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## NUTRIENT CONCENTRATIONS IN THREE *NEPENTHES* SPECIES (NEPENTHACEAE) FROM NORTH SUMATRA

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### ABSTRACT

MANSUR, M., SALAMAH, A., MIRMANTO, E. & BREARLEY, F. Q. 2022. Nutrient concentrations in three *Nepenthes* species (Nepenthaceae) from North Sumatra. *Reinwardtia* 21(2): 55–62. — *Nepenthes* is a genus of carnivorous plants that are unique ornamental plants, but their nutrient concentration relationships have not been studied much, especially in endemic species on the island of Sumatra. So far, the analysis of the nutrient concentration in *Nepenthes* is mostly limited to leaves. There are few reports of nutrient concentrations in the pitcher fluid and the soil around where it grows. Leaves, pitcher fluid, and soil around the growth sites of each species *i.e.*, *Nepenthes sumatrana*, *N. spectabilis*, and *N. tobaica*, from North Sumatra province were collected for nutrient analyses (N, P, K, Ca, Mg, and Na). The results showed that the nutrient concentrations in the leaves and pitcher fluid in the three *Nepenthes* species were generally low with those in the leaves greater than in the pitcher fluid. The concentration of nutrients in the leaves of *N. sumatrana* (lowland species) was least (except for N and Na) when compared to *N. spectabilis* and *N. tobaica* (highland species), likely reflecting the poorly fertile soil. In contrast, the nutrient concentration in the pitcher fluid of *N. sumatrana* was greater than *N. spectabilis* and *N. tobaica*. When compared across an extensive data set, we show that leaf N does not change with elevation, whereas P declines and the N:P ratio increases with elevation, suggesting that *Nepenthes* plants are obtaining sufficient N from prey at higher elevations.

**Key words:** *Nepenthes*, North Sumatra, nutrient concentration.

### ABSTRAK

MANSUR, M., SALAMAH, A., MIRMANTO, E. & BREARLEY, F. Q. 2022. Konsentrasi nutrisi pada tiga jenis *Nepenthes* (Nepenthaceae) dari Sumatra Utara. *Reinwardtia* 21(2): 55–62. — *Nepenthes* digolongkan ke dalam tumbuhan karnivora yang saat ini berfungsi sebagai tanaman hias unik, namun hubungan konsentrasinya belum banyak dipelajari khususnya pada jenis-jenis endemik pulau Sumatra. Sejauh ini, konsentrasi nutrisi yang dianalisis baru terbatas pada daun, belum ada yang melaporkan konsentrasi nutrisi yang ada di cairan kantong dan tanah tempat tumbuhnya. Sampel daun, cairan kantong, dan tanah di sekitar tempat tumbuh tiga jenis tanaman yaitu *Nepenthes sumatrana*, *N. spectabilis*, dan *N. tobaica*, dikoleksi dari provinsi Sumatra Utara untuk dipelajari kandungan unsur haranya. Hasil menunjukkan bahwa konsentrasi unsur hara pada daun dan cairan kantong pada ketiga jenis *Nepenthes* yang diteliti pada umumnya adalah rendah. Konsentrasi unsur hara pada daun *N. sumatrana* (jenis dataran rendah) adalah lebih rendah (kecuali unsur nitrogen dan natrium) jika dibandingkan dengan *N. spectabilis* dan *N. tobaica* (jenis dataran tinggi). Sebaliknya konsentrasi unsur hara pada cairan kantong *N. sumatrana* lebih tinggi dibandingkan dengan *N. spectabilis* dan *N. tobaica*. Untuk sampel daun, jika dibandingkan dengan seluruh kumpulan data yang luas, N daun tidak berubah dengan elevasi, sedangkan P menurun dan rasio N:P meningkat dengan elevasi, menunjukkan bahwa tumbuhan *Nepenthes* memperoleh N yang cukup dari mangsa di elevasi yang lebih tinggi.

**Kata kunci:** Konsentrasi unsur hara, *Nepenthes*, Sumatra Utara.

## INTRODUCTION

*Nepenthes* (Nepenthaceae) is a genus of dioecious climbing plants with the unusual yet fascinating habit of being carnivorous; they also function as a unique ornamental plant (Mansur, 2006). The Indonesian archipelago has the greatest number of *Nepenthes* species with diversity concentrated on the islands of Borneo (Kalimantan) and Sumatra. The Bukit Barisan Mountains, which stretch from South Sumatra to Aceh, are the largest contributor to the high biodiversity in Sumatra (Malik *et al.*, 2020), including of *Nepenthes* species (Lee *et al.*, 2006). Until now, 22 species of *Nepenthes* have been reported from North Sumatra province with 16 of them endemic to Sumatra (Mansur *et al.*, *in press.*).

*Nepenthes* often grow in marginal soils that are poor in nutrients, especially nitrogen (Clarke, 1997; Clarke, 2001; Moran & Clarke, 2010). Nitrogen, phosphorus and potassium often (co-) limit the growth of carnivorous plants (Ellison, 2006). For example, Brearley & Mansur (2012) reported that the foliar nitrogen concentrations of *N. ampullaria*, *N. gracilis*, *N. rafflesiana*, and *N. x hookeriana* were low in peat swamp forest (Sebangau National Park, Central Kalimantan). *Nepenthes* species occupy a broad elevation range from sea level to over 3,000 m elevation (*N. lamii* in New Guinea is the species found at the highest elevation) and so we might expect changes in foliar nutrient concentrations along such a broad gradient as has been seen in other tropical plant taxa (Tanner *et al.*, 1998; Bauters *et al.*, 2017). In addition to macronutrient elements, *Nepenthes* plants are also able to absorb metallic elements such as lead, as found in *N. macfarlanei* which grows in the montane forests of the Genting Highlands in Malaysia (Brearley, 2021).

The function of the pitchers of *Nepenthes* is to passively capture prey (Phillipps & Lamb, 1996), via nectar secreted by glands under the lid (Kurata & Kurata, 2009), through a slippery peristome (Bauer *et al.*, 2009) and through the aroma released (Clarke & Lee, 2004). These can all attract the prey that falls into the pitcher fluid (Bonhomme *et al.*, 2011) that may be highly viscoelastic (Gaume & Forterre, 2007). Once the prey is trapped, it will be broken down by the broad range of enzymes found in the pitcher fluid (Takeuchi *et al.*, 2011; Rottloff *et al.*, 2016), and the nutrients are absorbed through glands in the absorption zone in the pitcher walls (Clarke & Lee, 2004; Moran *et al.*, 2010).

The relationship between the nutrient concentrations in the genus of *Nepenthes* and its habitat has not been studied much, especially in species from Sumatra. The objective of this study was to determine nutrient concentrations in *N.*

*sumatrana* (lowland species), *N. spectabilis*, and *N. tobaica* (both highland species) (Fig. 1) that are protected by Indonesia's law (Permen LHK No. 20 of 2018). Based on the Red List issued by the IUCN, the conservation status of *N. sumatrana* is Critically Endangered (CR) and *N. spectabilis* is Vulnerable (VU), whereas *N. tobaica* is of Least Concern (LC). With this paper we hope to contribute to our understanding of nutrient relationships in *Nepenthes* and make a comparison between lowland and highland species using data from Sumatra as well as an extensive data comparison across the geographical range of *Nepenthes* species.

## MATERIALS AND METHODS

### Study site

Sampling was carried out in November 2019 in Aek Nabobar Village, Sidikalang, Central Tapanuli Regency (N: 01°35'41"; E: 098°53'43"; altitude 75 m asl) for *N. sumatrana* and in Lae Pandom Protected Forest, Gunung Sibutan, Karo Regency (N: 02°53'38"; E: 098°29'27"; altitude 1,750 m asl) for *N. spectabilis* and *N. tobaica*, both in North Sumatra Province. In Aek Nabobar village, *N. sumatrana* grows in the lowlands at an altitude between 20 to 75 m above sea level (asl) in open areas with shrubby habitats. In general, the habitat of *N. sumatrana* has high air temperature, soil pH, and light intensity, but low air humidity and soil moisture. On the other hand, the habitat of *N. spectabilis* and *N. tobaica* is in the Lae Pandom Protected Forest, Gunung Sibutan, Karo Regency at an altitude of around 1,750 m asl that has lower air temperature, light intensity and soil pH, but higher air humidity and soil moisture. Nutrient analysis was carried out at the Research Center for Ecology and Ethnobiology, National Research and Innovation Agency (BRIN), Indonesia.

### Sampling design

Following the approach of Brearley & Mansur (2012), Brearley (2021), and Mansur *et al.* (2021), three plant samples from each species of *Nepenthes* (*N. sumatrana*, *N. spectabilis*, and *N. tobaica*) were collected for their leaves, namely the second leaf from the tip of the stem (estimated to be six months old), pitcher fluid (10 to 40 ml depending on the pitcher sampled) and the soil (ca. 100 g from 10 to 20 cm depth) from the rooting zone where they grew. Morphological parameters of each plant sampled were also recorded (e.g. stem, leaf, and pitcher length and width) along with leaf chlorophyll concentration on the leaf sampled for nutrient concentrations using a Konica



Fig. 1. A. *Nepenthes sumatrana* (Miq.) Beck ex Tamin & M.Hotta. B. *Nepenthes spectabilis* Danser. C. *Nepenthes tobaica* Danser; from North Sumatra Province, Indonesia. Photos by M. Mansur.

Minolta SPAD-502 meter with the value used being the mean of three measurements per leaf.

### Experimental procedures

Leaf and soil samples were dried at 50°C for 5 days, then ground. The nutrient concentrations of soil and leaf nitrogen (N) were analyzed using a Yanako JM1000CN macro corder with a JMA 1000 autosampler. For other nutrients, leaves and soil were digested in a mixture of acids ( $\text{H}_2\text{SO}_4$ ,  $\text{HClO}_4$ , and  $\text{HNO}_3$ ) for 24 hours at 170°C, the concentrations of potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were analyzed using an atomic absorption spectrophotometer (Shimadzu AA-6200) while the concentration of phosphorus (P) used a spectrophotometer (Shimadzu UV Mini-1240) with the vanadomolybdate colorimetric method. Pitcher liquid was filtered and analysed using atomic absorption spectrophotometry (as above) or with the Nessler and vanadomolybdate colorimetric methods for N and P, respectively.

### Data comparison across an extensive elevational dataset

Data of the nutrient concentration of three species of *Nepenthes* were compared between leaves, pitcher fluid, and soils using one-way ANOVAs with post-hoc Duncan tests. Leaf nutrient concentration data were compiled from the literature (see Mansur *et al.*, 2021), to which were added additional data on leaf N and P from other studies (Moran & Moran, 1998; Clarke *et al.*, 2009; Moran *et al.*, 2001, 2003; Graefe *et al.*, 2011;

Bazile *et al.*, 2012). The elevation of each location was sourced from the appropriate paper, and linear regressions were calculated between elevation and the relevant leaf nutrient or the N:P ratio.

## RESULTS

### Morphology

The morphology of the three species studied differed, *N. sumatrana* growing in the lowlands and open areas, had shorter stems, but greater stem diameter, pitcher size and tendril length than *N. spectabilis* and *N. tobaica*. The pitcher of *N. sumatrana* is trumpet-shaped, while those of *N. spectabilis* and *N. tobaica* are cylindrical (Fig. 1). The leaf chlorophyll concentration (SPAD meter readings) of *N. sumatrana* was greater than *N. spectabilis*, but lower than *N. tobaica* (Table 1).

### Soil nutrient concentrations

Soil is not only a place for plants to grow but also a source of nutrients for the plants themselves (Turner, 2001). The concentration of nutrients contained in it determines its fertility and therefore populations of plants. The concentrations of N, P, Ca, and Na in the soil in the habitat of *N. sumatrana* (lowland) were lower and significantly different (Table 2) when compared to those in the habitat of *N. spectabilis* and *N. tobaica* (highland); in contrast the concentrations of K and Mg in the *N. sumatrana* habitat were greater than of *N. spectabilis* and *N. tobaica* and statistically significantly different (Table 2).

Table 1. Morphology of three *Nepenthes* species as measured from three samples in their natural habitat in North Sumatra province, Indonesia. Values are mean  $\pm$  standard error.

Parameter		<i>Nepenthes</i>		
		<i>sumatrana</i>	<i>spectabilis</i>	<i>tobaica</i>
Stem	Length (cm)	136 $\pm$ 41	215 $\pm$ 28	207 $\pm$ 23
	Diameter (mm)	10.4 $\pm$ 1.2	5.3 $\pm$ 0.3	3.3 $\pm$ 0.3
	Internode length (cm)	3.3 $\pm$ 0.8	5.3 $\pm$ 0.9	5.2 $\pm$ 1.0
Leaves	Length (cm)	33.7 $\pm$ 2.1	21.4 $\pm$ 1.6	11.9 $\pm$ 0.2
	Width (cm)	6.0 $\pm$ 0.7	4.0 $\pm$ 0.4	2.0 $\pm$ 0.1
	Thickness (mm)	0.45 $\pm$ 0.01	0.60 $\pm$ 0.03	0.41 $\pm$ 0.00
	Chlorophyll (SPAD meter units)	46.8 $\pm$ 2.1	37.6 $\pm$ 2.6	51.3 $\pm$ 3.5
Pitchers	Length (cm)	18.0 $\pm$ 1.6	18.5 $\pm$ 1.9	10.3 $\pm$ 0.2
	Bottom girth (cm)	12.5 $\pm$ 1.0	6.6 $\pm$ 0.8	8.6 $\pm$ 0.1
	Top girth (cm)	15.9 $\pm$ 0.1	7.4 $\pm$ 0.8	6.6 $\pm$ 0.1
	Tendrill length (cm)	38.0 $\pm$ 8.2	23.1 $\pm$ 4.2	12.2 $\pm$ 0.2

### Leaf nutrient concentrations

Leaves, apart from being a place for photosynthesis to occur, are also a store of nutrients (Turner, 2001). Results showed that the leaves of *N. sumatrana* contained the greatest concentration of N and significantly more than *N. spectabilis*, but not significantly different to *N. tobaica* (Table 2). On the other hand, the concentration of P in the leaves of *N. sumatrana* was lower and significantly less than *N. spectabilis*, but not significantly different from *N. tobaica*, while the concentration of K in the leaves of *N. sumatrana* was lower and different from *N. spectabilis* and *N. tobaica* (Table 2). Calcium and Mg in *N. sumatrana* leaves were lower and significantly different when compared to *N. spectabilis* and *N. tobaica*, whereas on the other hand the concentration of Na in the leaves of *N. sumatrana* was greater than in the leaves of *N. spectabilis*, but not significantly different from *N. tobaica* (Table 2).

### Pitcher fluid concentrations

The function of fluid in the pitcher of *Nepenthes* is as a nutrient solvent (Moran & Moran, 1998; Clarke, 2001), which helps in the process of breaking down trapped insects (Clarke *et al.*, 2009) and is carried out by enzymes secreted in the pitcher (Wang, 2009; Takeuchi *et al.*, 2011),

so that the presence of this fluid is very important for the fulfillment of *Nepenthes* plant nutrition. Nutrient concentrations were lower in the pitcher fluid compared to the leaves or soil (when all data were considered as percentages). Potassium was the most abundant nutrient in the pitcher fluid followed by Na and then Ca; other nutrients were at low concentrations. Concentrations of N, P, and K in the pitcher fluid of *N. sumatrana* were greater (although not always significantly) than in the pitcher fluid of *N. spectabilis* and *N. tobaica*. Likewise, *N. sumatrana* contained concentrations of micronutrients Mg, Ca, and Na in the pitcher fluid that were greater than *N. spectabilis* and *N. tobaica* (Table 2).

### Foliar nutrients in an extensive elevational dataset

Foliar N concentrations across our extensive elevational dataset were very variable (0.21 to 1.75 % N) but we found that there was no change in *Nepenthes* foliar N concentrations with elevation ( $r^2 = 0.04$ ,  $p = 0.23$ ). Phosphorus concentrations were less variable (0.06 to 0.26 % P) and showed a significant decline with elevation ( $r^2 = 0.17$ ,  $p = 0.023$ ); consequently there was an increase in the N:P ratio with elevation ( $r^2 = 0.23$ ,  $p = 0.007$ ) (Fig. 2).



Table 2. Concentrations of nutrients (% dry weight for leaves and soil, mg l<sup>-1</sup> for pitcher fluid) in three species of *Nepenthes* at the study site, North Sumatra province, Indonesia. Values are mean  $\pm$  standard error with letters indicating differences with a Duncan's test. BDL = Below detection limits.

Species		Nutrient						
		N	P	N:P	K	Ca	Mg	Na
Leaves	<i>sumatrana</i>	0.87 $\pm$ 0.10 (b)	0.10 $\pm$ 0.001 (a)	8.43 $\pm$ 1.12 (b)	1.67 $\pm$ 0.01 (a)	0.12 $\pm$ <0.01 (a)	0.07 $\pm$ <0.01 (a)	0.42 $\pm$ 0.01 (b)
	<i>spectabilis</i>	0.21 $\pm$ 0.06 (a)	0.11 $\pm$ 0.001 (b)	1.90 $\pm$ 0.54 (a)	1.75 $\pm$ 0.01 (b)	0.29 $\pm$ <0.01 (c)	0.11 $\pm$ <0.01 (c)	0.33 $\pm$ 0.01 (a)
	<i>tobaica</i>	0.58 $\pm$ 0.10 (ab)	0.11 $\pm$ 0.001 (ab)	5.37 $\pm$ 0.90 (ab)	1.77 $\pm$ 0.01 (b)	0.23 $\pm$ <0.01 (b)	0.11 $\pm$ <0.01 (b)	0.40 $\pm$ 0.01 (b)
Fluid	<i>sumatrana</i>	2.08 $\pm$ 0.13 (b)	8.30 $\pm$ 0.29 (b)	0.25 $\pm$ 0.01 (b)	1740 $\pm$ 49 (a)	396 $\pm$ 15 (b)	12.0 $\pm$ 1.18 (b)	1056 $\pm$ 42 (b)
	<i>spectabilis</i>	1.51 $\pm$ 0.14 (ab)	6.30 $\pm$ 0.29 (a)	0.24 $\pm$ 0.01 (b)	1640 $\pm$ 45 (a)	293 $\pm$ 14 (a)	5.5 $\pm$ 0.78 (a)	765 $\pm$ 42 (a)
	<i>tobaica</i>	1.13 $\pm$ 0.13 (a)	7.26 $\pm$ 0.34 (ab)	0.15 $\pm$ 0.01 (a)	1590 $\pm$ 30 (a)	304 $\pm$ 15 (a)	6.4 $\pm$ 1.15 (a)	873 $\pm$ 42 (a)
Soil	<i>sumatrana</i>	BDL	0.019 $\pm$ 0.001 (a)	NA	1.74 $\pm$ 0.01 (b)	1.02 $\pm$ <0.01 (a)	1.09 $\pm$ <0.01 (c)	0.47 $\pm$ 0.02 (a)
	<i>spectabilis</i>	1.17 $\pm$ 0.17 (b)	0.025 $\pm$ 0.001 (b)	46.9 $\pm$ 5.16 (a)	1.67 $\pm$ 0.01 (a)	1.30 $\pm$ 0.01 (b)	0.50 $\pm$ <0.01 (a)	0.63 $\pm$ 0.02 (b)
	<i>tobaica</i>	1.17 $\pm$ 0.17 (b)	0.024 $\pm$ 0.001 (b)	48.0 $\pm$ 5.36 (a)	1.68 $\pm$ 0.01 (a)	1.55 $\pm$ 0.01 (c)	0.76 $\pm$ <0.01 (b)	0.59 $\pm$ 0.02 (b)

## DISCUSSION

*Nepenthes sumatrana* grows in lowland areas (20 to 75 m asl) in open habitats with limestone soil and is often found overgrown with shrubs and ferns – at the study site, the vegetation and soil was quite degraded and prone to fire (Mansur *et al.*, in press.). In contrast, *N. spectabilis* and *N. tobaica* grow in a different habitat in highland secondary forests (1,750 m asl) with a mineral soil type overlain by an organic humus layer and under large trees that provide shaded canopy cover. The two habitats have different microclimates and soil properties, in particular, the organic carbon content of the soils differed markedly and was around 25% in the highland forest but less than 0.5% in the lowland location (M.M., unpublished data). The concentration of soil nutrients in the *N. spectabilis* and *N. tobaica* habitat is generally greater than the soil where *N. sumatrana* grows (N,

P, Na, and Ca), although the concentration of K and Mg in *N. sumatrana* habitat is greater than in the habitat of *N. spectabilis* and *N. tobaica*. However, this broad pattern was not reflected in the foliar nutrient concentrations as, in general, it was found that the nutrient concentration in the leaves and pitcher fluid in *N. sumatrana* had a higher concentration than the other two species indicating that soil nutrients are not always good predictors of foliar nutrients and that neither of them may be related to pitcher fluid nutrient concentrations. However, a measure of available soil nutrients would be more appropriate in future studies rather than the total nutrient concentrations as measured here. Broadly, the foliar nutrient concentrations in all three species were typical for *Nepenthes* as they were found in the centre of a PCA diagram presented by Mansur *et al.* (2021).

We know remarkably little about the determinants of pitcher fluid composition but this is im-

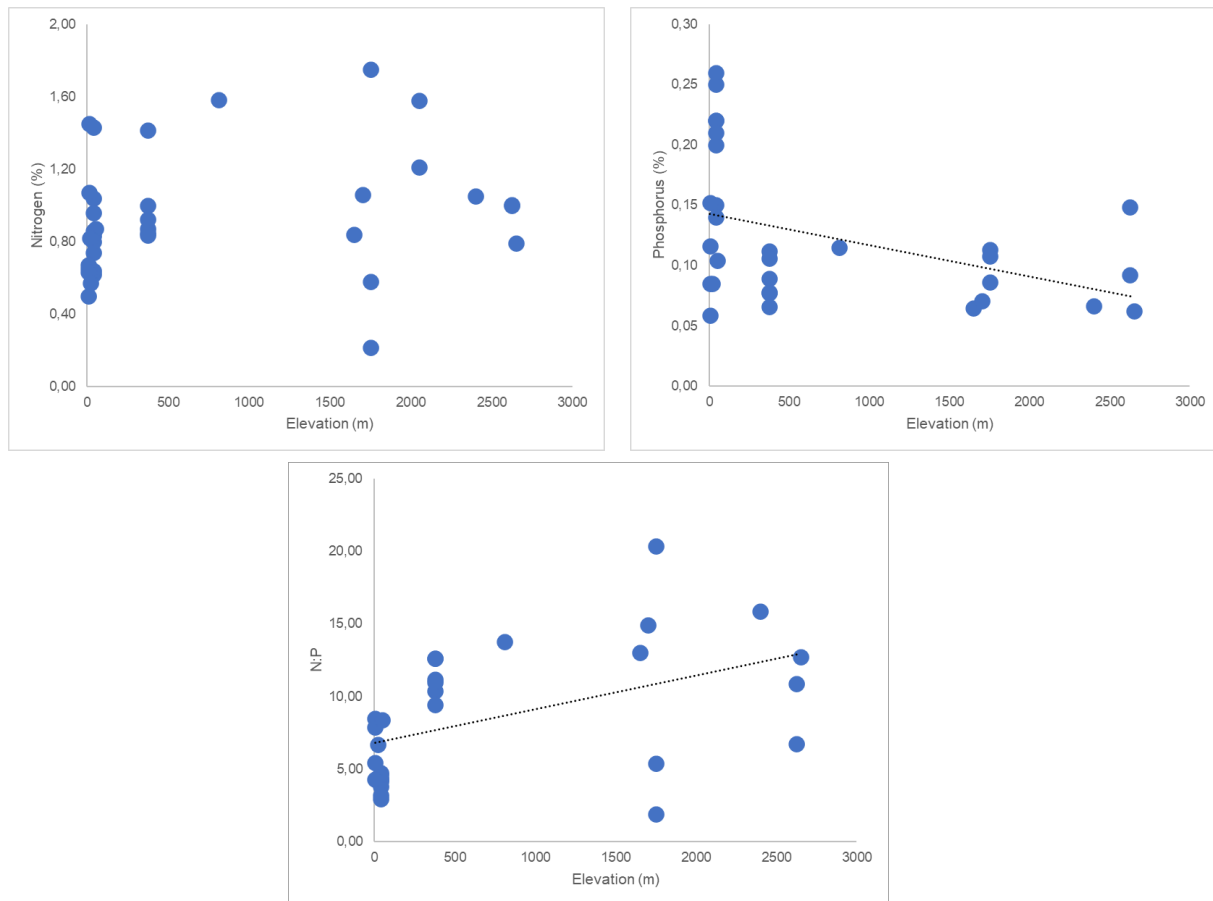


Fig. 2. Relationship between the concentration of foliar nitrogen (N), phosphorus (P), and the N:P ratio in a range of *Nepenthes* species across their geographical range. Each point represents the mean of each species sampled at each location.

portant due to the role of the pitcher fluid in influencing the microbial composition therein (Gilbert *et al.*, 2020) and its role in aiding in prey digestion through the production of a range of enzymes. Similar to other studies, K and Na were the dominant ions in the pitcher fluid (Buch *et al.*, 2013; Mansur *et al.*, 2021). Buch *et al.* (2013) found that N and P were at very low concentrations in the pitcher fluid they studied but these were under artificial experimental conditions so, in natural habitats, we would expect the pitcher fluid nutrient concentrations to be greater due to the addition of insect carcasses and other organic debris. The pitcher of *Nepenthes* has an important role in supplying nutrients that are not available in the soil and the greater concentrations of nutrients in the pitcher fluid of *N. sumatrana* may be because it has a larger pitcher size and is thus able to catch more insects than *N. spectabilis* and *N. tobaica*. This statement is also supported by the results of a study by Moran & Moran (1998) who reported that *N. rafflesiana* which lacks N (from prey sources) in its leaves can lead to decreased photosynthetic activity and reduced number of pitchers

of smaller sizes than control plants. It is not known if pitcher fluid has greater nutrient concentrations in species in more nutrient-rich habitats or if faster-growing species have lower nutrient concentrations due to more rapid uptake from the pitchers but this could be the focus of future research. Additionally, it would be informative to relate the pitcher and leaf nutrient concentrations to the composition and biomass of insects and other organic material collected in pitchers over a given time frame.

Across our extensive elevational dataset, we found that there was no change in foliar N with elevation – this suggests that *Nepenthes* species may obtain sufficient N from their prey with changing environmental conditions, or that there may be alterations to the stoichiometric ratio of their prey (*i.e.* changes in the N:P ratio) as elevation increases. As insects become less abundant at higher elevation, the importance of animal faeces may increase (Chin *et al.*, 2010) or *Nepenthes* may be able to increase soil N uptake via roots or even through recently-described underground pitchers to trap soil animals (Dančák

*et al.*, 2022). In contrast, foliar P concentrations did decrease with elevation and the N:P ratio increased indicating increasing phosphorus limitation of *Nepenthes* pitcher plants with elevation. This might more reasonably be considered to be a reduction in N limitation as the N:P ratios were very low overall with a median of 8.1 across our dataset. Declining P with elevation could be due to the presence of ultramafic sites at higher elevations in the dataset although this is unlikely as we would also expect declining K and Ca and increasing Mg (Proctor, 2003) which we did not see – additional data on nutrient concentrations of *Nepenthes* growing in ultramafic substrates would also help test this further.

## CONCLUSION

Nutrient concentrations in leaves and pitcher fluid in the three *Nepenthes* species studied (*N. sumatrana*, *N. spectabilis*, and *N. tobaica*) were generally low. The concentration of soil nutrients in the habitat of *N. sumatrana* in the lowland were generally lower than in the habitat of *N. spectabilis* and *N. tobaica* in the highland but nutrient concentrations in the leaves and pitcher fluid of *N. sumatrana* (lowland species) were often greater than *N. spectabilis* and *N. tobaica* (highland species). Further consideration of environmental controls over foliar nutrient concentrations and their linkages with pitcher fluid nutrients and how this is important for nutrient uptake processes are strongly warranted in additional *Nepenthes* species.

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