



Nitrate, proton, and ammonium uptake in fern gametophytes

Em Creasy, supervised by Dr. Barbara Hawkins and Dr. Patrick von Aderkas

Department of Biology, University of Victoria

February 26, 2024

Background

- Ferns (Polypodiophyta) possess two independent and alternating growth forms: the spore-producing sporophyte and the gamete-producing gametophyte.^{1,2}



- Fern sex is determined post-spore germination.¹
 - Gametophytes can be male, female or hermaphrodite.¹
 - Poor environmental conditions can prevent immature gametophytes from developing archegonia, resulting in males.³
- Nutrient acquisition occurs directly at the tissue surface in fern gametophytes.⁴
- Nitrogen is the most important mineral nutrient for plants. It is taken up primarily in the form of nitrate (NO_3^-) or ammonium (NH_4^+).⁵
- We measured instantaneous nutrient ion fluxes in *Polystichum munitum* (swordfern), *Christella dentata* (soft fern), and *Cyrtomium falcatum* (Japanese holly fern) gametophytes using a microelectrode ion flux measurement (MIFE) system.

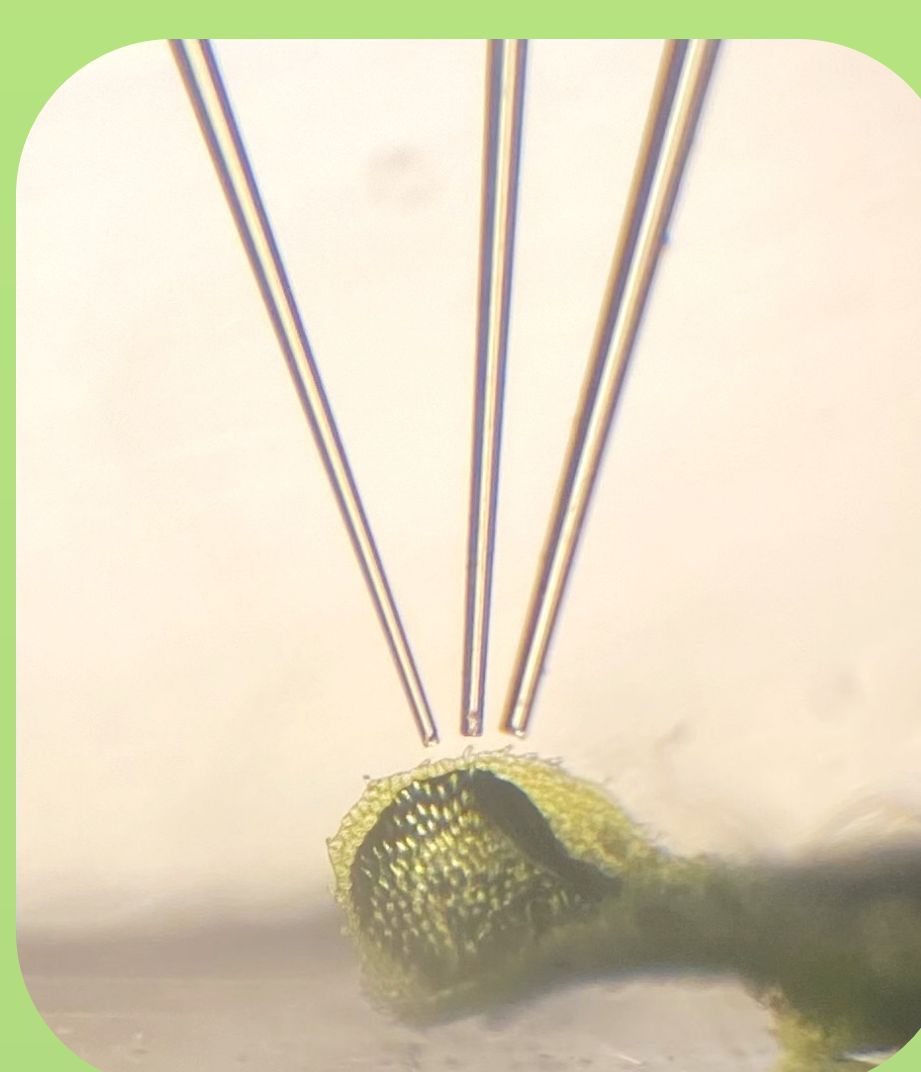
Objective

Characterize the major nutrient ion fluxes in fern gametophytes to determine if there is an effect of sex, pH, and/or generation on NH_4^+ , NO_3^- , and H^+ uptake.

Methods

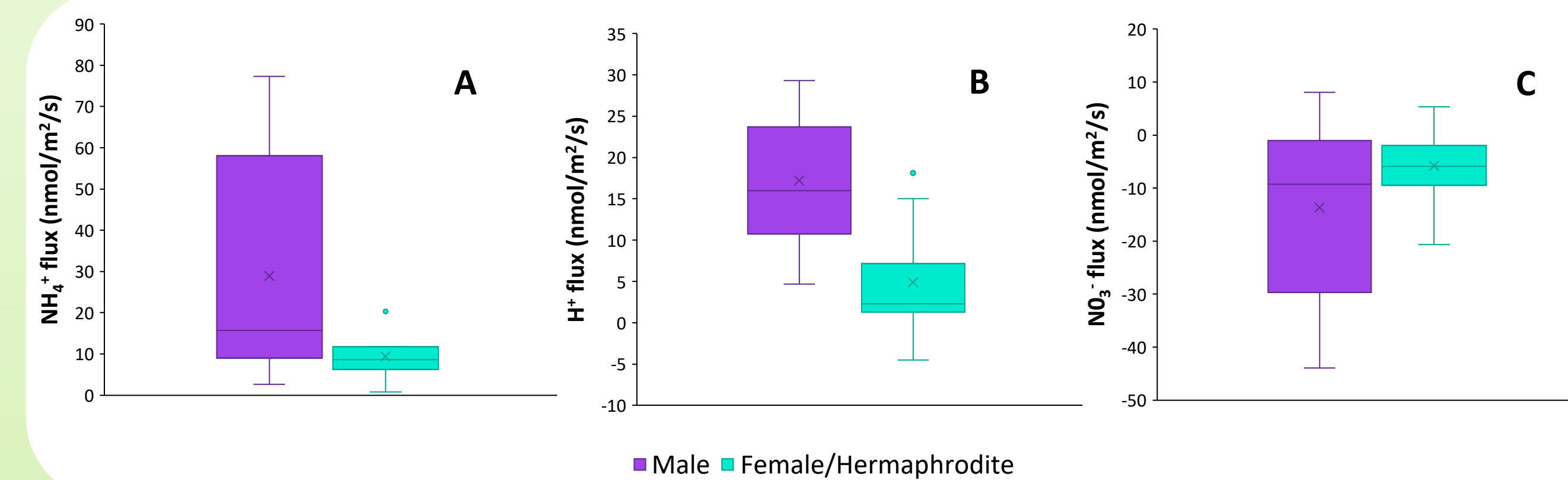
- All gametophytes were obtained from the von Aderkas collection maintained since Fall 2022 in a growth chamber set to 22°C with a 12-hour day/night cycle.
- NH_4^+ , NO_3^- , and H^+ fluxes were measured using the MIFE system in 500 μmol $\text{NH}_4^+\text{NO}_3^-$ solution for each of the following comparisons:
 - **Male vs female/hermaphrodite *P. munitum* gametophytes (at pH 5; n = 7-16)**
 - **pH 5 vs pH 7 in female *P. munitum* gametophytes (n = 10)**
 - **Sporophyte vs gametophyte in *C. dentata* and *C. falcatum* (n = 2-7)**
- Linear regressions and t-tests were performed in R. PROC GLMs were performed in SAS.

NH_4^+ , NO_3^- , and H^+ microelectrodes ~10 μm from female *P. munitum* tissue surface



Results

For all three ions, flux range is much greater for males (M) than females/hermaphrodites (F/H).



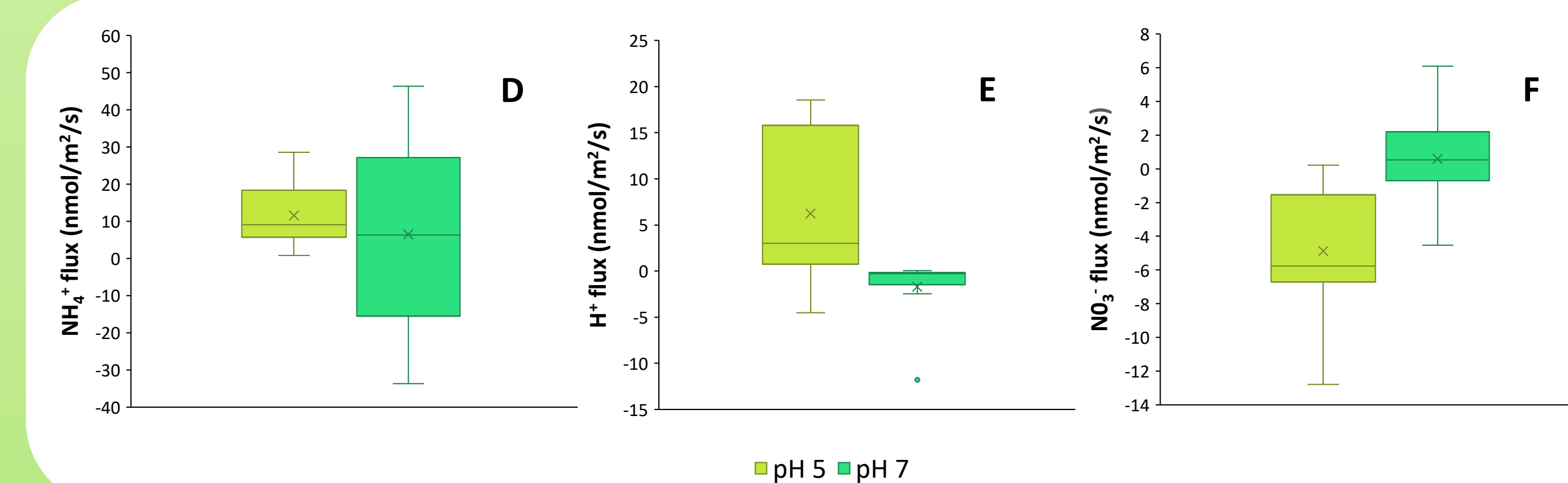
Interpreting box and whisker plots

- Minimum and maximum values are indicated by the ends of the whiskers
- The mean is indicated by the "X"
- The median is indicated by the centre line
- Outliers are indicated by points

A) On average, NH_4^+ influx is greater in M than F/H, but the difference is not significant ($p = 0.09$).

B) H^+ influx is significantly greater in M than F/H ($p = 0.00006$).

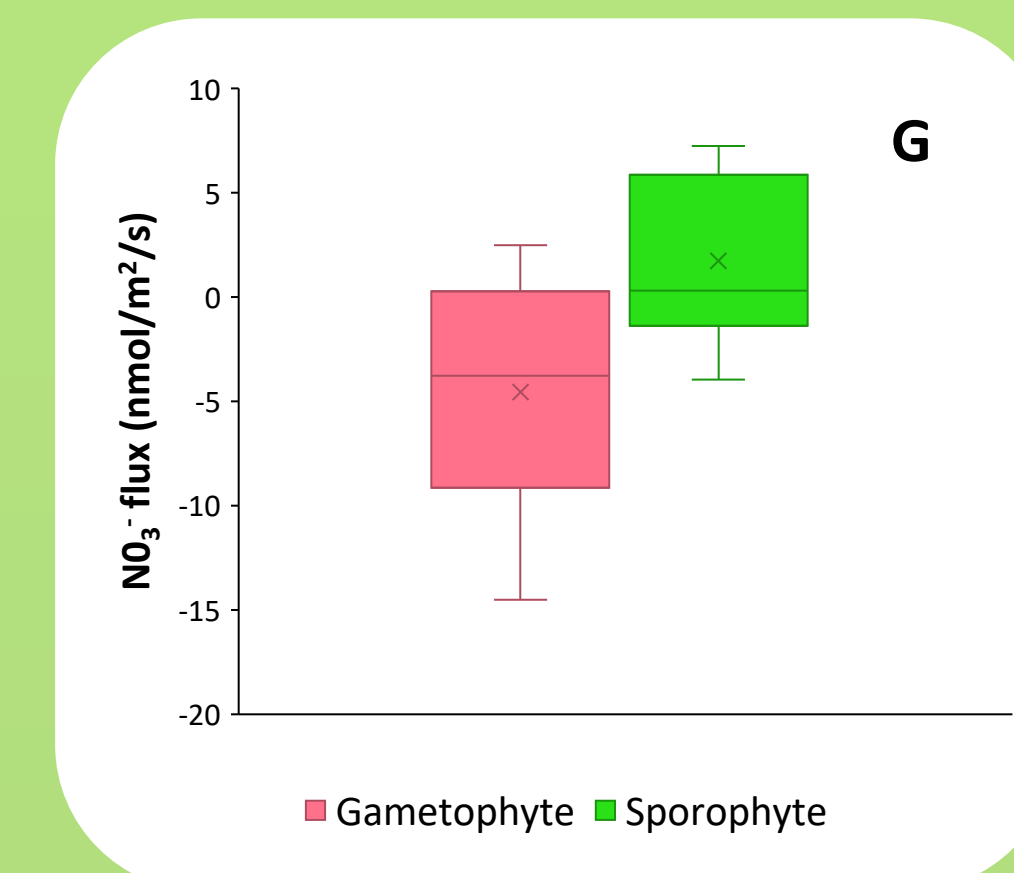
C) On average, NO_3^- efflux is greater in F/H than M, but the difference is not significant ($p = 0.08$).



D) There is no significant difference in NH_4^+ flux at pH 5 and pH 7 ($p = 0.6$); however, there is greater variability at pH 7.

E) H^+ influx is significantly greater at pH 5 than at pH 7 ($p = 0.02$).

F) NO_3^- efflux is significantly greater at pH 5 than pH 7 ($p = 0.003$).



G) NO_3^- efflux is significantly greater in gametophytes than sporophytes ($p = 0.05$) but does not significantly differ between species ($p = 0.3$).

There is no significant difference in NH_4^+ flux between *C. dentata* and *C. falcatum* ($p = 0.2$) or between generation ($p = 0.6$).

H^+ flux is significantly different between species ($p = 0.02$) but does not significantly differ between generation ($p = 0.3$). *C. falcatum* did not exhibit any H^+ flux.

Discussion

- The trend that male *P. munitum* gametophytes are taking up more NH_4^+ than females/hermaphrodites may suggest they have greater N requirements. This could be due to the male gametophytes being at earlier and more active stages of development.¹
- The majority of NO_3^- flux was efflux, whereas NH_4^+ was always taken up by gametophytes. This could be due to the tendency of NO_3^- to be lost in acidic environments and/or the potential high cation exchange capacity as demonstrated in bryophytes.⁶
 - The greater amount of NO_3^- efflux in gametophytes may be due to absence of a cuticle in this generation.²
- The total lack of H^+ flux in both *C. falcatum* gametophytes and sporophytes is surprising. This could be related to the leathery, glossy, and scale-like characteristics of this species' tissues.⁷

Future Directions

- Increase sample size as differences in NH_4^+ and NO_3^- in M versus F/H are close to significant
- Extrapolate results to estimate fluxes across entire surface area of the gametophyte
 - The greater size of F/H gametophytes is expected to result in greater total NH_4^+ uptake in F/H.



Acknowledgements

Heartfelt thanks to Samantha Robbins, Daisuke Yamakawa, and Dr. Barbara Hawkins for their MIFE expertise and troubleshooting help, and to Dr. Patrick von Aderkas, Alistair Knox, Kailey Strachan, Simar, Liberty, Olivia, and Jadyn for their incredible fern parenting skills and endless support.

This research was supported by the Jamie Cassels Undergraduate Research Awards, University of Victoria.

References

- Banks, J. A. (1999). Gametophyte development in ferns. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50(1), 163–186. <https://doi.org/10.1146/annurev.arplant.50.1.163>
- Farrar, D. R., Dassler, C., Watkins, J. E., & Skelton, C. (2008). Gametophyte ecology. In T. A. Ranker & C. H. Haufler (Eds.), *Biology and Evolution of Ferns and Lycophytes* (1st ed., pp. 222–256). Cambridge University Press. <https://doi.org/10.1017/CBO9780511541827.010>
- Miller, J. H. (1968). Fern gametophytes as experimental material. *The Botanical Review*, 34(4), 361–440. <https://doi.org/10.1007/BF02859133>
- Richardson, S. J., & Walker, L. R. (2010). Nutrient ecology of ferns. In K. Mehltrreter, L. R. Walker, & J. M. Sharpe (Eds.), *Fern Ecology* (1st ed., pp. 111–139). Cambridge University Press. <https://doi.org/10.1017/CBO9780511844898.005>
- Von Wirén, N., Gazzarini, S., & Frommer, W. B. (1997). Regulation of mineral nitrogen uptake in plants. *Plant and Soil*, 196(2), 191–199. <https://doi.org/10.1023/A:1004241722172>
- Hawkins, B. J., May, E., & Robbins, S. (2018). Nitrate and ammonium uptake in 21 common species of moss from Vancouver Island, British Columbia. *Botany*, 96(3), 201–208. <https://doi.org/10.1139/cib-2017-0154>
- Missouri Botanical Garden. (n.d.) *Cyrtotium falcatum* 'Rochfordianum'. <https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=243750&isprofile=0&>