

Nitrogen allocation in *Pentaclethra macroloba* cotyledons in five developmental stage classes

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Abstract

Nitrogen-fixation ability — the ability to incorporate atmospheric nitrogen into organic compounds — and toxicity to seed predators are important factors explaining the dominance of *Pentaclethra macroloba* in the forest of the La Selva Biological Station, Costa Rica. Because the nitrogen contained in the cotyledon — the fleshy seed leaf that serves as a nutrient source during seed germination — is necessary for growth and because herbivory rates (which may be related to concentrations of toxic nitrogenous compounds in cotyledons) have been shown to increase with seedling age, I hypothesized that nitrogen contained in the cotyledon would be increasingly allocated to different parts of the plant with age, and therefore predicted that *P. macroloba* cotyledon nitrogen concentration would decrease with cotyledon age. I collected eight *P. macroloba* seeds in each of five developmental stage classes and measured cotyledon mass, root mass, shoot mass, and cotyledon nitrogen concentration for each. Nitrogen concentration generally decreased as the seedling developed, but it increased at the stage class in which roots appeared. The pattern of decreased cotyledon nitrogen concentration seems to correlate with decreased root:shoot mass ratio, indicating that nitrogen from the cotyledon is allocated to other parts of the plant as development progresses.

INTRODUCTION

Pentaclethra macroloba is the dominant tree species in the tropical wet forest of the La Selva Biological Station, Costa Rica (Hartshorn, 1983). A member of the Fabaceae family, *P. macroloba* benefits from nitrogen-fixing rhizobia — a symbiotic bacterium — located in its root nodules. Members of this family depend on nitrogen-fixation to synthesize proteins, nucleic acids, and other nitrogen-containing compounds (Raven & Johnson, 1992). Nitrogen allocation is an important factor in explaining the dominance of *P. macroloba* on the nutrient-poor basaltic and old alluvial soils of La Selva. Two reasons that Hartshorn (1972) gives for the dominance of *P. macroloba* are that nutrient (i.e., nitrogen) poor soils are restrictive to potentially competing species, and that *P. macroloba* seeds are virtually immune to predation because they contain toxic nitrogenous compounds.

The nutrient source for young seedlings is chiefly the cotyledon (Hill et al., 1960). Given that: 1) *P. macroloba* seedlings must grow in nutrient poor soil; 2) nitrogen-fixing rhizobia are not yet established in seeds or seedling roots (Graham & Swenson, 2001); 3) seeds are not heavily preyed upon due to a high amounts of nitrogenous compounds; and 4) and rates of her-

bivory (not specified, however, as foliar or seed herbivory) increase from 5.5% to 20% as seeds develop into seedlings (Deem et al., 1998), I hypothesized that nitrogen concentration in the *P. macroloba* cotyledon (i.e., the nutrient source) is high in the seed and decreases with age of the plant as nitrogen from the cotyledon is allocated to different parts of the plant. I predicted that cotyledon nitrogen concentration would decrease with increased development of *P. macroloba* seedlings.

METHODS

I collected eight samples from each of five developmental stage classes of *Pentaclethra macroloba* haphazardly along the Camino Experimental Sur between 0 – 400 m from the La Selva Biological Station, Costa Rica. The five stage classes were: 1) closed cotyledons (seeds); 2) open cotyledons with no roots or shoots; 3) open cotyledons with roots and shoots shorter than 5 cm; 4) open cotyledons with roots and shoots longer than 5 cm, but without leaves; and 5) open cotyledons with roots, shoots longer than 5 cm, and with leaf shoots less than 10 cm. All samples were dried in an oven for 15 h at 70°C, and root, shoot, and single-cotyledon dry mass determined. To determine cotyledon nitro-

gen concentration, I milled a single cotyledon from each sample (n = 8 per stage class) and followed modified Kjeldahl and colorimetric analysis protocols (Brenes, 1999). The small sample size was due to an abbreviated time frame for the project.

I used a Box-Cox power transformation to normalize the nitrogen concentration data and compared mean nitrogen concentration per stage class using an ANOVA and a Tukey-Kramer HSD. I compared mean cotyledon mass per stage class using an ANOVA. I also log-transformed root:shoot ratio data and compared root:shoot ratio per stage class using an ANOVA and a Tukey-Kramer HSD.

RESULTS

Mean nitrogen concentration was 29.6, 8.7, 58.0, 58.4, and 25.1 parts per million (ppm) for stage classes 1-5, respectively (Fig. 1). Cotyledon nitrogen concentration differed significantly with stage class (ANOVA, $F = 14.95$, $df = 4$, $P < 0.0001$). Cotyledon mass did not differ significantly with stage class (ANOVA, $F = 2.39$, $df = 4$, $P = 0.069$), Table 1). Root:shoot ratio was 1.94 for

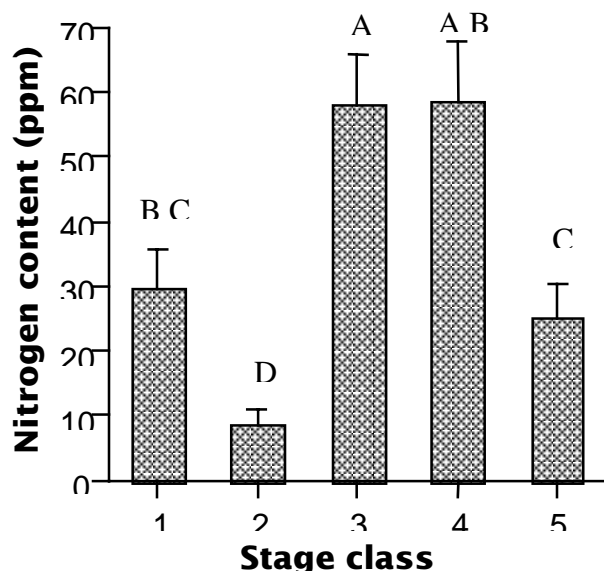


Figure 1. Mean nitrogen content (± 1 SE) for *Pentaclethra macroloba* cotyledons (n = 8) in five developmental stage classes: 1) closed cotyledons, 2) open cotyledons with no roots or shoots, 3) open cotyledons with roots and shoots shorter than 5 cm, 4) open cotyledons with roots and shoots longer than 5 cm, and 5) open cotyledons with roots, shoots longer than 5 cm, and leaves. A-D denote differences in statistical significance.

Stage Class	Mean cotyledon mass (g) ± 1 SE
1	2.00 \pm 0.22
2	1.63 \pm 0.14
3	1.61 \pm 0.13
4	1.50 \pm 0.12
5	1.35 \pm 0.15

Table 1. Mean single-cotyledon mass (n = 8) for *Pentaclethra macroloba* in five developmental stage classes: 1) closed cotyledons; 2) open cotyledons with no roots or shoots; 3) open cotyledons with roots and shoots shorter than 5 cm; 4) open cotyledons with roots and shoots longer than 5 cm; and 5) open cotyledons with roots, shoots longer than 5 cm, and leaves.

stage class 3, 0.96 for stage class 4, and 0.49 for stage class 5 (stage classes 1 and 2 were excluded from the analysis because individuals contained neither roots nor shoots). Root:shoot mass ratio differed significantly with stage class (ANOVA, $F = 15.51$, $df = 2$, $P < 0.0001$, Fig. 2).

DISCUSSION

Since cotyledon mass did not differ significantly between stage classes, the observed variations in nitrogen concentration between stage classes reflect differences in nitrogen dynamics. Initially, data in Figure 1 do not seem to support the hypothesis that cotyledon nitrogen concentration decreases with cotyledon age. There is a decrease in nitrogen concentration from the first to the second stage class, a large increase from the second to the third and fourth, and another decrease to the final stage class. A comparison of Figure 1 to Figure 2, however, shows that the increase in cotyledon nitrogen concentration from stage class 2 to 3 correlates with the appearance of roots. The young *Pentaclethra* tree may be taking up nitrogen and/or nitrogen-fixing rhizobia. The absence of root nodules on plants used in the study indicates that rhizobia were not fixing nitrogen in the samples (Graham & Swenson, 2001), suggesting that the seedlings were instead taking up nitrogen through their newly formed roots.

After this increase in nitrogen concentration with root germination, cotyledon nitrogen concentration appears to decrease as root:shoot ratio decreases (Fig. 2), supporting the idea that a growing plant demands more nitrogen. Nitrogen appears to be taken away from the cotyledon and is probably invested into plant tissue. In

addition, the cotyledons appear to be a temporary pool for nitrogen coming in through the roots. Perhaps by the time roots germinate, cotyledon nitrogen concentration is low in comparison to that of the soil, thus the potential difference for nitrogen diffusion is increased by the nitrogen deficient cotyledon tissue. Future study could investigate the ways in which *Pentaclethra* seedling-shoot-and-leaf-nitrogen concentration vary with cotyledon nitrogen concentration to determine total nitrogen allocation patterns for the seedlings. Further research into nitrogen allocation patterns for *Pentaclethra macroloba* may help explain its characteristic low levels of seed predation and its dominance of nutrient poor soils in the Neotropics.

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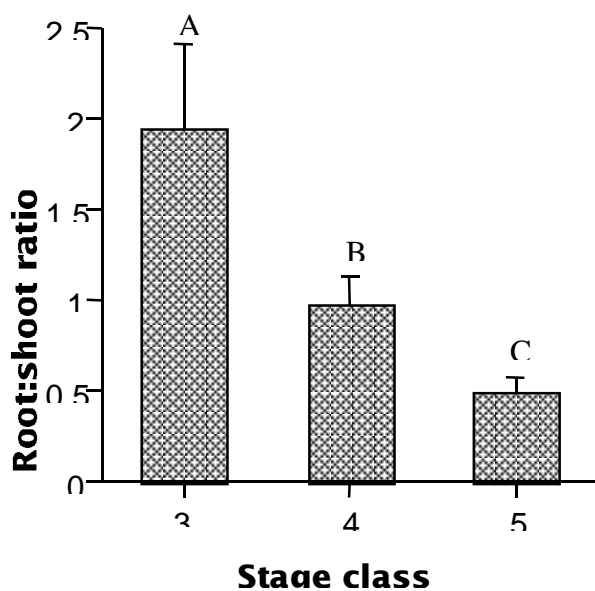


Figure 2. Mean root:shoot ratio for *Pentaclethra macroloba* seedlings in three development stage classes: 3 - 5 (see methods). A-C denote differences in statistical significance. Stage classes 1 and 2 not included because they did not contain roots or shoots.

Age Class	Replicate	Nitrogen conc. (ppm)	Root:shoot mass ratios
1	1	16.7	.
1	2	25.5	.
1	3	62.6	.
1	4	32.7	.
1	5	24.4	.
1	6	45.1	.
1	7	14.5	.
1	8	15.0	.
2	1	3.4	.
2	2	1.0	.
2	3	14.8	.
2	4	13.2	.
2	5	9.7	.
2	6	0.7	.
2	7	18.0	.
2	8	8.4	.
3	1	49.0	1.55
3	2	38.0	2.33
3	3	37.2	1.00
3	4	75.2	1.67
3	5	54.0	5.00
3	6	49.3	2.00
3	7	103.2	1.00
3	8	58.3	1.00
4	1	22.8	0.91
4	2	37.1	0.67
4	3	47.2	0.78
4	4	34.7	0.60
4	5	64.4	0.78
4	6	81.9	2.00
4	7	83.7	0.73
4	8	95.7	1.29
5	1	9.9	0.24
5	2	20.8	0.61
5	3	48.4	0.41
5	4	30.2	0.94
5	5	42.6	0.39
5	6	27.4	0.34
5	7	7.1	0.28
5	8	14.5	0.69

Table 2. Raw data for *Pentaclethra macroloba* cotyledon nitrogen concentrations and root:shoot mass ratios in five developmental stage classes: 1) closed cotyledons; 2) open cotyledons with no roots or shoots; 3) open cotyledons with roots and shoots shorter than 5 cm; 4) open cotyledons with roots and shoots longer than 5 cm; and 5) open cotyledons with roots, shoots longer than 5 cm, and leaves. Root:shoot data for stage classes 1 and 2 not included because they did not contain roots or shoots.

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Chris Leander '01 is a Biology major interested in many aspects of the life sciences. During his undergraduate years, he has explored interests in ecology and evolutionary biology. He is currently applying to medical school for enrollment in 2002 and plans to spend the next year working at home in Stormville, NY and at a medical clinic in rural Honduras.

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