

Root access to underground water sources by tropical canopy trees in the seasonally dry forests of the Yucatan peninsula

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Introduction

The seasonally dry forests (SDF) of the Yucatan peninsula represent a synapse between temperate and tropical environments in the Western hemisphere and overlay one of the largest known limestone karst systems on the planet. Karst systems are important sources of fresh water, and are responsible for the subterranean geography of the area. The 165,000 km² limestone platform is underlain by a pervasive underground aquifer which includes the world's largest underwater cave system (Baur-Gottwein et al. 2011).

Globally, SDF systems are threatened across 97% of their extant range, and the dry tropical forests of Latin America have experienced the greatest rates of deforestation, having been diminished by 12% (~220,000 km²) between 1980 and 2000 (Miles et al 2006). Additionally, only 5.7% (~100,000km) of the dry tropical forest in North and Central America are protected versus 14.2% in South East Asia and up to 37.8% in South America (Miles et al. 2006) making the potential for continued deforestation high, especially with the economic gain from resorts as a major driving factor.

Seasonality in SDFs is driven by precipitation rather than temperatures, and while annual rainfall remains high, precipitation is concentrated in rainy seasons punctuated by regular periods of

drought. Despite this, many canopy tree species exhibit an evergreen habit, likely a result of their capacity to access the subterranean aquifer. It is clear that plants are accessing this water source, as thick bundles of roots descending through the bedrock into open cavern space and radiating toward standing water are commonly observed in caves.

Despite the probable importance of access to this below ground water in determining above ground plant community structure, no definitive survey of the plant species that are capable of taking advantage of this resource has yet been published. In this study we use molecular genetic tools to address this knowledge gap and provide putative species identification for the plant species accessing the aquifer.

Hypotheses

H₁: Based on previous published reports and anecdotal evidence, we expect root bundles to be dominated by plants in the Moraceae.

H₂: Bedrock thickness will be a significant driver of root bundle community composition.

H₃: Root bundle community composition will vary among caves.



Sample collection

- o Morphologically distinct fine roots were sampled from bundles in four caves northwest of Akumal, Mexico.
- o We dried samples before returning to the United States
- o We also calculated vertical distances relative to ground level for every root bundle using a laser distance meter in order to calculate the thickness of bedrock roots had penetrated

DNA extraction, amplification, sequencing

- o We extracted DNA from the dried and cleaned root tissue samples using two different extraction protocols:
 - 1) MoBio PowerSoil DNA Extraction Kit (MoBio, Carlsbad, CA)
 - 2) A modified Doyle CTAB extraction (Doyle and Dickson, 1987) with 4x Ctab, 4% beta-mercaptoethanol, and ProteinaseK
 - o We cleaned genomic DNA using either MoBio PowerClean DNA kit or GeneClean Spin kits based on visual opacity
 - o We then amplified the RBCL and matK loci using the following primers and protocols:
 - 1) RBCL – F1 and R1, on a modified Sato protocol with annealing temperature reduced to 45

Data Analysis

- o We blasted our sequence data against the NCBI nucleotide database to establish probable species identities
- o Species designations were chosen from the top blast hits based on hit quality and documented regional occurrence in the Missouri Botanic Garden's Tropicos database.
- o In general, we had more confidence in matK hits because of its longer sequence reads
- o Root bundle community similarity was visualized using ordination analyses
- o PERMANOVA analysis was used to evaluate the effect of bedrock thickness on community similarity

Results

For the twenty root bundles sampled, 44 individual species identifications in fifteen plant families were distributed amongst the 133 tissue samples from the four different caves. The species richness and number of bundles sampled per cave are summarized in Table 1. About half of the 133 sampled root tissue belonged to species in the Moraceae and Fabaceae with the other fifty percent distributed throughout the thirteen other plant families (Figure 1). The PERMANOVA analysis (Table 2) returned an average p-value of about 0.5. The PCOA ordination plot is shown below (Figure 2).

Cave	Bundles sampled	Species richness
Grande	10	29
Big Ladder	8	18
Little Mayan	1	4
Water Pump	1	5