboveground biomass (AGB) at plot level (n= 160) in three different successional stages: very young, young , and mature forests. According to the ANOVA, differences in structural attributes and AGB between different successional stages are all statistically significant (p < 0.05).

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|  | **Very Young (n= 69)** | | **Young (n= 62)** | | **Mature (n= 29)** | |
| **Mean** | **S.D.** | **Mean** | **S.D.** | **Mean** | **S.D.** |
| Stem density (tree/ha) | 275.000 | 166.550 | 986.694 | 444.380 | 775.862 | 313.141 |
| Basal area (m/ha) | 6.875 | 4.432 | 24.756 | 9.336 | 43.422 | 13.288 |
| Lorey’s height (m) | 9.376 | 3.550 | 11.764 | 2.366 | 21.314 | 3.124 |
| AGB (Mg/ha) | 33.381 | 32.631 | 151.691 | 92.042 | 354.866 | 151.605 |

Table 5. Multiple regression model-AGB

Table 6. R2, CP and BIC of all models.

Table 7. GAM

**Figures**

Figure 1. Map

Figure 2. Structural attributes and tree size contribution to AGB and stem density.

Figure 3. Relationship between forest structure and diversity

Figure 4. Relationship between AGB and landscape composition (mosaic), and diversity and landscape composition (mosaic)

Figure 5. GAM result or surface plot

**Supporting Information**

Figure S1. Non-hierarchical cluster analysis – plots categories in three ‘successional stages’

Figure S2. Relationship between landscape composition value and forest disturbance

Figure S3. PCA of environmental variables