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The Effects of Varying Nitrogen Amounts on the Growth and Leaf Morphology of Cattail Species

Anissa Forero
University of Northern Iowa

Kenneth J. Elgersma
University of Northern Iowa

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Anissa Forero and Kenneth Elgersma
Department of Biology, University of Northern Iowa

Background

In Iowa, there are three types of cattails *(Typha spp.)*: *Typha latifolia* (broadleaf), *Typha angustifolia* (narrow leaf), and *Typha x glauca. T. latifolia* is native to Iowa, while *T. angustifolia* and *T. x glauca* are not native. Cattails are found in wetlands, highly productive ecosystems that provide many services such as water filtration and flood control.

Typha in a mesocosm

However, *Typha* are opportunistic and invasive: if left uncontrolled, *Typha* grows rapidly by clonal reproduction, quickly dominating the ecosystem, lowering overall diversity and hurting the wetland's conservation value. Being able to easily identify these taxa will improve the pace of future research.

The purpose of this study is to understand the effects of different nitrogen amounts on the growth and morphology of each *Typha* taxon. In order to achieve this, *Typha* was grown in 1.75-m mesocosms and in 25-cm pots with different amounts of fertilizer. Leaf length and width were measured, and a taxon was assigned based on those measurements. Leaves from these individuals were sampled, ground up for DNA extraction, purified, amplified by PCR, and sequenced. The data show how each taxon's leaf height and width responds to differing nitrogen amounts.

Research Question

1. **Does the amount of nitrogen input change leaf morphology of the three taxa of *Typha*?**
2. **How accurately does leaf morphology identify each *Typha* taxon?**

Methods

Two experiments were performed, a large-scale and a small-scale:

Experiment 1:

Mesocosms Methods

- Artificial wetlands (mesocosms) were created in 2011 using 1.75-m wide livestock tanks. Different amounts of nitrogen fertilizer were added to each tank, and one rhizome each of *T. angustifolia*, *T. latifolia*, and *T. x glauca* were planted in the spring of 2013.
- *Typha* leaf tissue samples were collected and measured in 2016, and then genetically identified.

Typha Genetics Methods

- 194 samples were randomly chosen to run DNA testing.
- 20 mg samples were ground up using a ball mill with lysis buffer.
- DNA was purified, bound to a silica membrane, and eluted under low ionic strength conditions with an elution buffer.
- PCR was used to amplify the DNA in a T-gradient machine and was sent to ISU for genotyping.

Experiment 2:

- Individuals from the three taxa were collected at different Iowa wetlands.
- Plants were grown in 25-cm diameter pots randomly assigned to 1 of 7 nitrogen levels.
- Leaf height and width were recorded, and plants were genetically identified using the same methods as above.

Results & Statistical Analysis

The following are results from the second experiment:

Fig. 1. Effects of Varying Nitrogen on the Leaf Width

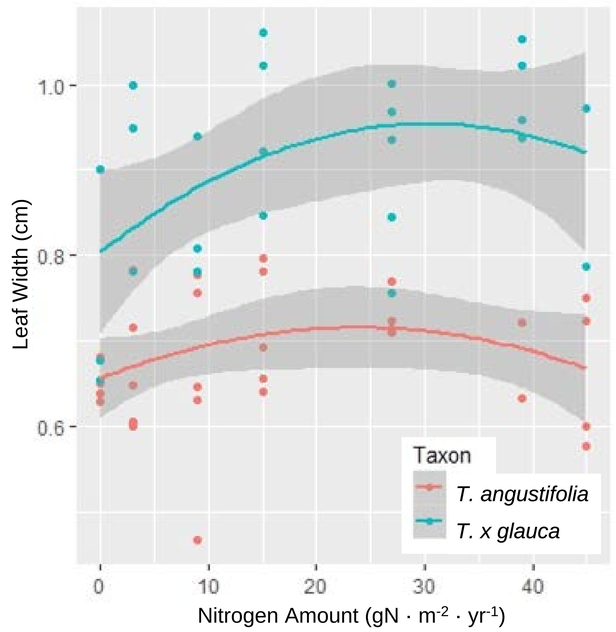
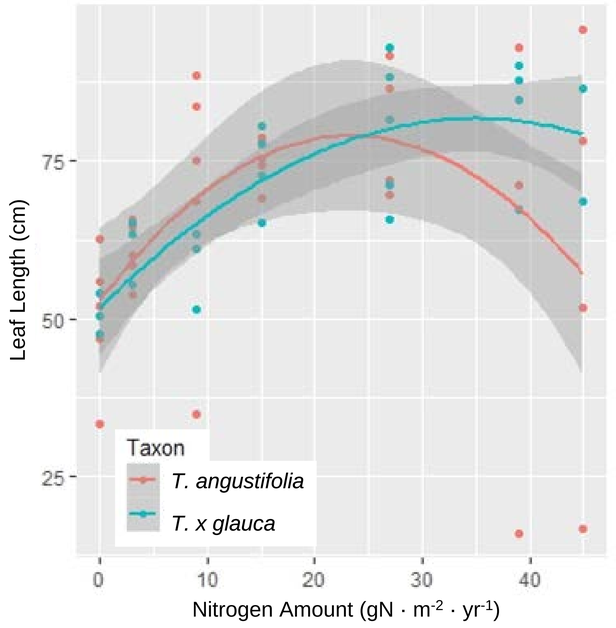


Fig. 2. Effects of Varying Nitrogen Amounts on Leaf Length



Due to such a small sample size of *T. latifolia*, this taxon was left out of figures 1 and 2, but is shown in figures 3 & 4.

Figure 3

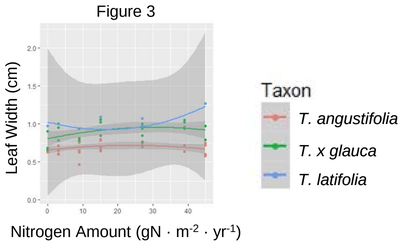


Figure 4

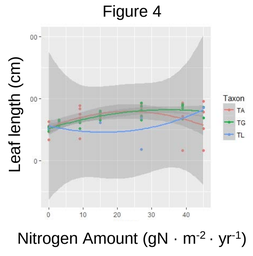
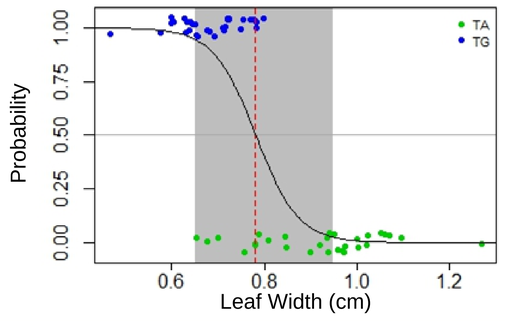


Fig. 5. Effectiveness of using leaf width to distinguish *Typha* taxa

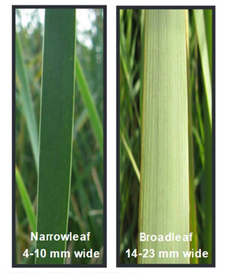


Conclusion & Discussion

From these results, we are able to conclude the following:

- Yes, a change in nitrogen input results in a change in both leaf width ($p = 0.03$) and length ($p = 0.0002$) of *T. angustifolia* and *T. x glauca*.
  - This contributes to the difficulty of identifying individuals; average leaf width may change from one location to the next.
- When identifying plants using leaf morphology, height is not useful; *T. angustifolia* and *T. x glauca* overlap at nearly every nitrogen level and are not significantly different ($p = 0.17$).
- *T. angustifolia* and *T. x glauca* have significantly different widths on average ($p < 0.0001$), but there is still some overlapping.
  - Using width is more trustworthy for identification.
- Leaf morphology can only be partially relied upon for identification
  - Genotyping of these individuals should be performed

- Experiment 1 is on-going
- Its larger sample size will allow for conclusions on *T. latifolia* as well as a better understanding of the range among each nitrogen level.



Identification by genetics utilizes fragments of DNA called microsatellites that have a unique base pair size among each taxon.

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