

NUTRIENT STOICHIOMETRY IN *PINGUICULA VULGARIS*: NUTRIENT AVAILABILITY, PLANT SIZE, AND REPRODUCTIVE STATUS

MARCOS MÉNDEZ^{1,2,4} AND P. STAFFAN KARLSSON^{1,3}

¹Department of Plant Ecology–Evolutionary Biology Center, Uppsala University, Villavägen 14, SE-752 36 Uppsala, Sweden

²Botanical Institute, Stockholm University, SE-106 91 Stockholm, Sweden

³Abisko Scientific Research Station, Royal Academy of Science, SE-981 07 Abisko, Sweden

Abstract. Current understanding of the extent, causes for, and consequences of variation in nutrient composition in plants is limited. Important questions to be addressed include to what extent nutrients covary, how flexible nutrient ratios are within a population or species, how reproduction influences nutrient ratios, and how much the ratios of nutrients to mass and nutrients to each other change through ontogeny. This information is needed to assess the physiological and ecological consequences of plant nutrient composition and to what extent plants function as balanced systems in acquisition and allocation of resources.

We studied the variation in nutrient stoichiometry (i.e., the ratio between contents of different nutrients within a plant) in relation to three factors: (1) environmental availability of nitrogen, (2) plant size, and (3) reproductive status. We investigated these questions in 11 populations of the carnivorous plant *Pinguicula vulgaris* in northern Scandinavia. Dry mass and N and P content were measured for reproductive and vegetative portions of flowering individuals and for winter buds corresponding to four reproductive states: control reproductive individuals, experimentally vegetative individuals (from which flower buds were removed), adult vegetative individuals, and individuals below the threshold size for reproduction.

[N], [P], and to a lesser extent, N and P content were positively related to soil N, but not to prey capture. Nutrient stoichiometry was also size dependent; in general, small plants were relatively enriched in N and relatively depleted in P compared to larger plants. Reproductive status affected not only size, but also nutrient stoichiometry of the resulting winter bud. Winter buds derived from reproductive individuals had a higher [N] and lower [P] than those of the different types of nonreproductive individuals. Our findings indicate that studies of nutrient stoichiometry in plants must go beyond links between environmental and plant nutrient concentrations to consider internal processes such as growth and reproduction.

Key words: carnivorous plant; *Pinguicula vulgaris*; plant nutrient stoichiometry; plant size; reproductive status; Scandinavia; size dependence; soil nutrient availability.

INTRODUCTION

Plants need carbon, water, and mineral nutrients to develop their vital functions (Larcher 1995). Although availability of those resources varies dramatically in space and time at a variety of scales, plants usually maintain tissue concentrations of carbon, water, and nutrients within much more restricted limits (Chapin et al. 1987). Using an economic analogy to study plant resource use, it has been proposed that plants act as balanced systems regarding acquisition and use of resources (Bloom et al. 1985, Chapin et al. 1987, Bazzaz 1997). Balanced, in this context, means that plants act in such a way that shortage or excess of a resource will

influence the way in which other resources are acquired and allocated, and that the resulting adjustment optimizes plant performance. Keeping a balance between resources entails homeostatic adjustment of resource concentrations by alteration of, among others, the root to shoot ratio, uptake efficiency of scarce or overabundant resources, and resource allocation patterns (Chapin et al. 1987). As the environment is always changing, the homeostatic ability of plants can be limited (Chapin et al. 1987). Thus, how constant should we expect plant nutrient concentration to be? Important questions that need to be addressed, following Bazzaz (1997), are to what extent nutrients covary, how flexible nutrient ratios are within a population or species, how reproduction influences nutrient ratios, and how much the ratios of nutrients to mass and nutrients to each other change through ontogeny.

In the following, we focus on dry mass (as an estimate of carbon) and two of the most important nutrients (namely N and P), which have been traditionally

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⁴ Present address: Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/ Tulipán s/n., E-28933 Móstoles, Madrid, Spain. E-mail: mmendez@escet.urjc.es

considered in studies of plant mineral nutrition (Bloom et al. 1985). A way to describe the relative content of nutrients in plants is to calculate nutrient ratios (e.g., Thompson et al. 1997). Here we adopt the term “nutrient stoichiometry” as a general description of a plant’s nutrient ratios. Nutrient stoichiometry is regularly used in interspecific comparisons within the study of food webs (e.g., Elser et al. 1996, Sterner and George 2000), but has also been used in intraspecific studies in plant ecophysiology (e.g., Loladze 2002).

Nutrient stoichiometry has potential important physiological and ecological consequences. For example, the leaf N:C ratio influences photosynthetic rate (Field and Mooney 1986). The P:C ratio is related to the maximum potential relative growth rate (Thompson et al. 1997). Nutrient stoichiometry in general (Ohlson and Staaland 2001), and N:C ratio in particular (Mattson 1980, Haukioja et al. 1991, Pérez-Harguindeguy et al. 2003), determines the quality of plant tissue as food for herbivores. The lignin : N ratio influences litter decomposition rate (Cornelissen 1996), which affects nutrient turnover in ecosystems. Tissue nutrient concentrations are also supposed to help identify ecological strategies of resource consumption and, thus, to predict the result of plant competitive interactions (Tilman 1982).

Variation in nutrient stoichiometry has been documented at several scales, including altitudinal and latitudinal variation (Körner 1989), changes between species of the same community (Garten 1978, Marris 1978, Ohlson and Staaland 2001), between and within populations of the same species (Benzing and Renfrow 1971, Thompson et al. 1997, Alonso and Herrera 2001, Ohlson and Staaland 2001, Orgeas et al. 2002), and seasonal changes within a species (Marris 1978, Shaver and Lechowicz 1985, Ohlson and Staaland 2001, Orgeas et al. 2002), including those related to organ age and senescence (Aerts 1996, Aerts and Chapin 2000, Orgeas et al. 2002). Within individuals and at a given time, nutrient stoichiometry also differs among plant organs (van Andel and Vera 1977, Benner and Bazzaz 1988, Ohlson and Malmer 1990, Andersson 1997). Despite all of this work, current understanding of the extent, causes for, and consequences of variation in nutrient composition in plants is limited (Poorter and Villar 1997, Alonso and Herrera 2001, Ohlson and Staaland 2001).

In the present paper we address three sources of variation in nutrient stoichiometry within plants, using two data sets gathered in 11 populations of the carnivorous plant *Pinguicula vulgaris* L. (Lentibulariaceae). In this way we aim at improving our understanding of the factors that can promote or limit the balances of nutrients in plants.

The first source of variation considered is environmental availability of nutrients. Usually, plant nutrient content has been studied in relation to nutrient availability in the soil (Ohlson 1986, Grimshaw and Allen

1987, Orgeas et al. 2002, Güsewell et al. 2003) or to other abiotic characteristics, e.g., pH and geological substrate (Thompson et al. 1997); CO₂ levels (Lutze and Gifford 1998, Marriott et al. 2001); salt spray (Benzing and Renfrow 1971). For carnivorous plants, plant nutrient content has also been related to prey capture and to the interaction between soil and prey nutrient availability (Adamec 1997). Although fertilization experiments induce an increase in nutrient tissue concentrations (Shaver and Lechowicz 1985), natural soil nutrient levels are often weakly correlated with plant nutrient stoichiometry (Garten 1978, Orgeas et al. 2002). Even when they significantly affect plant nutrient stoichiometry, soil nutrients can explain only a low percentage of variance in nutrient stoichiometry variation compared to other sources of variation, such as time (Orgeas et al. 2002). This poor correlation between soil and plant nutrient levels is due, in part, to differential absorption or exclusion of nutrients (Garten 1978), which is in agreement with the expectations of the “plant as a balanced system” hypothesis. Here we studied the relationship between nitrogen availability (both in soil and as prey) and nutrient stoichiometry.

The second, and previously mostly neglected, studied source of variation in nutrient stoichiometry is plant size. A “dilution” in N concentration with increasing size has been reported for some plants (Mooney and Chiariello 1984, Coleman et al. 1993, Ohnmeiss and Baldwin 1994, Lutze and Gifford 1998). To what extent such an effect is widespread in plants or present for other nutrients is currently unknown, despite its obvious bearing on plant nutrient balance and the potential relevance for photosynthetic performance and herbivory (see Field and Mooney 1986, Haukioja et al. 1991, Pérez-Harguindeguy et al. 2003). We examined the allometric variation in nutrient stoichiometry.

The third question deals with how nutrient stoichiometry is affected by reproduction. Reproduction has been predicted (Calow 1979) and empirically shown (Pakonen et al. 1988, Cipollini and Stiles 1991) to lead to resource depletion. In *P. vulgaris*, reproduction leads to smaller size in the following year and to a smaller nutrient pool (Thorén et al. 1996). In addition, reproduction increases N net losses (Eckstein and Karlsson 2001). This means that reproduction can entail not only resource depletion or decreased plant size, but also an imbalance among different nutrients. This imbalance can have consequences for future reproduction. Flowering is dependent not only on plant size, but also on sufficient N or P storage in some plant species (Wyka and Galen 2000). We assessed the differences in the total resource pool (in terms of dry mass, N, and P) and in nutrient stoichiometry for plants of *P. vulgaris* differing in reproductive status.

METHODS

Plant species and sampling sites

Pinguicula vulgaris is a perennial, herbaceous, carnivorous plant with circumboreal distribution. Its an-

nual cycle is as follows. The plant overwinters as a "winter bud" consisting of tightly packed scales around a meristem. In our field sites in northern Scandinavia, new leaves and roots develop from the winter bud in late May to early June. Leaves and roots last about four months and are shed at the end of each growing season. Flowering probability in *P. vulgaris* is size dependent (Worley and Harder 1999, Méndez and Karlsson 2004); plants below a certain threshold size do not flower. Flowering plants typically produce one (sometimes two to four) flower. In the second half of the growing season, while the seeds are still developing in the fruit capsule, the plant begins to form a new winter bud. At the end of the growing season, once the leaves and roots are shed, only the new winter bud and eventually the ripe seed capsule remain.

We carried out our study during summer 1997 at the Abisko Scientific Research Station, northern Sweden (68°21' N, 18°49' E, 380 m a.s.l.). In our study area, *P. vulgaris* is common in open habitats from nutrient-poor mires to rich calcareous soils. We sampled 11 populations covering the main habitats and altitudes in which *P. vulgaris* is present in northern Scandinavia: Norway 1, Norway 2, Abisko 2, and Abisko 2 at roadsides; Abisko 1, Paddus 1, and Paddus 2 at polygonal soils; Norway 3 and Katterjåkk at mires; and Njulla and Latnjajaure at alpine sites (for further details, see Méndez and Karlsson 2004: Table 1). The two most distant populations were ~80 km apart.

Sampling at flowering

During the flowering peak, we harvested 40–51 flowering individuals at each site. Individuals were chosen to cover the size range of reproductive plants within each population. Vegetative (leaves and roots) and reproductive (flowers) parts were oven-dried separately at 60°C for one week and the dry mass was obtained to the nearest 0.1 mg. N and P contents in vegetative and reproductive parts were obtained, after micro-Kjeldahl digestion, by means of a Flow Injection Analyzer (FIAstar 5010, Tecator, Höganäs, Sweden). N content could be reliably estimated in small samples (1–2 mg), but in order to get reliable results for P, samples larger than 5 mg were needed. Thus, in some of the analyses, original sample size was reduced due to these constraints, and no data were available for P in Norway 2, Abisko 1, Paddus 1, and Paddus 2.

Jointly with the plants, we took two soil samples per site as described in Méndez and Karlsson (2004). Samples were frozen until analysis. We extracted soil N with 0.2 mol/L KCl, and that extract was analyzed using a Flow Injector Analyzer as detailed in Méndez and Karlsson (2004). Due to the very heterogeneous nature of the soils among sites, N soil content was expressed on a per volume basis (excluding stones), as milligrams of N per liter.

From the flowering plants harvested, we took a subsample of 19–21 plants to estimate prey capture. We

counted all animals present in the leaves of each plant. Acarina were not considered as prey, because some of them are sapro-fagous, feeding on prey carcasses (Antor and García 1995). They also contribute a very small fraction of the mass of animals on the leaves (Karlsson et al. 1987). To account for individual differences in trapping surface, prey capture was expressed as prey number per unit size, estimated as the number of leaves times the length of the longest leaf (see Méndez and Karlsson 2004, for further details).

Sampling at the end of the growing season

At the beginning of the flowering period, we labeled 100 reproductive plants at each site. We left half of the plants (haphazardly chosen) as controls, and removed the flower bud(s) from the other half. The average dry mass removed in deflowered plants ranged from 0.5 to 2.4 mg (i.e., 10–40% of the final flower mass). In addition, we estimated the threshold size for reproduction at each population, as explained in Méndez and Karlsson (2004), and labeled 50 vegetative individuals that were below and above that size (not in Latnjajaure). At the end of the growing season, we harvested all of the winter buds from control, deflowered, and the two kinds of vegetative plants (35–50 winter buds per treatment were retrieved). We excluded from analysis those control plants that did not ripen a seed capsule. Winter buds were oven-dried at 60°C for one week and their dry mass was obtained to the nearest 0.1 mg. N and P content were obtained for the winter buds as previously explained. Sample sizes for P were reduced due to the constraints just explained, although data from all populations could be gathered for this data set.

Nutrient stoichiometry in relation to environmental N availability

We studied the relationship between nutrient stoichiometry and N availability for the samples taken at the flowering peak, by using redundancy analysis (RDA). RDA is an ordination method that summarizes overall variation in "species data" (nutrient stoichiometry in this case) in a few ordination axes that are linear combinations of the measured environmental variables. This is equivalent to a multivariate multiple regression of the nutrient stoichiometry variables on the environmental variables (ter Braak and Šmilauer 1998). We performed the analysis using CANOCO 4.0 (ter Braak and Šmilauer 1998). Using data taken at flowering peak, we calculated total (vegetative plus reproductive) dry mass, N and P content, as well as the following ratios: N : dry mass ([N] hereafter), P : dry mass ([P] hereafter), and P:N ratio. These variables were entered in RDA, and population (modeled as a dummy variable), average soil N content, and average prey capture (as a surrogate for prey N) were utilized as environmental variables. Previous studies have shown that prey-derived N uptake efficiency for *P. vulgaris* is ~41% (Hanslin and Karlsson 1996).

No colinearity was detected between the environmental variables. After deletion of missing data, 196 individuals from seven populations were utilized in this analysis.

Allometric changes in nutrient stoichiometry

Changes in [N], [P], and P:N ratio were studied by using reduced major axis (RMA), also called type II, regression, which is a statistical tool commonly used in allometric analyses (LaBarbera 1989). Pairs of variables were \log_{10} -transformed and regressed against each other. Isometry is indicated by a slope not significantly different from 1, and allometry by significant departures from 1. These departures were tested by means of a *t* test (Zar 1999). Allometric changes in nutrient stoichiometry were separately investigated for vegetative and reproductive structures from data taken at flowering peak, as well as for the four different kinds of winter buds (control reproductive, flower bud removed, vegetative, and under threshold size) taken at the end of the growing season. Data for each population were analyzed separately, but only summary results are shown (see Appendix for more detailed results). Analyses were performed only if sample size was >10. No sequential Bonferroni adjustment was utilized to correct significance (Moran 2003). Had we used a more restrictive *P* value, the number of significant relationships found would have decreased but the patterns still would hold the same.

Changes in nutrient stoichiometry as a result of reproductive status

We studied nutrient content as a result of reproductive status for the winter buds harvested at the end of the growing season by means of a discriminant analysis, performed with SPSS 4.0 (Norušis 1990). Dependent variables considered were winter bud dry mass, N and P, as well as [N], [P], and P:N ratio. In this way, the effect of plant size could be distinguished from that of nutrient stoichiometry. Grouping variables were the four kinds of reproductive states: reproductive, flower bud removed, vegetative, and under reproductive threshold. Data from all populations were pooled for analysis.

RESULTS

Nutrient stoichiometry

in relation to environmental nutrient availability

Soil N was 61.2 ± 29.3 mg/L ($n = 11$ populations; range = 29.2–116.4). Prey number per unit leaf size was 0.17 ± 0.09 ($n = 11$; range = 0.07–0.34); all values are expressed as mean \pm 1 SD.

The ordination produced by RDA was significant ($F_{12,184} = 17.099$, $P = 0.005$), indicating a significant relationship between environmental variables and nutrient stoichiometry. Forward selection of environmental variables identified soil N ($F_{1,184} = 31.58$, $P =$

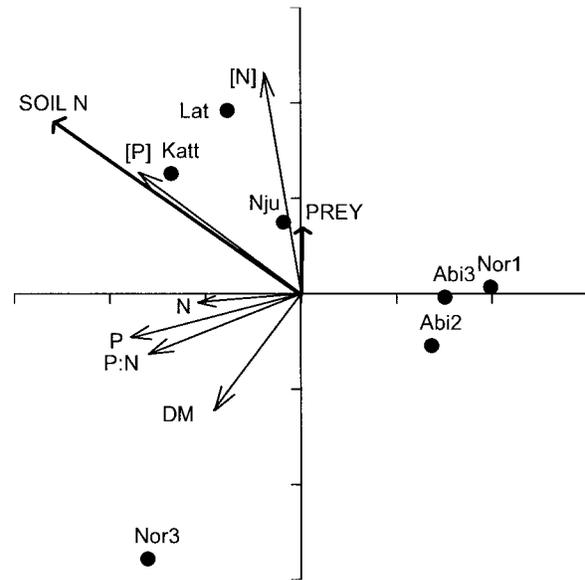


FIG. 1. Redundancy analysis (RDA)-derived biplot showing the relationships between *P. vulgaris* nutrient stoichiometry variables (thin arrows) and environmental variables (thick arrows for continuous variables, dots for categorical variables). Abbreviations are: DM, dry mass; Nor1, Norway 1; Nor3, Norway 3; Abi2, Abisko 2; Abi3, Abisko 3; Katt, Katterjåkk; Nju, Njulla; Lat, Latnjajaure.

0.005) and population (Norway 1, Norway 3, Katterjåkk and Njulla) ($F_{1,184} \geq 19.16$, $P \leq 0.015$) as significantly influencing the ordination. The sum of the canonical eigenvalues explained 29.7% of the variance in the data set.

The first canonical axis of the ordination was significant ($F_{1,184} = 36.291$, $P = 0.005$) and explained 16.1% of the variance. The intercorrelations of the environmental variables with this axis showed a correlation with soil N (-0.497) and population Norway 1 (-0.310) (Fig. 1). N content, P content, and P:N ratio decreased along the first canonical axis (Fig. 1). [N], [P], and, to a lesser extent, N and P content were positively related to soil N (Fig. 1).

Allometric changes in nutrient stoichiometry

Summary data for dry mass and nutrient content in the vegetative and reproductive parts of flowering plants, as well as in winter buds of individuals with different reproductive status, are provided in Table 1.

In total, 178 allometric comparisons were carried out (64 for N and dry mass, 57 for P and dry mass, 57 for P and N; Tables 2 and 3, Appendix). Four of those correlations (all affecting the P:N comparison) were nonsignificant (Table 2). Slopes of the allometric relationship between N and dry mass ranged from 0.70 to 1.24 (Table 2). For the allometric relationships between P and dry mass, the slopes ranged from 0.77 to 1.89 (Table 2). For the significant allometric relation-

TABLE 1. Dry mass, N, and P content, [N], [P], and P:N ratio (all values are means \pm 1 SD) for *Pinguicula vulgaris*: (A) vegetative and reproductive portions of flowering individuals; (B) winter buds produced by individuals of different reproductive status.

Tissue type and status	Dry mass (mg)	N content (μ g)	[N] (mmol/g)	P content (μ g)	[P] (mmol/g)	P:N (g/g)
A) Flowering individuals						
Vegetative portion	16.7 \pm 12.8 (540)	267 \pm 165 (536)	1.24 \pm 0.29 (536)	29 \pm 21 (524)	0.062 \pm 0.039 (524)	0.111 \pm 0.061 (524)
Reproductive portion	5.2 \pm 4.4 (541)	97 \pm 70 (536)	1.44 \pm 0.41 (536)	23 \pm 12 (196)	0.086 \pm 0.033 (196)	0.147 \pm 0.046 (196)
B) Winter buds						
Reproductive	11.1 \pm 8.3 (417)	221 \pm 140 (403)	1.40 \pm 0.26 (403)	36 \pm 23 (336)	0.095 \pm 0.026 (336)	0.155 \pm 0.037 (336)
Flower bud removed	18.2 \pm 10.1 (474)	301 \pm 155 (473)	1.23 \pm 0.25 (473)	48 \pm 25 (464)	0.089 \pm 0.026 (464)	0.161 \pm 0.036 (464)
Vegetative	11.6 \pm 6.5 (442)	201 \pm 90 (433)	1.30 \pm 0.26 (433)	31 \pm 14 (399)	0.087 \pm 0.026 (399)	0.151 \pm 0.030 (399)
Under threshold size	4.7 \pm 3.0 (435)	103 \pm 44 (356)	1.46 \pm 0.41 (356)	18 \pm 6 (169)	0.082 \pm 0.02 (169)	0.141 \pm 0.024 (169)

Note: Data from 11 populations in northern Scandinavia were pooled; sample sizes are given in parentheses.

ships between P and N, the slopes ranged from 0.87 to 1.46 (Table 2).

For 110 allometric analyses (62%), the hypothesis of isometry (slope = 1) could not be rejected (Table 3). Despite this dominance of isometric relationships, in all populations at least some structures showed allometric changes in nutrient stoichiometry (Appendix). In these cases, slopes for the N : dry mass relationship were usually less than 1 (30 cases; Table 3), while those for the P : dry mass and the P:N relationships were higher than 1 (13 and 11 cases, respectively; Table 3). In only three cases per comparison did the allometric analyses show the opposite trends to those previously described (Table 3). These relationships were only found for the vegetative structures in individuals harvested at blooming time and with flower bud(s) removed in our winter bud data set.

Changes in nutrient stoichiometry as a result of reproductive status

A discriminant analysis on the pooled data for the 11 populations produced two significant discriminant functions. The first discriminant function (Wilks' λ = 0.956, χ^2_{10} = 61.187, P < 0.001) explained 86.23% of the variance. Variables correlated with this function were P content, N content, dry mass, and P:N ratio (Table 4). All of them had positive coefficients in the

first function, except P content (Table 4). The second discriminant function (Wilks' λ = 0.992, χ^2_4 = 10.429, P = 0.034) explained an additional 11.45% of the variance. Variables correlated with this function were [N] and [P] (Table 4). [N] had a positive coefficient in this second function, whereas [P] had a negative coefficient. The centroids for the individuals under the reproductive threshold size, vegetative individuals, and deflowered individuals were ordered along the first discriminant axis (Fig. 2). The centroid for reproductive individuals, however, departed from the others also along the second discriminant axis (Fig. 2).

DISCUSSION

Nutrient stoichiometry in relation to environmental nutrient availability

Interspecific differences in nutrient stoichiometry have been interpreted as a reflection of the habitat characteristics in which the species or growth forms occur (Garten 1978, Marrs 1978). They also have been related to contrasting strategies of resource uptake and allocation (Lechowicz and Shaver 1982, Shaver and Lechowicz 1985, Grimshaw and Allen 1987, Thompson et al. 1997). Within species, several studies have found a correlation between nutrient stoichiometry and natural (Orgeas et al. 2002) or experimental (Lechowicz

TABLE 2. Range of values for reduced major axis (RMA) regression slopes testing allometric changes in nutrient stoichiometry for different structures of *Pinguicula vulgaris* plants.

Nutrient	Flowering individuals		Winter buds			
	Vegetative	Reproductive	Reproductive	Flower bud removal	Vegetative	Under threshold
N : dry mass	0.82–1.24 (11)	0.70–1.23 (11)	0.82–1.07 (11)	0.87–1.04 (11)	0.84–1.04 (10)	0.75–1.07 (10)
P : dry mass	0.80–1.50 (11)	0.92–1.53 (7)	0.93–1.20 (11)	0.77–1.21 (11)	0.89–1.17 (10)	1.07–1.89 (7)
P:N	0.87–1.41 (10)	1.02–1.28 (4)	0.99–1.28 (11)	0.87–1.36 (11)	0.92–1.32 (10)	0.97–1.46 (7)

Note: The number of populations studied is given within parentheses.

TABLE 3. Summary of the allometric relationships (N:DM, P:DM, P:N) tested in different structures of *P. vulgaris*, indicated by the number of allometric regressions with slope greater than, equal to, or lower than 1.

Ratio slope	Flowering individuals		Winter buds				Total
	Vegetative	Reproductive	Reproductive	Flower bud removal	Vegetative	Under threshold	
N:DM							
<1	4	3	6	5	7	5	30
1	4	8	5	6	3	5	31
>1	3	0	0	0	0	0	3
P:DM							
<1	1	0	0	2	0	0	3
1	6	6	10	7	8	4	41
>1	4	1	1	2	2	3	13
P:N							
<1	1	0	0	1	1	0	3
1	8	4	10	8	5	4	39
>1	1	0	1	2	4	3	11
NS	1	3	0	0	0	0	4

Note: Key to abbreviations: DM, dry mass; NS, nonsignificant relationship.

and Shaver 1982, Shaver and Lechowicz 1985, Wookey et al. 1995) variation in soil N conditions. In other cases, only some ratios were affected by soil conditions (Güsewell et al. 2003), or no relationship between soil and plant nutrient stoichiometry was found (Ohlson 1986, Bowman et al. 2003). A recent review for wetland plants revealed a poor correlation between plant nutrient stoichiometry and soil nutrient availability, although nutrient stoichiometry responded to experimental manipulation of nutrients (Güsewell and Koerselman 2002).

In our study, a significant influence of soil N on nutrient stoichiometry was found, although the percentage of variance explained by the RDA was modest. Curiously, prey capture did not influence nutrient stoichiometry. In *Pinguicula vulgaris*, prey addition has been shown to increase leaf [N] and [P] in some cases (Aldenius et al. 1983), but not in others (Karlsson and Carlsson 1984, Méndez and Karlsson 1999). Addition of N to soil increased winter bud [N] and [P] (Karlsson et al. 1991). In *P. vulgaris*, prey-derived N fosters nutrient uptake through roots (Karlsson et al. 1996, Ad-

mec 1997). This positive feedback could have affected our ability to disentangle the separate effects of soil and prey nutrients on nutrient stoichiometry. Another possibility is that prey number was a poor surrogate for prey-derived N.

In addition to soil N, population had a significant influence on nutrient stoichiometry. In other studies, population differences were mediated by soil N availability (Orgeas et al. 2002). In the present case, population could have had influence through other unmeasured environmental variables or through genetic differences.

Allometric changes in nutrient stoichiometry

Previous studies have documented a “dilution” of N content as plants grow bigger, both intra- (Mooney and Chiariello 1984, Coleman et al. 1993, Ohnmeiss and Baldwin 1994, Lutz and Gifford 1998) and inter-

TABLE 4. Results of the discriminant analysis testing for differences in nutrient stoichiometry between winter buds derived from individuals differing in reproductive status.

Variable	Standardized discriminant function coefficient		Correlation within function	
	Function 1	Function 2	Function 1	Function 2
Dry mass	0.221	-0.084	0.845	-0.286
N content	1.608	0.752	0.857	0.059
[N]	-0.109	1.112	-0.171	0.974
P content	-0.955	-0.610	0.903	0.068
[P]	0.062	-0.249	0.134	0.720
P:N	0.786	0.584	0.345	0.120

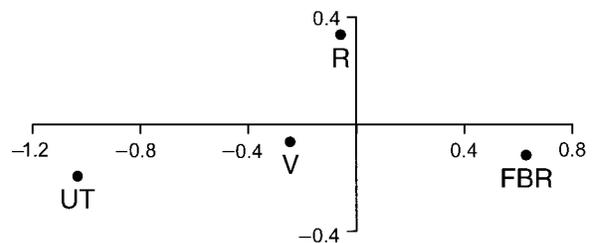


FIG. 2. Arrangement of four kinds of winter buds differing in reproductive status in the bidimensional discriminant space defined by variables related to nutrient stoichiometry. Dots represent the centroids of each group. The first discriminant axis was positively related to N content, dry mass, and P:N ratio, and was negatively related to P content. The second discriminant axis was positively related to [N] and negatively related to [P]. Abbreviations are: FBR, Flower buds removed (experimentally vegetative); R, reproductive; UT, under threshold size for reproduction; V, vegetative.

specifically (McJannet et al. 1995). Our study more often documented a "dilution" of N and/or a "concentration" of P, although there were a few exceptions to this pattern (Table 3). As far as we know, changes in [P] with plant size have not been reported previously.

Allometric variation in nutrient stoichiometry has not received much attention in the past. This makes it difficult to put our results in context. We will only draw attention to three points that require further research. Our first point refers to the underlying mechanisms. At a cellular level, N and P are involved in the same metabolic activities (Garten 1978, Thompson et al. 1997) and one would expect them to keep constant proportions. There are indications, however, that their stoichiometry is variable depending on growth rate (Ågren 2004). A dilution of N is also observed when plant growth exceeds the rate of nutrient input (Marrs 1978), but the concentration of P is difficult to explain. At an architectural level, increases in size entail changes in the proportion of organs or modules (stems to leaves, roots to shoots, and so on; e.g., Caloin 1987). As long as different organs or modules differ in chemical composition (e.g., lignin and cellulose vs. photosynthetic pigments or storage compounds), this can lead to allometric changes in nutrient stoichiometry. We presently lack a mechanistic explanation for the patterns found in *P. vulgaris*, but their consistency across reproductive, vegetative, and perennating structures makes us think that a common physiological process is involved. One possibility is that small and large *P. vulgaris* plants differ in their ability to capture carbon (through photosynthesis) or nutrients (through roots and/or prey), although we lack empirical data on this. Another possibility is related to the observed relationship between nutrient stoichiometry and growth rate. Ågren (2004) found that the N:C ratio increases linearly with growth rate, whereas the P:C ratio increases quadratically. These patterns would be consistent with our results for [P] and P:N if we were to assume that growth rate increases with plant size in *P. vulgaris*. All of these explanations, however, apply to growing plants and require some kind of carryover effect to explain the persistence of the pattern in the resulting winter buds.

Our second point refers to the generality of these patterns. Whatever the mechanisms involved, allometric changes in nutrient stoichiometry do not seem to be limited to plants, because there is at least one reported case in fishes (Sternler and George 2000). It remains to be determined how general these allometric changes are in plants and animals (see Marriott et al. [2001] for lack of a N dilution effect), and whether similar mechanistic explanations for both autotrophs and heterotrophs can be found.

Our third point deals with the functional meaning of this allometric change in stoichiometry. An ontogenetic change in nutrient stoichiometry has been documented (e.g., tobacco plants; Mooney and Chiariello 1984). Our results can be interpreted as ontogenetic changes

if one considers them as a space per time substitution. At the moment it is not possible to assess the function of those purported ontogenetic changes. They could simply reflect different optimal nutrient stoichiometry in different life stages of a plant. On the other hand, they could be interpreted in the light of a change in the identity of the limiting nutrient with size. Relationships between interspecific differences in nutrient stoichiometry and the resource niche have been made by several authors (Garten 1978, Tilman 1982). In recent times Koerselman and Meuleman (1996) proposed utilizing the N:P ratio to assess the most limiting nutrient for plant productivity. Although their proposal is valid at a community level (Koerselman and Meuleman 1996), it is tempting to apply those ideas at an intraspecific level, too, and speculate that an allometric change in N:P ratio could mean a change in the most limiting resource for plant growth. Güsewell et al. (2003) have made some attempts in this direction. This idea deserves further experimental study.

Changes in nutrient stoichiometry after reproduction

Several studies have found differences between vegetative and reproductive individuals in nutrient (Benzing and Renfrow 1971, Pakonen et al. 1988, Cipollini and Stiles 1991) or carbohydrate levels (Cipollini and Stiles 1991). These findings agree with theoretical predictions of a negative effect of current reproduction on subsequent resource level (Calow 1979). As long as future flowering probabilities are dependent on resource level, changes in nutrient stoichiometry derived from reproduction might act as proximate mechanisms influencing future reproductive performance.

Our results partially support the arguments just outlined. Discriminant analysis showed that winter buds produced by three types of nonreproductive individuals shared a similar nutrient stoichiometry and differed mainly in size and absolute nutrient pool, whereas winter buds derived from reproductive individuals had lower [P]. However, winter buds derived from reproductive individuals showed high, instead of low, [N]. This is paradoxical, as Eckstein and Karlsson (2001) showed higher N losses in reproductive individuals. Higher N leaf resorption efficiency in reproductive vs. vegetative individuals, reported by Eckstein and Karlsson (2001), could have been involved, but those differences were slight and not significant (Eckstein and Karlsson 2001). An anonymous referee suggested that reproduction could entail a disproportionate investment of sugars with respect to mineral nutrients. Although reproductive structures of *P. vulgaris* are enriched in N and P with respect to vegetative ones, it is, in fact, true that the somatic cost of reproduction is lower in terms of N or P than in terms of dry mass (Marcos Méndez and P. Staffan Karlsson, unpublished data).

In *Rhododendron lapponicum*, Karlsson and Jacobson (2001) found that the probability of becoming reproductive increased with increasing [N] and with de-

creasing [P]. This finding stresses the potential importance of studying nutrient stoichiometry, and not only “resource accumulation,” in relation to flowering probabilities. Ongoing research (Marcos Méndez and P. Staffan Karlsson, *unpublished data*) indicates the influence of previous reproductive history, aside from plant size, as a determinant of flowering probabilities in *P. vulgaris*. The present results provide a mechanistic basis for these historical effects. However, the present study investigated consequences of reproduction for nutrient stoichiometry and, being based on destructive sampling, did not allow a follow-up study of future flowering. Such a study, although technically complicated for a small plant like *P. vulgaris*, could nevertheless be very informative.

Sources of variation in nutrient stoichiometry in plants

Our study revealed three sources of variation in nutrient stoichiometry that are potentially general in herbaceous plants, although two of them (plant size and reproductive status) have received little attention in the previous literature. In particular, these two sources of variation offered insight into the questions about nutrient stoichiometry raised by Bazzaz (1997): nutrient ratios are flexible within a species, are influenced by reproduction, and possibly show ontogenetic changes. They also stress that any consideration of nutrient stoichiometry in plants must go well beyond a search for links between environmental and plant nutrient concentrations, as implicitly assumed by the “plant as a balanced system” hypothesis. First, climate can underlie the altitudinal and latitudinal trends found in nutrient content (Körner 1989). Second, intrinsic traits of plants, such as size, and past events in an individual life, such as reproduction, add to the factors that influence the balance of nutrients in plant tissues (Fig. 3).

Environmental and internal sources of variation potentially interact in a complicated way to produce the observed patterns of nutrient stoichiometry. Because nutrient stoichiometry has often been considered adaptive (Bloom et al. 1985, Weih and Karlsson 2001), it will be crucial to determine which sources of variation favor, and which of them hinder, achievement of such optimal stoichiometry. Our study gives two clues. First, the optimal balance might be different for different individuals, depending on size or other ontogenetic considerations. Second, reproductive events could disturb that balance. The relative importance of each source of variation, as well as their interactions, need also to be addressed in a diversity of plant and animal systems in order to advance the current understanding of the stability of, and variation in, nutrient stoichiometry.

Finally, it remains to be established whether the undeniable variation in plant nutrient stoichiometry is compatible with the homeostasis predicted by the

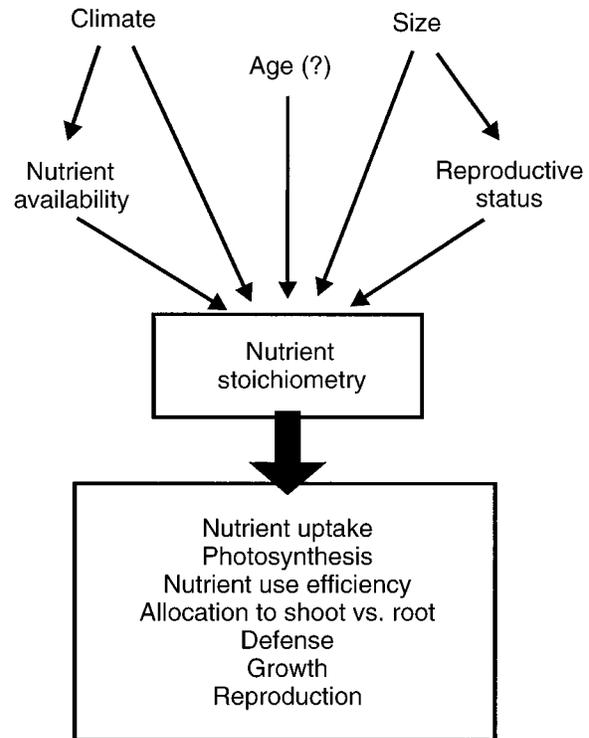


FIG. 3. A conceptual model for the factors influencing nutrient stoichiometry in plants, and the resulting plant functions.

“plant as a balanced system” hypothesis or results from the inability of plants to maintain such homeostasis. Although this hypothesis has made some testable predictions (Bloom et al. 1985, Chapin et al. 1987), we lack clear predictions of how constant or variable nutrients need to be in order to support or reject the hypothesis. A broader knowledge of sources of variation in nutrient stoichiometry, combined with experimental tests of the homeostatic abilities of plants, will help in this task. Internal sources of variation, and not only environmental nutrient availability, should be incorporated into experimental designs testing the “plant as a balanced system” hypothesis.

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APPENDIX

A table summarizing the allometric relationships (N : dry mass, P : dry mass, P:N) tested in different structures of *Pinguicula vulgaris* is available in ESA's Electronic Data Archive: *Ecological Archives* E086-053-A1.