

Assessment of the diversity and viability of a centuries-old wetland seedbank

Catherine Yan, Shelby Riskin

Department Ecology and Evolutionary Biology, University of Toronto

Introduction

Wetlands are crucial ecosystems with roles in water quality, carbon storage, and biodiversity. However, industrialization and urbanization can bring substantial changes to wetlands, rendering them distinct and degraded from their centuries-old counterparts. To restore these ecosystems, understanding their original state before urbanization is essential. Therefore, exploring the ancient wetland seedbank becomes crucial, as it shapes vegetation and reflects wetland history.

A recent restoration project along Lake Ontario has led to the re-exposure of long-buried soil and seeds⁽²⁾, providing a chance to explore the centuries-old wetland seedbanks. Our project primarily focuses on investigating these aged seeds in the soil, comparing seed diversity in different soil types, and discerning differences between present-day and historical seed compositions. Our main questions are: what is the diversity and viability of old wetland seeds? Do they resemble today's wetland vegetation? We hypothesize that different soil types will yield varying seed distributions, yet wetland plants (especially sedges) will dominate as today's wetland.

Methodology

We analyzed 21 unique soil samples collected from the industrial area at the mouth of the Don River. They represented two soil types, one peat (10 samples) and one mineral soil (11 samples).

Seed Collection: For each sample, we selected a volume of 15 cm x 15 cm x 10 cm soil. Employing the floatation method⁽⁵⁾, we extracted seeds and other organic material from the soil. We scattered the selected soil samples within a water-filled bucket and employed a 0.25 mm mesh sieve to gather the floating materials.

Seed cleaning & photographing: To remove adherent mud, we cleaned all seeds by applying a four-hour soak in 3% H₂O₂ after extraction. Then we photographed and scaled each seed under a microscope using ToupView.

Seed germination test: We selected 48 seeds from two peat soil samples and soaked the test seeds in a 200 ppm gibberellic acid solution for 24 hours to increase their germination chance⁽⁶⁾. We used rockwool cubes to facilitate germination and supplemented the seeds with 500 ppm 10-52-10 fertilizer to support root growth.



Fig 6. Seeds and other organic materials collected from floatation.

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Results

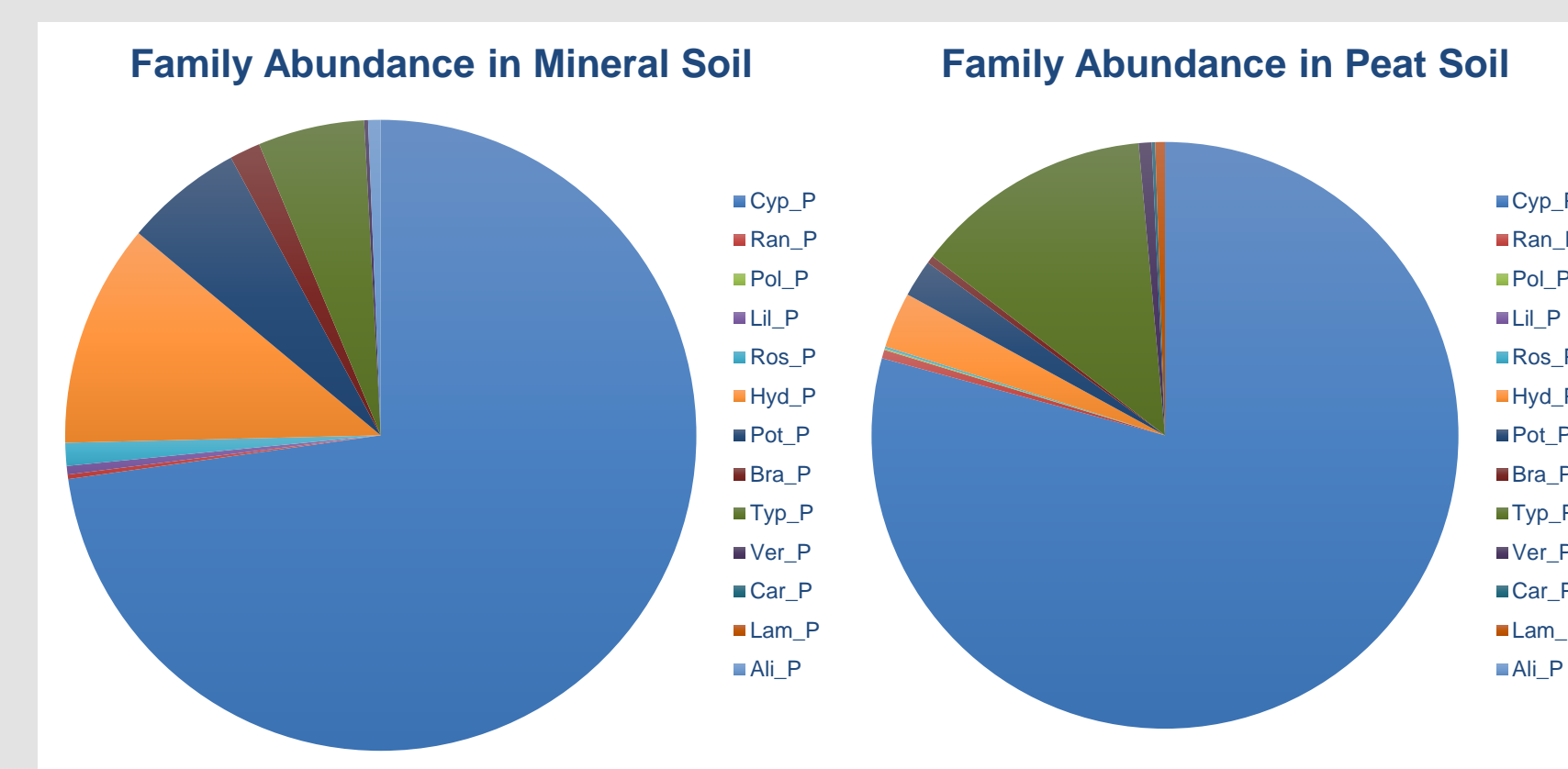


Fig 1. Seeds abundance categorized by family in mineral and peat soil. Cyp = *Cyperaceae*; Ran = *Ranunculus*, Pol = *Polygonaceae*, Lil = *Liliaceae*, Ros = *Rosaceae*, Hyd = *Hydrocharitaceae*, Pot = *Potamogetonaceae*, Bra = *Brassicaceae*, Typ = *Typhaceae*, Ver = *Verbenaceae*, Car = *Caryophyllaceae*, Lam = *Lamiaceae*, Ali = *Alismataceae*

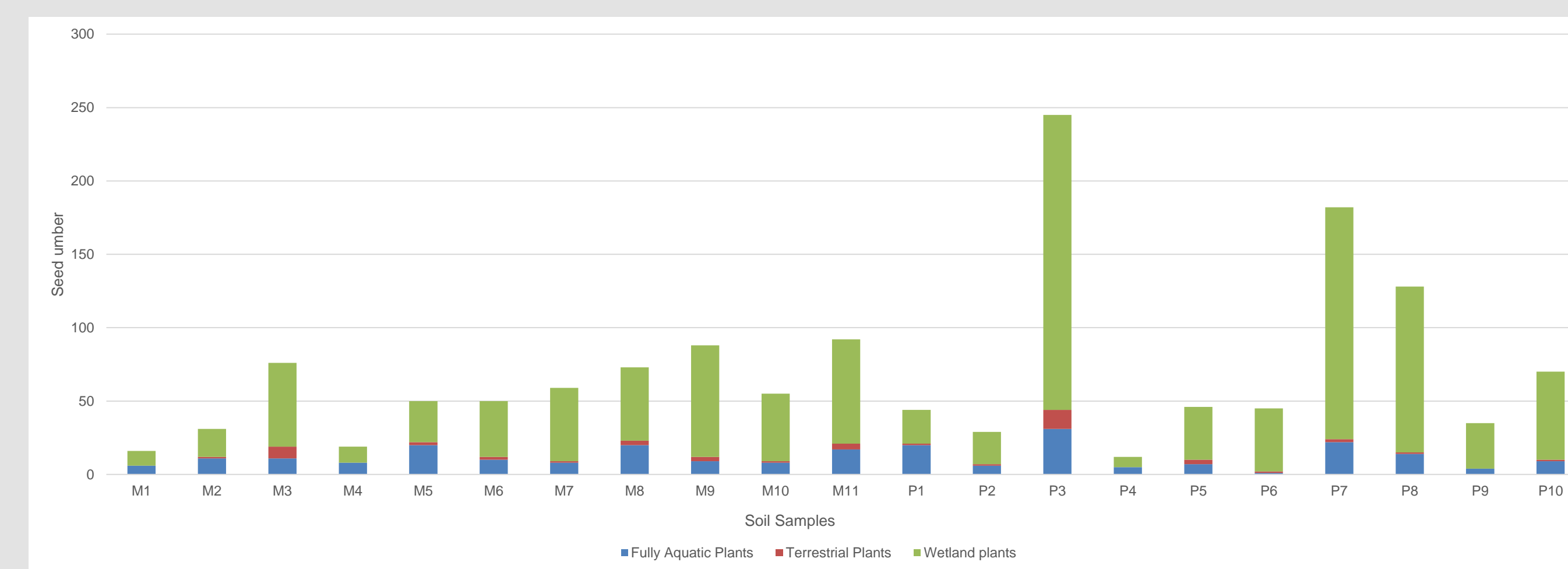


Fig 2. The fully aquatic plants, terrestrial plants, and wetland plants diversity. The seeds diversity are significantly different among the three types based on an ANOVA test ($p < 0.0001$). Wetland plants are significantly more than the others (both $p < 0.0001$), while terrestrial and fully aquatic plants are similar ($p = 0.535$).

Fully aquatic: *Hydrocharitaceae*, *Potamogetonaceae*, *Typhaceae*, *Lamiaceae*, *Characeae*; terrestrial: *Ranunculus*, *Liliaceae*, *Rosaceae*, *Brassicaceae*, *Verbenaceae*, *Lamiaceae*; wetland: *Cyperaceae*, *Polygonaceae*, *Caryophyllaceae*.

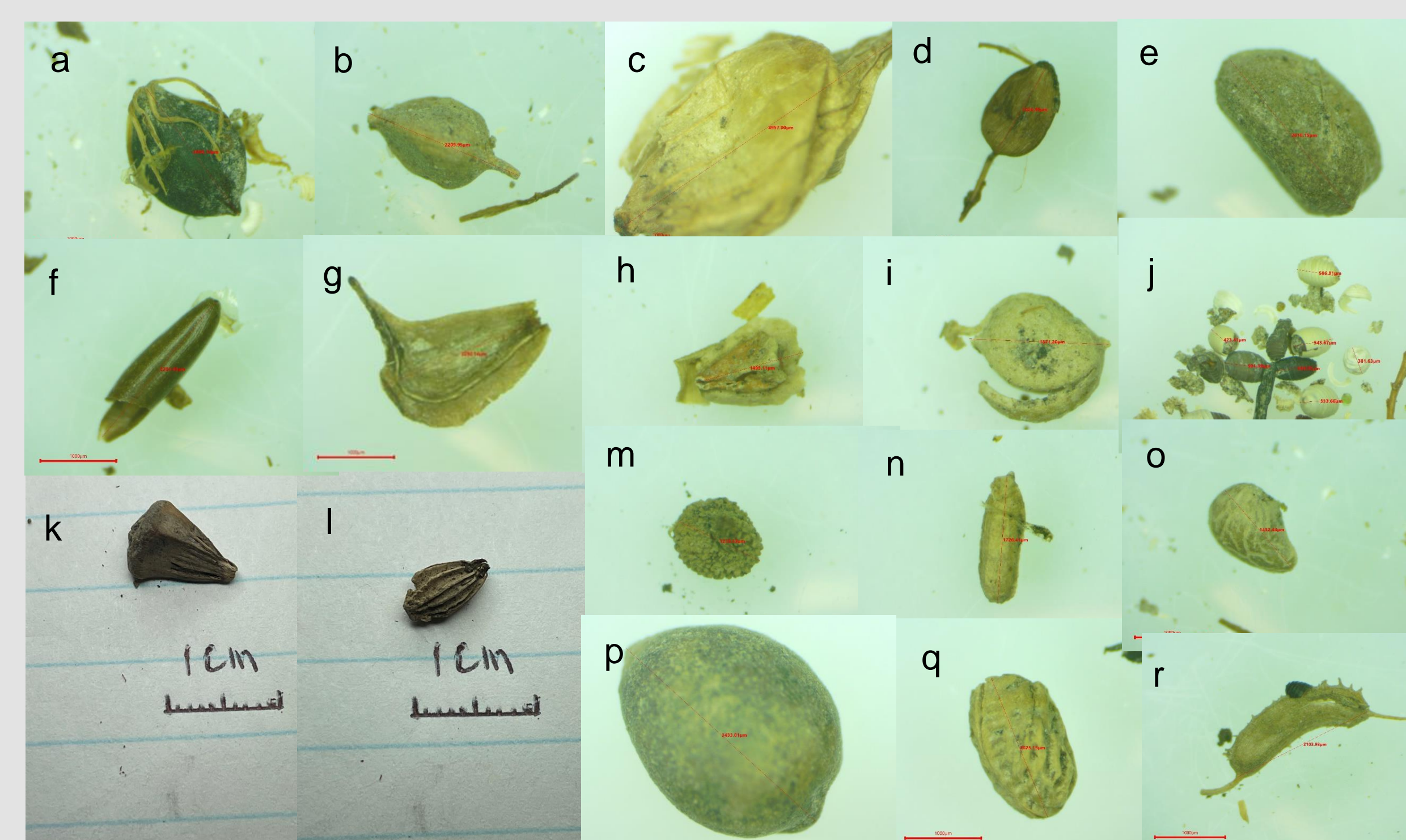


Fig 4. Representative seeds from the soil samples: (a) *Schoenoplectus tabernaemontani*; (b) *Carex comosa*; (c) *Carex sp.* with perigynium; (d) *Carex sp.*; (e) *Conringia orientalis*; (f) *Najas sp.*; (g) *Sagittaria latifolia*; (h) *Lycopus americanus*; (i) *Potamogeton sp.* (j) *Chara sp.* (k, l) *Sparganium eurycarpum*; (m) *Stellaria media*; (n) *Verbena hastata*; (o) *Potentilla pensylvanica*; (p) *Polygonatum biflorum*; (q) *Rubus sp.*; (r) *Zannichellia palustris*⁽³⁾. Scale = 1 mm (a-j, m-r); Scale = 1 cm (k, l).

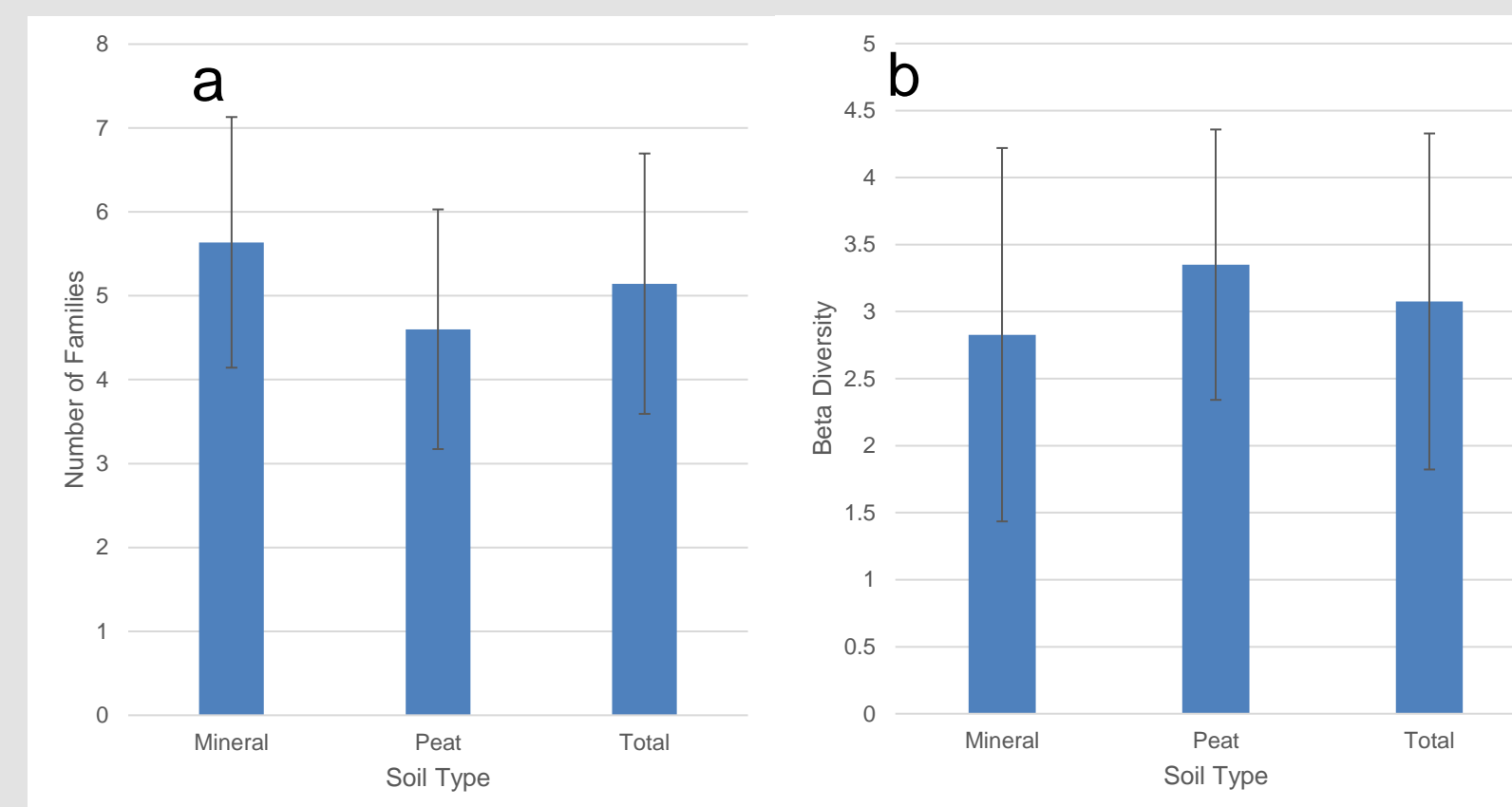


Fig 3 (a). The number of seeds families in mineral, peat, and all soil samples. The seed genus numbers in mineral and peat soil are similar based on a t-test (p -value = 0.14, $\alpha = 0.05$).

Fig 3 (b). We calculated the beta diversity in all samples through dividing the number of families by total number of families (14). The average beta diversity of all the samples is 3.07619.

Family / Genus	Mineral soil	Peat soil	Total
<i>Cyperaceae</i>	460	693	1153
<i>Carex sp.</i> without perigynium	114	303	417
<i>Carex sp.</i> with perigynium	22	196	218
<i>Eleocharis sp.</i>	80	10	90
<i>Scirpus sp.</i>	31	12	43
<i>Schoenoplectus sp.</i>	185	108	293
<i>Cyperus sp.</i>	6	12	18
<i>Ranunculaceae</i>	2	2	4
<i>Ranunculus sp.</i>	2	2	4
<i>Polygonaceae</i>	0	1	1
<i>Polygonum sp.</i>	0	1	1
<i>Liliaceae</i>	2	0	2
<i>Polygonatum biflorum</i>	2	0	2
<i>Rosaceae</i>	8	2	10
<i>Rubus sp.</i>	6	1	7
<i>Potentilla sp.</i>	2	0	2
<i>Hydrocharitaceae</i>	69	20	89
<i>Najas sp.</i>	69	20	89
<i>Potamogetonaceae</i>	36	7	43
<i>Potamogeton sp.</i>	30	7	37
<i>Zannichellia sp.</i>	7	0	7
<i>Brassicaceae</i>	13	9	22
<i>Conringia orientalis</i>	13	9	22
<i>Typhaceae</i>	20	90	110
<i>Sparganium eurycarpum</i>	20	90	110
<i>Verbenaceae</i>	1	8	9
<i>Verbena sp.</i>	1	8	9
<i>Caryophyllaceae</i>	0	1	1
<i>Stellaria sp.</i>	0	1	1
<i>Lamiaceae</i>	1	3	4
<i>Lycopus sp.</i>	1	3	4
<i>Alismataceae</i>	5	1	6
<i>Sagittaria sp.</i>	5	1	6
<i>Characeae</i>	Many	Many	Many
<i>Chara sp.</i>	Many	Many	Many
<i>Cristatella mucedo</i>	0	2	2
Shell	21	0	21
Total	807	1057	1864

Results - Seed list

Table 1. Identified seeds and animal lists by family and Genus from mineral and peat soil samples.

Conclusion

- There are 14 families and 21 genera of seeds with 1864 seeds from 21 samples, with more seed abundance in peat soil. There are also 21 shells in mineral soil and 2 bryozoan (*Cristatella mucedo*) statoblasts in peat soil.
- Cyperaceae* is the most common family represented in the seedbank with the highest abundance.
- The seeds range from fully aquatic, to terrestrial, and wetland plants with wetland plant species represented more than either fully terrestrial or fully aquatic plants.
- Though the soil types have different properties, the number of families and beta diversity of seeds are similar in peat and mineral soil.
- After a two-week germination test, there was no successful germination.
- All identified seeds represent native wetland plants and they are still found in wetlands in Ontario today⁽⁴⁾.
- Based on a previous carbon dating for a subset of 6 seeds and our project, our wetland samples are likely from 200 years ago when the Ashbridge's Bay Marsh was relatively unimpacted by urbanization.

Acknowledgements

I would like to thank Shelby Riskin, Melanie Sifton, and Tom Gludovacz for helping with conceptualizing the project and assistance along the way. I would also like to thank The Centre of Global Change Science for funding this project.