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Quantifying the nitrogen allocation and resorption for an orchid pseudobulb in relation to nitrogen supply

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ABSTRACT

As the main organ for storing water and nutrients, pseudobulbs are of central importance to the growth and survival of orchids. Quantifying nitrogen allocation and resorption of pseudobulbs is important for understanding the survival and adaptation strategies of orchids, but studies in this area are still lacking due to the complicated integration effect between pseudobulbs of different ages. In this study, nitrogen allocation to each organ and nitrogen resorption of *Pleione aurita*, an epiphytic orchid characterized by annually renewed pseudobulbs, were examined quantitatively under three nitrogen levels using ^{15}N labeling. We found the old pseudobulb can act as a sink for exogenous nitrogen, although it had a much lower sink activity than the developing new pseudobulb. The single leaf accounted for the largest proportion of both exogenous and pseudobulb-stored nitrogen among all organs and in three nitrogen treatments, and the fruit tended to use pseudobulb-stored nitrogen preferentially regardless of exogenous nitrogen supply. The resorption of nitrogen in the leaf and pseudobulb was highly efficient, but decreased with increasing nitrogen supply. Overall, positive biomass accumulation was only observed in plants with exogenous nitrogen supply, while all fruit-bearing plants lost more than 30% of their biomass after a growing season regardless of nitrogen supply. The *P. aurita* plants exhibited a strong low nitrogen tolerance with only a slight loss of biomass for those nitrogen deficient and fruitless plants. Surprisingly, an increase in the nitrogen pool of the whole-plant was always observed regardless of biomass loss in fruit-bearing plants after nitrogen addition. Our results suggest an important role of the pseudobulb in the nitrogen economy of *P. aurita*, and the high dependence of the reproduction process on the pseudobulb-stored nitrogen. The findings will help to understand the adaptive strategies of orchids, and contribute to the conservation of endangered species.

1. Introduction

Many orchids have conspicuous storage organs. Corms, rhizomes, or tuberoids may occur in terrestrial orchids while pseudobulbs, which are somewhat enlarged or thickened succulent stems, are common in epiphytic orchids (Göbel *et al.*, 2020; Zimmerman, 1990). It is estimated that roughly half of all orchid genera have pseudobulbs, and the evolution of these specialized structures seems to coincide with a shift from terrestrial to epiphytic habit (Zotz, 2016). Considering the astounding richness of species in the family Orchidaceae, which is comprised of approximately 28,000 species (Christenhusz and Byng, 2016), there are probably thousands of species throughout the world that equipped with pseudobulbs.

As an organ that stores water, carbohydrates, and minerals, pseudobulbs are of central importance to the growth and survival of orchids (He, 2018; Ng and Hew, 2000). Serving as water reservoirs, pseudobulbs play a crucial role in coping with frequently encountered water deficits in epiphytic orchids. The presence of pseudobulbs facilitates a slow reduction in water content of leaves and thus maintains the whole-plant water balance during drought stress (He *et al.*, 2013; Li and Zhang, 2019; Yang *et al.*, 2016). Pseudobulbs are also involved in the regulation of leaf photosynthesis, growth, and flower initiation of orchids by storing a massive amount of carbohydrates and minerals (He, 2018). This specialized organ can act as both a source and sink of carbohydrates and minerals under different circumstances. For example, carbohydrates and minerals in old pseudobulbs can be remobilized at the beginning of

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growth to support the development of new shoots and inflorescences (Hew and Ng, 1996; Yong and Hew, 1995a; Zimmerman, 1990). Acting as sink, the pseudobulb is one of the main receivers of assimilates from leaves, and can retrieve large amounts of mineral nutrients from leaves prior to their abscission (He *et al.*, 2011; Yong and Hew, 1995b; Zimmerman, 1990). Under water deficit conditions, polysaccharides in the pseudobulb can be remobilized and transferred to maintain a constant ratio of sucrose/monosaccharides in the leaf (Stancato *et al.*, 2001). A pseudobulb itself is also shown to have the ability to recycle respiratory carbon that would otherwise be lost, thus contributing positively to whole-plant carbon economy (Hew and Yong, 1994; Rodrigues *et al.*, 2013).

Nitrogen is one of the essential macronutrients in plants and has great influence on almost every aspect of plant life. Nitrogen can be transported from old to young pseudobulbs and between leaves and pseudobulbs. Thus, nitrogen concentration in pseudobulbs usually exhibits significant developmental or seasonal variation (Hew and Ng, 1996; Zimmerman, 1990). Nitrogen translocation and resorption are critical for orchids because their epiphytic habitats are usually regarded as nutrition limited. Due to the small volume of substrate, orchids living in epiphytic habitats may largely depend on stem flow for nutrients. The low fertility tolerance of orchids is closely associated with the development of the pseudobulb (Ng and Hew, 2000). These nutrient-related functions indicate that the pseudobulb is an important nitrogen pool for orchids, while similar functions in non-pseudobulb orchids are achieved by other storage organs, such as succulent leaves (Lin *et al.*, 2019; Susilo *et al.*, 2013). Although researchers have focused on the function of this exchange center of orchids, quantitative studies of the nitrogen allocation in pseudobulbs are still lacking. This is probably due to the complicated integration effect between pseudobulbs, which enables plants to exchange multiple resources between each pseudobulb. Most pseudobulbs live much longer than their leaves, and even when the leaves attached to them have dropped, the leafless pseudobulbs can survive and function for years before they eventually die. As a result, the pseudobulbs accumulate over time, given the annual development of young pseudobulbs, and thus an orchid may possess several pseudobulbs of different ages (Li and Zhang, 2019; Zimmerman, 1990). The integration of many young and old pseudobulbs makes it difficult to distinguish the nutrient resource and contribution of any particular pseudobulb.

Members in the genus *Pleione* are world-famous ornamental plants and important medicinal plants (Cribb and Butterfield, 1999; Teoh, 2016). Unlike many other epiphytic orchids, *Pleione* plants renew their pseudobulbs annually and have a conspicuous dormant stage. The lifespan of a *Pleione* pseudobulb is usually less than 2 years, which leads to at most two different ages of pseudobulbs coexisting in a plant (Zhang *et al.*, 2017; Fig. 1). The relatively simple arrangement of pseudobulbs and a dormant stage facilitates the accurate determination of the biomass and nitrogen content in the original pseudobulbs. When using a stable isotope as a tracer, the newly absorbed nitrogen can be easily determined and distinguished from that stored in the original (old) pseudobulbs.

In this study, we investigated the physiological performance, biomass allocation and nitrogen resorption of *P. aurita* under three nitrogen levels. ^{15}N labeling was used to compare the contributions of exogenous nitrogen and stored nitrogen, and their allocation to each organ. Our main objectives were to (1) explore the nitrogen requirements of *Pleione* plants; and (2) quantify the flows of exogenous nitrogen and pseudobulb stored nitrogen to each organ.

2. Materials and methods

2.1. Plant materials and growth conditions

Pseudobulbs of *Pleione aurita* P. J. Cribb & H. Pfennig were used in the present study. Details of life history of this species have been described in our previous study (Zhang *et al.*, 2017). Each pseudobulb was weighed and labeled during its dormancy, when it was leafless and

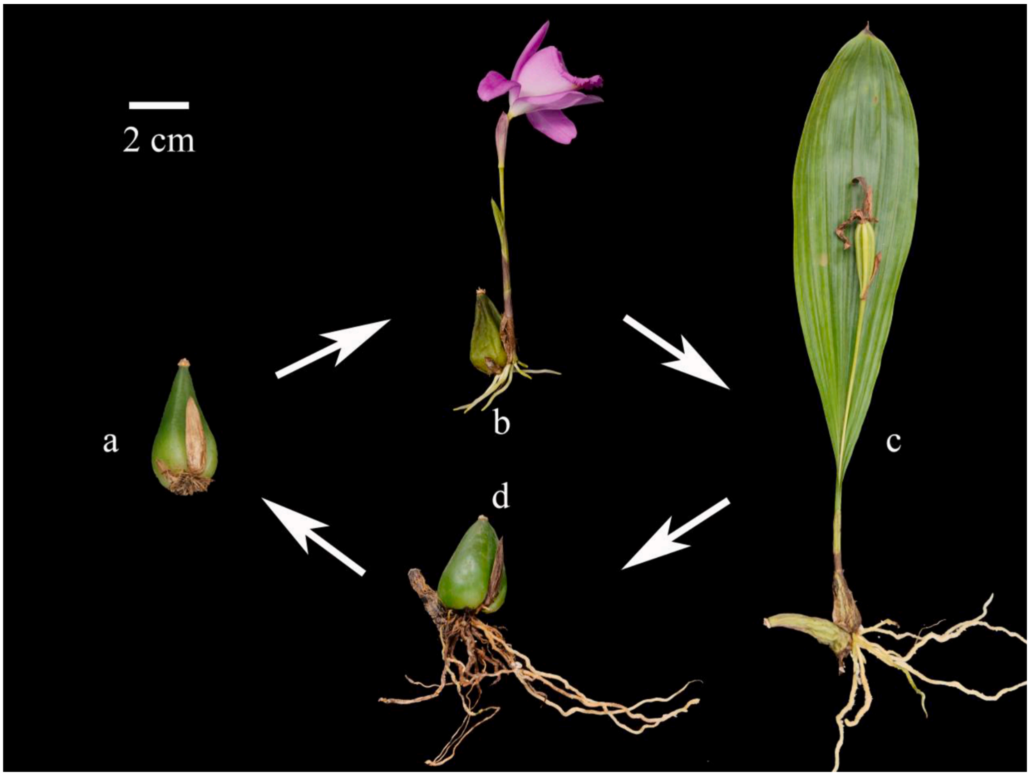

Fig. 1. Development of *Pleione aurita* over a whole growing season.
a, Dormant pseudobulb; **b**, flowering stage; **c**, full leaf expansion; and **d**, leaf shedding.

rootless (Fig. 1a). The dry mass of each pseudobulb was estimated by multiplying its fresh weight by the mean ratio of dry weight to fresh weight. The dormant pseudobulbs were then grown in plastic nursery trays containing a pure perlite medium, which is a form of amorphous volcanic glass and nitrogen-free. Each tray contained 30 plants and these nursery trays were then placed in a greenhouse at Kunming Institute of Botany, Chinese Academy of Sciences (1990 m, E102°41′, N25°01′). When the growth phase began in April 2019, conditions in the greenhouse included a light intensity maintained at half full sunlight, temperature at 18–30°C, relative humidity above 60%, and watering at 7-day intervals. Only adult individuals (flowering-sized pseudobulbs) were used in our study.

2.2. Nitrogen treatment

Nitrogen addition was achieved by spraying 500 ml ammonium nitrate solution on each tray weekly from the flowering stage, which is the onset of leaf and roots growth (Fig. 1b), to the full expansion of the leaf (Fig. 1c), and the nitrogen supply lasted for 8 weeks. Ammonium nitrate labeled with ^{15}N ($^{15}NH_4{}^{15}NO_3$, 10 atom %) was used in this experiment. The high nitrogen group (HN) was supplied with 0.818 g ammonium nitrate/L solution each week, and the low nitrogen group (LN) was supplied with 0.414 g ammonium nitrate/L solution each week, while the control group (CK) was treated with an equal volume of water. In total, three nitrogen levels were involved in our study and 90 individuals were used in each treatment. Half of the plants in each treatment were hand-pollinated during flowering to ensure fruit setting.

2.3. Measurements of leaf traits and photosynthetic gas exchange

Leaf area was measured at full expansion (Fig. 1c) with a leaf area meter (3100, Li-Cor, USA). Leaf mass per unit area (LMA) was expressed as the leaf dry mass divided by leaf area. To eliminate the impact of individual size on our results, a leaf area index, which was defined as the leaf area per unit mass of the original pseudobulb, was used to compare leaf area between treatments. Light-saturated photosynthetic rate (P_{max}) was measured with a portable photosynthesis system (LI-6400, Li-Cor, USA), and recordings were made at a saturating light intensity of 600 μmol photons m^{-2} s^{-1} under the CO_2 concentration of 400 μmol mol^{-1} between 9:00 am–12:00 am.

2.4. Analysis for biomass, ^{15}N isotope and nitrogen resorption

Samples were taken at full leaf expansion, when both the leaf and fruit reach their full size (Fig. 1c), and at leaf shedding (Fig. 1d). All samples were rinsed with deionized water, oven-dried at 80°C to obtain their constant weight, and ground to fine powders before analysis. For a given plant, the biomass allocation to the organ was calculated as dry mass of each organ divided by total dry mass. Since every pseudobulb was weighed and labeled, and the original dry mass before planting was estimated, the total biomass was also expressed as a proportion of the original pseudobulb dry mass (% original pseudobulb) when compared between nitrogen treatment groups, and between plants with and without fruit.

The nitrogen concentration (N%) and ^{15}N abundance (atom %) for each organ were determined with an Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Germany). Nitrogen derived from fertilizer (NDFF) was calculated using the following equation: NDFF(%)=100(a-b)/(c-b), where a is the abundance of ^{15}N atoms in the sample, b is the natural abundance of ^{15}N atoms (0.366%), and c is the abundance of ^{15}N atoms in the fertilizer (Susilo *et al.*, 2013). Since the medium we used is nitrogen-free, nitrogen derived from reserves of the original pseudobulb (NDFR) can be obtained by subtracting NDFF from 100%.

The amount of nitrogen absorbed from fertilizer (ANA) for an organ was calculated using the following equation: N% × DW × NDFF, where the DW is the dry weight for a given organ. Allocation of fertilizer nitrogen to each organ was calculated using the ANA divided by the whole-plant absorbed nitrogen, where the whole-plant absorbed nitrogen is the sum of the ANA for each organ. Similarly, the amount of nitrogen derived from reserves (ANR) was calculated by N% × DW × NDFR, and the allocation of stored nitrogen to each organ was calculated using the ANR divided by the whole-plant stored nitrogen, where the whole-plant stored nitrogen is the sum of the ANR for each organ.

Nitrogen resorption efficiency (NRE), defined as the proportional withdrawal of nitrogen during senescence, was calculated as the ratio of the difference in the N pool between mature and senesced organs to the N pool of mature organs (Lü *et al.*, 2012; van Heerwaarden *et al.*, 2003). Nitrogen resorption proficiency (NRP) was defined as the level to which the nitrogen concentration was reduced in senesced materials, and usually expressed as the nitrogen concentration of the senesced organ, with a low nitrogen concentration of the senesced organ corresponding to a high resorption proficiency (Killingbeck, 1996). After a growing season, the annual nitrogen gain or loss for a plant can be expressed as the ratio of total nitrogen content in the newly produced pseudobulb(s) and the original pseudobulb, with a value exceeding 100% indicating an annual nitrogen net gain. The total nitrogen for a given pseudobulb was estimated by multiplying its dry mass by the mean nitrogen concentration for each treatment.

2.5. Data analysis

One-way ANOVA was used to examine differences between nitrogen treatments, with means discriminated by LSD multiple comparison tests, and independent *t*-tests were used to examine differences between plants with and without fruits. Two-way ANOVA (with nitrogen level and fruiting as the main factors) was also used to analyze the effects of the nitrogen level and fruiting on the biomass and leaf traits. Data analyses were conducted using the SPSS 16.0 program (SPSS Inc., USA).

3. Results

3.1. Biomass and leaf traits

All fruit-bearing plants had lower biomass at the full leaf expansion stage when compared with those without a fruit. However, the difference was significant only in the HN group (n=8). There was no difference in biomass among nitrogen treatments regardless of fruit bearing (Fig. 2A). The high nitrogen supply increased the leaf area for HN plants while fruit bearing decreased the leaf area and LMA regardless of nitrogen supply (Fig. 2B, C). The P_{max} increased significantly after nitrogen addition but did not differ between the two nitrogen addition treatments. Fruitless and fruit bearing plants had a similar P_{max} for each nitrogen level (Fig. 2D).

3.2. Biomass allocation at the leaf full expansion stage

The single leaf accounted for the largest proportion of total biomass, while the roots accounted for the smallest proportion of total biomass in all treatments (Fig. 3). For fruitless plants, the newly produced pseudobulb(s) accounted for the second largest proportion of total biomass (Fig 3A), whereas in fruit-bearing plants, the old pseudobulb accounted for the second largest proportion of total biomass (Fig. 3B). Nitrogen addition significantly decreased the biomass proportion of the old pseudobulb in both fruit-bearing and fruitless plants (Fig 3).

3.3. Allocation of fertilizer and stored nitrogen

Among the organs, the leaf had the highest nitrogen concentration while the old pseudobulb had the lowest. Nitrogen concentration in the roots, new pseudobulb and leaf increased significantly with increasing nitrogen addition. Nitrogen addition also increased nitrogen

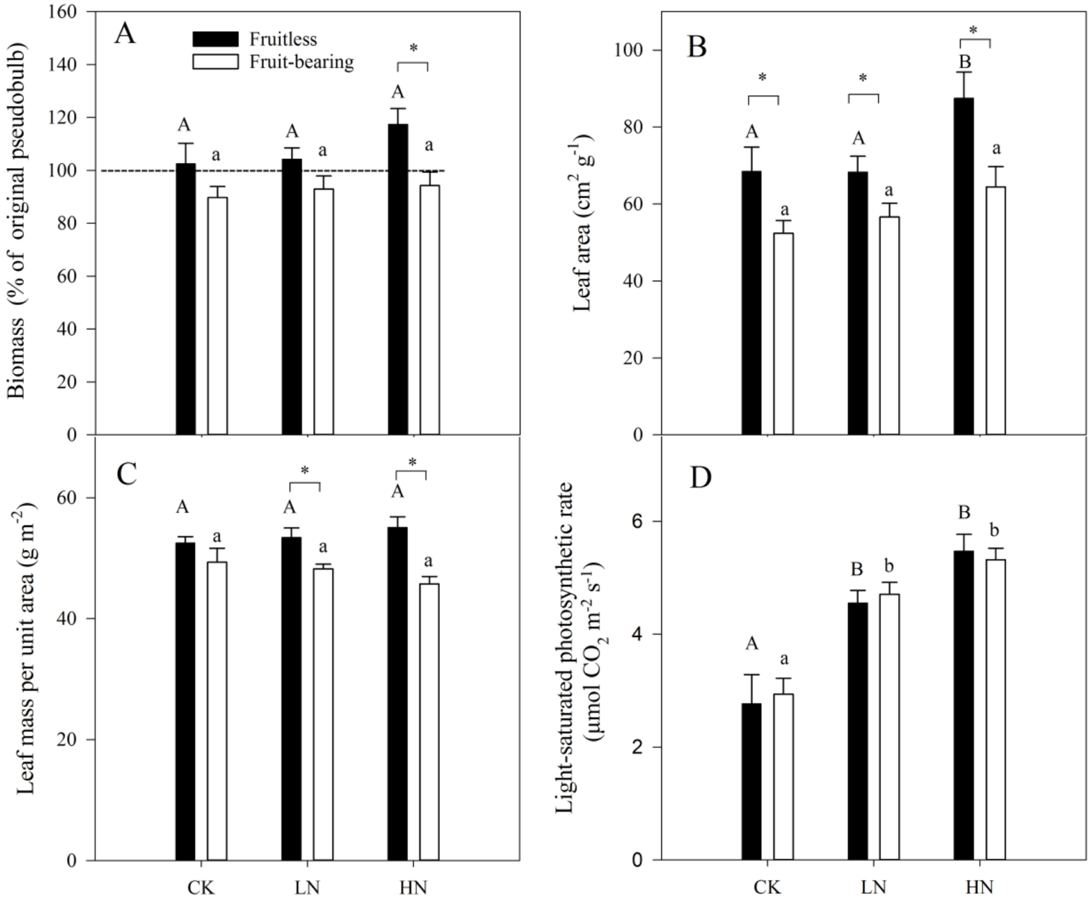

Fig. 2. Biomass and leaf traits of *Pleione aurita* under three nitrogen levels at full leaf expansion stage.
Data are means ± SE ($n = 6$–8). Means with different letters above the bars of the same color are statistically different ($p<0.05$) as determined by LSD multiple comparison test. * indicates a significant difference between two bars ($p<0.05$) as determined by an independent t-test.

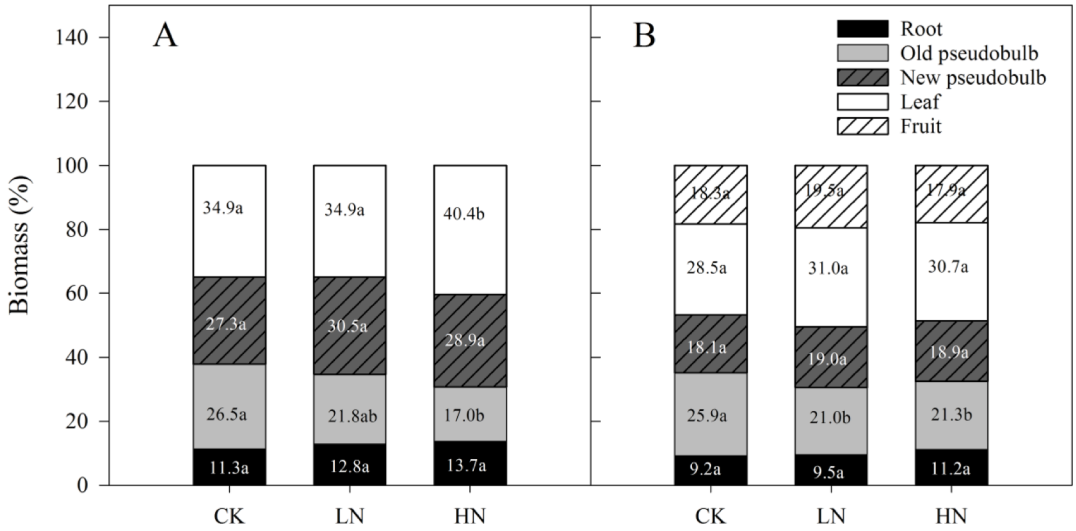

Fig. 3. Biomass allocation for fruitless plants (A) and fruit-bearing plants (B) of *Pleione aurita* under three nitrogen levels at full leaf expansion stage.
Data are means of eight individuals. Mean numbers followed by different letters inside the bars are statistically different ($p<0.05$) for each organ as determined by an LSD multiple comparison test.

concentration in the old pseudobulb, but the two nitrogen addition treatments did not differ significantly. All three nitrogen treatments showed a similar nitrogen concentration in the fruit (Fig. 4). The roots, new pseudobulb and leaf had a relatively higher proportion of fertilizer nitrogen than the old pseudobulb and fruit. The proportion of fertilizer nitrogen increased with the increasing of nitrogen addition in all organs (Fig. 4).

In terms of the allocation of the newly absorbed fertilizer nitrogen,

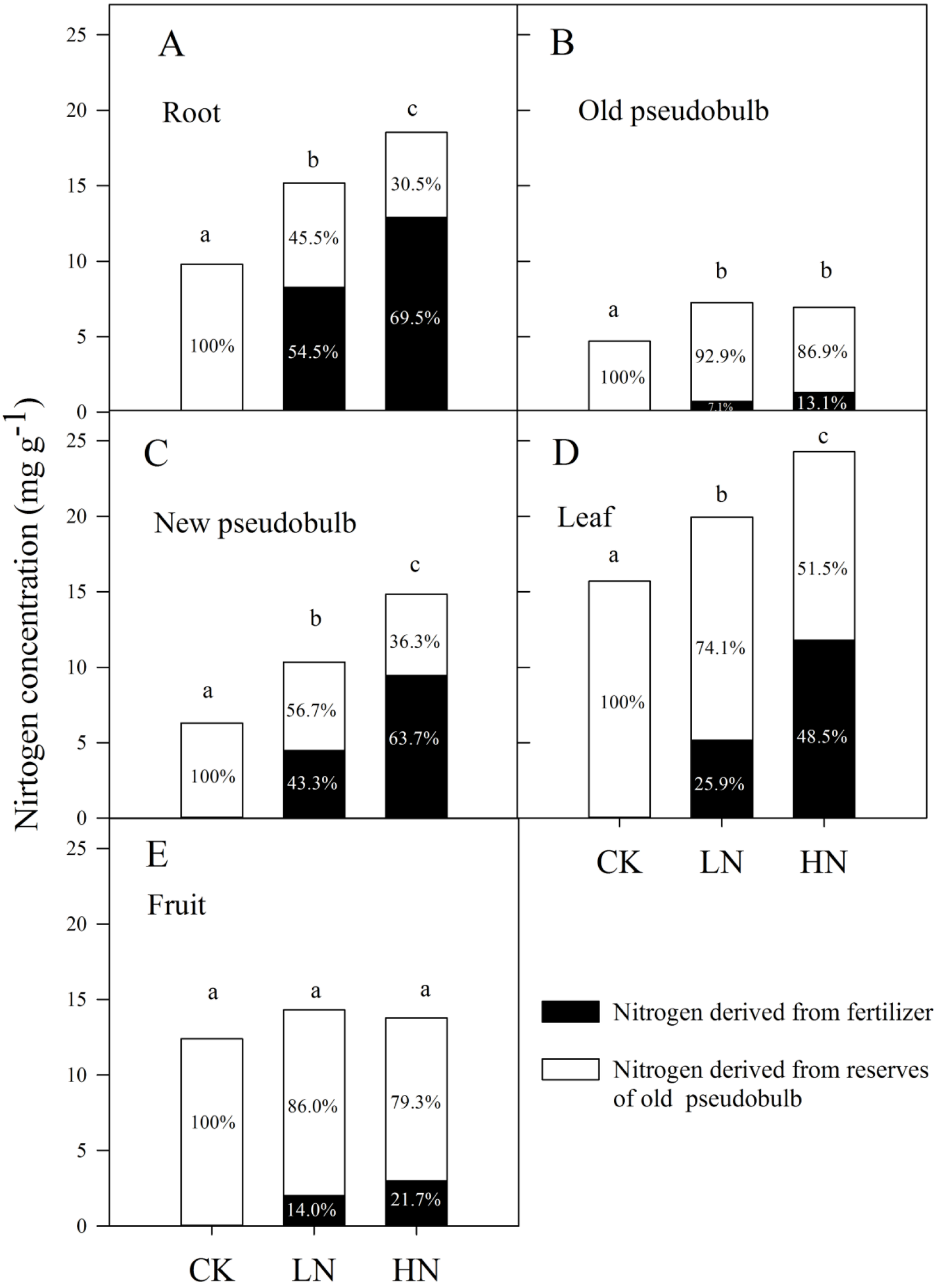

Fig. 4. Nitrogen concentration of each organ of *Pleione aurita* under three nitrogen levels at full leaf expansion stage.
The bars show the means of the total nitrogen concentration and the numbers inside the bars indicate the percentage of fertilizer nitrogen or pseudobulb-stored nitrogen. Data are means of three samples, with each sample being a mix from three individual plants. Means with different letters above the bars for each organ are statistically different ($p<0.05$) as determined by an LSD multiple comparison test.

more than 40% of the absorbed nitrogen was allocated to the leaf. It seems that an increasing proportion of newly absorbed nitrogen was allocated to the leaf if the plants were subjected to a higher nitrogen level. The old pseudobulb can also act as a weak sink with approximately 3–4% of the newly absorbed nitrogen allocated to it (Fig. 5). Fruit bearing substantially decreased the allocation of newly absorbed nitrogen to the new pseudobulb rather than to the leaf and root (Fig. 5).

With the values of more than 56% for fruitless plants and 44% for fruit-bearing plants, the single leaf also received the largest proportion of nitrogen from reserves of the original pseudobulb. There was a trend that high nitrogen supply also increased the allocation of stored nitrogen to the leaf in fruitless plants. The presence of fruit, which accounted for approximately 23% total nitrogen, substantially decreased the allocation of nitrogen to the new pseudobulb and the leaf (Fig. 6).

3.4. Nitrogen resorption efficiency and proficiency

Fruit-bearing plants were used to investigate the NRE and NRP for leaves and pseudobulbs. The NRE decreased with the increasing of nitrogen availability. With more than 78% of nitrogen recycled in the control group, leaves of *P. aurita* exhibited a highly efficient resorption of nitrogen. It is noteworthy that an even higher proportion of nitrogen was recycled in the pseudobulb than in the leaf (Table 1). Nitrogen concentration in the litter increased with increasing nitrogen addition, indicating the decrease of NRP after nitrogen addition (Table 1).

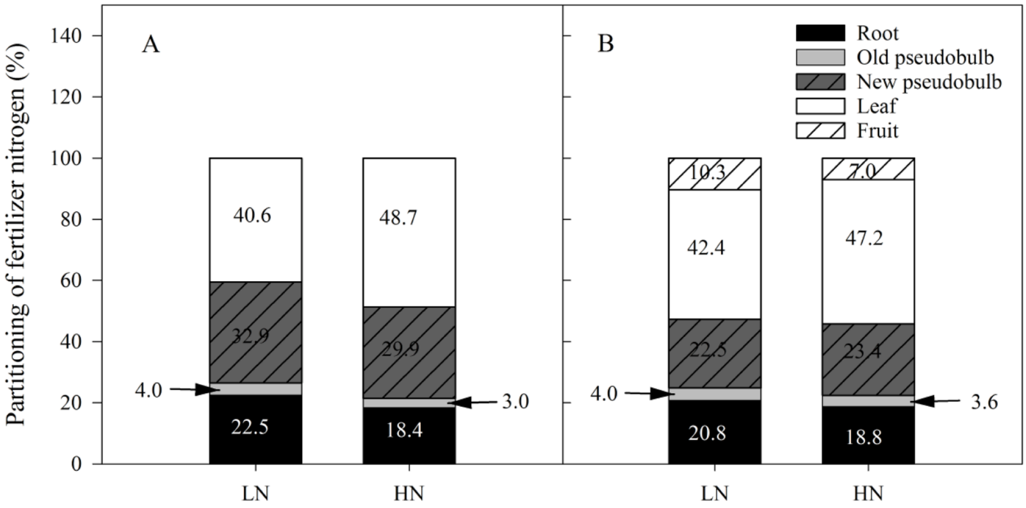

Fig. 5. Fertilizer nitrogen allocation for fruitless (A) and fruit-bearing (B) individuals of *Pleione aurita* in two nitrogen addition groups at full leaf expansion stage. Numbers inside the bars indicate the percentage of total absorbed fertilizer nitrogen in each organ. Data are means of three samples, with each sample being a mix from three individual plants.

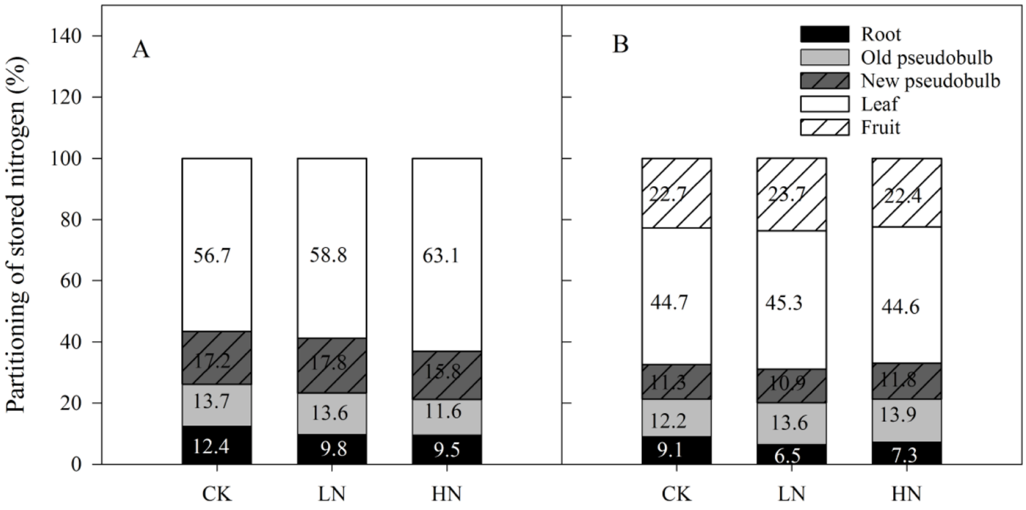

Fig. 6. Allocation of pseudobulb-stored nitrogen for fruitless (A) and fruit-bearing (B) individuals of *Pleione aurita* under three nitrogen levels at full leaf expansion stage.
Numbers inside the bars indicate the percentage of total pseudobulb-stored nitrogen in each organ. Data are means of three samples, with each sample being a mix from three individual plants.

Table 1
Nitrogen resorption efficiency and proficiency for *Pleione aurita* under three nitrogen levels (treatments).

Treatment	Nitrogen resorption efficiency (%)		Nitrogen resorption proficiency (mg g^{-1})	
	Leaf	Pseudobulb	Leaf	Pseudobulb
CK	78.8±0.5**a**	90.9±0.3**a**	4.6±0.1**a**	3.5±0.1**a**
LN	73.5±0.5**b**	79.2±1.3**b**	6.7±0.14**b**	4.6±0.3**b**
HN	67.3±0.6**c**	81.8±1.1**b**	10.2±0.2**c**	6.2±0.2**c**

Different letters in each column indicate statistically different mean values ($p < 0.05$), as determined by LSD multiple comparison tests.

3.5. Plant annual biomass and nitrogen gain

At the end of the growing season, a plant shed its roots, old pseudobulb, leaf and fruit, leaving the new pseudobulb(s), which accounted for the whole-plant biomass. For the fruitless plants, the biomass increased with the increasing of nitrogen addition. However, for the fruit-bearing plants, they had significantly lower biomass compared with their respective fruitless plants in all treatments (Fig. 7A). When compared with their respective original pseudobulbs, the biomass of fruitless plants in the two nitrogen addition groups (LN and HN) had a positive growth (values for biomass > 100%) after a growing season, while it had a slight decrease in the control group for the fruitless plants. Interestingly, all fruit-bearing plants had a decrease in biomass after a growing season regardless of exogenous nitrogen addition (Fig. 7A).

By comparing the biomass and nitrogen concentration of the original pseudobulb and the newly produced pseudobulb(s) after a growing season, the whole-plant level of annual nitrogen gain and loss can be estimated. Higher total nitrogen content was observed in all fruitless plants when compared with fruit-bearing individuals. This parameter for the two nitrogen addition groups (LN and HN) indicated positive growth (values for whole-plant nitrogen content > 100%) after a growing season, i.e. a net annual gain of nitrogen after exogenous nitrogen addition. While without the exogenous nitrogen addition, no increase in nitrogen content was observed in both fruitless and fruit-bearing plants in the control group. Nevertheless, there was only a slight loss of nitrogen (~2% on average) for fruitless plants but considerable loss (~22% on average) for fruit-bearing plants (Fig. 7B). Both nitrogen addition and fruiting decreased the ratio of carbon to nitrogen (C/N) in the newly produced pseudobulb after a growing season (Fig. 7C).

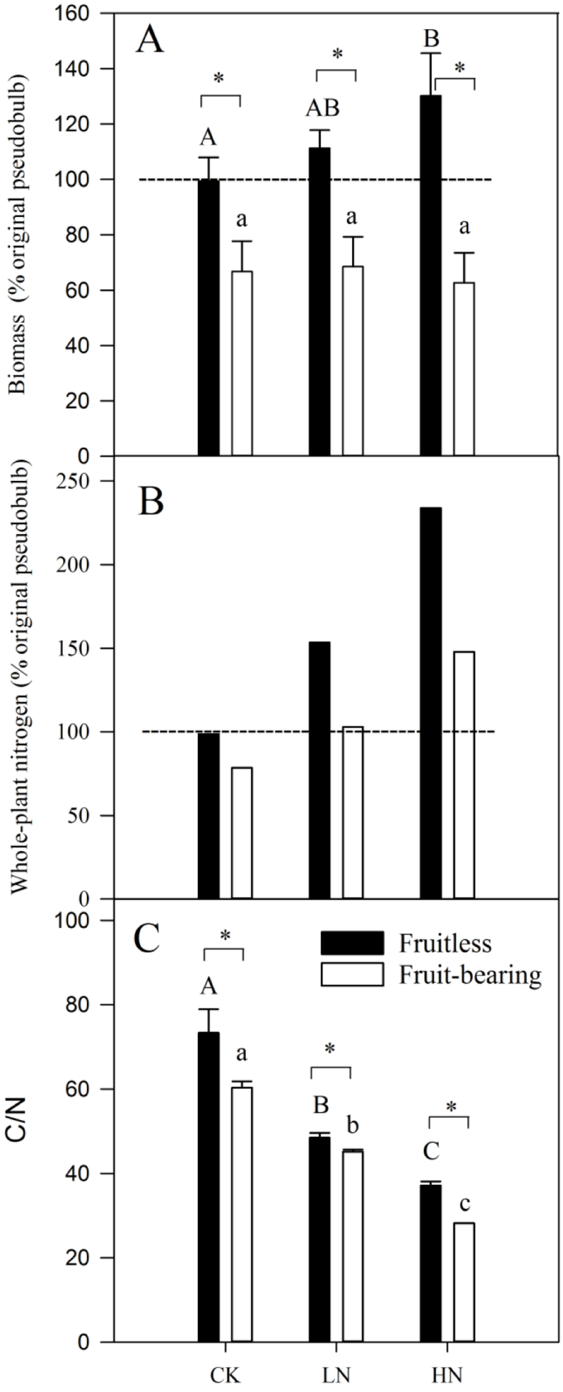

Fig. 7. Annual biomass (A) and nitrogen (B) loss or gain for *Pleione aurita* plants under three nitrogen levels, and the ratio of carbon and nitrogen in the dormant pseudobulbs (C).

Values exceeding 100% in plate A and B indicate an annual gain of biomass or nitrogen. Different letters above the bars indicate statistical differences ($p<0.05$) among nitrogen treatments as determined by an LSD multiple comparison test. * indicates the significant difference between two bars ($p<0.05$) as determined by independent *t*-test.

3.6. *Effects of nitrogen level and fruiting on plant traits*

According to the results of the two-way ANOVA, leaf area, whole-plant nitrogen content, and C/N were influenced by both nitrogen level and fruiting. Biomass at the leaf full expansion stage and the end of growing season and LMA were mostly influenced by fruiting, while P_{max} was mostly influenced by the nitrogen level. No interaction effect between the nitrogen level and fruiting was detected for any of the above-mentioned traits (Table 2).

4. Discussion

4.1. *Sink-source transition and the competition for resources in the pseudobulb*

The life cycle of the *Pleione* pseudobulb studied in our experiment exhibited a remarkable transition process from a sink to a source organ. The developing leaf was the largest sink of both pseudobulb-stored and fertilizer nitrogen in all nitrogen treatments (Figs. 5 and 6). During leaf expansion, the assimilates accumulated in the newly produced pseudobulb, and the new pseudobulb served as the only sink of nutrients in the old pseudobulb and leaf before their abscission. The fully developed new pseudobulb then served as the sole source for the new growth of the flower, roots, leaf, and new pseudobulb in the next year. The life circle of the *Pleione* orchid is simlar to other plants characterized by a conspicuous storage organ (e.g. Millard *et al.*, 1989; Ruiters and Mckenzie, 1994). Previous studies have indicated the interdependence of old and young pseudobulbs in terms of the whole-plant carbon economy (Yong and Hew, 1995a,b), and here we have demonstrated that the old pseudobulb can act as a sink for newly absorbed nitrogen, although with a significantly lower sink activity than a developing pseudobulb. Translocation of nutrients from the old to the young corms is important for a terrestrial orchid, but it does not meet all the needs of the new growth (Whigham, 1984). Similarly, the biomass increased after a growing season only when there was exogenous nitrogen addition in our experiment.

If there is no exogenous nitrogen addition, all nitrogen in the newly produced fruit has to be obtained from the old pseudobulb. However, around 80% of the nitrogen in the fruit still originated from the old pseudobulb even under the circumstance of sufficient nitrogen supply. This proportion was much higher than those in the newly produced roots, pseudobulb, and leaf (Fig.5), indicating the prior use of pseudobulb-stored nitrogen by the fruit. Trade-offs between vegetative growth and reproduction are usually prominent when resources are limited (Bloom *et al.*, 1985; McConnaughay and Coleman, 1999). Most of the *Pleione* species are hysteranthous, which means they produce flowers prior to the leaves (see Fig. 1b), and the fruit is already developing during expansion of the leaf. Thus, competition for the nitrogen resource in the pseudobulb between the developing fruit and leaf may be inevitable, especially when nitrogen supply is limited. As both carbon and nitrogen resources are involved in the construction of leaf (Griffin *et al.*, 1993), there is probably also a carbon limitation during leaf construction for fruit-bearing plants, as reflected by the decreased C/N ratio in pseudobulbs after nitrogen addition, and the lower leaf area even under the circumstance of sufficient nitrogen supply. In fact, the dry matter in the old *Pleione* pseudobulb almost depleted once the leaf was fully expanded (Zhang *et al.*, 2017), then the old pseudobulb acted

Table 2
Summary statistics from the two-way ANOVA for effects of nitrogen level and fruiting on traits of *Pleione aurita*.

Trait	Nitrogen level	Fruiting	Interaction
Leaf area (LA)	5.4**	8.0**	1.7 ns
Leaf mass per unit area (LMA)	0.6 *ns*	20.3***	0.9 *ns*
Light-saturated photosynthetic rate (P_{max})	40.8***	0.1 *ns*	0.2 *ns*
Biomass at leaf full expansion	2.3 *ns*	4.5*	1.3 *ns*
Biomass after a growing season	0.9 *ns*	22.6***	1.5 *ns*
Whole-plant N after a growing season	12.9***	10.8**	1.8 *ns*
C/N after a growing season	148.5***	27.0***	3.0 *ns*

Data are *F*-ratios followed by probability values (*** $p<0.001$, ** $p<0.01$, * $p<0.05$, *ns*=not significant).

more as a water reservoir than a carbohydrate and mineral reserve before its death. Since the fruitless and fruit-bearing plants had similar photosynthetic rates, the lower biomass accumulation in the pseudobulb of fruit-bearing plants can probably be attributed to the lower leaf area in the latter group. Nitrogen depletion or addition did not change the allocation pattern of the reproduction organ, as reflected by the indistinguishable biomass proportion and nitrogen concentration of fruit among the three nitrogen treatments (Figs 3 and 4), implying that multiple resources were needed for fruit setting. Reproduction of orchids is limited by both pollinators and resources (Tremblay *et al.*, 2005; Zimmerman and Aide, 1989). Production of fruits usually leads to a decrease in resource allocation to vegetative growth and future reproduction in orchids, as reflected by the decrease in leaf area, size of storage organ or future flowering probability (Primack and Hall, 1990; Snow and Whigham, 1989; Zimmerman and Aide, 1989). In our experiment, the estimation of reproductive cost of the studied *P. aurita* was achieved by measuring the biomass and nitrogen content before and after the reproduction process, and then comparing the results with those fruitless plants. Our results suggested that more than 30% of the pseudobulb biomass and ~20% of the stored nitrogen was allocated to reproduction.

4.2. Nitrogen absorption and resorption

The rate of mineral uptake by orchids is usually lower than in most other higher plants (Hew *et al.*, 1993). Besides the roots, the leaves of some orchid species have also been proven to have the ablility to take up nitrogen (e.g. Susilo *et al.*, 2013). During the two months of nitrogen addition in our experiment, ammonium nitrate solution was sprayed onto the whole plant and nitrogen determination in each organ was conducted a week after the a fertilizer application. Thus, we assume that in our experiment the majority of nitrogen was taken up by the roots while a small amount of nitrogen was directly taken up by the single leaf. Both the old and newly produced pseudobulbs were able to take up exogenous nitrogen in our experiment. It is reasonable to consider that the newly produced pseudobulbs would take up large amount of nitrogen for further use. However, there seems no point for the old pseudobulb to act as a sink since it will whither after a few months. There are two explanations about why weak sink activity was detected in the old pseudobulb. The first is that the old pseudobulb was still active when the nitrogen was supplied, and the second and more important possibility is that the ability for nitrogen uptake by the old pseudobulb would be of great advantage to the whole-plant nitrogen economy, especially for those species with long-lived pseudobulbs. In other orchid species, old tissue (i.e. remaining stalks of old inflorescences) was also proven to have the ability to take up ^{14}C-assimilate (Yong and Hew, 1995b).

There was a substantial decrease in the whole-plant biomass for all fruit-bearing plants after a growing season, but surprisingly, an increase of whole-plant nitrogen was always observed after nitrogen addition, regardless of the loss of biomass. This highlights the function of nutrition storage in the pseudobulb. Resorption efficiency often decreases with improved nutrition and different organs may respond differentially to nitrogen addition (Mao *et al.*, 2013). More than 78% of the nitrogen in the leaf can be recycled, which is much higher than the average of 47% to 62% for terrestrial plants (Aerts, 1996; Vergutz *et al.*, 2012; Yuan and Chen, 2009) and the average of 35% for epiphytic plants (Zotz, 2004), even if the potential of substantial underestimation of real resorption efficiency due to the changes of leaf mass or leaf area during senescence (van Heerwaarden *et al.*, 2003) was taken into consideration. The high NRE in our study is probably a very special case because this parameter displays great variability depending on different species, growth forms, study sites and soil fertility (Aerts, 1996; Mao *et al.*, 2013; Vergutz *et al.*, 2012). Leaves often have higher NRE than non-storage stems (Freschet *et al.*, 2010; Lü *et al.*, 2012). However, pseudobulbs showed higher resorption efficiency and proficiency than leaves under all nitrogen treatments in our study, suggesting an important contribution of the nitrogen recycling by pseudobulbs to the whole plant.

The tested epiphytic orchid *P. aurita* showed a highly efficient nitrogen recycling capacity, especially under the circumstance of nitrogen depletion. For those plants not engaged in a fruiting process, only a small proportion (1–3% on average) of the biomass and nitrogen was lost during growth without exogenous nitrogen. The biomass and nitrogen loss for this epiphytic orchid mainly resulted from the reproductive event rather than its ability for nitrogen resorption.

4.3. Epiphytic habitats and the presence of a pseudobulb

With no root contact with the soil, water and nutrients for epiphytic plants may largely depend on atmospheric depositions, which is characterized by pulse supply (Benzing, 1990). Different epiphytic taxa have developed various adaptations to ensure the continuous supply of water and nutrients. Many bromeliads produce water-impounding foliage to gain a certain independence from the intermittent water supply in tree canopies (Zotz and Thomas, 1999). While in orchids, the velamen radicum, a spongy tissue around the roots, facilitates the retention of charged particles in the roots to provide a more continuous supply of mineral elements (Zotz and Winkler, 2013). Furthermore, the presence of pseudobulbs in epiphytic orchids further promotes the storage of nutrition that roots uptake. The sink-source transition of a pseudobulb is similar to the charge and discharge of a battery. Pseudobulbs mobilize carbohydrates and minerals to support new growth while retrieving them from senescing organs and storing them in the newly formed pseudobulbs. In the present study, by relying solely on the nitrogen stored in the pseudobulb, plants exhibited a very strong tolerance to nitrogen depletion with only a slight biomass loss after a growing season.

5. Conclusion

In this study, nitrogen allocation and resorption in the epiphytic orchid *P. aurita* in relation to nitrogen supply were quantified. We found both the old and newly produced pseudobulbs can act as a sink for exogenous nitrogen, but with much lower sink activity in the former. The leaf took up the largest proportion of both fertilizer and old pseudobulb-stored nitrogen, and the fruit used pseudobulb-stored nitrogen preferentially regardless of exogenous nitrogen supply. Resorption of nitrogen in the leaf and pseudobulb was found to be more efficient than most other plants but decreased with increasing nitrogen availability. Overall, all fruit-bearing plants lost more than 30% of their biomass after a growing season regardless of nitrogen supply, but with an increase in the whole-plant nitrogen content even in fruit-bearing plants after nitrogen addition. Our results suggested an important role of the pseudobulb in the nitrogen economy of *P. aurita*, and the high dependence of the reproduction process on the pseudobulb-stored nitrogen rather than an exogenous supply.

CRediT authorship contribution statement

Wei Zhang: Methodology, Investigation, Writing – original draft, Resources, Funding acquisition. **Shi-Bao Zhang:** Conceptualization, Validation, Writing – review & editing, Supervision, Funding acquisition. **Ze-Xin Fan:** Conceptualization, Validation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

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

Benzing, D.H., 1990. Vascular Epiphytes. General Biology and Related Biota. Cambridge University Press, Cambridge.

Bloom, A.J., Chapin, F.S., Mooney, H.A., 1985. Resource limitation in plants - an economic analogy. Annu. Rev. Ecol. Syst. 16, 363–392.

Christenhusz, M.J.M., Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa 261, 201–217.

Cribb, P., Butterfield, I., 1999. The Genus *Pleione*. Second edition Royal Botanic Gardens. Kew, Richmond.

Freschet, G.T., Cornelissen, J.H.C., van Logtestijn, R.S.P., Aerts, R., 2010. Substantial nutrient resorption from leaves, stems and roots in a subarctic flora: what is the link with other resource economics traits? New Phytol 186, 879–889.

Göbel, C.Y., Schlumpberger, B.O., Zotz, G., 2020. What is a pseudobulb? Toward a quantitative definition. Int. J. Plant Sci. 181, 686–696.

Griffin, K.L., Thomas, R.B., Strain, B.R., 1993. Effects of nitrogen supply and elevated carbon dioxide on construction cost in leaves of *Pinus taeda* (L) seedlings. Oecologia 95, 575–580.

He, J., 2018. Physiological roles of the green pseudobulb in tropical epiphytic orchids. Adv. Plants Agric. Res. 8, 75–77.

He, J., Norhafis, H., Qin, L., 2013. Responses of green leaves and green pseudobulbs of CAM orchid *Cattleya laeliocattleya* Aloha Case to drought stress. Journal of Botany, 710539, 2013.

He, J., Tan, B.H.G., Qin, L., 2011. Source-to-sink relationship between green leaves and green pseudobulbs of C_3 orchid in regulation of photosynthesis. Photosynthetica 49, 209–218.

Hew, C.S., Lim, L.Y., Low, C.M., 1993. Nitrogen uptake by tropical orchids. Environ. Exp. Bot. 33, 273–281.

Hew, C.S., Ng, C.K.Y., 1996. Changes in mineral and carbohydrate content in pseudobulbs of the C_3 epiphytic orchid hybrid *Oncidium* Goldiana at different growth stages. Lindleyana 11, 125–134.

Hew, C.S., Yong, J.W.H., 1994. Growth and photosynthesis of *Oncidium goldiana*. J. Hortic. Sci. Biotech. 69, 809–819.

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

Lü, X.T., Freschet, G.T., Flynn, D.F.B., Han, X.G., 2012. Plasticity in leaf and stem nutrient resorption proficiency potentially reinforces plant-soil feedbacks and microscale heterogeneity in a semi-arid grassland. J. Ecol. 100, 144–150.

Li, J.W., Zhang, S.B., 2019. Physiological responses of orchid pseudobulbs to drought stress are related to their age and plant life form. Plant Ecol 220, 83–96.

Lin, J.A., Susilo, H., Lei, J.Y., Chang, Y.C.A., 2019. Effects of fertilizer nitrogen shortly before forcing through flowering on carbon-nitrogen composition and flowering of *Phalaenopsis*. Sci. Hortic. 252, 61–70.

Mao, R., Song, C.C., Zhang, X.H., Wang, X.W., Zhang, Z.H., 2013. Response of leaf, sheath and stem nutrient resorption to 7 years of N addition in freshwater wetland of Northeast China. Plant Soil 364, 385–394.

McConnaughay, K.D.M., Coleman, J.S., 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80, 2581–2593.

Millard, P., Robinson, D., Mackiedawson, L.A., 1989. Nitrogen partitioning within the potato (*Solanum tuberosum* L) plant in relation to nitrogen supply. Ann. Bot. 63, 289–296.

Ng, C.K.Y., Hew, C.S., 2000. Orchid pseudobulbs - 'false' bulbs with a genuine importance in orchid growth and survival ! Sci. Hortic. 83, 165–172.

Primack, R.B., Hall, P., 1990. Costs of reproduction in the pink lady's slipper orchid: a four-year experimental study. Am. Nat. 136, 638–656.

Rodrigues, M.A., Matiz, A., Cruz, A.B., Matsumura, A.T., Takahashi, C.A., Hamachi, L., Felix, L.M., Pereira, P.N., Latansio-Aidar, S.R., Aidar, M.P.M., Demarco, D., Freschi, L., Mercier, H., Kerbauy, G.B., 2013. Spatial patterns of photosynthesis in thin- and thick-leaved epiphytic orchids: unravelling C_3-CAM plasticity in an organ-compartmented way. Annal. Bot. 112, 17–29.

Ruiters, C., Mckenzie, B., 1994. Seasonal allocation and efficiency patterns of biomass and resources in the perennial geophyte *Sparaxis grandiflora* subspecies *fimbriata* (Iridaceae) in lowland coastal fynbos, South Africa. Ann. Bot. 74, 633–646.

Snow, A.A., Whigham, D.F., 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). Ecology 70, 1286–1293.

Stancato, G.C., Mazzafera, P., Buckeridge, M.S., 2001. Effect of a drought period on the mobilisation of non-structural carbohydrates, photosynthetic efficiency and water status in an epiphytic orchid. Plant Physiol. Biochem. 39, 1009–1016.

Susilo, H., Peng, Y.C., Lee, S.C., Chen, Y.C., Chang, Y.C.A., 2013. The uptake and partitioning of nitrogen in *Phalaenopsis* Sogo Yukidian 'v3′ as shown by ^{15}N as a tracer. J. Am. Soc. Hortic. Sci. 138, 229–237.

Teoh, E.S., 2016. Medicinal Orchids of Asia. Springer International Publishing, Cham.

Tremblay, R.L., Ackerman, J.D., Zimmerman, J.K., Calvo, R.N., 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biol. J. Linn. Soc. 84, 1–54.

van Heerwaarden, L.M., Toet, S., Aerts, R., 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., Manzoni, S., Porporato, A., Novais, R.F., Jackson, R.B., 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol. Monogr. 82, 205–220.

Whigham, D.F., 1984. Biomass and nutrient allocation of *Tipularia discolor* (Orchidaceae). Oikos 42, 303–313.

Yang, S.J., Sun, M., Yang, Q.Y., Ma, R.Y., Zhang, J.L., Zhang, S.B., 2016. Two strategies by epiphytic orchids for maintaining water balance: thick cuticles in leaves and water storage in pseudobulbs. Aob Plants 8 plw046.

Yong, J.W.H., Hew, C.S., 1995a. The importance of photoassimilate contribution from the current shoot and connected back shoots to inflorescence size in the thin-leaved sympodial orchid *Oncidium goldiana*. Int. J. Plant Sci. 156, 450–459.

Yong, J.W.H., Hew, C.S., 1995b. Partitioning of ^{14}C assimilates between sources and sinks during different growth stages in the sympodial thin-leaved orchid *Oncidium goldiana*. Int. J. Plant Sci. 156, 188–196.

Yuan, Z.Y., Chen, H.Y.H., 2009. Global-scale patterns of nutrient resorption associated with latitude, temperature and precipitation. Glob. Ecol. Biogeogr. 18, 11–18.

Zhang, W., Huang, W., Zhang, S.B., 2017. The study of a determinate growth orchid highlights the role of new leaf production in photosynthetic light acclimation. Plant Ecol 218, 997–1008.

Zimmerman, J.K., 1990. Role of pseudobulbs in growth and flowering of *Catasetum viridiflavum* (Orchidaceae). Am. J. Bot. 77, 533–542.

Zimmerman, J.K., Aide, T.M., 1989. Patterns of fruit production in a Neotropical orchid: pollinator vs resource limitation. Am. J. Bot. 76, 67–73.

Zotz, G., 2004. The resorption of phosphorus is greater than that of nitrogen in senescing leaves of vascular epiphytes from lowland Panama. J. Trop. Ecol. 20, 693–696.

Zotz, G., 2016. Plants on Plants–The Biology of Vascular Epiphytes. Springer International Publishing, Cham.

Zotz, G., Thomas, V., 1999. How much water is in the tank? Model calculations for two epiphytic bromeliads. Ann. Bot. 83, 183–192.

Zotz, G., Winkler, U., 2013. Aerial roots of epiphytic orchids: the velamen radicum and its role in water and nutrient uptake. Oecologia 171, 733–741.