

## 2. Methods

### Harvard Forest Leaf Sampling

During the summer of 2019 leaf samples were collected from the Harvard Forest (HF) in Petersham, Massachusetts. This is classified as a temperate forest dominated by Hemlock (*Tsuga canadensis*), various Birch sp. (*Betula* sp.), Red Maple (*Acer rubrum*), Red Oak (*Quercus rubra*), and American Beech (*Fagus grandifolia*). This forest experiences freeze, thaw dynamics, and is located at an elevation of ~1200 ft above sea level. Data collected from HF climate monitoring stations shows mean air temperature to be 46.382 (44.60 min - 49.60 max) °F and average annual precipitation as 43.136 (22.780 min - 72.460 max) inches during 2019. Collection sites were randomly chosen, targeting depositional environments comparable to fossil localities. Leaves are found within the sediment layers or overbank deposits and thus mimic compression fossils within the geologic record. Three depositional environments were targeted: low-transport swamp, mid-transport small tributary, high-transport river, and an upland location. At the swamp, small tributary, and dynamic river, three quarries were sampled to capture lateral variability. Each quarry was blindly sampled to approximately 400 leaves, resulting in ~1200 leaf samples per depositional environment. The upland location was only sampled once for direct comparison to the neighboring dynamic river. A total of 4115 leaves were sampled within the HF forest.

Leaf layers were identified within each depositional environment and randomly sampled. Leaves were isolated from the sediment using a small trowel and 3mm sieve, leaves were excavated from the sediment, placed in plastic bags, and transported back to the HF facility for further cleaning and drying. Fine sediment was not always present within this sampling location and thus, overbank deposits were also sampled. A section of the depositional environment was chosen and a 50m tape was randomly run through the environment. Samples were blindly taken every 10 meters, placed in a bag, and later sub-sampled to 400. All leaves were further cleaned of sediment and debris back at the HF facility. Once leaves were cleaned, they were pressed flat and dried in a converted incubator at 60°C until dry (approximately 48hrs). Leaves were then identified to species, when possible, and transported back to the University of Wyoming for further analysis.

#### low-transport swamp

Three quarries (HF1901.1, 1901.2, 1901.3) were sampled within the swamp with each site approximately 200m in distance from one another. HF1901.1 is located just off of French road within the HF complex. 50m transects were created through dense canopy and swamp with leaves randomly sampled every 5m. To allow for blind sampling, two random handfuls were collected and placed in the sampling bag for further cleaning and analysis. The canopy coverage within this section of swamp ranged from 68-79% with pockets of standing water. As we moved through the transect the swamp became drier and the canopy began to slightly open.

The second lateral sample of HF, HF1901.2 was a much wetter environment with consistent standing water compared to the previous site which only had "isolate" pools of standing

water. Overall, the vegetation seemed similar at this locality but with more ferns and aquatic plants. The canopy ranged from 55-76% in cover and as with the other site, this location is on the margin of the overall larger swamp area. These sampling locations illustrate how fragmented and varied swamp ecosystems are. The higher quantity of standing water within this location allows for an abundance in mosquito's, forest frogs, tad pols, and other aquatic biota.

The last sampling locality within the HF swamp, HF1901.3 occurred along a boardwalk which runs through the swamp, unlike the margin sampling locations of HF1901.1 and HF1901.2. Canopy cover here was slightly more open with much more stagnant water and deep pools for leaves to collect in. The range of canopy cover was 49-60% with very dense leaf material in pools with dense mats of root material on the edges or "banked" areas created by the vegetation and leaf litter. Very anoxic environment with strong sulfur aroma.

### **mid-transport small tributary**

The small tributary sampled (HF1902.1, 1902.2, 1902.3) is a stream of class 2 or 3 and branches from the larger river somewhere above the HF property. This environment lacks fine sediment thus an adopted method was needed to sample leaf litter in an appropriate manner for comparison to the fossil record. HF1902.1 was sampled by running a 50m transect through the waterway with samples taken every 5m from snags, sediment, and/or leaf litter found directly next to the moving water. Large handfuls were randomly grabbed within a 1m sampling area and later subsampled to 400 leaves. Although the sediment was very-coarse, there was a small amount of fine-grained sediment accumulation where leaf accumulation created a snag, presumably decreasing water velocity, allowing for fine sediment to drop from the suspended load. Canopy cover here ranged from 55-67%. Utilizing the same protocol, HF1902.2 was sampled 100m downstream from HF1902.1. This location has much more fine-sediment accumulation than the previous site but still not enough to sample leaves isolated within the sediment. Canopy cover was similar to the previous site and ranged from 48-60%. The last section of sampling within the small tributary, HF 1902.3, occurred ~100m upstream from HF1902.2. This site seems to have less tree fall and dense vegetation over the river and was easier to run the 50m transect through the waterway. The topography of this section of the river was more dynamic than previous sites, creating steps, deep pools, and small waterfalls. Canopy cover at this location ranged from 62-76% which is slightly higher than the previous sites suggest a more established canopy with less understory growth, evident by an ease in running-out the transect.

### **high-transport fluvial**

The dynamic river sampled (HF1903.1, 1903.3) is on the Moccasin Brook and (HF1903.2) East branch of the Swift River, off of Quaker drive and highway 122 South of Petersham, MA town central. HF1903.1 and HF1903.3 had enough sediment accumulation to allow for the collection of leaves from within the sediment while HF1903.2 was sampled utilizing the methods described in the section 2.3. Low topography at HF1903.1 allowed for the accumulation of very-fine to fine-grained sediment within a small horseshoe shaped (~5m wide) deposit directly next to the walking trail. Leaves were isolated from the sediment

using a sieve and small trowel. This deposit was highly anoxic with a strong sulfuric aroma while excavating leaves. Once leaves were isolated from the sediment, they were rinsed in the Swift River of sediment and placed in a sample bag, later subsampled to 400 leaves. Canopy cover at this depositional environment was 59.17%. Downstream of sampling location are large boulders with very-coarse to coarse river bottom however, the sampling location was dominated by fine sediment of mud and clay. Following the same methods, the second site allowing itself for the isolation of leaves from the sediment is HF1903.3, which is 300m upstream of HF1903.1. Similar to its downstream equivalent, HF1903.3 is a small horseshoe shaped deposit dominated by large boulders framing the horseshoe, allowing for the catchment of fine sediment and leaves to accumulate.

Unlike HF1903.1 and HF1903.3, HF1903.2 is a floodplain deposit 100m downstream of HF1903.1. This floodplain deposit is located directly adjacent to the Swift River. The canopy is comprise of Eastern Hemlock, various Birch sp., and some occasional red maples. The sediment is very sandy with large rocks and boulders. Due to the floodplain composition, it suggest that this section of the Swift River is highly dynamic with high velocity flooding events occurring throughout the year. Understory plants are lacking except directly next to the river where moss, fungi, ferns, and other small water loving plants have colonized. All growth next o the stream is a few inches in height, suggesting that disturbance events occur frequently. Methods used in section 2.1.2. were used to sample the floodplain deposit. Canopy cover ranges from 45-54%.

### upland locality

One upland locality was sampled ~50m east of the Quaker drive parking area. This site was collected to compare upland samples to the dynamic river samples across a gradient. Methods from section 2.1.2. were replicated here for consistency. Forest canopy is dominated by Eastern Hemlock and Red Oak with canopy cover ranging from 54-59%. Randomly sampled leaves were highly dry and densely packed on the forest floor.

## SERC Leaf sampling

During the summer of 2019 leaf samples were collected from the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland. Collection sites were randomly chosen, targeting depositional environments comparable to fossil localities. Leaves are found within the sediment layers and thus mimic compression fossils within the geologic record. Three depositional environments were targeted: low-transport swamp, mid-transport small tributary, and high-transport freshwater spring which drained into the brackish waters of the Chesapeake Bay. At each depositional environment, three quarries were sampled to capture lateral variability. Each quarry was blindly sampled to approximately 400 leaves trapped within the sediment, resulting in 1200 leaf samples per depositional environment. A total of ~3600 leaves were sampled within the SERC forest.

Leaf layers were identified within each depositional environment and randomly sampled. Using a small trowel and #3mm sieve, leaves were excavated from the sediment, placed in plastic bags, and transported back to the SERC facility for further cleaning and drying. Each

quarry was cleaned of fine sediment using a strainer and freshwater until the majority of the fine sediment was removed from the leaves without causing further breakage. Once leaves were cleaned, they were pressed flat and dried in an oven at 70°C until dry (approximately 24hrs). Leaves were then identified to species, when possible, and transported back to the University of Wyoming for further analysis.

### low transport swamp

Three quarries (MD1901.1, 1901.2, 1901.3) were sampled within the swamp/marsh with each site approximately 200m in distance from one another. The forest is dominated by American Beech (*Fagus grandifolia*), Dogwood (*Cornus florida*), White oak (*Quercus alba*), Sweetgum (*Liquidambar styraciflua*), and Hickory (*Carya alba*) likely signaling an older growth forest with less disturbance. The swamp/marsh area is flooded by a small creek comprised of muddy sediment just below the 109-water monitoring station. All localities are similar in dominant plant species with a slight elevation increase (~1000ft) from the most upland site (MD1901.2) to the lowest level site (MD1901.3). The most downstream site (MD1901.3) has the most understory vegetation comprised of various fern species, indicating a less disturbed environment. Leaf packs, highly dense mats of leaf material within the sediment, were collected along 50m transects, randomly sampling leaf mats every 10m. If the sampling location fell in an area of the marsh with sensitive animal populations, we offset the location by 1m.

### mid-transport small tributary

The small tributary (MD1902.1, 1902.2, 1902.3) is located on the southern end of the property containing very fine sediment, comprised of clay and silt with a highly incised riverbank. Deposit is ~5m in depth with varied leaf sizes and large sticks, twigs, and woody plant parts above and below leaf packs. Leaf packs are located within anoxic environments with high fossilization potential. Canopy above MD1902.1 is comprised of American Beech (*Fagus grandifolia*), Tulip Poplar (*Liriodendron tulipifera*), White (*Quercus alba*) and Red Oak (*Quercus rubra*). Approximately 150m downstream of MD1902.1 another leaf pack was sampled as our second quarry within this location, MD1902.2. The tributary is much wider here but still dominated by clay and silty sediment. Less large sticks and twigs were present at this location, as the velocity of the water decreases with a wider stream. Canopy above this site is comprised of mainly American Beech (*Fagus grandifolia*), Red Maple (*Acer rubrum*), and Hornbeam (*Carpinus caroliniana*) with the understory made up of brambles (*Rosaceae Rubus* sp.) and various tree species saplings. This locality seems to be flooded often with lots of leaf litter. The last quarry within the mid-transport small tributary, MD1902.3, is located ~100m upstream from MD1902.2. The two sites are separated by a swamp/marsh. Very variable deposition and range of sediment clasts from coarse grained to very fine silt. Samples were collected along band deposits with a small point bar. Varied leaf sizes suggesting more transport within this quarry compared to others downstream. Canopy is mainly Hornbeam (*Carpinus caroliniana*) with a well-developed understory of young saplings and herbaceous and grass species.

## high-transport fresh-water drainage

Unlike the previous sites, MD1903 is highly complex with leaves undergoing higher levels of transport. These quarries are fresh waterways draining the surrounding forest into the brackish Chesapeake Bay. These freshwater drainages run through tall grasses before depositing leaves on the mudflats, experiencing tidal drying sequences daily. These leaves are in highly anoxic fine sediment. MD1903.1 has a less apparent spring and leaves are less abundant than the other quarries within this depositional environment with various oak species dominating this site and Hornbeam (*Carpinus caroliniana*). This site has more woody debris than the other two quarries and is the furthest away (~300m) from the source forest likely feeding this drainage. The most abundant site is MD1903.2 with leaves often coming from the sediment retaining their color. The source forest is ~200m behind the grass area which the drainage winds through. Leaves are nearly completely intact with Red (*Quercus rubra*) and Chestnut oak (*Quercus montana*) dominating the sample.

## La Selva Leaf sampling

During the winter (dry season) of 2019 leaf samples were collected from the Organization of Tropical Studies La Selva research station, Costa Rica. This low-land tropical wet ecosystems is home to over 700 plant species. The station is 1,600 hectares and located within a wet lowland tropical rain forest bound by the Rio Sarapiquí and Rio Puerto Viejo, both dynamic rivers. Sites sampled were chosen to encompass various habitats and depositional environments. Three habitats were sampled including a dynamic river within a secondary growth forest, a tributary river within an ecological reserve (undisturbed) and a swamp within an old-growth forest. It should be noted that during the wet season all sampling locations would be under water with the exception of the swamp which would be more saturated but not necessarily underwater. Each habitat was sampled three times to allow for spatial variability. Sampling of leaves was done in two different methods. A total of ~3600 leaves were sampled across all depositional environments. Once collected, leaves were pressed and dried at 70°C for 12-48hrs depending on the habitat sampled and saturation of leaves.

## low transport swamp

The swamp is located within an old-growth forest and at the edge of the research property. Canopy cover within the swamp was calculated using a densiometer and ranged from 84.5-87.5%. Transects were utilized and sampling occurred along a boardwalk. Two 50m transects were used (LS1903.1, LS1903.3) and a 44m transect (LS1903.2) due to reaching the edge of the property line. Each transect was sampled every 5m for a four-minute interval. During the interval, the meter before and after the marker was sampled on the East and West of the transect. For example, at the 5m mark the area between 4-5m and 5-6m were sampled. The lower limit was always sampled on the West facing side and the upper limit was always sampled to the East facing direction. Blind sampling was not possible due to the venomous snake hazard within the leaf litter so each leaf was carefully turned over and then collected. One swamp location crossed two fresh water streams and when the sampling interval crossed the water, we collected leaves deposited in the bank to the West and East. Leaf samples were collected in sample bags, rinsed of sediment to the best of our ability and transported back

to the lab facilities within the research station. Each transect was then subsampled to 400 leaves.

### **mid-transport tributary**

Leaf layers were randomly identified within the sediment and collected by cutting into the river bank with a trowel and #4 sieve, often leaf layers were submerged in the water. LS1902.1 leaf layers were located 10cm below the surface of the water and a #4 sieve was used to scoop sediment into the sieve, leaves were washed of sediment and placed in a sampling bag. Productive leaf layer appeared to only be about 30cm thick with leaves deposited in very fine sediment (clay-silt) and anoxic environment. Very little to no sand at the site. Total diameter of quarry sampled was approximately 3m in width. Canopy cover at this locality was ~59%.

The second locality sampled within the tributary was located 50m downstream from LS1902.1, LS1902.2 had much coarser sediment than LS1902.1. Sediment ranged from large cobbles to sand and as such, leaves were more varied than LS1902.1 with small leaves indicating high levels of transport (autochthonous) and larger leaves representing the local or allochthonous signal. At this location, leaves were sampled from above and below the water line with the majority of the leaves coming from below the water line. Canopy cover here was slightly higher at 73%.

The last location sampled within the small tributary was another ~50m downstream from LS1902.2, LS1902.3 is very muddy, clay/silt sized sediment. Leaves are deposited in layers of fine sediment below a layer of sticks and woody plant material suggesting flooding events persisted in this tributary. Productive leaf layer is 20cm thick and the overall quarry is ~4m wide. Canopy cover here is 72%.

### **high-transport dynamic river**

All samples taken from the dynamic river were isolated from the sediment using a #4 sieve and towel. Leaves were washed clean of debris to the best of our ability (avoiding additional damage) and subsampled to 400. The first location sampled within the high-transport dynamic river, LS1901.1 is the most downstream location within this depositional environment. Productive leaf layers span a 3m thick sediment outcrop above the waterline. Layers of leaves are found within a mixture of clay and fine sand with dense woody debris above and below productive layers. Samples were taken from three sediment layers and average together. Each layer of leaves is approximately 20cm thick. Canopy cover here is approximately 75% within the forest but much more open at the river's edge.

The second locality sampled within the dynamic river is located 80m southeast of LS1901.1 (upstream). Sediment is much finer here with beautiful lamination. Productive leaf layers are intermixed with sand (fine-very fine) and clay layers. The sand is dark brown while the clay is grey. Compared to LS1901.1, leaves found at this lateral equivalent LS1901.2, are much larger. The sampling location was set back from the water 6m with canopy cover ranging from 61-74%.

The last locality sampled, LS1901.3 is a true sandbar with leaf layers deposited within fine sand and clay layers. Much less clay at this location than previous sites. North facing section of sandbar has more leaves preserved than the southern end. As you move South on the productive layer, it pinches out. The direction of flow is North which explains why leaves are more predominately preserved on the North side of the sand bar as the velocity of the water slows and deposits the light material of clay particles and leaf material. Canopy cover here is the most open with a range of 55-62% cover. Leaves from this location are the least well preserved and are highly damaged. Additionally, leaves were much less abundant at this site and required extensive sampling.

## Fossil plant-insect methods

A comprehensive literature search was preformed to identify all previously published papers on plant-insect interactions since the Cretaceous. Records with >300 leaf specimens were used and any publications with fewer than 300 leaf specimens were not analyzed. A total of 63 locations met all the criteria set by the authors. Insect herbivory was quantified for percent: total, specialized, mine, gall, hole, margin, skeletonization, surface, and piercing and sucking damage. To account for uneven sampling diversity was standardized to 300 leaves for: total, specialized, mine, gall, and plant diversity. Lastly, a Shannon diversity index was calculated along with Pielou's J for evenness. Mean annual temperature (MAT), mean annual precipitation (MAP), latitude (High-North, Mid-North, Low, Mid-South, High-South), and depositional environment were also collected for all fossil datasets.