Wild versus introduced species: the efficiency of forest eDNA surveys

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Abstract

We applied airborne environmental DNA (eDNA) technology to research plant biodiversity in a 20 hectare (50-acre) uneven aged, mixed hardwood, remnant urban old–growth forest. We collected airborne eDNA samples from two sites (A and B) in order to determine whether the sites differed in the numbers of introduced versus wild species. Using a *rbcL* D mini–barcode, we sequenced the eDNA samples to identify what species were collected. 4,656–12,461 high–quality sequences were obtained for each site and negative control. Our data indicated that site A harbored more wild species (57%) than introduced species (42.1%), a pattern mirrored at site B (54.5% versus 45.5%). Despite the variation observed between sites, the numbers of wild versus introduced species is less than 4%. Site A had many more species than site B (26 and 17, respectively).

Introduction

Airborne environmental DNA (eDNA) uses genetic remnants collected from the air to detect plant species. It is a relatively new concept allowing plant and animal communities to be surveyed without much effort.

We applied airborne eDNA to the study of plant biodiversity. The location for our study at the New York Botanical Garden is a 20 hectare (50-acre) uneven aged, mixed hardwood, remnant urban old–growth forest that has never been cleared (Atha et al., 2016). The forest contains 261 extant wild species and 229 introduced species (490 species total).

Two locations differing in distance from the Bronx River and amount of sun exposure were sampled. We expect the type and number of eDNA sequencing reads to reflect the number of migrating propagules from the Bronx River and the variety of habitats (e.g. shady versus sunny).

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Fig. 1. Airborne eDNA collection sites A and B.

Materials & Methods

We collected airborne eDNA samples from the NYBG forest at two sites (A, B) near the Bronx River (Figs. 1 and 2). We recovered the eDNA from Sterivex filters (Fig. 3) and each sample was amplified using *rbcL* D primers. Amplification products were combined by site and AmpliconEZ sequenced (150-PE; GENEWIZ).

We removed sequencing failures from the raw data, filtered out low–quality sequences, removed primer sequences, and merged identical sequences within each sample. Then we compared assembled sequences to a reference database of the forest species. Then for each site and sequence type, we subtracted the number of reads in the negative controls from the positive samples.

Fig. 2. Airborne eDNA collection Sterivex filter and pump.

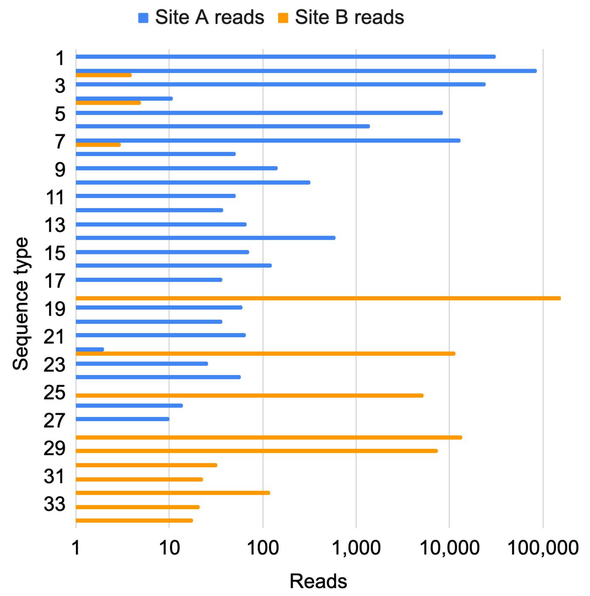

Fig. 4. eDNA reads from sites A and B by sequence type.

Results

Sites A and B are 50 m apart. Both are under tree canopy, site A is 70 m from the Bronx River, while site B is 100 m (Fig. 1).

After quality filtering and merging, there were 12,461 unique sequence reads from site A, 4,656 from site A negative control, 7,701 from site B, and 7,035 from site B negative control. For all of these reads, a total of 34 unique identifications were produced from the *rbcL* D reference database (Table 1; Fig. 4).

Some species are found in abundance at one site, while the other site has fewer reads. For instance, at site A, *Allium vineale* had 86,013 reads compared to site B with only 4 reads (sequence type 2 in Fig. 4). Despite the close proximity between the sites, they had distinct species complements without much overlap. Sites A and B had more sequencing reads from wild species (57.89% and 54.55%, respectively) than introduced ones (42.11% and 45.45%, respectively; Table 1). Site A had more species than site B (26 and 17, respectively; Table 1).

Table 1. Sequence types from sites A and B.

	Site A	Site B
Total sequence types	26	17
Wild sequence types	57.89%	54.55%
Introduced sequence types	42.11%	45.45%

Discussion

Our filter–based airborne eDNA collection was successful as evidenced by high read counts and diverse sequence types despite collecting in late November—when plants are largely dormant. In comparison to Johnson et al. (2021), we collected 34 sequence types from two filters in six minutes as opposed to a maximum of 25 sequence types from nine dust traps over two weeks.

Both sites had similar proportions of wild versus introduced species—with about a 4% difference (Table 1). Our hypothesis was that site A, located closer to the Bronx River, would have more introduced species, while site B would have more native species. Our eDNA collections had similar proportions, disproving our hypothesis. However, species and numbers differed between sites; site A having more species than B and with low overlap between sites.

References

Atha et al. 2016. DOI: 10.1007/s12228-016-9417-5
Johnson et al. 2021. DOI: 10.1186/s12862-021-01947-x

Fig. 3. eDNA extraction.