Nutrient stoichiometry of *Nepenthes* species from a Bornean peat swamp forest

Francis Q. Brearley • School of Science and the Environment • Manchester Metropolitan University • Chester Street • Manchester • M1 5GD • United Kingdom • f.q.brearley@mmu.ac.uk
Muhammad Mansur • Botany Division • Research Center for Biology • Indonesian Institute of Sciences • Cibinong Science Center • Jalan Raya Jakarta-Bogor, Km 46 • Cibinong 16911 • Indonesia • mansurhalik@yahoo.com

Keywords: Indonesia, micronutrients, nitrogen, phosphorus, wetlands.

Abstract

To shed light on the nature of nutrient limitation in carnivorous plant communities, we collected data on foliar nutrient concentrations and ratios from three *Nepenthes* species and one natural hybrid growing in peat swamp forests of southern Borneo. Due to their low foliar nitrogen concentrations and very low nitrogen:phosphorus ratios, our data strongly suggest that these species are limited by nitrogen availability.

The carnivorous habit and uptake of nutrients from invertebrate prey items is an evolutionary response to the nutrient-poor environments in which some plants find themselves. To determine patterns of nutrient use and nutrient limitation in carnivorous plants, nutrient concentrations and stoichiometry (ratios between nutrients) can be assessed (Koerselman & Meuleman 1996; Willby *et al.* 2001; Olde Venterink *et al.* 2003; Güsewell 2004). From a review of the literature, Ellison (2006) suggested that most carnivorous plants are phosphorus (P) or nitrogen (N) + P limited but he presented minimal data from *Nepenthes* pitcher plants. Osunkoya *et al.* (2007) went some way towards rectifying this deficit by providing data on N, P, and potassium (K) concentrations from six species in Brunei that suggested that *Nepenthes* are actually N limited due to their very low N concentrations and low N:P ratios. However, this is currently the only study with multi-element data from *Nepenthes* species. In this paper we provide extra much-needed information on *Nepenthes* leaf nutrients from three species and one natural hybrid found in a peat swamp forest in Indonesian Borneo to improve generalizations about nutrient relationships in this fascinating plant genus.

In the Sebangau peat swamp forest in Central Kalimantan, Indonesia (2°18'S; 113°55'E), three species of *Nepenthes, N. ampullaria* Jack, *N. gracilis* Korth. (see Fig. 1), and *N. rafflesiana* Jack are found along with one natural hybrid, *N.* × *hookeriana* Lindl. (Mansur 2008). Leaf material (all leaves were estimated to be less than one year old) was collected from individuals of these four 'species' in April-May 2006. About 40 mg of leaf material was digested in 2.5 ml concentrated sulphuric acid with a lithium sulphate/selenium (100:1) catalyst at 375°C for 4 hours, diluted with deionized water, and analyzed for nutrient concentrations on a Dionex ICS-2000 Ion Chromatography System (for N) or a Varian Vista AX Inductively Coupled Plasma Optical Emission Spectrometer (for all other elements). Differences in nutrient concentrations between species were analyzed by one-way ANOVAs implemented in Minitab 15.1 and data was $\log_{10}$ transformed where needed to improve homogeneity of variances.

Figure 1: *Nepenthes gracilis* growing in peat swamp forest at Sebangau, Central Kalimantan, Indonesia.

Table 1 shows the nutrient concentrations of the four 'species'. Nitrogen, phosphorus, and calcium all showed generally low concentrations whereas potassium and magnesium were around, or above, average (Grimshaw *et al.* 1989; Pugnaire 2001; Broadley *et al.* 2004). There were no significant differences in N concentrations between the 'species'. Phosphorus concentrations were greatest in *N. gracilis* and *N. rafflesiana* as were K concentrations (although differences for K were not significant). *N. ampullaria* showed the lowest concentrations for many of the nutrients, including the micronutrients.

Based on prior analysis of species and life-forms from temperate regions, many from wetlands (Koerselman & Meuleman 1996; Willby *et al.* 2001; Olde Venterink *et al.* 2003; Tessier & Raynal 2003), our data suggest N limitation for all the 'species' found as they all had very low N concentrations (less than 0.85% in all individuals). In addition, the foliar N:P ratios of 3.3 to 9.3 were exceptionally low (significantly lower values were found in *N. gracilis* and *N. rafflesiana* than *N. ampullaria* and *N.* × *hookeriana*) where the critical value of N:P is 14, below which N is considered limiting to plant growth (Koerselman & Meuleman 1996) although this value may be lower in drier forests (Tessier & Raynal 2003). P concentrations were also very low in *N. ampullaria* at levels that are considered limiting (<1 mg g^{-1}; Willby *et al.* 2001) but the low N:P ratio still suggests greater limitation by N in this species. There was no indication of K being a limiting nutrient in any of the 'species' examined (>0.8%, N:K <2:1, and K:P >3.4; Willby *et al.* 2001; Olde Venterink *et al.* 2003).

Our results therefore corroborate the data of Osunkoya *et al.* (2007) who found average nutrient concentration across six species in Brunei to be N = 0.81%, P = 1.9 mg g^{-1}, and K = 1.08% with an N:P ratio of less than 5; Osunkoya *et al.* (2007) also collected leaves from peat swamp forest but these were combined with plants from heath forest for analysis. Their N and P values were higher than ours although their K values were lower and overall they considered that *Nepenthes* species

Table 1. Nutrient concentrations and stoichiometric ratios for nine plant nutrients in four 'species' of *Nepenthes* pitcher plants in the Sebangau peat swamp forest, Central Kalimantan, Indonesia. Different letters within a row indicate statistically significant differences (p <0.05) using Tukey's tests (on log_{10} transformed data where required).

	N. ampullaria	*N. gracilis*	*N.* × *hookeriana*	*N. rafflesiana*
N (%)	0.50 ± 0.07 (a)	0.65 ± 0.07 (a)	0.67 ± 0.08 (a)	0.63 ± 0.05 (a)
P (mg g^{-1})	0.59 ± 0.07 (a)	1.52 ± 0.36 (b)	0.85 ± 0.14 (ab)	1.16 ± 0.09 (b)
K (%)	2.61 ± 0.21 (a)	4.70 ± 0.29 (a)	2.29 ± 0.33 (a)	5.90 ± 1.68 (a)
N:P	8.49 ± 0.41 (b)	4.65 ± 0.59 (a)	8.09 ± 0.39 (b)	5.42 ± 0.32 (a)
N:K	0.19 ± 0.01 (ab)	0.14 ± 0.01 (a)	0.30± 0.01 (b)	0.16 ± 0.06 (a)
K:P	45.1 ± 2.3 (a)	34.6 ± 5.3 (a)	27.1 ± 0.6 (a)	49.8 ± 13.9 (a)
Ca (mg g^{-1})	0.57 ± 0.09 (a)	1.51 ± 0.86 (a)	6.86 ± 1.13 (b)	1.67 ± 0.31 (a)
Mg (mg g^{-1})	2.05 ± 0.38 (a)	5.10 ± 0.36 (b)	6.61 ± 0.58 (b)	9.01 ± 2.47 (b)
Fe (µg g^{-1})	7.7 ± 0.8 (a)	40.5 ± 10.6 (b)	60.9 ± 7.9 (b)	73.4 ± 31.0 (b)
Mn (µg g^{-1})	9.7 ± 1.6 (a)	57.3 ± 23.9 (b)	251 ± 24 (c)	172 ± 31 (c)
Ni (µg g^{-1})	0.41 ± 0.05 (a)	2.13 ± 0.74 (ab)	4.99 ± 1.93 (b)	2.45 ± 0.99 (ab)
Zn (µg g^{-1})	5.9 ± 0.6 (a)	21.1 ± 6.2 (a)	66.5 ± 9.3 (b)	17.2 ± 4.1 (a)

were N limited in contrast to other carnivorous plant genera which appear to be P or N + P limited (Ellison 2006).

Most micronutrient concentrations in the *Nepenthes* leaves were normal to low for plant tissues and there were no remarkable concentrations. It was interesting to note that, for *N.* × *hookeriana*, only in some cases (P, Mg, Fe) were the values intermediate between that of its 'parent' species *N. ampullaria* and *N. rafflesiana*. In terms of other nutrients (Ca and other micronutrients) it had the highest concentrations; perhaps reflecting some aspect of hybrid vigor (Chen 2010).

In conclusion, our results suggest that N is the limiting nutrient for *Nepenthes* in contrast to other carnivorous plants that seem to be P or N limited. This hypothesis may not include montane *Nepenthes* that, somewhat surprisingly, appear to have higher foliar N concentrations (Clarke *et al.* 2009). However, the only way to fully determine the nature of nutrient limitation is through fertilization experiments although this presents difficulties of its own as differences have been found in plant responses to fertilization through the leaves as compared to the roots (Adamec 1997). More work is clearly required on the mineral nutrition and nutrient stoichiometry of Asian pitcher plants.

Acknowledgements: CIMTROP are thanked for research permission and David McKendry and Paul Warren are thanked for assistance with nutrient analyses.

References

Adamec, L. 1997. Mineral nutrition of carnivorous plants: a review. Bot. Rev. 63: 273-299.

Broadley, M.R., Bowen, H.C., Cotterill, H.L., Hammond, J.P., Meacham, M.C., Mead, A., and White, P.J. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. J. Exp. Bot. 55: 321-336.

Chen, Z.J. 2010. Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci. 15: 57-71.
Clarke, C.M., Bauer, U., Lee, C.C., Tuen, A.A., Rembold, K., and Moran, J.A. 2009. Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant. Biol. Lett. 5: 632-635.
Ellison, A.M. 2006. Nutrient limitation and stoichiometry of carnivorous plants. Plant Biol. 8: 740-747.
Grimshaw, H.M., Allen, S.E., and Parkinson, J.A. 1989. Nutrient elements. In Allen, S.E. (ed.) Chemical Analysis of Ecological Materials, 2nd Edition, pp. 81-159. Blackwell Scientific Publications, Oxford, UK.
Güsewell, S. 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164: 243-266.
Koerselman, W., and Meuleman, A.F.M. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33: 1441-1450.
Mansur, M. 2008. Penelitian ekologi Nepenthes di Laboratorium Alam Hutan Gambut Sabangau, Kereng Bangkirai, Kalimantan Tengah. J. Teknol. Lingkung. 9: 67-73.
Olde Venterink, H., Wassen, M.J., Verkroost, A.W.M., and de Ruiter, P.C. 2003. Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. Ecology 84: 2191-2199.
Osunkoya, O.O., Daud, S.D., Di-Giusto, B., Wimmer, F.L., and Holige, T.M. 2007. Construction costs and physico-chemical properties of the assimilatory organs of *Nepenthes* species in northern Borneo. Ann. Bot. 99: 895-906.
Pugnaire, F. 2001. Variability of inorganic nutrient concentrations in leaves. New Phytol. 150: 506-507.
Tessier, J.T., and Raynal, D.J. 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J. Appl. Ecol. 40: 523-534.
Willby, N.J., Pulford, I.D., and Flowers, T.H. 2001. Tissue nutrient signatures predict herbaceous-wetland community responses to nutrient availability. New Phytol. 152: 463-481.