**­Abstract:**

Although water is the most abundant molecule in the Earth, its availability is playing a critical role in restricting the global plant distribution and productivity. Future projections suggests that tropical ecosystems are expected to experience increased death due to drought. With the increasing human land use, forest habitat changes, further increasing the impacts of drought in the forest edges. Forest saplings, the future of forest are the first to get hit by these impacts. In this study, we examined the how human land-use and drought interactively shape the sapling community using their functional traits. We found that species with denser stems and thicker leaves having lower survival in drought treatments but not different with forest habitat. Drought response, measured as the individuals survived in the drought to control was decreasing with increased thickness of leaves and denser stems in the forest edge compared forest interior. Our results show that pattern of survival explained through their functional traits is not the same as the adult individuals, highlighting the importance of ontogeny difference in species response to drought and human land use for the conservation plans.

**1 Introduction:**

**Importance of tropical system**

Tropical forests are the most complex, productive, and species-rich terrestrial ecosystems globally (klein, ricklefs). Despite covering less than 7% of the Earth’s land surface, these forests provide invaluable ecosystem services essential for the maintainance of local, regional, and global natural ecosystems, as well as for human wellbeing and survival (oduro, brandon). Tropical forests harbor approximately 50% of all living organisms on earth (swanborough) and contain about 60% of the world’s vascular plants (FAO and UNEP). Furthermore, they play significant role in regulating global climate and weather patterns (Brandon) amd contribute carbon cycle (le quere), thereby enhancing soil quality and biodiversity (Batjes, Mcdowell)

**Threat to them due to global change and land use**

Especially in the tropics, dry periods can vary widely from a few weeks in every other year in tropical wet forests, to several months in seasonally dry forests (Markesteijn 2010, Trewin 2014).-ron

Drought-induced mortality is expected to increase with projected changes in climate, and

tropical ecosystem may be particularly vulnerable to these changes (Duffy et al. 2015; Allen et al. 2017). -Ron

Fragments experience edge effects that alter the microclimate (De Frenne et al. 2015; 2019; Zellweger et al. 2020) and forest-climate feedbacks that can enhance local climatic stress (Laurance 2004)(Arroyo-Rodríguez et al., 2017; Laurance, 2004).-meghna

**Importance of saplings and literature study on traits mediating their response**

**2 Methods:**

**2.1 Study site:**

**2.2 Experimental design**

We conducted this study on 16 native rainforest saplings (Appendix Table A1). Species were chosen according to: (1) availability and germination of sufficient seeds, (2) sufficient degree of seedling shade-tolerance for regeneration in the understory. Each species’ sapling was planted in pairwise combination of control and drought at 13 different locations. The rain-sheltered layers without compromising for light availability was chosen to simulate the drought treatment. This set up is replicated in both the forest interior and edge to study edge effects (Figure 1).

**2.3 Plots monitoring**

Soil moisture was measured every month using hydrosense.

**2.4 Trait collection**

We followed protocols recommended by (Perez-Hargingeguy et al. 2013) for the collection and processing of samples to quantify leaf functional traits. Three to five alive individuals from both forest edge and interior of each species were harvested, brought back to field station on the same day, and water saturated overnight by immersing the petiole, root, and branch in container filled with water. Water saturated leaves were weighed to determine saturated fresh weight, scanned with a desktop scanner for quantifying leaf area (LA), and then oven–dried at 70 °C for 72 hours to determine dry weight. Leaf mass per unit area (LMA) was quantified as the ratio of dry weight to area, and leaf dry matter content as the ratio of dry weight to saturated fresh weight. A portion of stem, main root and fine root were taken and used water displacement method to estimate the volume followed by oven-dried at 70 °C for 72 hours to determine dry weight. Stem specific density (SSD) was estimated as the ratio of dry weight to volume. Main root specific density (MRSD), and fine root specific density (FRSD) were estimated as the dry weight of main root and fine root to their volumes.

**2.5 Statistical analysis:**

We used regression model to test soil moisture difference as a function of treatments (control and drought) and forest habitat (edge and interior). To address question 1 and 2, first we performed principal component analysis (PCA) on traits to obtain composite phenotypes defined by trait combinations. Generalised linear mixed effects model to model saplings’ survival (time after the drought period) as a function of the treatment (control and drought), forest habitat (edge and interior), and their traits. Linear mixed effects model was used to model saplings’ drought response (number of individuals survived in drought treatment to control) as a function of forest habitat (edge and interior) and the traits. All analyses were performed using R v 3.4. We used packages nlme (Pinheiro et al. 2022)⁠ for mixed effects models with Gaussian error structure lme4 (Bates et al. 2015)⁠ for models with Bernoulli error, FactoMineR (Lê et al. 2008)⁠ for PCA.

**3 Results:**

**3.1 Soil moisture:**

**3.2 Principal Component Analysis of Traits:**

Principal Component Analysis (PCA) was conducted on the measured traits; explaining 65.0% of the total variance (Dim1: 46.4%, Dim2: 18.6%). Dim1 was associated with Leaf Mass per Area (LMA), Stem Specific Density (SSD), and Leaf Dry Matter Content (LDMC), while Dim2 was influenced by Fine Root Specific Density (FRSD) and Leaf Area.The PCA biplot (Figure 1) shows that LMA, SSD, and LDMC are positively correlated and Leaf Area negatively correlated. FRSD was orthogonal to this.

**3.3 Trait mediation on saplings’ survival:**

With PC1: In our three-way interaction of traits, forest habitat and treatments, there were no individual significant effects of PC1(estimate=0.13; p=0.54), treatments (estimate=-0.37; p=0.45), and forest habitat (-0.17; p=0.25). Compared edge, in the interior, survival decreased with increasing in PC1 (estimate= -0.67; p=0.08). Similarly, the survival decreased in drought treatments with increasing PC1 (estimate= -1.10; p=0.01). There was no interaction between treatment and forest habitat (estimate=-0.76; p=0.38). We noticed that survival decreased (estimate=-0.28; p=0.04) with increasing PC1 in drought treatment of forest interior as compared to control treatment forest edge.

With PC2: With increasing in PC2, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing PC2 (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

With LMA: With increasing in LMA, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing LMA (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

With LDMC: With increasing in LDMC, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing LMA (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

With LA: With increasing in LMA, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing LMA (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

With SSD: With increasing in LMA, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing LMA (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

With MRSD: With increasing in LMA, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing LMA (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

With FRSD: With increasing in LMA, the survival decreased (estimate=-0.90; p=0.02) and the survival was lower in the interior of forest (estimate=-1.43; p=0.07). There was no difference in the survival in drought (estimate=-1.86; p=-0.13). In the interior of forest, the survival decreased with increasing LMA (estimate=-0.96; p=0.01). In the drought treatment survival decreased marginally with increasing PC2 (estimate=-0.27; p=0.05). There was no difference in the survival drought based on the forest habitat (estimate= 0.30; p=0.13). The survival decreased (estimate=-0.56; p=0.05) with increasing PC2 in the drought treatment of forest interior as compared to control treatment of forest edge.

**3.4 Trait mediation on saplings’ drought response:**

With PC1: With increase in PC1, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With PC2: With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With LMA: With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With LDMC: With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With LA; With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With SSD: With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With MRSD: With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

With FRSD: With increase in PC2, the drought response did not change (estimate=; p=). The drought response decreased with increasing PC1 in the forest edge as compared interior (estimate=; p=)

**4 Conclusion:**

**Notes for reference:**

* **Rishiddh’s paper: https://www.authorea.com/users/752337/articles/723135-anatomical-traits-explain-comparative-drought-response-of-seedlings-from-wet-tropical-forests**
* **Land use paper : https://www.sciencedirect.com/science/article/pii/S2351989422000531#bib38**