Oikos 126: 32–39, 2017
doi: 10.1111/oik.03455

Subject Editor: Jotaro Urabe. Editor-in-Chief: Gerlinde de Deyn. Accepted 22 April 2016

To recycle or steal? Nutrient resorption in Australian and Brazilian mistletoes from three low-phosphorus sites

Marina C. Scalon, Ian J. Wright and Augusto C. Franco

M. C. Scalon (marina_scalon@yahoo.com.br) and I. J. Wright, Department of Biological Sciences, Macquarie University, Sydney, NSW, 2109, Australia. Present address for MCS and A. C. Franco, Laboratório de Ecofisiologia Vegetal, Depto de Botânica, Inst. de Ciências Biológicas, Univ. de Brasília, Brasília – DF, Brazil.

Resorption is the process by which nutrients are withdrawn from leaves prior to leaf fall. Mistletoes are generally thought not to rely on nutrient resorption; being xylem-tapping parasites, they instead derive the nutrients required for new growth from their host plant, at little or no cost. We measured nutrient (N, P, K, Ca, Mg) resorption in 18 parasitic mistletoe–host species pairs distributed across three sites with notably low-P soil, also quantifying relationships with leaf lifespan (LL) and specific leaf area (SLA). There was little or no evidence of N, Ca or Mg resorption. By contrast, on average ~30% of P and ~20% of K were resorbed prior to leaf fall. Longer LL in mistletoes was associated with lower N and P concentrations in mistletoes and in host leaves. We provide evidence that, even though mistletoes are relatively inefficient in terms of nutrient resorption compared to non-parasite species, on low-P soils their ecological and evolutionary strategies for conserving phosphorous involve modulation of both leaf lifespan and P concentration in senesced leaves.

Nutrient resorption is the process of mobilizing and withdrawing nutrients from old leaves prior to leaf fall, whether for immediate or later use in actively growing tissues. Reducing nutrient losses diminishes the need for acquiring new nutrients from the soil, so having higher nutrient resorption might be especially advantageous in habitats where soil nutrients are scarce (Aerts 1996). Indeed, the ability to attain low residual nutrient concentration in senesced leaves, together with long leaf lifespan (LL) and low specific leaf area (SLA, the ratio of leaf area to dry mass), are considered the main plant adaptations for enhancing nutrient conservation in infertile habitats (Escudero et al. 1992, Aerts and Chapin 1999, Wright and Westoby 2003). Therefore, for species with short leaf longevity and high SLA, the retranslocation process is especially important for nutrient conservation (Aerts and Chapin 1999).

The unit-cost of acquiring nutrients from the soil may vary according to site nutrient availability (Bloom et al. 1985), so that if the soil is artificially nutrient impoverished, plants are reported to accelerate the senescing process (Ono et al. 1996). The resorption process also has a cost, and the balance between use of soil-derived and resorption-derived nutrients should be set by their relative costs (Wright and Westoby 2003). The relative costs not only vary according to the amount of nutrient, but also depend on the compounds from which they are derived. For example, N can occur in multiple forms in the soil (nitrate, ammonium and organic N) and different species can differ in their preference over one of these forms, depending on the relative costs to absorb and assimilate it (Aerts and Chapin 1999). Similarly, the cost of resorbed nutrients depends on which compounds are broken down and remobilised during the senescence process (Lambers et al. 1998).

As should be clear from the preceding paragraphs there are two chief indices of nutrient resorption, i.e. proportional resorption from green to senesced leaves, (hereafter N_{resorp}, P_{resorp} etc, referring to specific nutrients), and the residual concentrations of nutrients in senesced leaves (hereafter, $[N]_{sen}$, $[P]_{sen}$, etc, referring to specific nutrients). Proportional resorption, also known as resorption "efficiency" (Killingbeck 1996), describes the percentage of nutrient invested in foliage that was conserved, thus summarizing nutrient demand and withdrawal. Residual nutrient concentrations presumably reflect plant biochemical limitations as well as adaptive strategies to minimise nutrient losses (Killingbeck 1996). On average, very significant proportions of leaf nitrogen (~62%), phosphorus (~65%) and potassium (~70%) are resorbed from leaves before they are shed, while plants resorb far less of elements such as Ca and Mg, averaging ~11% and ~28%, respectively (Vergutz et al. 2012).

Clearly, both high proportional resorption and low residual nutrient concentrations decrease the demand for new nutrient uptake and – when associated with a long leaf lifespan – form part of an overall, highly "conservative" nutrient use strategy (Aerts and Chapin 1999). Although intuitively one might expect that species typical of low nutrient soils or plants grown experimentally at lower soil fertility would show higher proportional nutrient resorption, this is not generally true (Aerts and Chapin 1999) – because both green-leaf and residual nutrient concentrations typically shift with soil nutrient availability, and in parallel. This was the case in a survey of 73 perennial species in eastern Australia, where species from low-P soils showed dramatically lower [N] and [P] in both green and senesced leaves, but did not differ in N_{resorp} or P_{resorp} from species growing on richer soils (Wright and Westoby 2003). However, because of the small variation of leaf nutrient resorption across species from nutrient-rich and nutrient-poor habitats, leaf longevity is suggested to be a more important adaptation to lower fertility than the resorption process itself, as concluded by many studies with different functional types of plants (Escudero et al. 1992, Reich et al. 1992, Aerts and Chapin 1999). Moreover, the negative effects of increasing LL, such as investing heavily on leaves that are more robust with a longer time to pay off their construction costs, were suggested to be surpassed by its positive effect on prolonging nutrient retention in the biomass in a study of woody species in central Spain (Escudero et al. 1992).

Nitrogen and phosphorus are commonly assumed to be the most limiting (crucial and expensive to acquire) nutrients for primary production in terrestrial environments (Vitousek and Howarth 1991, Elser et al. 2007). Other key nutrients for plant growth, such as Ca, K and Mg may also be expensive to obtain and could influence ecosystem processes, such as vegetation structure, primary production and nutrient cycling (Vitousek and Howarth 1991, Eviner and Chapin III 2003). Because nutrients are limiting resources and because tradeoffs exist between the energy expended on acquiring and maintaining nutrients and the energy used for other metabolic purposes, it is expected that natural selection will drive evolutionary shifts favouring more efficient nutrient use in plants. Different strategies may be involved in this process, such as optimal differential accumulation through the canopy (Niinemets 2007), investment in defence against herbivory (Mattson 1980), tight control over leaf senescence (meaning that nutrients can be resorbed prior to losing leaves; Reich et al. 1992), and evolutionary "choices" about which compounds in leaves are broken down and withdrawn during the resorption process and which are left behind (Wright and Westoby 2003).

Parasitic plants, such as xylem-tapping mistletoes, can be found in all biomes. Mistletoes do not have a conventional root system; instead, they develop a connection to their host xylem – the haustorium – through which they acquire all mineral nutrients and water (Calder and Bernhardt 1983). The energetic costs required for nutrient acquisition in mistletoes are presumably far lower than that in plants that access nutrients in the soil. Non-parasitic plants need to invest a very considerable proportion of their carbon budget to building and maintaining root systems, fostering symbiotic relationships with N-fixing bacteria and/or mycorrhizae, or building specialized structures such as cluster roots and/or exuding substantial amounts of organic acids or carboxylates to help access tightly-bound nutrients on clay particles (Lambers et al. 1998). For mistletoes then, presumably it would only be advantageous to resorb nutrients from old leaves for use in new growth if the costs of doing so are lower than simply acquiring more nutrients from the xylem of their hosts. A number of studies have reported an absence of nutrient resorption in mistletoes (Pate et al. 1991, March and Watson 2007, 2010). However, these studies were each limited to only one mistletoe species in a specific location.

In this paper we measured green-leaf and residual nutrient concentrations in 10 mistletoe species growing on 18 different host species, sampled from sites located in Australia and Brazil. We aimed to 1) quantify proportional nutrient resorption and residual nutrient concentrations, focusing on N, P, Ca, Mg and K; 2) investigate relationships between nutrient resorption and two key functional traits, leaf lifespan (LL) and specific leaf area (SLA); and 3) ascertain whether resorption varied a) between vegetation types, and b) in relation to host green-leaf nutrient concentration. Based on the arguments given above, we hypothesized that:

I) Mistletoes would show very low proportional nutrient resorption compared to what is known for non-parasitic plants.
II) Nutrient resorption strategies would be related to the 'leaf economics spectrum' (Wright et al. 2004) in that mistletoe species with longer LL (and lower SLA) would withdraw nutrients down to lower residual levels during resorption – in parallel to what is known for Australian non-parasitic plants (Wright and Westoby 2003).
III) Host green-leaf nutrient concentration, acting as a proxy for nutrient concentration in the host xylem, would be related to mistletoe resorption patterns and leaf lifespan. Specifically, lower green-leaf nutrient concentrations in host leaves would indicate higher proportional resorption and longer leaf lifespans in mistletoes.

Material and methods

We sampled mistletoes at three different sites, two in Australia and one in Brazil (Table 1). Sites were located in National Parks and differed in rainfall, seasonality and nutrient

Table 1. Site locations, climates and soil properties (mean ± SD). Mean annual precipitation (MAP) and mean annual temperatures (MAT) were obtained from the CRU CL2.0 global gridded dataset (New et al. 2002).

Site vegetation	Location	Coordinates	MAT (°C) (min–max)	MAP (mm)	Total P (ppm)	Total N (%)
Brazilian savanna	IBGE Ecological Reserve (Brasília, DF, Brazil)	15°55′S, 47°51′E	25.8 (14.4–31.2)	1478.0	207.1 ± 12.3	0.140 ± 0.028
Australian savanna	Howard Springs (Darwin, NT, Australia)	12°30′S, 130°45′E	27.6 (23.2–32.0)	1668.6	62.6 ± 3.1	0.067 ± 0.02
Australian closed woodland	Ku-ring-gai NP Royal NP (Sydney, NSW, Australia)	33°63′S, 151°26′E 34°17′S, 151°05′E	17.2 (13.7–21.7)	1266.6	191.4 ± 25.7	0.079 ± 0.012

availability. Soil samples were collected from the surface layer (0–10 cm) in four different locations at each site. Total soil N (%) and P (ppm) were determined by combustion and emission spectrometry following nitric–perchloric acid digestion, respectively.

Site descriptions

The Cerrado (Brazilian Savanna) site corresponded to a typical cerrado sensu stricto area, characterized by the strong seasonality of the rainfall, low availability of nutrients associated with high soil acidity and fire disturbance (Eiten 1972, Haridasan 2001).

The Australian Savanna site also consisted of tropical savanna with marked seasonality (Williams et al. 1997), and well drained, highly weathered, laterised soils extremely low in nutrients (Hutley et al. 2000). The two Australian closed woodland sites were located in distinct parks, both being characterized by sandstone soils with low nutrient content, temperate climate and rainfall evenly distributed throughout the year (NPWS 2000, 2002).

Leaf trait measurements

In this study, each of the 18 different mistletoe–host (M-H) species pairs was considered a distinct observation unit (Table 2). Mature, fully expanded leaves of hosts and mistletoes and senesced leaves of mistletoes were collected from five to seven replicates (different individual mistletoes growing on different individual hosts) per M-H species pair over a 1–2 year period at each site, on at least two different occasions. Senesced leaves were identified as those which could be removed by gently flicking the branch, indicating that an abscission layer was present, and thus no further nutrient resorption was possible (Wright and Westoby 2003).

Fresh leaves were scanned and one-sided leaf areas were measured using Image J software (Abràmoff et al. 2004). After oven-drying for 72 h at 65°C, leaves were weighed and the specific leaf area (SLA, $cm^2\ g^{-1}$) was calculated as the ratio between fresh area and dry mass. The dried leaves combined from each replicate were ground and sent for analysis, totalling at least five mature and five senescent leaf nutrient determinations for each M-H pair. Australian samples were sent to the Analytical Service Unit from the School of Agriculture and Food Science at The University of Queensland for macronutrient determination. Total N (%) was measured using a CHN combustion analyser. Total P ($mg\ kg^{-1}$), K (%), Ca (%) and Mg (%) were measured using an ICP-OES analyser, following nitric perchloric acid digestion. Brazilian samples were sent to Laboratorio de Agroquimica e Meio Ambiente at Universidade Estadual de Maringa (PR/Brazil) for N and P ($g\ kg^{-1}$) determination by Kjedahl digestion and UV-Vis spectroscopy, respectively. The other nutrients (Ca, Mg and K, $g\ kg^{-1}$) were determined by atomic absorption spectrometry. The same procedures were used for host leaves.

Age-related changes in mass-based concentrations of leaf nutrients do not account for structural changes in soluble carbon and may underestimate proportional resorption. Therefore, resorption measured on an area basis is preferred because it controls for these structural changes (Chapin III and Moilanen 1991). We calculated resorption as the proportion of nutrients in senesced leaves ($[Nut]_{sen}$) relative to the green leaves ($[Nut]_{green}$), on a leaf area basis (Eq. 1). Residual nutrient concentrations in senesced leaves were also expressed on an area basis.

$$\text{Resorption (\%)} = \left(1 - \frac{[Nut]sen}{[Nut]green}\right) \times 100 \qquad (1)$$

We also collected data on leaf lifespan (LL, years) for all mistletoes sampled in the Australian closed woodland and for three out of the six mistletoe–host pairs sampled in the Brazilian savannas sites (Table 2). Four branches of at least five individual plants were used to measure LL, based on leaf turnover rates (Wright et al. 2002). All leaves from each branch were sequentially numbered and revisited every three to four months for at least one complete year (12–18

Table 2. Species list of mistletoes used in this study and the host it was parasitising. Leaf lifespan (LL) data were collected for species in bold.

Site	Mistletoe	Host	Host family
Brazilian Savanna (Brasília)	*Passovia ovata*	*Stryphnodendron adstingens*	Fabaceae
	Passovia ovata	*Miconia albicans*	Melastomataceae
	Psittacanthus robustus	*Qualea grandiflora*	Vochysiaceae
	Phoradendron crassifolium	*Tapirira guianensis*	Anacardiaceae
	Passovia ovata	*Dalbergia miscolobium*	Fabaceae
	Passovia ovata	*Piptocarpha rotundifolia*	Compositeae
Australian Savanna (Darwin)	*Amyema sanguinea*	*Corymbia porrecta*	Myrtaceae
	Amyema sanguinea	*Eucalyptus tetrodonta*	Myrtaceae
	Amyema sanguinea	*Corymbia blesseri*	Myrtaceae
	Amyema miquelli	*Eucalyptus miniata*	Myrtaceae
	Dendrophthoe odontocalyx	*Grevillea pteridifolia*	Proteaceae
	Decaisnina signata	*Xanthostemon paradoxus*	Myrtaceae
AustralianClosed Woodland (Sydney)	***Muellerina eucalyptoides***	*Eucalyptus hemastoma*	Myrtaceae
	Muellerina eucalyptoides	*Eucalyptus moluccana*	Myrtaceae
	Muellerina eucalyptoides	*Eucalyptus* sp.	Myrtaceae
	Amyema congener	*Allocasuarina littoralis*	Casuarinaceae
	Dendrophtoe vitellina	*Eucalyptus* sp.	Myrtaceae
	Dendrophtoe vitellina	*Angophora costata*	Myrtaceae

months). LL was calculated as the inverse of the mortality rate (number of dead leaves per number of leaves at beginning of census/ period of time).

Data analyses

Leaf trait data were generally strongly right-skewed and thus were $\log_{10}$-transformed to achieve approximately normal distributions (Shapiro–Wilk normality test, $p > 0.05$); with the exception of the proportional nutrient resorption indices (%), which needed no such transformation. Paired t-tests were used to compare leaf nutrient concentrations and SLA between green and senesced mistletoe leaves across the various M-H species pairs, and to compare nutrient concentrations between mistletoes and their hosts. To investigate whether mistletoe proportional resorption differed between sites we used two-way ANOVA, the two factors being leaf age class ($n = 2$ groups: green and senesced; 18 M-H pairs per group) and site ($n = 3$ groups: Australian savanna, Australian closed woodland, Brazilian savanna; six mistletoe–host pairs per group). We used Pearson correlations to quantify relationships between mistletoe nutrient resorption and mistletoe functional traits (SLA and LL), and between mistletoe nutrient resorption and host leaf nutrient concentrations. All data analyses were run in R ver. 3.1.0 (<www.r-project.org>).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.cs3h1> (Scalon et al. 2016).

Results

Nutrient concentrations and resorption processes

Nitrogen concentrations (area-basis) in green and senesced leaves varied around six-fold among the various mistletoe species (Fig. 1, Supplementary material Appendix 1 Table A1). Proportional N resorption, N_{resorp} (calculated on a leaf area basis), ranged from 26.8% in *Amyema sanguinea* parasitising *Eucalyptus tetrodonta* all the way down to –45.2% in *Passovia ovata* parasitising *Stryphnodendron adstingens* (i.e. N accumulated during leaf lifetime). In most cases N_{resorp} was close to zero; indeed, the overall mean was –4.3%, and not significantly different from zero (Fig. 2, $p = 0.666$). N_{resorp} site-mean values were also not significantly different from zero (ranging from –1.1% to –7.1%, Supplementary material Appendix 1 Table A1), and did not differ across sites (Fig. 2; $p = 0.900$).

Phosphorus concentrations (area-basis) in green and senesced leaves varied around four-fold among the various mistletoe species (Supplementary material Appendix 1 Table A1). In all sites, green-leaf [P] was significantly higher than senescent [P] (Fig. 1). In contrast to what we found for nitrogen, there was clear evidence of P resorption in mistletoes, P_{resorp} ranging from 5.3% to 62.4% among species (mean 29.8%). Each of the three site-mean P_{resorp} values were significantly higher than zero (all $p < 0.001$), ranging from 18.2% to 36.1% but, again, these were not significantly different among sites ($p = 0.285$), since there was substantial variation among mistletoe–host pairs within each site (Supplementary material Appendix 1 Table A1).

On average, calcium accumulated in senesced leaves while potassium was resorbed, averaging –29.7%, and 25.4% proportional resorption, respectively (Fig. 2). There was no difference between green and senescent leaves in Mg concentration or in specific leaf area for individual sites (ANOVA, all $p > 0.1$) or considering all species together (Fig. 2).

On a leaf-area basis, Brazilian Cerrado mistletoes showed significantly higher green-leaf and senesced-leaf N_{area}, P_{area} and Ca_{area} than mistletoes from other sites, while those from the Australian closed woodland showed higher K_{area} (Fig. 1, Supplementary material Appendix 1 Table A1). However, there was not a strong pattern of nutrient resorption related to individual sites, which suggests that environmental differences did not affect nutrient use and resorption patterns of mistletoes (Fig. 2).

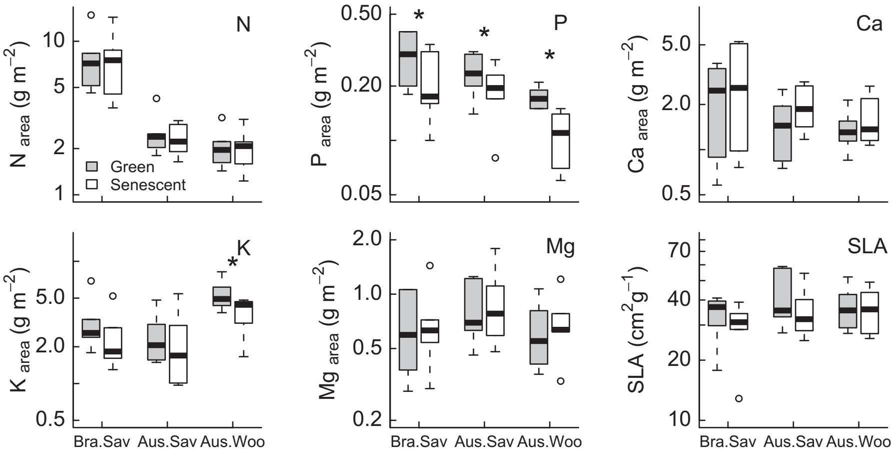

Figure 1. Boxplots of nitrogen (N), phosphorus (P), calcium (Ca), potassium (K) and magnesium (Mg) concentration on an area basis, and specific leaf area (SLA), for mistletoes at the three studied sites, for green leaves (grey box) and senesced leaves (white box). The data are plotted on a log scale. The continuous line within the box shows the median and error bars show 10th and 90th percentiles. Outliers are represented by small open circles. The symbol * denotes significant differences between the green and senesced leaves (paired t-tests, $p < 0.05$).

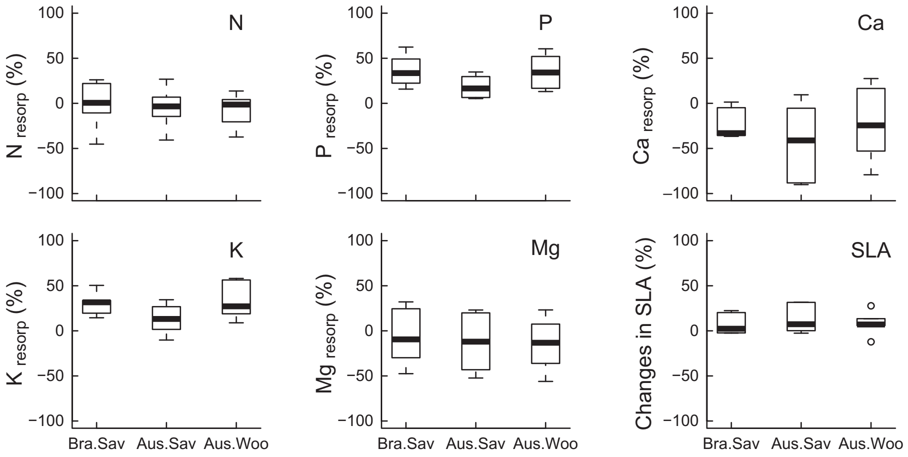

Figure 2. Boxplots of nitrogen (N), phosphorus (P), calcium (Ca), potassium (K) and magnesium (Mg) proportional resorptions (on a leaf area basis) and changes in specific leaf area (SLA) of mistletoes at the three studied sites. The continuous line within the box shows the median, and error bars show 10th and 90th percentiles. Outliers are represented by small open circles. There was no significant difference between sites (ANOVA, all $p > 0.05$).

Relationships among functional traits

Mistletoes leaf lifespan averaged 2.2 years, and it was shorter for the three Brazilian species (ranging from 1.3 to 1.7 years) than the six Australian species (ranging from 2.0 to 3.3 years). On average, mistletoes with longer LL had lower N and P concentrations in both green and senesced leaves (Fig. 3, Table 3). By contrast, SLA was generally unrelated to green-leaf and residual nutrient concentrations, except that it was weakly, positively correlated with green-leaf [P]. P_{resorp} was unrelated to either of SLA or LL (Table 3). N_{resorp} was unrelated to SLA but unexpectedly showed a negative relationship with LL ($r^2 = 0.57$; Fig. 3c).

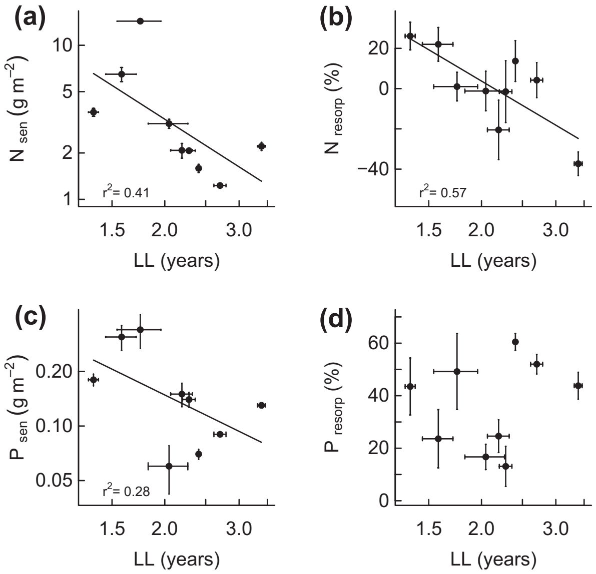

Figure 3. Relationship between leaf lifespan (LL) and N and P concentrations in senesced leaves (a, c), and proportional N and P resorption (b, d). Except for proportional N and P resorption, the data are plotted on a log scale. Correlations statistics are given in Table 3.

Mistletoes showed, on average, higher green leaf N (mean ± SD: 2.01 ± 0.89 and 4.16 ± 3.32 g m^{-2} for hosts and mistletoe, respectively) and P (mean ± SD: 0.098 ± 0.026 and 0.254 ± 0.135 g m^{-2} for hosts and mistletoe, respectively) concentrations compared to their hosts (paired t-tests, all $p < 0.001$). The N concentration of host leaves were strongly related to both the green and residual N concentrations of mistletoe leaves ($r^2 = 0.57$ and 0.62, respectively; Table 3, Supplementary material Appendix 1 Fig. A1). The same was not true for mistletoe and host leaf P concentrations (Table 3, Supplementary material Appendix 1 Fig. A1). Host N and P leaf concentrations were negatively related to mistletoe leaf lifespan ($r^2 = 0.55$ and 0.71 respectively, $p < 0.05$; Fig. 4), indicating that mistletoes growing on N and P-poor hosts tend to show longer leaf lifespan. Contradicting our expectations, the nutritional status of the host was not related to mistletoe nutrient resorption efficiency (i.e. there was no correlation between host leaf nutrient concentration and proportional resorption in mistletoes; Table 3, Fig. 4). No significant relations were found for the other nutrients (Ca, K and Mg), either with LL, SLA or with host leaf nutrient concentration.

Table 3. Pearson correlation (r^2 and p-values) between nitrogen and phosphorus concentrations in green leaves (N_{green} and P_{green}), N and P concentrations in senesced leaves (N_{sen} and P_{sen}), proportional resorption (N_{resorp}, P_{resorp}) and leaf traits (specific leaf area - SLA; and leaf lifespan - LL), for mistletoes and hosts. Significant correlations (p-values lower than 0.05) showed in bold.

	Mistletoe		Host	
Nutrients	SLA ($n = 18$)	LL ($n = 9$)	N_{host}	P_{host}
N_{green}	0.11, 0.178	**0.71, 0.004**	**0.57, < 0.001**	–
N_{sen}	0.10, 0.219	**0.41, 0.042**	**0.62, < 0.001**	–
P_{green}	**0.29, 0.023**	**0.58, 0.018**	–	0.12, 0.162
P_{sen}	0.04, 0.423	**0.28, 0.049**	–	0.17, 0.093
N_{resorp}	0.05, 0.385	**0.57, 0.017**	0.01, 0.889	–
P_{resorp}	0.12, 0.171	0.05, 0.636	–	0.09, 0.218

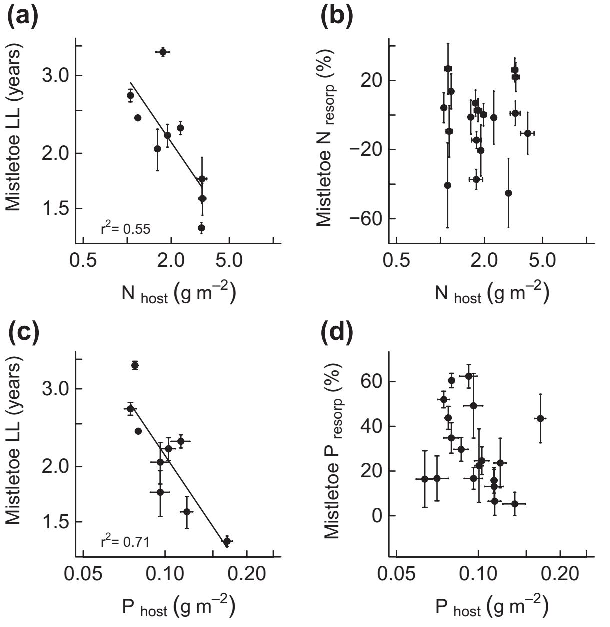

Figure 4. Relationship between hosts N and P leaf concentration and mistletoe leaf lifespan (a, c) and mistletoe N and P resorption (b, d). Except for proportional N and P resorption, the data are plotted on a log scale. Error bars are shown and r^2-values are given when $p < 0.05$.

Discussion

Nutrient resorption and implications for the N-parasitism hypothesis

Contradicting ideas that N resorption might be important for all plants independent of life form (Eckstein et al. 1999), but in line with previous work on Australian mistletoes from *Amyema* genus (Pate et al. 1991, March and Watson 2010), our study shows that in fact very little N is resorbed from senesced leaves in mistletoes. Considering our results, we conclude that nitrogen is not a limiting nutrient for mistletoes in the studied sites, otherwise selection would have favoured the optimization of N use, and diminished N loss by high proportional N resorption. The data presented here are far more comprehensive than in previous studies, containing seven genera of mistletoes from three vegetation types on two continents, thereby providing strong support for a general lack of N resorption in mistletoes. This general lack of N resorption is strong evidence against the "N-parasitism hypothesis", at least for the sites under consideration here. This hypothesis was originally suggested by Schulze et al. (1984) in order to explain the very high transpiration rates and low photosynthetic water use efficiency seen in mistletoes, the idea being that the high transpiration rates is driven by the need to acquire sufficient N from the xylem sap of the hosts.

Our three sites are representative of systems with very old soils where soil P rather than N is generally thought to be most limiting to plant production. Our results suggest that this was probably even more strongly the case for mistletoes than for other species (which generally would be expected to resorb around 50% of leaf N). Indeed, perhaps on these low-P soils, it could be the need for P (along with heterotrophic C) that largely drives the high transpiration rates in mistletoes, hence the concept of 'P-parasitism' (rather than 'N-parasitism') should be considered.

Calcium is generally conserved in senesced leaves of terrestrial plants because it is a structural element in cell walls and usually correlates positively with Mg because the association of them in metabolism and photosynthesis, both acting as activators of metabolic reaction enzymes (Epstein and Bloom 2005). Indeed, the accumulation of Ca from green to senesced leaves often observed in evergreen species presumably reflects accumulation of cell wall material over the life of a leaf (Van Heerwaarden et al. 2003). However, recently Vergutz et al. (2012) showed that Ca and Mg might also be resorbed in many species, which was not the case for mistletoes (Fig. 1, 2).

By contrast, K_{resorp} averaged 24.5%. In mistletoes, K is usually found in disproportionally higher concentrations compared to non-parasitic host plants (Schulze et al. 1984, Scalon et al. 2013). It has been suggested that K accumulation could be an active process in mistletoes (Lamont and Southall 1982) because K^+ plays an important role in neutralizing anions, stabilizing pH and osmotic potential, and maintaining cell turgor and membrane integrity (Amtmann and Rubio 2012). However, perhaps a more plausible hypothesis is that K accumulates passively due to its high phloem mobility and the lack of appropriate sinks (Glatzel 1983). If passive accumulation is the case, the resorption process of highly mobile ions should be unnecessary, although the positive K_{resorp} observed here suggests that the higher concentrations of K in mistletoes leaves may be a physiological requirement. Nonetheless, 24.5% K resorption is much lower than the previously reported average of 70% from a global dataset (Vergutz et al. 2012), and somewhat lower than the P_{resorp} that we observed (average ~30%). Another possibility to consider is that K is not being actively resorbed but rather being leached from old leaves prior to leaf fall, driven by rainfall. Concerns over the overestimation of K_{resorp} due to ignorance of the effect of leaching was previously pointed out by Wang et al. (2003) while studying nutrient resorption of a mangrove species in China, because K is suggested to be one of the inorganic nutrients leached in greatest quantities (Tukey Jr 1970, Potter 1991). By contrast, organic bounded nutrients, such as N and P are not readily leached from leaves (Tukey Jr 1970, Chapin III and Moilanen 1991, Aerts and Chapin 1999) and their resorption values are presumably far more reliable.

Differences in nutrient economy between mistletoes versus non-parasitic plants

Non-parasitic plants have two pathways for acquiring nutrients used in producing new tissue: root uptake and retranslocation from old organs. As discussed by Wright and Westoby (2003), the balance between use of soil-derived and resorption-derived nutrients should be set by their relative costs. Expanding on this concept, mistletoes also have two nutrient sources: resorption of nutrients from old leaves, or host-derived nutrients (i.e. acquiring nutrients directly from the host xylem). If nutrients in the host xylem are abundant, the costs of acquiring them should be very inexpensive compared to resorption-derived nutrients. By contrast, if any nutrient is found in low concentration in the host xylem and also limits mistletoe nutritional requirements, resorbing it from old leaves might be cheaper than acquiring it from the host. In addition, mistletoes face physiological constraints and lack the ability to develop natural strategies seen in non-parasitic plants to deal with low nutrient concentration, such as allocating more carbon to expand the root system, associating with symbiotic bacteria or fungi from the soil, or even modifying the soil environment to enhance nutrient availability (Aerts and Chapin 1999). Therefore, they are subject to whatever the host plant has to offer, and may have to tolerate differences in xylem chemistry and deal with nutrient deficiency (Glatzel and Geils 2009). All sites in this study are low-P environments, with very low soil nutrient availability (Table 1). The host plants are adapted to survive in these conditions, e.g. constructing green leaves with very low [P], ~50% lower compared to the average of 1.4 mg g^{-1} from 496 plant species distributed globally and reported by Vergutz et al. (2012) (here, site-mean host green-leaf P concentration ranged from 0.4 to 0.7 mg g^{-1}).

The resorption process may have been favoured by natural selection in these species, as evidenced by the lack of relationship between mistletoe and host leaf P concentration in contrast with the strong positive relationship between host and N leaf concentration (Table 3, Supplementary material Appendix 1 Fig. A1). The correspondence between host and mistletoe leaf N concentration was already reported in a global analysis considering more than 160 mistletoe–host pairs (Scalon and Wright 2015), but the fact that leaf P concentration of mistletoes does not reflect that of their hosts suggests that the maintenance of higher P concentration may be achieved through the resorption process.

Resorption and functional trait relationships

Nutrients can be conserved in the plant biomass by resorption or by extending the time the plant organ lives (Eckstein et al. 1999). Therefore, in species with short leaf longevity, the retranslocation process is very important for nutrient conservation (Jonasson 1989, Aerts and Chapin 1999). Indeed, our results show that species with longer LL showed lower nutrient concentrations in senesced leaves (Fig. 3), suggesting that selection has minimized nutrient losses in mistletoes both via extending LL and via lower nutrient residual concentrations. The same relationship between N and P concentration and LL was found in non-parasitic Australian plants (Wright and Westoby 2003).

While LL was negatively associated with N and P concentrations, we did not find any strong relationship between nutrient resorption and specific leaf area (Table 3). Even though mass loss during senescence is expected (Vergutz et al. 2012), here we found similar SLA in senesced leaves and green leaves. We interpret this as reflecting the balance between two processes: a general tendency for SLA to decrease with leaf age in evergreen species (Wright et al. 2006), and the loss of mass due to resorption, most nutrients being transported as part of organic compounds (Aerts 1996). Possibly a third factor comes into play here. According to Van Heerwaarden et al. (2003), leaf area is not stable during leaf senescence and leaf shrinkage may lead to up to 6% of underestimation of leaf nutrient resorption. Still, since underestimation may be consistent across all nutrients, our results do not change, but it is also possible that the real absolute resorption values will be slightly higher than the values reported in this study.

Conclusion

Overall, our results showed that P and K, but not N, Mg and Ca, are resorbed by mistletoe species sampled across two continents. The lack of N resorption together with the fact that P is on average 30% resorbed suggest that N is not a limiting nutrient for mistletoes, contradicting the N-parasitism hypothesis. Our results suggest that mistletoes growing in low-P environments and parasitising hosts adapted to deal with very low P availability actively modulate P use, so that the resorption process may have been favoured by natural selection in these species. Our findings also show that, even though mistletoes are relatively inefficient in terms of nutrient resorption, they still exhibit well understood syndromes of leaf-level adaptations that determine nutrient residence times, i.e. teaming longer leaf lifespans with lower residual nutrient concentrations in senesced leaves.

Acknowledgements – Warm thanks to Lindsay Hutley and Matthew Norwood for providing lab space, logistical support and access to the Australian Savanna site. We also thank Izabelly Sant'Ana, Allyson Eller, Julieta Garcia-Russell, Julia Cooke and Fabricius Domingos for the valuable help in the field; and Rachael Gallagher, Fabricius Domingos and Raquel Miatto for providing useful feedback on earlier versions of this manuscript.

Funding – MCS was supported by a scholarship from 'Conselho Nacional do Desenvolvimento Cientifico e Tecnologico' (CNPq) and by Macquarie University. IJW acknowledges the support of the Australian Research Council. ACF is recipient of a CNPq Research Productivity fellowship.

Permits – We acknowledge Australian National Parks and RECOR/IBGE offices for permits and logistic support.

References

Abràmoff, M. D. et al. J. 2004. Image processing with ImageJ. – Biophotonics Int. 11: 36–43.

Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns? – J. Ecol. 84: 597–608.

Aerts, R. and Chapin, F. S. 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. – Adv. Ecol. Res. 30: 1–67.

Amtmann, A. and Rubio, F. 2012. Potassium in plants. – eLS Wiley.

Bloom, A. J. et al. 1985. Resource limitation in plants: an economic analogy. – Annu. Rev. Ecol. Syst. 16: 363–392.

Calder, M. and Bernhardt, P. 1983. The biology of mistletoes. – Academic Press.

Chapin III, F. S. and Moilanen, L. 1991. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. – Ecology 72: 709–715.

Eckstein, R. et al. 1999. Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. – New Phytol. 143: 177–189.

Eiten, G. 1972. The cerrado vegetation of Brazil. – Bot. Rev. 38: 201–341.

Elser, J. J. et al. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – Ecol. Lett. 10: 1135–1142.

Epstein, E. and Bloom, A. 2005. Mineral nutrition of plants: principles and perspectives, 2nd edn. – Sinauer.

Escudero, A. et al. 1992. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. – Oecologia 90: 80–87.

Eviner, V. T. and Chapin III, F. S. 2003. Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. – Annu. Rev. Ecol. Evol. Syst. 34: 455–485.

Glatzel, G. 1983. Mineral nutrition and water relations of hemiparasitic mistletoes: a question of partitioning. Experiments with *Loranthus europaeus* on *Quercus petraea* and *Quercus robur*. – Oecologia 56: 193–201.

Glatzel, G. and Geils, B. W. 2009. Mistletoe ecophysiology: host–parasite interactions. – Botany 87: 10–15.

Haridasan, M. 2001. Nutrient cycling as a function of landscape and biotic characteristics in the cerrado of central Brazil. – In: McClain, M. E. et al. (eds), Biogeochemistry of the Amazon basin and its role in a changing world. Oxford Univ. Press, pp. 68–83.

Hutley, L. et al. 2000. Evapotranspiration from Eucalypt open-forest savanna of Northern Australia. – Funct. Ecol. 14: 183–194.

Jonasson, S. 1989. Implications of leaf longevity, leaf nutrient re-absorption and translocation for the resource economy of five evergreen plant species. – Oikos 56: 121–131.

Killingbeck, K. T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. – Ecology 77: 1716–1727.

Lambers, H. et al. 1998. Plant physiological ecology. – Springer.

Lamont, B. B. and Southall, K. J. 1982. Distribution of mineral nutrients between the mistletoe, *Amyema preissii*, and its host, *Acacia acuminata*. – Ann. Bot. 49: 721–725.

March, W. A. and Watson, D. M. 2007. Parasites boost productivity: effects of mistletoe on litterfall dynamics in a temperate Australian forest. – Oecologia 154: 339–347.

March, W. A. and Watson, D. M. 2010. The contribution of mistletoes to nutrient returns: evidence for a critical role in nutrient cycling. – Austral Ecol. 35: 713–721.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. – Annu. Rev. Ecol. Syst. 11: 119–161.

New, M. et al. 2002. A high-resolution data set of surface climate over global land areas. – Climate Res. 21: 1–25.

Niinemets, U. 2007. Photosynthesis and resource distribution through plant canopies. – Plant Cell Environ. 30: 1052–1071.

NPWS 2000. Royal National Park, Heathcote National Park and Garawarra State Recreation Area Plan of Management. – NSW National Parks and Wildlife Service, Sydney.

NPWS 2002. Ku-ring-gai Chase National Park and Lion Island, Long Island and Spectacle Island Nature Reserves. – Office of Environment and Heritage, Sydney.

Ono, K. et al. 1996. Interaction between nitrogen deficit of a plant and nitrogen content in the old leaves. – Plant Cell Physiol. 37: 1083–1089.

Pate, J. et al. 1991. Partitioning of dry matter and mineral nutrients during a reproductive cycle of the mistletoe *Amyema linophyllum* (Fenzl.) Tieghem parasitizing *Casuarina obesa* Miq. – J. Exp. Bot. 42: 427–439.

Potter, C. S. 1991. Nutrient leaching from *Acer rubrum* leaves by experimental acid rainfall. – Can. J. For. Res. 21: 222–229.

Reich, P. et al. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. – Ecol. Monogr. 62: 365–392.

Scalon, M. C. and Wright, I. J. 2015. A global analysis of water and nitrogen relationship between mistletoes and their hosts: broad-scale tests of old and enduring hypotheses. – Funct. Ecol. 29: 1114–1124.

Scalon, M. C. et al. 2013. A comparative study of aluminium and nutrient concentrations in mistletoes on aluminium-accumulating and non-accumulating hosts. – Plant Biol. 15: 851–857.

Scalon, M. C. et al. 2016. Data from: To recycle or steal? Nutrient resorption in Australian and Brazilian mistletoes from three low-phosphorus sites. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.cs3h1>.

Schulze, E. D. et al. 1984. Carbon, water and nutrient relations of two mistletoes and their hosts: a hypothesis. – Plant Cell Environ. 7: 293–299.

Tukey Jr, H. 1970. The leaching of substances from plants. – Annu. Rev. Plant Physiol. 21: 305–324.

Van Heerwaarden, L. et al. 2003. Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. – Oikos 101: 664–669.

Vergutz, L. et al. 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. – Ecol. Monogr. 82: 205–220.

Vitousek, P. M. and Howarth, R. W. 1991. Nitrogen limitation on land and in the sea: how can it occur? – Biogeochemistry 13: 87–115.

Wang, W. Q. et al. 2003. Seasonal changes in element contents in mangrove element retranslocation during leaf senescene. – Plant Soil 252: 187–193.

Williams, R. et al. 1997. Leaf phenology of woody species in a north Australian tropical savanna. – Ecology 78: 2542–2558.

Wright, I. J. and Westoby, M. 2003. Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. – Funct. Ecol. 17: 10–19.

Wright, I. J. et al. 2002. Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. – J. Ecol. 90: 534–543.

Wright, I. J. et al. 2004. The worldwide leaf economics spectrum. – Nature 428: 821–827.

Wright, I. J. et al. 2006. Gradients of light availability and leaf traits with leaf age and canopy position in 28 Australian shrubs and trees. – Funct. Plant Biol. 33: 407–419.

Supplementary material (available online as Appendix oik-03455 at <www.oikosjournal.org/appendix/oik-03455>). Appendix 1.