



# Different reutilization of mineral nutrients in senescent leaves of aquatic and terrestrial carnivorous *Utricularia* species



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## ABSTRACT

Nutrient reutilization (recycling) from senescent tissues in aquatic and terrestrial plants is important especially in nutrient-poor environments. The hypothesis was verified that N and P are reutilized efficiently from senescent shoots in both aquatic and terrestrial plants, while K is reutilized only in terrestrial plants. The efficiency of N, P and K reutilization in old traps and shoot segments in the submerged carnivorous plant *Utricularia reflexa* and the leaves of non-carnivorous *Ceratophyllum submersum* was compared with that in the leaves of five terrestrial *Utricularia* species. Oxygen-based dark respiration rates of *U. reflexa* traps, measured as a criterion of physiological activity, exhibited a marked polarity, while a weak respiration polarity of leaves was not significant. No distinct polarity of trap and shoot N contents were found in *U. reflexa* shoots. When the correction factor of 0.71 was applied to compensate for the assumed dry weight (DW) decrease in old traps and leaf nodes, the mean reutilization efficiency for N was 19% in traps and 37% in leaf nodes and for P, 67% in traps and 52% in leaf nodes. However, in spite of the correction factor, the K reutilization efficiency in both types of organs was negative. In *Ceratophyllum* leaves, the corrected N, P and K reutilization efficiencies were similar to *U. reflexa* leaves. In five terrestrial *Utricularia* species, the N, P and K contents in adult leaves always significantly (usually at  $p < 0.01$ ) exceeded that in senescent leaves. The following corrected reutilization efficiencies were found: 35–82% for N, 56–84% for P and 48–91% for K. These efficiencies are comparable with those in the aquatic species only for N and P, but are quite different for K. It may be concluded that reutilization of K (unlike N and P) in leafy shoots generally occurs only in terrestrial and not in aquatic plants. The lack of K reutilization in aquatic plants might be due to the direct  $K^+$  uptake from the water column.

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## 1. Introduction

The rootless carnivorous plant genus *Utricularia* L. (bladderwort, Lentibulariaceae) includes about 220 species. While most of these are wetland, terrestrial or epiphytic, about 50 are submerged aquatic or amphibious species (Taylor, 1989; Jobson et al., 2003; Guisande et al., 2007). The typical aquatic or amphibious species from the generic *Utricularia* and *Vesiculina* sections have linear, modular and fairly regular shoot structures, consisting of nodes with dissected leaves (reminiscent of whorls) and thin, cylindrical internodes (Friday, 1989; Taylor, 1989; Sattler and Rutishauser, 1990; Rutishauser, 1993). These linear shoots can be monomorphic and bear traps on green photosynthetic shoots or dimorphic, with traps (exclusively or dominantly) held on special, pale carnivorous

shoots. The terrestrial *Utricularia* species from the other sections usually have rhizomatous shoot structures with flat, lanceolate or even circular, peltate leaves on long, thin petioles growing in nodes from short filiform stems. Similarly, several epiphytic species have robust lanceolate or ovoid leaves and all traps are borne on special, linear carnivorous shoots usually growing in bromeliad leaf cisternae (Taylor, 1989). In this paper, the term “leaf” refers to the dissected or flat photosynthetic organs which grow in nodes perpendicular to the “stem” (stolon).

Aquatic *Utricularia* species usually grow in shallow, standing, humic (dystrophic) waters, which are generally poor in inorganic nitrogen (N) and phosphorus (P), but commonly also in potassium (K) (Adamec, 1997, 2008, 2009, 2011; Guisande et al., 2007). However, these waters are usually rich in free  $CO_2$ . These species take up all necessary nutrients by their shoots, either directly from the water or from prey. The observed very rapid growth of aquatic *Utricularia* in these nutrient-poor waters requires a combination of several ecophysiological adaptations that enable the plants to access the very limited supplies of mineral nutrients. Their principal

Abbreviations: RD, dark respiration rate; DW, dry weight; FW, fresh weight.

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adaptations include a very high net photosynthetic rate, carnivory, efficient mineral nutrient reutilization (recycling/resorption) from senescent shoots (*i.e.*, efficient nutrient economy) and a very high nutrient uptake affinity from water (Kosiba, 1992a,b; Adamec, 1997, 2006, 2008, 2009, 2011, 2013; Englund and Harms, 2003).

Traps of aquatic *Utricularia* species—hollow bladders 1–5 mm long—are physiologically very active organs, having high aerobic dark respiration rates (RD) and large photosynthetic and energetic (maintenance) costs (Knight, 1992; Adamec, 2006; Adamec, 2011). These maintenance costs include a substantial carbohydrate supply to the trap fluid to support the commensal microbial communities that exist there (Sirová et al., 2010). The structural investment in carnivory, *i.e.*, the proportion of trap dry weight (DW) to the total shoot DW, greatly depends on the *Utricularia* ecological group: aquatic species allocate between 4% and 62% DW to traps (mean usually *ca.* 30–40%; Friday, 1989; Englund and Harms, 2003; Adamec, 2008, 2009, 2011), the epiphytic *Utricularia quelchii* devotes 30%, but terrestrial species only devote between 0.14% and 0.85% (Porembski et al., 2006). Additionally, the traps also have a high mineral cost (Adamec, 2008, 2010). Adamec (2010) found that in four aquatic *Utricularia* species, trap P and K contents (per unit DW) were markedly greater than in adult carnivorous shoot segments, while the opposite was observed for N, Ca and Mg contents. In these four species, the structural investment in carnivory ranged between 24% and 50%, the mineral cost of traps was 12–47% of the total N content and as much as 20–63% P and 43–69% K. However, aquatic *Utricularia* species are able to regulate the structural costs (and hence also the energetic and mineral costs) to match variations in habitat factors, mainly water chemistry, prey availability and the level of irradiance. Tissue N or P contents in young shoots were found to regulate the investment in trap biomass through a negative feedback mechanism (for the review, see Adamec, 2011).

Aquatic *Utricularia* species with linear shoots exhibit very distinct growth polarity: their very rapid apical shoot growth (1.5–4.2 leaf nodes  $d^{-1}$ ) is associated with permanent, very rapid senescence and decay of basal shoot segments so that the main shoot length remains approximately constant with the new biomass allocated to branches (“conveyor-belt” growth system; Adamec, 2009, 2011; Adamec et al., 2010). The associated physiological polarity of the linear shoots has been found to include a polarity of RD and foliar mineral nutrient content (N, P, K and Ca; Friday and Quarmby, 1994; Adamec, 2008, 2013). While the foliar/shoot N and P contents steeply decrease from the shoot apex towards the senescent shoot base, the opposite is seen with K and Ca shoot contents which are highest at the shoot base. Exactly the same polarity of the N, P, K and Ca contents was also found in the ecologically similar aquatic carnivorous plant *Aldrovanda vesiculosa* (Droseraceae; Adamec, 2000). Thus, the same polarity pattern presumably occurs in all aquatic carnivorous plants, confirming that both N and P are efficiently reutilized from senescent shoot segments to the growing shoot apices but K and Ca are not (Adamec, 2000, 2008, 2011). In general, the efficiency of nutrient reutilization (*i.e.*, recycling, nutrient resorption) in senescent shoots (leaves) is considered an important ecophysiological trait and a high efficiency is an adaptation to nutrient-poor environments (Killingbeck, 1996; Aerts et al., 1999; Adamec, 2002). Terrestrial plants are known to reutilize N, P and K from senescing leaves/shoots relatively efficiently, but Ca is not reutilized at all (*e.g.*, Marschner, 1995). However, with the exception of three aquatic carnivorous species, namely *Utricularia australis* (Adamec, 2008), *Utricularia purpurea* (Moeller, 1980) and *A. vesiculosa* (Adamec, 2000), and the non-carnivorous *Potamogeton pectinatus* (Lytle and Smith, 1995), reutilization characteristics have not been studied in submerged aquatic plants.

In this study, the N, P and K reutilization characteristics in traps and trap-free shoot segments in the aquatic *Utricularia reflexa* were

compared with those in the leaves of five terrestrial *Utricularia* species. This was to confirm the hypothesis that N and P are reutilized efficiently from senescent shoots in both ecological plant groups, while K is reutilized only in terrestrial plants. A rootless submerged plant, *Ceratophyllum submersum*, was used as a non-carnivorous aquatic control to test this hypothesis. Moreover, in *U. reflexa*, RD was measured in halved traps and trap-free leaves of different ages to characterize the physiological polarity of the shoots. Possible reasons for different K reutilization efficiencies in aquatic vs. terrestrial plants are discussed.

## 2. Material and methods

### 2.1. Plant material

Dark respiration and N, P and K reutilization were measured in traps and leaves of aquatic *U. reflexa* Oliver (collected in Okavango Delta, Botswana). The plants were grown indoors in a naturally-lit 3 L aquarium in water approx. 20 cm deep (Sirová et al., 2003). A litter of robust sedges was used as a well-trieved substrate and natural water chemistry conditions were approximately simulated. The pH of the water ranged from 5.8 to 5.9, the total alkalinity was *ca.* 0.2 to 0.3 meq  $L^{-1}$ , the free  $CO_2$  concentration was *ca.* 0.8 to 1.2 mM and the electrical conductivity was 14.6  $mS\ m^{-1}$ . The water was considered oligo-mesotrophic (in  $\mu g\ L^{-1}$ :  $NO_3^-$ -N, 0;  $NH_4^+$ -N, 37;  $PO_4$ -P, 27) and slightly dystrophic. The concentration of metallic cations in the water was (in  $mg\ L^{-1}$ ):  $K^+$ , 1.26;  $Na^+$ , 5.99;  $Ca^{2+}$ , 16.1;  $Mg^{2+}$ , 1.1. To promote plant growth, small zooplankton (a mixture of dozens of crustaceans of body size 0.6–1.5 mm) were added to the culture *ca.* every other day and feeding was interrupted approx. 1 week before the experiment. See Adamec et al. (2010) for mineral content of the zooplankton. Adult *U. reflexa* plants used for the experiments were 25–30 cm long, with 32–40 leaf nodes and their largest traps were 4–6 mm long.

The evergreen free-floating, rootless aquatic, *Ceratophyllum submersum* L. (hornwort, Ceratophyllaceae), was used as a non-carnivorous control plant. These plants (collected from Třeboň region, Czech Rep.) were grown outdoors in a 1  $m^2$  plastic container in water 40 cm deep, the substrate was a 5 mm thick layer of garden loam and the container was partly shaded for cooling. The pH of the water ranged from 9.1 to 9.3, total alkalinity was *ca.* 1.0 meq  $L^{-1}$ , the free  $CO_2$  concentration was only *ca.* 1 to 2  $\mu M$  and the electrical conductivity was 16.5  $mS\ m^{-1}$ . The water was considered oligo-mesotrophic (in  $\mu g\ L^{-1}$ :  $NO_3^-$ -N, 0;  $NH_4^+$ -N, 33;  $PO_4$ -P, 8.8). The concentration of metallic cations in the water was (in  $mg\ L^{-1}$ ):  $K^+$ , 0.30;  $Na^+$ , 2.37;  $Ca^{2+}$ , 4.70;  $Mg^{2+}$ , 0.58). Adult *C. submersum* plants were 50–60 cm long with 20–30 leaf nodes.

Five terrestrial and/or epiphytic *Utricularia* species were used for comparison of the N, P and K reutilization efficiencies with *U. reflexa*. These were *Utricularia alpina* Jacq., *Utricularia humboldtii* Schomb., *Utricularia nelumbifolia* Gardner, *U. quelchii* N.E.Br. (all originating from the northern part of South America) and *Utricularia dichotoma* Labill. (collected from N.S.W., Australia). All were grown in 13 × 13 cm plastic pots containing a mixture of peat and washed sand (*ca.* 2:1 v/v) in a naturally lit, partly shaded greenhouse at 22–30 °C and 70–90% relative humidity. Water purified by reverse osmosis was used to keep the soil permanently very wet.

### 2.2. Dark respiration measurements

To study the physiological polarity of linear *U. reflexa* shoots, aerobic dark respiration was measured in 2–6 separated, partly halved traps and 4–10 leaves with traps removed. These were all collected from leaf nodes at three different positions and therefore ages. The traps and trap-free leaves were used from the 3rd to the

**Table 1**

Aerobic respiration rate of halved traps and leaves of *Utricularia reflexa* of different ages at 25 °C. The old traps and leaves were the last functional ones. Means  $\pm$  SE intervals are shown;  $n=6$ . RD values expressed both on FW and DW basis.

Organ	Organ age	DW (% FW)	RD per FW (mmol kg <sup>-1</sup> h <sup>-1</sup> )	RD per DW (mmol kg <sup>-1</sup> h <sup>-1</sup> )
Traps	3rd–5th mature nodes (young)	4.51	**6.86 $\pm$ 0.51 <sup>a</sup>	**152 $\pm$ 11.3 <sup>a</sup>
	9th–10th mature nodes (medium)	5.68	*5.44 $\pm$ 0.41 <sup>ab</sup>	*95.8 $\pm$ 7.3 <sup>b</sup>
	>30th mature nodes (old)	6.27	**3.86 $\pm$ 0.40 <sup>b</sup>	61.5 $\pm$ 6.4 <sup>c</sup>
Leaves	3rd–5th mature nodes (young)	13.8	12.3 $\pm$ 0.98 <sup>a</sup>	89.5 $\pm$ 7.1 <sup>a</sup>
	9th–10th mature nodes (medium)	14.7	9.85 $\pm$ 1.47 <sup>a</sup>	66.8 $\pm$ 10.0 <sup>a</sup>
	>30th mature nodes (old)	12.0	8.96 $\pm$ 1.34 <sup>a</sup>	74.6 $\pm$ 11.1 <sup>a</sup>

Different letters within each organ type denote statistically significant differences at  $p < 0.05$  (1-way ANOVA, Tukey HSD test). The asterisks on the left denote statistically significant difference between traps and leaves of the same age; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  (1-way ANOVA).

5th adult leaf nodes (denoted as 'young'), the 9th to the 10th nodes ('medium'), and from the 31st to the 40th nodes ('old'), which were the last functional nodes with traps. The fresh weight (FW) of the trap or leaf samples was 4–25 mg. Generally, RD was measured in a solution of 1 mM NaHCO<sub>3</sub> with 0.1 mM KCl (ca. 90% O<sub>2</sub> saturation) in a 2-mL stirred chamber kept at 25.0  $\pm$  0.1 °C. A Clark-type oxygen sensor and a pen recorder (for details, see Adamec, 2006) was used. RD was measured in darkness for 12–15 min. After the measurements, the FW of the blotted samples and DW (80 °C drying) of the pooled samples were estimated. All measurements were repeated six times using material from different plants and RD is expressed in mmol kg<sup>-1</sup> h<sup>-1</sup> for both FW and DW.

### 2.3. N, P and K reutilization, chemical analyses and statistical treatment

To estimate the polarity in the nutrient content and the reutilization efficiency (also termed resorption efficiency; Killingbeck, 1996), tissue N, P and K contents were determined in 20–30 traps and 1–3 trap-free shoot segments for *U. reflexa*, in plants parallel to those used for the DR measurements (see above;  $n=6$ ). The positions of the sampled traps and shoot segments were the same as for the RD measurements. Sufficient biomass for one sample (DW 4–6 mg) was pooled from 1 to 3 parallel plants. All traps were halved, quickly rinsed in distilled water to remove commensal communities from the trap interior, blotted dry and dried at 80 °C. Tissue N, P and K contents were also determined in 6–20 leaves (without stems; DW 4–5 mg) collected from the 7th to 8th adult leaf nodes ('young') and three senescent, last-living leaf nodes ('old') from the same plant of *C. submersum*. Evidently, the senescent leaf nodes were formed during the previous growing season. Six parallel samples from different plants were used. The collected leaves were thoroughly cleared of macroscopic filamentous algae using forceps. Adult green leaves ('adult') and yellowish-brown senescent ones (mixed samples from 1 to 4 leaves; 'senescent') were collected from different colonies of *U. alpina*, *U. humboldtii*, *U. nelumbifolia*, *U. quelchii* and *U. dichotoma* (DW 3–5 mg;  $n=6$  for each species). The sampled material was dried at 80 °C and ground by forceps into small pieces.

N, P and K contents of the dried, ground organs were estimated in diluted samples after acid mineralization (for all analytical details, see Adamec, 2002, 2010). N and P analyses were determined colorimetrically by an automatic FIAStar 5010 Analyzer (Tecator, Sweden), while K<sup>+</sup> concentrations were estimated by atomic absorption flame spectrometry using a Varian AA240FS (Agilent, Santa Clara, CA, USA). The results of tissue N, P and K contents in both young/adult and old/senescent organs are always expressed as % DW and presented as such. As no data are available for the relative decrease of DW in senescent organs of the species used in this study, the assumed decrease of DW of senescent leaves relative to that in young/adult leaves was arbitrarily chosen to be 29% for all species (as the mean estimated in leaves of terrestrial carnivorous

plants, Adamec, 2002; see also Güsewell, 2005). Thus, a correction factor of 0.71 was used to multiply the estimated values of nutrient content for senescent leaves to calculate the true reutilization efficiency (as a % of the nutrient content in the young organs).

Significant differences in RD and tissue nutrient contents (and molar N:P and N:K ratios) within the same organ type (traps or leaves), as a function of organ age, as well as differences between the traps and leaves of the same age were found using 1-way ANOVA. Significant differences in the tissue nutrient contents between the adult and senescent leaves (corrected in the senescent ones by the factor of 0.71) in the terrestrial *Utricularia* species were found using a two-tailed Student *t*-test for independent samples. Means  $\pm$  1 SE are shown.

### 3. Results

The dark respiration rates of *U. reflexa* traps on both the FW and DW basis exhibited a marked and statistically significant polarity, declining from young to old leaf nodes (Table 1). The rate of RD per unit DW in old traps was only 40.5% of that in young traps. However, a weak polarity of RD of leaves was not significant at  $p < 0.05$  on the FW or DW basis. The RD rate of traps of all ages per unit FW was significantly lower than that in leaves but the RD rate per DW for young and medium traps significantly exceeded that of leaves.

No distinct polarity in trap and shoot N content was found in *U. reflexa* shoots and the values were comparable in traps and leaf nodes (Table 2). However, when the correction factor of 0.71 for the DW decrease in old traps and leaf nodes was applied, the mean reutilization efficiency for N was 19% and 37% in traps and leaf nodes, respectively. The tissue P content in young and medium traps (0.18 and 0.11% DW) highly significantly exceeded that in leaf nodes of the same age (0.12 and 0.08% DW) and exhibited a significant polarity along the shoots. Therefore, the mean corrected P reutilization efficiency was 67% in traps and 52% in leaf nodes. K content in traps (2.8–6.1% DW) highly significantly exceeded that in leaf nodes of the same age (2.0–3.6%). In marked contrast to P, the greatest K content, both in traps and leaf nodes, was found in the old organs. Even with the correction factor, the reutilization efficiency in both types of organs was negative. The molar N:P and N:K ratios reflected the trends in the tissue contents for the given nutrients: significant polarity of the N:P ratio was found only in the traps, while significant polarity of the N:K ratio, but with an opposite sign, was found in both organ types. Like *U. reflexa* shoots, foliar N and P contents in old leaves of *C. submersum* were significantly lower than that in young leaves, suggesting an efficient reutilization of 53% N and 60% P (Table 2). The foliar K content in old leaves was, however, significantly higher (by 33%) than that in young leaves. After the DW correction in old leaves, a low reutilization efficiency of 5.2% was estimated. The molar N:P and N:K ratios differed significantly between young and old leaves.

In five terrestrial *Utricularia* species, values of the N, P and K contents of both adult and senescent leaves were very variable

**Table 2**  
The comparison of tissue N, P and K content in traps and shoots of *Utricularia reflexa* of different age and in young and senescent leaves of *Ceratophyllum submersum*. RE-, reutilization efficiency of the given nutrient in senescent organs expressed as the decline of nutrient content relative (in %) to that of the young organ. Negative values of nutrient reutilization efficiency indicate higher nutrient content in senescent organs after correction. Due to an assumed decrease of DW of senescent leaves relative to that in adult leaves by 29% (Adamec, 2002), the correction factor of 0.71 was used to multiply the shown estimated values of nutrient content for senescent leaves to calculate the true reutilization efficiency. Different types of organs or species are separated by dotted line. Means  $\pm$  SE intervals are shown;  $n = 6$ .

Organ	Organ age	N content (% DW)	RE-N (%)	P content (% DW)	RE-P (%)	K content (% DW)	RE-K (%)	N:P (molar)	N:K (molar)
Traps	3rd–5th nodes (young)	<sup>ns</sup> 0.752 $\pm$ 0.045 <sup>ab</sup>	–	<sup>**</sup> 0.180 $\pm$ 0.004 <sup>a</sup>	–	<sup>**</sup> 3.10 $\pm$ 0.10 <sup>a</sup>	–	<sup>**</sup> 9.22 $\pm$ 0.46 <sup>a</sup>	<sup>**</sup> 0.683 $\pm$ 0.052 <sup>a</sup>
	9th–10th nodes (med.)	<sup>*</sup> 0.621 $\pm$ 0.022 <sup>a</sup>	–	<sup>**</sup> 0.114 $\pm$ 0.003 <sup>b</sup>	–	<sup>**</sup> 2.82 $\pm$ 0.20 <sup>a</sup>	–	<sup>**</sup> 12.1 $\pm$ 0.71 <sup>b</sup>	<sup>**</sup> 0.628 $\pm$ 0.047 <sup>a</sup>
	>30th nodes (old)	<sup>*</sup> 0.857 $\pm$ 0.052 <sup>b</sup>	19.1	<sup>ns</sup> 0.085 $\pm$ 0.004 <sup>c</sup>	66.5	<sup>**</sup> 6.11 $\pm$ 0.23 <sup>b</sup>	–39.9	<sup>ns</sup> 22.3 $\pm$ 0.84 <sup>c</sup>	<sup>**</sup> 0.394 $\pm$ 0.022 <sup>b</sup>
Shoot	3rd–5th nodes (young)	0.772 $\pm$ 0.034 <sup>a</sup>	–	0.116 $\pm$ 0.006 <sup>a</sup>	–	2.35 $\pm$ 0.04 <sup>a</sup>	–	14.8 $\pm$ 0.52 <sup>a</sup>	0.918 $\pm$ 0.040 <sup>a</sup>
	9th–10th nodes (med.)	0.709 $\pm$ 0.022 <sup>a</sup>	–	0.080 $\pm$ 0.002 <sup>b</sup>	–	1.96 $\pm$ 0.13 <sup>a</sup>	–	19.7 $\pm$ 0.89 <sup>a</sup>	1.03 $\pm$ 0.055 <sup>a</sup>
	>30th nodes (old)	0.686 $\pm$ 0.034 <sup>a</sup>	36.9	0.078 $\pm$ 0.010 <sup>b</sup>	52.3	3.56 $\pm$ 0.33 <sup>b</sup>	–7.6	21.5 $\pm$ 3.48 <sup>a</sup>	0.555 $\pm$ 0.042 <sup>b</sup>
<i>Cerat.</i>	Young leaves	1.53 $\pm$ 0.11 <sup>a</sup>	–	0.112 $\pm$ 0.007 <sup>a</sup>	–	2.42 $\pm$ 0.10 <sup>a</sup>	–	30.1 $\pm$ 0.69 <sup>a</sup>	1.76 $\pm$ 0.089 <sup>a</sup>
<i>Cerat.</i>	Old leaves	1.02 $\pm$ 0.08 <sup>b</sup>	52.7	0.063 $\pm$ 0.003 <sup>b</sup>	60.1	3.23 $\pm$ 0.08 <sup>b</sup>	5.2	35.7 $\pm$ 2.2 <sup>b</sup>	0.888 $\pm$ 0.081 <sup>b</sup>

Different letters within each organ type and species denote statistically significant difference at  $p < 0.05$  (1-way ANOVA, Tukey HSD test). The asterisks on the left side of the columns denote statistically significant difference between traps and leaves of the same age; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  (1-way ANOVA).

among single species but the relationship between the foliar contents of all three nutrients in adult and senescent leaves was quite unambiguous and consistent (Table 3). The N, P and K contents of adult leaves in all species always significantly (usually at  $p < 0.01$ ) exceeded that in the senescent leaves, which indicate very efficient reutilization of N, P and K. In summary, the N content in adult leaves ranged between 0.61% and 1.96% DW, and only between 0.23% and 0.81% in senescent leaves; the P content in adult leaves was between 0.05% and 0.14% DW, and only between 0.02% and 0.04% in senescent leaves; the K content in adult leaves was between 1.3% and 2.6% DW and only between 0.18% and 0.97% in senescent leaves. Thus, assuming a 29% reduction of DW of senescent leaves (Adamec, 2002), the following reutilization efficiencies apply: 35–82% for N, 56–84% for P, and 48–91% for K. These efficiencies are comparable with those in the aquatic species for N and P only but are quite different for K (cf. Table 2).

#### 4. Discussion

One of the aims of this study was to characterize the physiological polarity of *U. reflexa* shoots. A marked and significant polarity of RD was found in traps (for both FW and DW), but not in leaves of the same age (Table 1). This confirms that traps in aquatic

*Utricularia* species age faster than leaves alone, i.e., the trap life-span is lower and their turnover is faster. This phenomenon was described in detail in field-grown *Utricularia vulgaris* (Friday, 1989); the mean trap life-span was only 19 d, while for leaves it was 25–30 d. Moreover, the relatively weak RD polarity of leaves along linear shoots of aquatic *Utricularia* species is in accordance with an absence of polarity in the net photosynthetic rate in the trap-free leaves of these species (Adamec, 2013). This study showed that for *U. vulgaris* and *U. australis*, even old leaves (from 50 to 60th leaf nodes) without decayed traps kept a very high net photosynthetic rate (ca. 60–64% of the maximum). The relatively low RD rates of large traps in *U. reflexa*, when compared with those in leaves (Table 1), are in contrast with the results for six aquatic *Utricularia* species in which the RD rates of mature traps (both on FW and DW) exceeded those for leaves by 2–2.5 times (cf. Adamec, 2006). The metabolic (maintenance) cost of *Utricularia* traps is probably also dependent on trap size or may be highly species specific.

In this paper, nutrient reutilization in aquatic *U. reflexa*, *Ceratophyllum demersum* and five terrestrial *Utricularia* species was estimated by the standard procedure of comparing the tissue N, P and K content in young/adult and old/senescent leaf nodes or leaves. However, as senescent terrestrial organs generally contain a significantly lower DW than young or adult ones (e.g., DW decrease

**Table 3**  
The comparison of tissue N, P and K content in adult and senescent leaves in five terrestrial *Utricularia* species. Reutilization efficiency of the given nutrient in senescent leaves expressed as the decline of nutrient content relative (in %) to that in the adult leaves. Due to an assumed decrease of DW of senescent leaves relative to that in adult leaves by 29% (Adamec, 2002), the correction factor of 0.71 was used to multiply the shown estimated values of nutrient content for senescent leaves to calculate the true reutilization efficiency. Both the reutilization efficiency and the statistical significance between the adult and senescent leaves were calculated from these corrected values.

Species	Nutrient	Foliar content (% DW)		Reutilization (% of adult)
		Adult	Senescent	
<i>U. alpina</i>	N	0.608 $\pm$ 0.039	0.227 $\pm$ 0.034 <sup>**</sup>	73.5
	P	0.063 $\pm$ 0.013	–	–
	K	1.56 $\pm$ 0.17	0.716 $\pm$ 0.285 <sup>**</sup>	67.4
<i>U. humboldtii</i>	N	1.14 $\pm$ 0.13	0.559 $\pm$ 0.026 <sup>**</sup>	65.2
	P	0.121 $\pm$ 0.019	–	–
	K	2.35 $\pm$ 0.09	0.678 $\pm$ 0.187 <sup>**</sup>	79.6
<i>U. nelumbifolia</i>	N	1.62 $\pm$ 0.14	0.422 $\pm$ 0.033 <sup>**</sup>	81.5
	P	0.135 $\pm$ 0.023	0.037 $\pm$ 0.008 <sup>**</sup>	80.6
	K	1.30 $\pm$ 0.12	0.944 $\pm$ 0.188 <sup>**</sup>	48.2
<i>U. quelchii</i>	N	0.661 $\pm$ 0.063	0.602 $\pm$ 0.045 <sup>*</sup>	35.3
	P	0.045 $\pm$ 0.006	0.028 $\pm$ 0.004 <sup>**</sup>	56.0
	K	1.47 $\pm$ 0.18	0.180 $\pm$ 0.077 <sup>**</sup>	91.3
<i>U. dichotoma</i>	N	1.96 $\pm$ 0.14	0.809 $\pm$ 0.167 <sup>**</sup>	70.7
	P	0.095 $\pm$ 0.009	0.021 $\pm$ 0.002 <sup>**</sup>	84.2
	K	2.62 $\pm$ 0.21	0.971 $\pm$ 0.217 <sup>**</sup>	73.7

<sup>\*\*</sup>,  $p < 0.01$ ; <sup>\*</sup>,  $p < 0.05$  (two-tailed Student *t*-test). Means  $\pm$  SE intervals are shown;  $n = 5-6$ .

by 28–30% in *Drosera* and *Dionaea* (Adamec, 2002), by 8–38% in 10 wetland graminoid species (Güsewell, 2005), the calculation of the reutilization efficiency in senescent organs should be corrected for this DW decrease. Evidently, a similar DW decrease in senescent leaves/shoots also occurs in aquatic plants, but these data are presently unknown. It can be deduced from the decreased DW:FW ratio in *U. australis* leaves that this DW decrease might be at least 20% (Adamec, 2013) and at least 17% in *Aldrovanda* (Adamec, 2000). Thus, the mean correction factor of 0.71 (i.e., 100–29%; Adamec, 2002) was used equally for all aquatic and terrestrial species in the present study.

Aquatic *U. reflexa* did not show a distinct polarity of N content in trap-free leaf nodes but a weak, significant polarity of N content occurred, peaking in old traps (Table 2). This might be caused by a marked DW decrease in the old traps as the corrected value of N reutilization efficiency was 19%. Unlike N, trap P content was significantly higher than in leaves and exhibited a much steeper polarity, which declined from young traps and resembled the polarity of trap RD. On the other hand, the marked polarity of K content in both traps and leaves followed the opposite trend. Generally, the data on N, P and K contents in young *U. reflexa* traps and leaves found in this study were exactly the same as those reported by Adamec (2010) for the same species and organs. Moreover, they support the view that foliar N, P and K contents in aquatic *Utricularia* species are partly species-specific and genetically fixed. The polarity pattern of N, P and K contents in young and old leaves in the *C. submersum* control confirmed the efficient reutilization of both N and P, and the almost zero reutilization of K in aquatic plants (Table 2). The nutrient content in adult leaves of the five terrestrial *Utricularia* species varied by 2–3 times between the species, but in all species, a very efficient N, P and K reutilization was found (Table 3). This efficient N, P and K reutilization is similar to that found in leaves of other terrestrial carnivorous plants from the *Drosera* and *Dionaea* genera (Adamec, 2002) and, thus, confirms this principal and essential adaptation of terrestrial carnivorous plants to nutrient-poor soils (cf. Aerts et al., 1999).

It is generally accepted for terrestrial plants that the efficiency of N and P reutilization in senescent leaves reflects the plant's ecological status, the trophic level of the soil environment, the leaf life-span, organ type, the plant's functional traits, seasonal climatic differences and partly also taxonomic differences (Killingbeck, 1996; Adamec, 1997, 2002; Aerts et al., 1999; Güsewell, 2005). Moreover, a very effective K reutilization is common in terrestrial plant leaves (e.g., Marschner, 1995; Adamec, 2002). However, only a few studies have so far been conducted to describe the efficiency of nutrient reutilization in submerged plants, mainly in carnivorous species. Lytle and Smith (1995) compared the autumn and winter foliar nutrient content in rooted *Potamogeton pectinatus* in Utah, USA, and found an efficient reutilization of N, P and S, but only a very weak (ca. 19%) K reutilization and even accumulation of Mg and Ca. In the very slowly growing aquatic *U. purpurea*, Moeller (1980) reported that summer K content in all adult shoot segments was about constant and this also held for the oldest ones grown in the given season. In *U. australis* grown at 30 natural sites within a wide K<sup>+</sup> concentration range (totally 0.48–10.2 mg L<sup>-1</sup>), K content in senescent shoot segments was about 10% higher than that in young shoot segments (Adamec, 2008). Similarly in *Aldrovanda*, K content was around the same in adult leaf whorls of different age, from young up to the turgid 'last living whorls' (Adamec, 2000). The shoot K content dropped dramatically (from 2.30% to 0.65% DW) in the next leaf whorl considered the 'first dead one'. Thus, this sharp gradient of foliar K content in senescent *Aldrovanda* shoots proves the ability of foliar tissues to concentrate K<sup>+</sup> in the 'last living whorls', which is then rapidly lost.

In spite of the rather low ambient K<sup>+</sup> concentrations in which both *U. reflexa* and *Ceratophyllum* were growing in this study (1.26

and 0.30 mg L<sup>-1</sup>, respectively), the K reutilization in old leaves in both aquatic species was zero (Table 2). Thus, the plants lost all their K content in their senescent shoots and all K<sup>+</sup> necessary for rapid plant growth had to be taken up *de novo*. Moreover, these low ambient K<sup>+</sup> concentrations indicate that a high-affinity, active K<sup>+</sup> transport has to operate on leaf cell membranes on account of the energy consumption to facilitate the necessary growth K<sup>+</sup> uptake from the ambient water (e.g., Britto and Kronzucker, 2008; Szczerba et al., 2009; Wang and Wu, 2010). In rooted aquatic plants, unlike the uptake of N and P, K<sup>+</sup> uptake by leafy shoots from the water greatly prevails over that by roots from sediments (Barko, 1982; Barko et al., 1991). In the natural habitats of some *Utricularia* species in dystrophic waters, the ambient K<sup>+</sup> concentrations can be as low as between 0.01 and 0.1 mg L<sup>-1</sup> (see Adamec, 1997) and, together with N and P, can co-limit plant growth. In rootless aquatic carnivorous plants, all K<sup>+</sup> is taken up from the ambient water by leafy shoots or from prey by traps but the contribution of prey K to the total K<sup>+</sup> gain by the plants is unknown. Although a significant K<sup>+</sup> uptake from prey was proven in field-grown *U. australis* (Adamec, 2008), a zero K<sup>+</sup> uptake from prey occurred in the same species in a greenhouse-growth experiment when a profound K<sup>+</sup> uptake from prey was simultaneously found in *Aldrovanda* (Adamec et al., 2010).

Unlike aquatic *Utricularia* species, five terrestrial *Utricularia* species very efficiently reutilized K (mean efficiency 48–91%) in senescent leaves (Table 3). Thus, the ability of plants to reutilize K is not confined taxonomically to genera, but only ecologically to functional plant traits, and only in terrestrial plants. Although submerged aquatic plants have greatly reduced vascular bundles in their stems (usually total loss of xylem; e.g., Sculthorpe, 1967), their ability to reutilize N and P in their senescent shoots via functional phloem is not limited (Table 2; Moeller, 1980; Lytle and Smith, 1995; Adamec, 2000, 2008).

Why is K not reutilized from senescent shoots of aquatic species as it is in terrestrial ones? There are several hypothetical possibilities explaining this phenomenon which could come together. One of the reasons might be the maintenance of a relatively high net photosynthetic rate in old, basal shoot segments in submerged plants, which was found by Adamec (2013) in two *Utricularia* species. Yet this reason should apply only for rapidly growing species. As reviewed by Jeschke (1976), K<sup>+</sup> uptake by the leaves of aquatic plants depends strongly on light and photosynthetic electron flow and a high photosynthetic rate also requires a high foliar K content to maintain itself and the general metabolic rate (i.e., enzyme activation; Wang and Wu, 2010). As a marked decrease in N, P and chlorophyll *a* content also occurs in the senescent leaves of aquatic plants (Table 2; Adamec, 2013), this hypothesis may not be valid. The comparison of rapidly growing aquatic *Aldrovanda* and *Utricularia* with slowly growing *Ceratophyllum* shows that the basal shoot decay rate in itself is not important either. Another explanation of the absence of K reutilization might be based on the maintenance of cell turgor and electrophysiological equilibria (i.e., the magnitude of cell membrane electric potential) in cells of senescent shoot bases and these reasons could prevent a greater reduction of K<sup>+</sup> concentration in the cytosol or vacuoles of cells (*sensu* Britto and Kronzucker, 2008). Thus, the high cellular K<sup>+</sup> concentration could be a prerequisite for the efficient reutilization of both growth-limiting N and P in senescent shoots. Finally, an alternative explanation might assume that K<sup>+</sup> concentration in fresh waters inhabited by submerged plants was usually sufficiently high (ca. 2–10 mg L<sup>-1</sup>) so as to suppress any significant evolutionary selective pressure to evolve a K reutilization mechanism similar to that for N and P. In light of molecular research on K<sup>+</sup> transporters and channels in plant cells (Britto and Kronzucker, 2008; Szczerba et al., 2009; Wang and Wu, 2010), it is possible to assume that relevant K<sup>+</sup> transporters and channels, which take part in phloem

loading and unloading, are not expressed in senescent shoots of aquatic plants.

In conclusion, reutilization of K (unlike N and P) in leafy shoots generally occurs only in terrestrial and not in aquatic plants although the reasons for this phenomenon are unclear. An experimental approach to better explain the K reutilization differences between terrestrial and aquatic plants could be to compare the K reutilization characteristics in submerged and emergent leaves of the same individual plants (e.g., in *Salvinia*) or to compare both submerged and emergent forms of some amphibious species.

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