BSJ

Species-specific nitrogen resorption proficiency in legumes and nonlegumes

Shimpei Oikawa[1] · Yusuke Matsui[1] · Michio Oguro[2] · Masanori Okanishi[3] · Ryo Tanabe[1] · Tomoki Tanaka[1] · Ayaka Togashi[1] · Tomoyuki Itagaki[4]

Abstract

Nitrogen (N) resorption from senescing leaves enables plants to reuse N, thereby making them less dependent on current N uptake from the environment. Therefore, N resorption is important for survival and fitness, particularly for plants growing under low N supply. We studied N resorption from senescing leaves of 25 legumes and 25 nonlegumes in a temperate region of Japan to test the hypothesis that high N resorption has not evolved in legumes that fix atmospheric N_2. The extent of N resorption was quantified by N resorption proficiency that is measured as the level to which leaf N concentration was reduced during senescence, i.e., the lower the senesced leaf N concentration, the lower the N loss through leaf fall and higher the N resorption proficiency. In support of the hypothesis, senesced leaf N concentration was higher in legumes than in nonlegumes, but there was considerable overlap between the groups. The higher senesced leaf N concentration of legumes was associated with a lower proportion of leaf N resorbed during senescence, particularly in species with higher leaf N concentrations. According to a hierarchical partitioning analysis, there was a large contribution of species to the total variance in the senesced leaf N concentration as opposed to a minor contribution of functional group (legume/nonlegume). This study reveals that legumes are not proficient at resorbing N from senescing leaves but that N_2-fixation might not be the single most important determinant of N resorption.

✉ Shimpei Oikawa
  shimpei.oikawa.dx@vc.ibaraki.ac.jp

[1] Graduate School of Science and Engineering, Ibaraki University, Mito 310-0056, Japan

[2] Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

[3] Graduate School of Science, Misaki Marine Biological Station, The University of Tokyo, 1024 Koajiro, Misaki, Miura, Kanagawa 238-0225, Japan

[4] Graduate School of Life Sciences, Tohoku University, Sendai 980-8578, Japan

Introduction

Nitrogen (N) resorption from senescing leaves is an adaptive strategy of plants for N conservation. It enables plants to reuse N, thereby making them less dependent on current N uptake from the environment, leading to a higher growth, survival, and fitness, particularly under low N supply. Nitrogen resorption also plays a role in influencing ecosystem N cycling as N that is not resorbed returns to soils later on as litterfall and fertilize ecosystems. The extent of N resorption from senescing leaves has been quantified by the N concentration in senesced leaves; the lower the N concentration in the senesced leaves, the lower the N loss through leaf fall and higher the N resorption proficiency (Killingbeck 1996). Many researchers have investigated the relationship between senesced leaf N concentration and soil fertility, most of them reporting that the senesced leaf N concentration was lower in species growing in N-poor environments than species growing in N-rich environments (e.g., del Arco et al. 1991;

Drenovsky et al. 2019; Hayes et al. 2014; Lal et al. 2001; Rejmánková 2005). Thus, natural selection for N conservation may have acted to reduce N concentration in senesced leaves (Killingbeck 1996).

These observations have led to the hypothesis that legume (Fabaceae) species that form root nodules and are capable of fixing atmospheric N_2 have not evolved efficient N resorption, if the energetic cost of resorbing leaf N is higher than that of fixing atmospheric N (Killingbeck and Whitford 2001). The tendency for N concentration of green leaves to be higher in legumes than in nonlegumes was reported in literature data compilations (Adams et al. 2016; Waterman 1994) while no consistent difference has been found in the proportion of N resorbed from their green leaves during senescence (Hayes et al. 2014; Norris and Reich 2009). Therefore, it is expected that N concentration in senesced leaves is higher in legumes than in nonlegumes. However, there has been mixed support for the hypothesis (e.g., Chuyong et al. 2000; Hayes et al. 2014; Inagaki et al. 2011; Killingbeck and Whiford 2001; Lal et al. 2001; Liang et al. 2015; Norris and Reich 2009; Yuan et al. 2005), which may be attributable partly to the small number of legume species studied.

Biological categories such as growth habit or taxon also can be the predictor variables which affect N resorption from senescing leaves (Drenovsky et al. 2019; González-Zurdo et al. 2015; Norris and Reich 2009; Vergutz et al. 2012; Yuan and Chen 2009). It would be particularly interesting to see if N resorption is different among legume subfamilies. As far as is known, most species of subfamilies Papilionoideae and Mimosoideae form N_2-fixing root nodules, whereas very few species of subfamily Caesalpinioideae are known to nodulate[1] (de Faria et al. 1989; Doyle 1998; Sprent 2001). If the availability of N is different among the subfamilies, they may exhibit different extents of N resorption, leading to a large variation in N resorption within the group.

In this study, we investigated N resorption from senescing leaves of 25 legumes and 25 sympatrically growing nonlegumes in a temperate region of Japan. The samples were collected from relatively small areas, thereby minimizing potential influence of climates on N resorption. Our aim was to test the following hypotheses: (1) senesced leaf N concentration is higher (i.e., N resorption proficiency is lower) in legumes than in nonlegumes. (2) The higher N concentration in senesced leaves of legumes is associated with a higher N concentration in green leaves. In addition, the relative importance of functional group (legume/nonlegume),

legume subfamily, species and growth habit to senesced leaf N concentration was explored using a hierarchical partitioning approach.

Materials and methods

Selection of species and sites

Study sites were chosen to include legume species that are common in temperate regions in Japan (Table 1). Most species in papilionoid and mimosoid subfamilies are known to nodulate. For the caesalpinioid species, previous reports found no nodulation, with the exception of *Chamaecrista nomame* (Doyle and Luckow 2003; Sprent 2001), which is consistent with our field observation of the roots in these species. Nonlegume species that grew sympatrically with the legume species and varied in growth habit and phylogenetic position were chosen (Table 1). In this paper, plant systematics follows Yonekura and Kajita (2003). All wild species, with the exceptions of *Glycine max* subsp. *soja* and *Senna obtusifolia*, were sampled at road verges, abandoned fields, riparian forests, and shaded understory and edges of secondary deciduous forests. *G. max* subsp. *soja* and *S. obtusifolia* were obtained from an experimental garden at Ibaraki University. Crop species were raised at the experimental garden following standard agronomic practice in this region. For *G. max* subsp. *max*, *Phaseolus vulgaris*, *Psophocarpus tetragonolobus*, *Vigna angularis* var. *angularis*, *Cucurbita maxima*, *Raphanus sativus* var. *hortensis*, *Spinacia oleracea*, the seeds were sown in germination trays, and the seedlings were transplanted to a plowed field. The remaining two crops (*Apios americana* and *Colocasia esculenta*) were raised from tubers in the field. All crops were grown under full sunlight and were watered and sprayed with insecticides whenever necessary. The mean annual air temperature taken at a meteorological observatory at Mito, Ibaraki (36° 23′N, 140° 28′E) was 14.8 °C in 2015, 14.8 °C in 2016 and 15.5 °C in 2017. The annual precipitation was 1227 mm, 1426 mm and 1103 mm, respectively.

Leaf sampling and nitrogen measurements

Leaves that were discolored (often brown, yellow and red in color) and detached easily from the plants by gentle flicking were defined as "fully senescent" for the majority of study species, and collected from various positions within the leaf canopy. For *Adenocaulon himalaicum*, *Colocasia esculenta*, *Commelina communis*, *Miscanthus sinensis*, *Raphanus sativus* var. *hortensis* and *Spinacia oleracea*, leaves that were discolored and apparently physiologically disconnected from the plants were cut off with scissors and collected. Leaves presenting any symptoms

---

[1] Recently, a new subfamilial classification of Fabaceae was proposed (Azani et al. 2017), but in this study, we adopt the traditional classification system in which the information on difference in nodulation status among subfamilies is well organized.

Table 1 The 25 legume (Fabaceae) species and the 25 non-legume species used in this study, along with their family, legume subfamily, growth habit, sampling site, year of sampled and the number of leaves sampled

Species	Family	Legume subfamily	Growth habit	Site	Year sampled	No. of green leaves	No. of dead leaves
Albizia julibrissin Durazz.	Fabaceae	Mimosoideae	Tree	1	2015–2017	13	15
Amorpha fruticosa L.	Fabaceae	Papilionoideae	Shrub	2	2015–2017	20	20
Apios americana Medik.	Fabaceae	Papilionoideae	Herb	3	2016–2017	6	8
Caesalpinia decapetala (Roth) Alston var. *japonica* (Siebold et Zucc.)	Fabaceae	Caesalpinioideae	Shrub/liana	1	2015–2017	11	16
Caragana sinica (Buc'hoz) Rehder	Fabaceae	Papilionoideae	Shrub	4	2015–2016	12	16
Cercis chinensis Bunge	Fabaceae	Caesalpinioideae	Tree	4	2015–2017	15	10
Chamaecrista nomame (Makino) H.Ohashi	Fabaceae	Caesalpinioideae	Herb	2	2016–2017	8	7
Desmodium paniculatum (L.) DC.	Fabaceae	Papilionoideae	Herb	2	2015–2017	10	10
Dumasia truncata Siebold et Zucc.	Fabaceae	Papilionoideae	Herb	5	2016–2017	7	9
Euchresta japonica Hook.f. ex Maxim.	Fabaceae	Papilionoideae	Shrub	6	2015	9	9
Gleditsia japonica Miq.	Fabaceae	Caesalpinioideae	Tree	7	2015–2017	14	17
Glycine max (L.) Merr. subsp. *max* (cv. Tachinagaha)	Fabaceae	Papilionoideae	Herb	3	2015–2017	15	14
Glycine max (L.) Merr. subsp. *soja* (Siebold et Zucc.) H.Ohashi	Fabaceae	Papilionoideae	Herb	3	2015–2017	10	14
Indigofera pseudotinctoria Matsum.	Fabaceae	Papilionoideae	Shrub	2	2015–2017	15	13
Lespedeza bicolor Turcz.	Fabaceae	Papilionoideae	Shrub	2	2015–2017	10	14
Lespedeza cuneata (Dum.Cours.) G.Don	Fabaceae	Papilionoideae	Herb	2	2015–2017	13	10
Lespedeza pilosa (Thunb.) Siebold et Zucc.	Fabaceae	Papilionoideae	Herb	2	2016–2017	6	6
Phaseolus vulgaris L. (cv. Kentucky Wonder)	Fabaceae	Papilionoideae	Herb	3	2015–2017	13	6
Psophocarpus tetragonolobus (L.) DC.	Fabaceae	Papilionoideae	Herb	3	2015–2017	15	12
Pueraria lobata (Willd.) Ohwi	Fabaceae	Papilionoideae	Shrub/liana	2	2015–2017	18	18
Robinia pseudoacacia L.	Fabaceae	Papilionoideae	Shrub	8	2015–2017	17	16
Senna obtusifolia (L.) H.S.Irwin et Barneby	Fabaceae	Caesalpinioideae	Herb	3	2016–2017	10	7
Vigna angularis (Willd.) Ohwi et H.Ohashi var. *angularis* (cv. Tanbadainagon)	Fabaceae	Papilionoideae	Herb	3	2015–2017	13	13
Vigna angularis (Willd.) Ohwi et H.Ohashi var. *nipponensis* (Ohwi) Ohwi et H.Ohashi	Fabaceae	Papilionoideae	Herb	1	2015–2017	5	13
Wisteria floribunda (Willd.) DC.	Fabaceae	Papilionoideae	Shrub/liana	5	2015–2017	11	12
Acer crataegifolium Siebold et Zucc.	Sapindaceae		Tree	5	2015–2017	30	20
Adenocaulon himalaicum Edgew.	Asteraceae		Herb	7	2017	5	5
Akebia trifoliata (Thunb.) Koidz.	Lardizabalaceae		Shrub/liana	5	2016–2017	7	5
Aucuba japonica Thunb. var. *japonica*	Aucubaceae		Shrub	5	2015–2017	15	16
Calystegia pubescens Lindl. f. *major* (Makino) Yonek.	Convolvulaceae		Herb	4	2015	5	6
Camellia japonica L.	Theaceae		Tree	5	2015–2016	9	10
Carpinus tschonoskii Maxim.	Betulaceae		Tree	5	2015–2017	19	28
Castanea crenata Siebold et Zucc.	Fagaceae		Tree	5	2015–2017	13	9
Cerasus jamasakura (Siebold ex Koidz.) H.Ohba	Rosaceae		Tree	5	2015–2017	10	18
Chenopodium album L.	Amaranthaceae		Herb	4	2015–2017	15	13
Colocasia esculenta (L.) Schott (cv. Yatsuga-shira)	Araceae		Herb	3	2016–2017	8	4
Commelina communis L.	Commelinaceae		Herb	2	2017	5	5
Cornus controversa Hemsl. ex Prain	Cornaceae		Tree	5	2016–2017	9	7
Cucurbita maxima Duchesne ex Lam. (cv. Kurikabocha)	Cucurbitaceae		Herb	3	2015–2017	9	4
Daphniphyllum macropodum Miq.	Daphniphyllaceae		Tree	5	2016–2017	5	9

Table 1 (continued)

Species	Family	Legume subfamily	Growth habit	Site	Year sampled	No. of green leaves	No. of dead leaves
Dendropanax trifidus (Thunb.) Makino ex H.Hara	Araliaceae		Tree	5	2015–2017	8	11
Eurya japonica Thunb. var. *japonica*	Ternstroemiaceae		Shrub	6	2015	7	6
Mallotus japonicus (L.f.) Müll.Arg.	Euphorbiaceae		Tree	1	2015–2017	14	12
Miscanthus sinensis Andersson	Poaceae		Herb	1	2016–2017	10	10
Padus grayana (Maxim.) C.K.Schneid.	Rosaceae		Tree	5	2015–2017	5	14
Quercus glauca Thunb.	Fagaceae		Tree	5	2015–2017	13	13
Quercus myrsinifolia Blume	Fagaceae		Tree	5	2015–2017	11	15
Raphanus sativus L. var. *hortensis* Backer (cv. Haruyutaka)	Brassicaceae		Herb	3	2015–2017	15	16
Solidago altissima L.	Asteraceae		Herb	1	2015–2017	10	16
Spinacia oleracea L. (cv. Asagiri)	Amaranthaceae		Herb	3	2015	5	5

Site: 1 road verges, Hitachiohmiya, Ibaraki [36° 36′N, 140° 18′E], 2 road verges, Mito, Ibaraki [36° 25′N, 140° 22′E], 3 an experimental garden, Mito, Ibaraki [36° 24′N, 140° 26′E], 4 abandoned fields, Mito, Ibaraki [36° 24′N, 140° 26′E], 5 secondary deciduous forests, Mito, Ibaraki [36° 25′N, 140° 22′E], 6 secondary decidulus forests, Kamogawa, Chiba [35° 09′N, 140° 08′E], 7 secondary deciduous forests, Shirosato, Ibaraki [36° 32′N, 140° 19′E], 8 riparian forests, Mito, Ibaraki [36° 22′N, 140° 27′E])

Nomenclature follows Yonekura and Kajita (2003)

of shrinkage and damage by herbivory or other factors, or those that had fallen on the ground were avoided. Collection dates ranged from August to December as the timing of leaf senescence varied among species. For each species, 4–28 fully senesced leaves were collected from several clumps or individuals, which were living within a 0.5 km radius in most cases, for three years (2015–2017, Table 1). Fresh green leaves were also sampled from the outer canopy between June and August (5–30 leaves per species). Care was taken to select fully expanded leaves that did not show symptoms of senescence, damage by herbivores, or any disease.

Leaf blades (hereafter "leaves") were scanned on a flatbed scanner (GT-X750, Seiko Epson Corporation, Nagoya, Japan) and their projected area was measured with an image-processing software (Image J, National Institutes of Health, Maryland, USA). Leaf samples were weighed after oven-drying at 70 °C for at least 48 h, and the N concentration (mass-based) was determined using an N/C analyzer (Sumigraph NC-95A; Sumika-Bunseki, Osaka, Japan). Area-based leaf N concentration was calculated by multiplying mass-based N concentration by leaf dry mass per unit leaf area (LMA). Quantification of N resorption using senesced leaf N concentration can be confounded by changes in the measurement basis (i.e., leaf mass and area) during leaf senescence. A literature survey by van Heerwaarden et al. (2003a) suggested that senescence-related changes in leaf mass were particularly large. Thus, to account for senescence-related changes in leaf mass, we corrected the mass-based N concentration by multiplying it with the mass loss correcting

factor (*m*, van Heerwaarden et al. 2003a, Vergutz et al. 2012):

$$m = 1 - \left(1 - \frac{\text{Dry mass of senesced leaf}}{\text{Dry mass of green leaf}} \right). \qquad (1)$$

Senesced leaf N concentration was related to green leaf N concentration and the proportion of leaf N resorbed during senescence (r_N) using the following equation:

Senesced leaf N concentration
$$= \text{Green leaf N concentration} \times (1 - r_N). \qquad (2)$$

Data analyses

Although most statistical analyses assume all observations to be independent, interspecific comparisons of N resorption could violate this assumption if closely related species have similar trait values due to their shared phylogenetic history (Felsenstein 1985). In this study, we treated the observations for each species to be independent because our preliminary analyses did not show significant phylogenetic signals in the senesced leaf N concentration (Electronic supplementary material 1).

Mean leaf N concentration of each functional group (legume or nonlegume) was calculated as the sum of the mean leaf N concentration of species in each group, divided by the number of species. Mean leaf N concentration of each subfamily (Papilionoideae or Caesalpinioideae) was calculated as well; it was not calculated for Mimosoideae as we

had only one species. Mean leaf N concentration of woody (tree, shrub, and liana) and herbaceous species was also calculated for each group. Data on leaf N concentration over the years were pooled as we observed no consistent interannual trend among species. Welch's t test was used to compare leaf variables (leaf N concentration and LMA) between legumes and nonlegumes, Papilionoideae and Caesalpinioideae, and woody and herbaceous species. Pearson's correlation coefficient test was used to find whether senesced leaf N concentration varied as a function of green leaf N concentration or r_N, and the difference in the regression slope between functional group was tested using analysis of covariance (ANCOVA). All statistical analyses were conducted using an open source statistical software R, version 3.5.1 (R Core Team 2019).

A hierarchical partitioning analysis was used to determine the relative importance of four categorical variables to senesced leaf N concentration: functional group (legume/nonlegume), subfamily (Papilionoideae/Caesalpinioideae), species and growth habit (woody/herbaceous species). This analysis investigated the average increase in the goodness-of-fit of all models with a particular variable compared with the equivalent model without that variable. We specified a linear model using a gamma distribution with r^2 value as the measure of goodness-of-fit. Analyses were carried out using the *hier.part* function in the *hier.part* package of R (Walsh and Mac Nally 2013).

Results

Senesced leaf N concentration per unit mass was significantly higher in legumes than in nonlegumes ($t = 4.24$, $P < 0.001$), but with considerable overlap between the functional groups (Fig. 1a). Quantification of N resorption using senesced leaf N concentration per unit mass can be largely confounded by senescence-related changes in leaf mass (see "Materials and methods"), but we found that the correction factor (m, Eq. 1) was similar between the legumes (mean ± standard deviation, $m = 0.99 \pm 0.31$) and the nonlegumes ($m = 0.93 \pm 0.26$), indicating that the corrected values (Fig. 1a) were useful for comparing between the functional groups. The difference between functional groups was marginally significant when N concentration was expressed on an area basis ($t = 1.73$, $P = 0.090$, Fig. 1b). Green leaf N concentration was higher in legumes than in nonlegumes when expressed on a leaf mass basis ($t = 3.63$, $P < 0.001$, Fig. 1d), but it was not different between the groups when expressed on a leaf area basis ($t = 0.16$, $P = 0.877$, Fig. 1e). The LMA was lower in legumes than in nonlegumes for both senesced leaves ($t = -2.51$, $P = 0.016$, Fig. 1c) and green leaves ($t = -3.00$, $P = 0.005$, Fig. 1f).
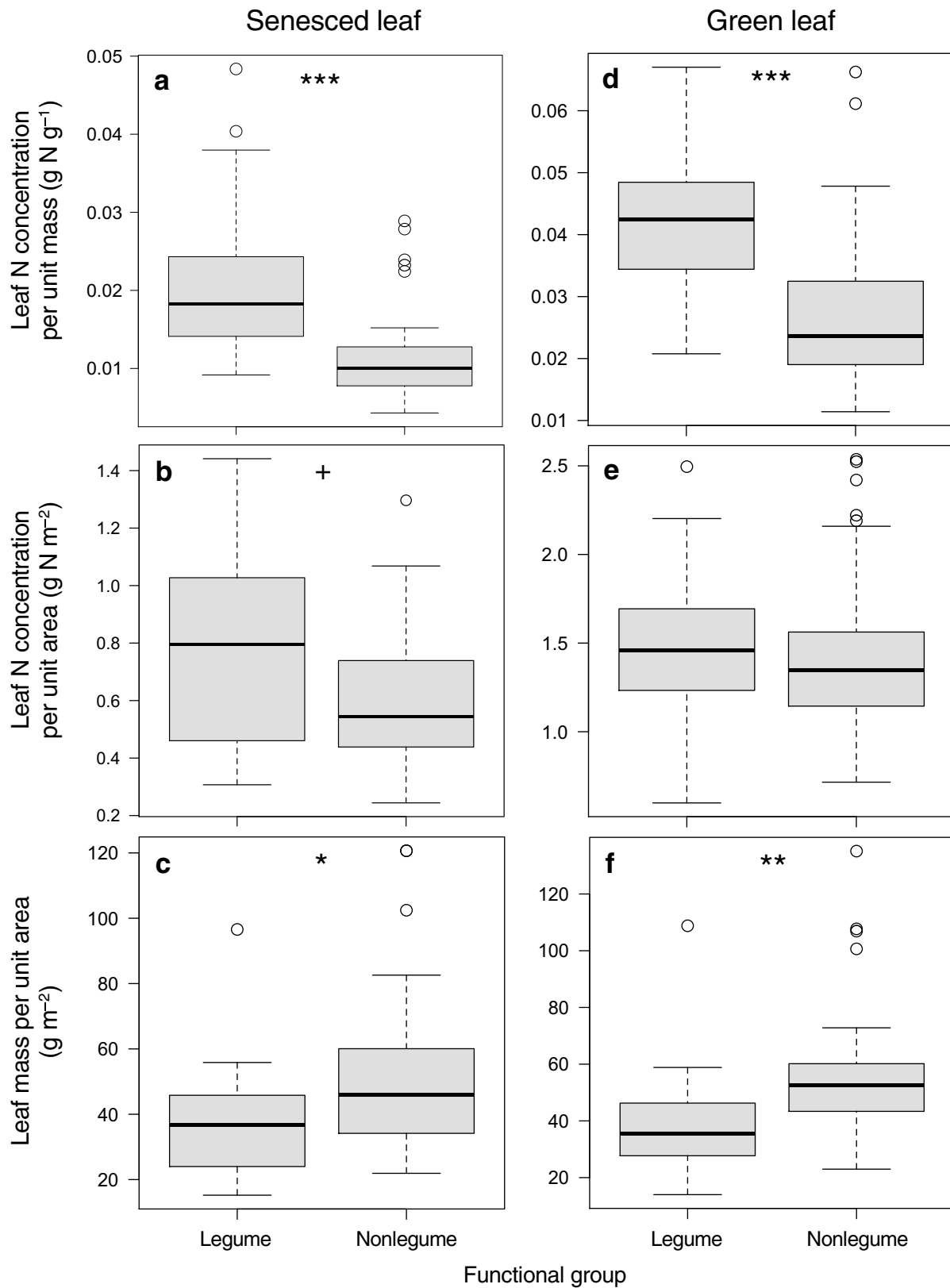
The senesced leaf N concentration per unit area was lower in caesalpinioid species than in papilionoid species, but the replication of Caesalpinioideae was low (5 species) and the difference was not statistically significant ($t = -0.38$, $P = 0.717$, Table 2). *Albizia julibrissin*, the only species in this study belonging to the subfamily Mimosiodeae, showed the highest senesced leaf N concentration across all species (1.44 g m^{-2}). Senesced leaf N concentration was marginally significantly higher in woody than herbaceous species in legume ($t = -1.90$, $P = 0.070$), and there was no significant difference in nonlegume ($t = 0.15$, $P = 0.886$) (Table 3).

Senesced leaf N concentration was positively correlated with green leaf N concentration in both legumes ($r = 0.54$, $P = 0.006$) and nonlegumes ($r = 0.49$, $P = 0.013$) (Fig. 2a). The slope of the regression line was steeper in legumes than in nonlegumes (significant green leaf N concentration-by-group interaction in ANCOVA, $P < 0.001$), indicating that r_N (Eq. 2) was lower in legumes than in nonlegumes when species with high leaf N concentration were compared. Senesced leaf N concentration negatively correlated with r_N in both legumes ($r = 0.71$, $P < 0.001$) and nonlegumes ($r = 0.57$, $P = 0.010$), and the slope of the regression line was not significantly different between the functional groups ($P = 0.255$, Fig. 2b).

Using hierarchical partitioning to assess the importance of each of the four categorical variables (functional group, subfamily, species and growth habit) on the total variance in senesced leaf N concentration per unit area, we found that functional group and subfamily had low independent contributions (2.0% and 7.6%, respectively) (Fig. 3). The joint contributions of functional group and subfamily were 3.8% and 9.8%, respectively. The total variance in senesced leaf N concentration was largely explained by species (the independent and the joint contributions were 89.7% and 10.3%, respectively), in agreement with the observation that the interspecific difference in senesced leaf N concentration was very large in each functional group (Fig. 1a). The independent and the joint contributions of growth habit were negligible.

Discussion

Our results supported the hypothesis that legumes retain more N in their senesced leaves than nonlegumes do, consistent with the synthesis of existing data from different terrestrial biomes worldwide (Drenovsky et al. 2019; Vergutz et al. 2012). The present study investigated many sympatrically growing species subjected to similar climatic conditions, by which confounding effects of temperature and rainfall on N resorption (Yuan and Chen 2009) would have been minimized. While we focused on Fabaceae, the third largest flowering family, Stewart et al. (2008) studied many

◄**Fig. 1** Nitrogen concentration expressed on a leaf mass basis (**a**, **d**) and on a leaf area basis (**b**, **e**), and leaf mass per unit area (**c**, **f**) of senesced leaf and green leaf of legumes ($n=25$) and nonlegumes ($n=25$). Solid black lines represent the medians, top and bottom of boxes the first and last quartiles, whiskers the interquartile distance multiplied by 1.5 and open circles the outliers. Senesced leaf N concentration expressed on a leaf mass basis was corrected for changes in leaf mass during leaf senescence. ***, **, * and + indicate a significance level of $P<0.001$, $P<0.010$, $P<0.050$ and $P<0.100$, respectively

actinorhizal species (mostly *Alnus*) and arrived at the same conclusion. Taken together, we conclude that selection pressures for N conservation have been lower in N_2-fixing plants than in non-N_2-fixing plants.

The higher senesced leaf N concentration in legumes was associated with a lower proportion of green leaf N resorbed during leaf senescence (r_N). Some studies also found that the r_N was lower in legumes than in nonlegumes (Norris and Reich 2009; Yuan et al. 2005) but others did not (Finzi et al. 2001; Hayes et al. 2014). We further found that the difference in r_N between functional groups (i.e., the difference in senesced leaf N concentration between legumes and nonlegumes at a given green leaf N concentration, Fig. 2a) increased with increasing green leaf N concentration, and both the r_N and the senesced leaf N concentration were similar between legumes and nonlegumes when their green leaf N concentration was low (see also Electronic supplementary material 2). This suggests that N resorption is as critical for legumes as nonlegumes when plant N status is poor, and may explain the inconsistent results among studies. Occasionally, r_N is referred to as "N resorption efficiency" and has been used to measure N resorption ability (e.g., Aerts 1996; Aerts and Chapin 2000), but we analyzed it as an explanatory factor of "N resorption proficiency" (senesced leaf N concentration, Eq. 2). N resorption proficiency is more appropriate for quantifying N resorption because it is not subject to the temporal variation in green leaf N concentration, and thus to the timing of sampling (Killingbeck 1996; Kobe et al. 2005; Norris and Reich 2009; van Heerwaarden et al. 2003b). In addition, previous studies found that N resorption proficiency was more responsive to plant N status than N resorption efficiency (Killingbeck 1996; Oikawa et al. 2017; Ratnam et al. 2008). Thus, we considered N resorption proficiency as a suitable metric for detecting differences in N resorption ability between legumes and nonlegumes.

We found that the extent of N resorption was lower in legume species with greater plant N status than other species with lower N status, but which steps in a series of N resorption processes (e.g., degradation of leaf proteins, phloem loading and abscission of leaves) are the targets of selection remains unclear. Recently, we quantified levels of leaf protein degradation that occurred preceding N resorption, and

found that legumes retained more proteins (in particular, the cell wall proteins) in their senesced leaves than nonlegume did (Ryo Tanabe and Shimpei Oikawa, unpublished results). The greater cell wall proteins in senesced leaves were attributable to the lower protein degradability of legumes, and no difference in the cell wall protein in green leaves between legumes and nonlegumes was found. Further, the degradability of other proteins was not different between the groups. These observations may suggest that symbiotic N_2-fixation was less costly than degradation of cell wall proteins, and therefore, the r_N of legumes was lower, with more N remaining in the senesced leaves.

Senesced leaf N concentration was higher in legumes than in nonlegumes, whereas green leaf N concentration was not different between functional groups when they were expressed on an area basis (Fig. 1b, e). On the other hand, the N concentration per unit mass was higher in legumes than in nonlegumes for both senesced and green leaves (Fig. 1a, d). Adams et al. (2016) also found that the difference in green leaf N concentration between legumes and nonlegumes was more evident when N concentration was expressed on a leaf mass basis than on a leaf area basis. Area-based and mass-based N concentrations are linked by LMA: area-based N concentration = mass-based N concentration × LMA. In the present study, the average LMA was lower in legumes than in nonlegumes because 7 of the 25 nonlegumes had sclerophyllous, evergreen leaves, whereas there was only 1 evergreen species among the legumes as evergreen legumes were very rare in the study area. The lower LMA of the legume species offset the higher mass-based N concentration, and the area-based N concentration was close to that of nonlegumes. Thus, area-based N concentration should be interpreted carefully when distribution of evergreen species between legumes and nonlegumes is uneven.

It should be emphasized that the contributions of species to the total variance in the senesced leaf N concentration was overwhelmingly greater than those of other predictor variables (Fig. 3), in agreement with the large interspecific differences in the senesced leaf N concentration in each functional group (Fig. 1). What are the factors explaining the interspecific differences in senesced leaf N concentration? The interspecific differences in green leaf N concentration and r_N would explain part of the difference in senesced leaf N concentration (Fig. 2). Species with high senesced leaf N concentration, green leaf N concentration and low r_N are abundant in nutrient-rich (Drenovsky et al. 2019; Li et al. 2020; Vergutz et al. 2012) and high-temperature and high-rainfall environments (Yuan and Chen 2009; Zhao et al. 2017). In the current study, however, air temperature and rainfall would not be very different among species because the sampling was conducted within relatively narrow geographic areas. The soil N status also did not differ greatly
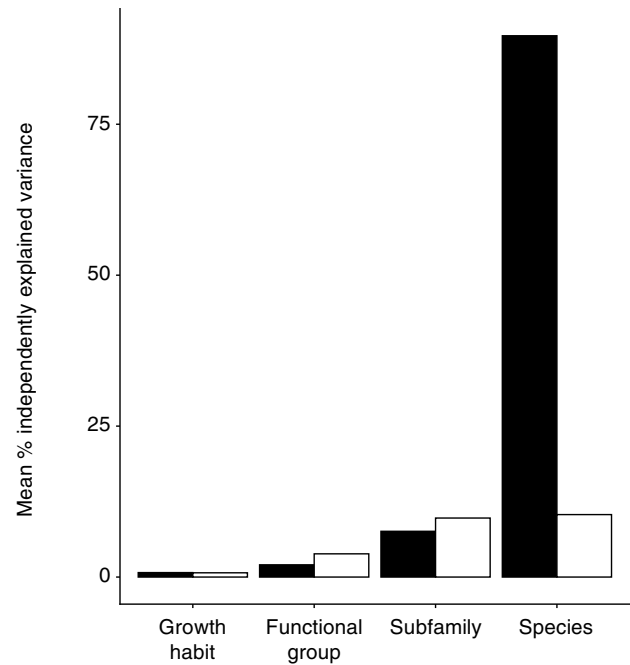
Table 2 Nitrogen concentration of fully senesced leaf of Papilionoideae and Caesalpinoideae

	Papilionoideae	Caesalpinioideae
Number of species	19	5
Senesced leaf N concentration (g N m^{-2})	0.74 ± 0.07	0.69 ± 0.13

Mean ± standard error. Leaf N concentration showed no significant difference between the subfamilies (Welch's t test, $P < 0.05$)
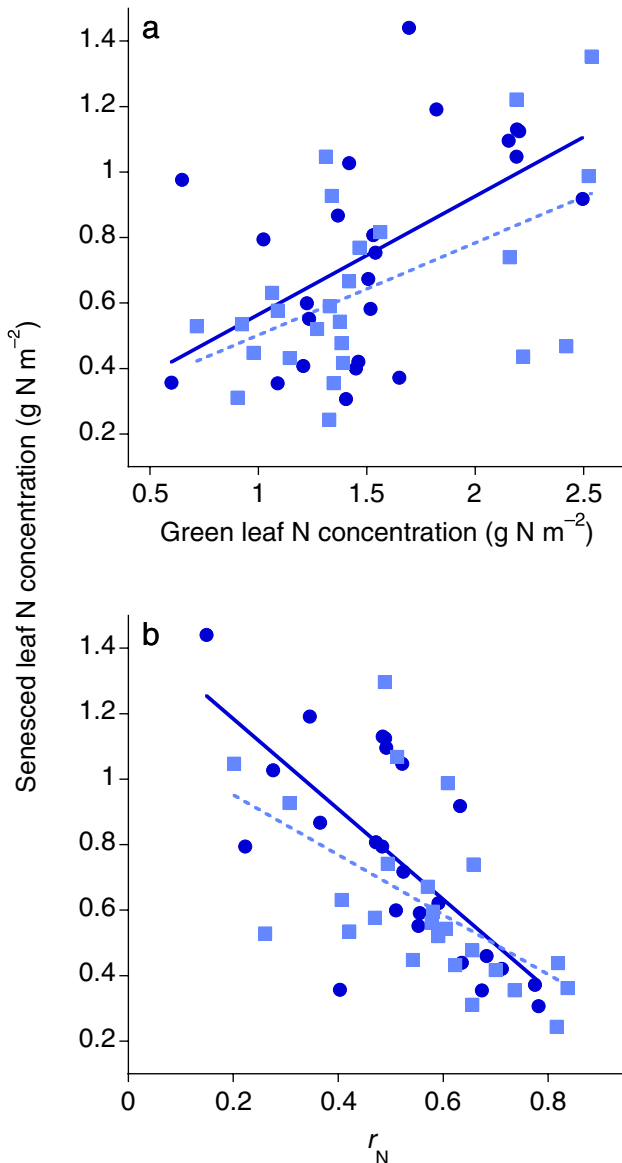
Fig. 2 **a** Senesced leaf nitrogen concentration of legume (circles) and nonlegume (squares) in relation to green leaf nitrogen concentration. **b** Senesced leaf nitrogen concentration in relation to proportion of green leaf nitrogen resorbed during senescence (r_N). Each point represents the mean value for each species

Fig. 3 Percentage of the variance in senesced leaf nitrogen concentration explained by four categorical variables; functional group (legume/nonlegume), subfamily (Papilionoideae/Caesalpinioideae), species and growth habit (woody/herbaceous species). Bars indicate the independent (black) and joint (white) effects

among study sites, and no significant correlation between the soil N availability and green leaf N concentration, r_N, or senesced leaf N concentration was detected in the current study (Electronic supplementary material 3), as was observed in an understory shrub guild in the United States (Killingbeck and Costigan 1988). Unfortunately, we have no data that allows further speculation the interspecific variation in N resorption.

There was no significant difference detected in the senesced leaf N concentration between subfamilies (Table 2), and the contribution of subfamily to the total variance in the senesced leaf N concentration was very small (Fig. 3). However, the nodulating caesalpinioid species *C. nomame* showed similar levels of N resorption (0.79 g N m^{-2}) to other nodulating legume species (mean, 0.78 g N m^{-2}), and the senesced leaf N concentration of non-nodulating caesalpinioid species (mean, 0.68 g N m^{-2}) was comparable to that of nonlegume species (mean, 0.62 g N m^{-2}). These observations would also support the idea that N resorption proficiency is low in N$_2$-fixing species. The lack of a statistically significant difference might be partly due to the small number of available caesalpinioid species in the study area. The tropics may offer excellent opportunities to further test this hypothesis, because majority of caesalpinioid species are distributed in the tropics, similar to those in Papilionoideae and Mimosoideae (Sprent 2001).

Table 3 Nitrogen concentration of fully senesced leaf of woody (tree, shrub, and liana) and herbaceous species

	Legume		Nonlegume	
	Woody species	Herbaceous species	Woody species	Herbaceous species
Number of species	12	13	15	10
Senesced leaf N concentration (g N m^{-2})	0.89 ± 0.08	0.66 ± 0.08	0.61 ± 0.07	0.63 ± 0.08

Mean ± standard error. Leaf N concentration showed no significant difference between woody and herbaceous species in either group (Welch's t test, $P < 0.05$)

In conclusion, legume species were not proficient at resorbing N from senescing leaves. This agrees with a generally accepted view that N-rich legume litters contribute significantly to N input in natural- and agricultural ecosystems. However, much of the variation in senesced leaf N concentration was explained by species, and the range of legumes and nonlegumes overlapped considerably. Our results suggest that species identity may be a stronger predictor of N resorption level, and that examining N_2-fixer vs non-N_2-fixer dichotomy may not always be appropriate when trying to understand the ecological and economical significance, and underlying mechanisms contributing to N resorption.

Compliance with ethical standards

References

Adams MA, Turnbull TL, Sprent JI, Buchmann N (2016) Legume are different: leaf nitrogen, photosynthesis, and water use efficiency. Proc Natl Acad Sci USA 113:4098–4103

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

Aerts R, Chapin FS III (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67

Azani N, Babineau M, Bailey CD et al (2017) A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon 66:44–77

Chuyong GB, Newbery DM, Songwe NC (2000) Litter nutrients and retranslocation in a central African rain forest dominated by ectomycorrhizal trees. New Phytol 148:493–510

de Faria SM, Lewis GP, Sprent JI, Sutherland JM (1989) Occurrence of nodulation in the Leguminosae. New Phytol 111:607–619

del Arco JM, Escudero A, Garrido MV (1991) Effects of site characteristics on nitrogen retranslocation from senescing leaves. Ecology 72:701–708

Doyle JJ (1998) Phylogenetic perspectives on nodulation: evolving views of plants and symbiotic bacteria. Trends Plant Sci 3:473–478

Doyle JJ, Luckow MA (2003) The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. Plant Physiol 131:900–910

Drenovsky RE, Pietrasiak N, Short T (2019) Global temporal patterns in plant nutrient resorption plasticity. Glob Ecol Biogeogr 28:728–743

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125:1–15

Finzi AC, Allen AS, DeLucia EH, Ellsworth DS, Schlesinger WH (2001) Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. Ecology 82:470–484

González-Zurdo P, Escudero A, Mediavilla S (2015) N resorption efficiency and proficiency in response to winter cold in three evergreen species. Plant Soil 394:87–98

Hayes P, Turner BL, Lambers H, Laliberté E (2014) Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. J Ecol 102:396–410

Inagaki M, Kamo K, Miyamoto K, Titin J, Jamalung L, Lapongan J, Miura S (2011) Nitrogen and phosphorus retranslocation and N: P ratios of litterfall in three tropical plantations: luxurious N and efficient P use by *Acacia mangiu*m. Plant Soil 341:295–307

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

Killingbeck K, Costigan SA (1988) Element resorption in a guild of understory shrub species: niche differentiation and resorption thresholds. Oikos 53:366–374

Killingbeck K, Whiford W (2001) Nutrient resorption in shrubs growing by design, and by default in Chihuahuan Desert arroyos. Oecologia 128:351–359

Kobe RK, Lepczyk CA, Iyer M (2005) Resorption efficiency decreases with increasing green leaf nutrients in a global data set. Ecology 86:2780–2792

Lal CB, Annapurna C, Raghubanshi AS, Singh JS (2001) Effect of leaf habit and soil type on nutrient resorption and conservation in woody species of a dry tropical environment. Can J Bot 79:1066–1075

Li L, Li X, Liu B, Lei J, Yue Z, Li C (2020) Imbalanced stoichiometric patterns in foliar nutrient resorption response to N and P addition in grazing alpine grassland. Acta Oecol 102:103505

Liang D, Zhang J, Zhang S (2015) Patterns of nitrogen resorption in functional groups in a Tibetan alpine meadow. Folia Geobot 50:267–274

Norris MD, Reich PB (2009) Modest enhancement of nitrogen conservation via retranslocation in response to gradients in N supply and leaf N status. Plant Soil 316:193–204

Oikawa S, Ehara H, Koyama M, Hirose T, Hikosaka K, Chen CP, Nakamura H, Sakai H, Tokida T, Usui Y, Hasegawa T (2017) Nitrogen resorption in senescing leaf blades of rice exposed to free-air CO_2 enrichment (FACE) under different N fertilization levels. Plant Soil 418:231–240

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ratnam J, Sankaran M, Hanan NP, Grant RC, Zmbatis N (2008) Nutrient resorption patterns of plant functional groups in a tropical savanna: variation and functional significance. Oecologia 157:141–151

Rejmánková E (2005) Nutrient resorption in wetland macrophytes: comparison across several regions of different nutrient status. New Phytol 167:471–482

Sprent JI (2001) Nodulation in legumes. The Royal Botanic Gardens, Kew

Stewart JR, Kennedy GJ, Landers RD, Dawson JO (2008) Foliar-nitrogen and phosphorus resorption patterns differ among nitrogen-fixing and nonfixing temperate-deciduous trees and shrubs. Int J Plant Sci 169:495–502

van Heerwaarden LM, Toet S, Aerts R (2003a) Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. Oikos 101:664–669

van Heerwaarden LM, Toet S, Aerts R (2003b) Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. J Ecol 91:1060–1070

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol Monogr 82:205–220

Walsh C, Mac Nally R (2013) Package 'hier.part'. https://cran.r-project.org/web/packages/hier.part/index.html

Waterman PG (1994) Costs and benefits of secondary metabolites to the Leguminosae. In: Sprent JI, McKey D (eds) Advances in legume systematics 5: the nitrogen factor. Royal Botanic Gardens, Kew, pp 129–149

Yonekura K, Kajita T (2003) BG plant index (Y-List, https://ylist.info)

Yuan ZY, Chen HYH (2009) Global trends in senesced-leaf nitrogen and phosphorus. Glob Ecol Biogeogr 18:532–542

Yuan ZY, Li LH, Han XG, Huang JH, Jiang GM, Wan SQ, Zhang WH, Chen QS (2005) Nitrogen resorption from senescing leaves in 28 plant species in a semi-arid region of northern China. J Arid Environ 63:191–202

Zhao G, Shi P, Wu J, Xiong D, Zong N, Zhang X (2017) Foliar nutrient resorption patterns of four functional plants along a precipitation gradient on the Tibetan Changtang Plateau. Ecol Evol 7:7201–7212

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.