Can Root Samples Reflect Biodiversity of Above Ground Forest?

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Abstract

The Thain Family Forest, located in The New York Botanical Garden, Bronx, NY, was once part of a larger hemlock, oak forest. A densely populated urban area surrounding the forest poses a constant threat to its original bio-diversity. Core soil samples were collected at the margin of the forest. The species of the roots were identified using barcode to prove that below ground plant material could characterize the above ground ecosystem. Six species were identified. These results were compared to an existing above ground barcode survey and an identical type of survey conducted in the center of the forest. The rate of success for data collection was 58%.

Figure 1: River defining edge and survey site

Introduction

The Thain family forest, a part of the New York Botanical Garden (NYBG), is believed to be one of oldest remnants of the original hemlock (*Tsuga canadensis*) and oak (*Quercus spp.)* forest that once covered the New York City area (Fig. 1). Founded in 1891, the New York Botanical Garden is now surrounded by a densely populated area. Changes have been noted in the forest checklist since 1891 (Atha et al., 2016). For example, the species *Castanea dentata* was completely eliminated by the disease chestnut blight; there was an increase in populations of *Acer rubrum, Prunus serotina, and Cornus florida* (Rudnicky, 1989). "However, such a large difference illustrates that there are fewer stems in the NYBG Forest than would be expected in natural forests." (Rudnick, 1989*).* The site is managed but surrounded by a highly populated urban area where disturbances to plant life is common. This study will focus on a marginal area of the forest. It is thought that "[...] increasing fragmentation of natural habitat by human disturbances in the direction toward urban centers will tend to reduce species richness (number of species) in that direction" (McKinney, 2002).

Roots cannot be used to morphologically distinguish most species, but root samples should reflect the plant biodiversity of the whole ecosystem. DNA barcoding allows accurate identification of below ground plant materials (Randall, 2014). Sampling took place in the New York Botanical Garden on a small scale. The objective of this research was to collect root samples to see if below ground plant materials can characterize the above ground ecosystem. Results were crosschecked with an existing DNA barcode reference database, constructed using above ground materials (Little, unpublished). This data set was compared to a concurrent survey in the center of the Thain Family Forest using the same techniques to demonstrate the effectiveness of DNA barcodes.

Materials and Methods

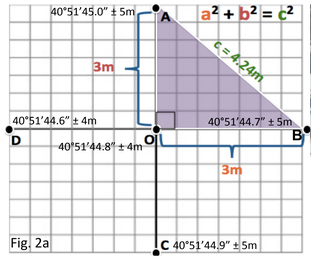

Fig. 2a

Fig. 2b

Fig. 2a and b: map of transect and core sample coordinates A,B,C,D,O.

Survey of Land Two transects were laid out in a perpendicular fashion (Fig. 2). First, 30.5 by 2 cm diameter sterile pipes were used for collection. These pipes were hammered in the ground. Consequently, the pressure in the pipe prevented the soil sample from falling out. The pipe was retrieved from the ground by banging the pipes in four directions. Five samples were taken in each direction, along each transect, three meters from the origin at points A,B,C,D,O. (Fig. 3a). The roots in the soil were separated from other components by filtration with sterile water.

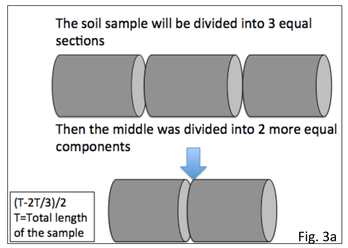

Fig. 3a

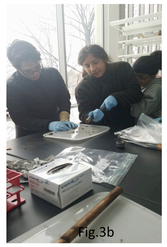

Fig.3b

Fig. 3a and b: figure dimensions, struggling to remove the core sample from the pipe.

DNA Extraction and PCR Root tissue was homogenized in an extraction buffer. Silica resin was bound to the DNA, allowing isolation from the solution. The silica bound DNA was purified with a wash buffer multiple times. The silica was removed by adding distilled water to the solution. The PCR reaction contained: 1x buffer (from 10x concentrate: 200 mM tris [pH 8.8 at 25°C], 100 mM KCl, 100 mM$(NH_4)_2$ SO_4, 20 mM, $MgSO_4$•7H2O, 1% [v/v] Triton X-100, 50% [w/v] sucrose, 0.25% [w/v] cresol red), 0.2 mM dNTPs, 0.25µg/mL BSA, 0.5 µM foward primer (rbclF: ATGTCACCACAAACAGAGACTAAAGC; Levin et al. 2003), 0.5 µM reverse primer (rbcl-R: GAAACGGTCTCTCCAACGCAT; Fazekas et al. 2008), 0.2 units *Taq*, and 1 µL DNA. During PCR, the reaction was heated to 95°C for 150 s. Afterwards, the reaction was heated to 95°C for 30 s and cooled to 58°C for 120 s ten times. Then the reaction was heated to 88°C for 30 s and cooled to 55°C for 120 s 25 times. Lastly, it was cooled to 58°C for 600 s.

The sequence chromatograms were assembled into contigs and analyzed using Sequencer v5.2.4. After editing, contigs were examined for the correct size and stop codons. Sequence quality was evaluated using B_{30} (Little 2010), a reference database that contains sequences of all the plants in the Thain Family Forest. They were compared with the reference database by using BRONX v2.0 (Little 2011). This program identified the closest matching sequences to find the species of the sample. In this case, the raw scores should be above 450 and have a sequence quality score (B_{30}) of 0.5 or higher.

Results

18 of 31 of the samples extracted had amplifiable DNA (Table 1). 16 of the sequences had high B_{30} sequence quality scores, with the highest being 0.965. 83% of the sequences had high BRONX raw scores (Fig. 4). Five species were reliably identified: *Populus deltoids, Liriodendron tulipifera, Fagus grandifolia, Pinus strobus, Poa annua.* Additional genera were identified, *Carya* and *Quercus,* but the species could not be conclusively determined.

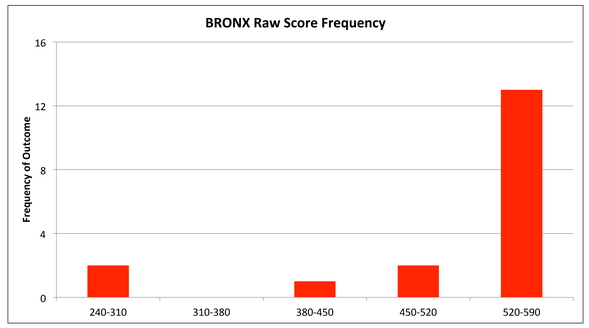

Figure 4: Graph of BRONX Raw Score frequencies.

Sample Number	Raw Score	Sequence Quality Index	Genus Species
O01	563	0.965	*Populus deltoids*
O03	563	0.737	*Liriodendron tulipifera*
O04	265	0.289	*Fagus grandifolia*
O06	562	0.904	*Pinus strobus*
O07	562	0.541	*Pinus strobus*
O09	588	0.639	*Pinus strobus*
O10	543	0.952	*Liriodendron tulipifera*
O11	545	0.739	*Fagus grandifolia*
O12	562	0.886	*Pinus strobus*
O14	588	0.838	*Pinus strobus*
O15	433	0.389	*Carya glabra, Quercus alba, Q. coccinea, Q. macrocarpa, Q. palustris, Q. rubra, Q. velutina*
O17	543	0.945	*Liriodendron tulipifera*
O18	543	0.944	*Liriodendron tulipifera*
O27	291	0.512	*Poa annua*
O28	474	0.592	*Poa annua*
O29	538	0.637	*Poa annua*
O30	487	0.561	*Poa annua*
O31	543	0.947	*Liriodendron tulipifera*

Table 1: Identification of sequenced samples.

Species	Margin*	Center*	Thain Family Forest
Populus deltoids	1	0	1
Liriodendron tulipifera	1	1	1
Fagus grandifolia	1	0	1
Pinus strobus	1	1	1
Poa annua	1	1	1
Morus alba	0	1	1
Prunus persica	0	1	1

Table 2: Comparison of species identification from Margin and Center samples. *Margin sample O15 was not included due to poor quality sequence data. ** Center samples O02 and O14 were not included since they could not be identified with certainty.

Discussion

There were five species that were reliably identified: *Populus deltiodes*, *Pinus strobus*, *Fagus grandfolia*, *Lirodendron tulipifera*, and *Poa annua*. Sample O04 was identified as *Fagus grandfolia* and had low-sequence quality (B_{30} = 0.289) and a BRONX raw score of 265 making its identification unreliable. However, *Fagus grandfolia* was a reliably identified in sample O11. Sample O15 could be one of seven different species, six of which were *Quercus* including *Q. alba*, *Q. coccinea*, *Q. macrocarpa*, *Q. palusris*, *Q. rubra,* and *Q. velutina,* but is a low-quality sequence (B_{30} = 0.389).

Sample O27 was identified as *Poa annua* with a BRONX raw score of 291 and a sequence quality index of 0.512. One possibility is that this particular species is not in the reference barcode library and therefore an unreported species for the forest. Other explanation includes that this particular sample is a *Poa annua* with a mutation in the barcode sequence, or that the sequence quality was just too low to be useful.

The frequency of species from the 18 sequenced root samples were the follows: 5.6% were *Populous deltiods*, 27.8% were *Pinus strobus*, 5.6% were *Fagus grandifolia*, 27.8% were *Liriodendron tulipifera*, 22.2% were *Poa annua,* and 11% were not identifiable. Only 27.8% of the species collected are invasive species, the only one being *Poa annua* a native of Eurasia, often known for being "high genetic variability and tolerance of low mowing height" (ISSG.org) hence it is a common invasive species (Atha, 2016). *Populus deltolds* and *Fagus grandifola* were frequent in the sample as supported the above ground survey of the forest (Atha, 2016).

The species of plants found in the center transect of the Thain Family Forest were: *Liriodendron tulipifera*, *Pinus strobus*, *Poa annua*, *Morus alba*, and *Prunus persica*. The margin and center had three species in common: *Liriodendron tulipifera*, *Pinus strobus*, and *Poa annua*. *Populus deltoids* and *Fagus grandifolia* did not appear in the center transect and *Morus alba* and *Prunus persica* were not found in the margin transect. However, the number of samples is not sufficient to conclude which location has more diversity.

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