ANNALS OF
BOTANY
Founded 1887

Plant carnivory beyond bogs: reliance on prey feeding in *Drosophyllum lusitanicum* (Drosophyllaceae) in dry Mediterranean heathland habitats

M. Paniw*, E. Gil-Cabeza and F. Ojeda

Departamento de Biología, CASEM, Universidad de Cádiz, Campus Río San Pedro, E-11510 Puerto Real, Spain
*For correspondence. E-mail maria.paniw@uca.es

● **Background and Aims** In a cost–benefit framework, plant carnivory is hypothesized to be an adaptation to nutrient-poor soils in sunny, wetland habitats. However, apparent exceptions to this cost–benefit model exist, although they have been rarely studied. One of these exceptions is the carnivorous subshrub *Drosophyllum lusitanicum*, which thrives in Mediterranean heathlands on dry sandstone soils and has relatively well-developed, xeromorphic roots. Here, the roles of leaf (carnivory) and root (soil) nutrient uptake in growth promotion of this particular species were assessed.
● **Methods** In a greenhouse experiment, plants were fed with laboratory-reared fruit flies (*Drosophila virilis*) and received two concentrations of soil nutrients in a factorial design. Above-ground plant growth and final above- and below-ground dry biomass after 13 weeks were recorded. Nutrient uptake via roots was also evaluated, using stable nitrogen isotope analysis.
● **Key Results** Insect feeding resulted in significantly higher growth and above- and below-ground biomass compared with soil fertilization. No additional benefits of fertilization were discernable when plants were insect-fed, indicating that roots were not efficient in nutrient absorption.
● **Conclusions** The first evidence of strong reliance on insect prey feeding in a dry-soil carnivorous plant with well-developed roots is provided, suggesting that carnivory *per se* does not preclude persistence in dry habitats. Instead, the combination of carnivory and xeromorphic root features allows *Drosophyllum* to thrive on non-waterlogged soils. New evidence is added to recent research emphasizing the role of root systems of carnivorous plants in explaining their distribution, partly challenging the cost–benefit hypothesis.

Key words: Carnivorous plant root, dry-soil carnivorous plant, insect prey, pyrophyte, soil nutrient uptake, stable isotope analysis.

INTRODUCTION

Intensively studied by Darwin (1875) in his treatise *Insectivorous plants*, plant carnivory is arguably the most captivating adaptation to nutrient-poor soils (Adamec, 1997; Ellison and Gotelli, 2001; Król *et al.*, 2012). The uptake and assimilation of nutrients via modified leaf structures has evolved at least nine times independently across the angiosperms (Givnish, 2015), with ~600 extant species of carnivorous plants in the world's flora (Król *et al.*, 2012; Givnish, 2015). The nutrition of carnivorous plants has been studied in various species, with a strong focus on sundews (*Drosera* spp.: Darwin, 1878; Karlsson and Pate, 1992; Adamec, 2002; Thorén *et al.*, 2003; Millett *et al.*, 2012), butterworts (*Pinguicula* spp.: Karlsson and Carlsson, 1984; Karlsson *et al.*, 1991; Hanslin and Karlsson, 1996) and pitcher plants (*Nepenthes/Sarracenia* spp.: Schulze *et al.*, 1997; Moran *et al.*, 2001; Gotelli and Ellison, 2002; Butler and Ellison, 2007; Farnsworth and Ellison, 2008). These studies have supported the hypothesis that carnivorous plants benefit from captured prey insects by acquiring mineral nutrients, mainly nitrogen and phosphorus (Ellison, 2006; Farnsworth and Ellison, 2008).

Most carnivorous plants are restricted to nutrient-poor, wet soils in sunny habitats (Ellison and Gotelli, 2001; Brewer *et al.*,

2011; Pavlovič and Saganová, 2015). These environmental associations led Givnish *et al.* (1984) to propose a cost–benefit model for the evolution of plant carnivory and its general restriction to sunny, infertile wetlands. According to this model, the net benefit of carnivory, i.e. the photosynthetic gain in terms of leaf production minus the cost of producing and maintaining specialized prey-trapping structures, is predicted to be largest when soil nutrient availability is the major limiting factor to plant growth but light and soil water are readily available. Several studies have since investigated nutrition in carnivorous plants, demonstrating that species vary widely in their capacity to assimilate mineral nutrients from soil (Adamec, 1997, 2010; Schulze *et al.*, 1997; Ellison, 2006; Król *et al.*, 2012). Support for the cost–benefit model comes in particular from studies showing that reliance on prey nutrients decreases with increasing soil nutrient availability (e.g. Benzing, 1987; Karlsson and Pate, 1992; Millett *et al.*, 2012) or shade (Givnish *et al.*, 1984; Schulze *et al.*, 2001).

More recently, extensions or alternatives to the cost–benefit model have been proposed (Benzing, 2000; Brewer *et al.*, 2011; Abbott and Brewer, 2016). Brewer *et al.* (2011), for instance, hypothesized that the characteristic, weakly developed and low-porosity roots, rather than low soil fertility *per se*, might explain the general restriction of carnivorous plants to

boggy, waterlogged soils and their disadvantage in drier, non-waterlogged soils. However, carnivorous plant species that thrive in dry habitats and appear to contradict the predictions of the cost–benefit model have received far less attention in the literature, despite potentially providing significant novel insights into the evolution of plant carnivory (Givnish *et al.*, 1984; Givnish, 2015). One prominent example is the subshrub *Drosophyllum lusitanicum* (Drosophyllaceae). This species (*Drosophyllum* hereafter) is the only extant species of the family Drosophyllaceae (Heubl *et al.*, 2006) and is endemic to the western Iberian Peninsula and northern Morocco (Garrido *et al.*, 2003). Across its range, *Drosophyllum* is restricted to fire-prone Mediterranean heathlands on acid, nutrient-poor, dry soils that are subject to a moderate summer drought (Adlassnig *et al.*, 2006; Paniw *et al.*, 2015).

Unlike most other carnivorous plant species, many *Drosophyllum* individuals maintain their complex, sticky mucilage on leaves to capture prey even under unfavourable conditions in the dry summer months (Adlassnig *et al.*, 2006; Adamec 2009). Another difference between *Drosophyllum* and most other carnivorous plant species is that the root system of the former is relatively well developed, consisting of a branched tap-root with xeromorphic features (Carlquist and Wilson, 1995; Adlassnig *et al.*, 2005, 2006; Adamec, 2009). Despite being one of the few carnivorous plant species with deep, large root systems, no research has been done on the putative role of roots for soil nutrient uptake in this species (Adlassnig *et al.*, 2005, 2006). The taxonomic uniqueness and habitat particularity of *Drosophyllum* make the species a valuable system for investigating the importance of leaves versus roots in nutrient acquisition and growth promotion of carnivorous plants in dry habitats.

Here, we studied plant nutrition in *Drosophyllum* plants through leaves (prey insects) and roots (soil nutrients) and the effect of nutrient uptake from the two sources on above-ground growth and above-ground (leaf) and below-ground (root) biomass allocation. Given the scarcity of fine lateral roots in this species (Adamec, 2009), we hypothesized that leaf nutrient uptake from trapped insects will determine plant growth, with a low contribution, if any, of soil nutrient uptake from roots, despite their considerable size and depth (Adlassnig *et al.*, 2005). To test this hypothesis, we performed a full-factorial greenhouse experiment in which we fed juvenile plants growing on a substrate mixture of siliceous sand and peat moss via leaves (fruit flies) and/or soil (Hoagland's nutrient solution). We recorded above-ground growth as well as final dry biomass of above-ground (leaves) and below-ground (roots) plant parts and compared them between treatments. Since the Hoagland's nutrient solution used had an anomalously high $\delta^{15}N$ value (see Materials and methods), we measured $\delta^{15}N$ values in the above-ground (leaves) and below-ground (roots) tissue of plants from the different treatments to ascertain the ability of the plants to absorb mineral nutrients from the roots.

MATERIALS AND METHODS

Growth of plants and experimental design

We grew *Drosophyllum* plants in the University of Cádiz greenhouse from seeds collected in July 2014 from 80 individuals randomly chosen at five sites (16 individuals per site). We mixed all seeds to provide a homogeneous pool and, on 2 February 2015, we randomly took 200 seeds from the pool and exposed them to dry heat (100 °C) for 5 min to break seed dormancy (Correia and Freitas, 2002). We then sowed these seeds in seedling trays with a 1:1 mixture of siliceous sand and peat moss and selected the first 120 emerged seedlings for the experiment. The seedlings emerged 20–26 d after sowing and were then individually transplanted into 0·5-L clay pots containing the same mixture of siliceous sand and peat moss. This low-fertility soil mixture is commonly used in nutrient addition experiments for carnivorous plants (e.g. Butler and Ellison, 2007) and approximates the low-fertility conditions of Mediterranean heathland soils (Ojeda *et al.*, 2010). The pH of this substrate, measured in a saturated soil paste, was ~4·5, similar to the pH of Mediterranean heathland soils (Ojeda *et al.*, 2010).

We grew the 120 potted seedlings in the greenhouse at ambient temperature, but never exceeding 25 °C, and keeping relative humidity around 70–90 % throughout the whole experiment, resembling ambient conditions of natural *Drosophyllum* populations during the spring growing season (M. Paniw, unpubl. res.). During the night, the lowest temperature recorded in the greenhouse was 15 °C. Pots were kept moist via a sprinkling system mounted above the pots that sprayed decalcified water during daytime for 30 s at 2-h intervals. We used decalcified water because soil Ca is toxic to most carnivorous plants (Adlassnig *et al.*, 2005), including *Drosophyllum* (Adlassnig *et al.*, 2006). We maintained the temperature regime and periodic sprinkling throughout the study. In addition, before initiating the nutrient addition experiment, we watered the pots three times a week with 50 mL of decalcified water. On 12 March 2015, 14 d after being transplanted, the seedlings were large enough (five to seven leaves of length 5·0 ± 0·3 cm, mean ± s.d.) to start the feeding experiment, which extended for 11 weeks until 27 May 2015, lasting a total of 91 d after seedling emergence.

The experiment was performed in a full-factorial design with insect feeding [two treatment levels: insect feeding (F) and no insect feeding (NF)] and soil fertilization [three treatment levels: high (H), low (L) and zero (O)] as fixed factors. The 120 potted seedlings were randomly divided into two equal-sized groups, one of which, the F treatment, was supplied with fruit flies (*Drosophila virilis*; ~0·3 mg dry weight per fly) and the other, the NF treatment, was not. Each plant of the F treatment received three flies per leaf in the first 2 weeks of the experiment, increasing the number of flies by two more per leaf each additional week until the sixth week, when the number of flies per leaf increased to four more each week. The *D. virilis* fruit flies used throughout the experiment were reared in a carbohydrate-rich medium under standard culture conditions and were kept frozen in vials at −20 °C prior to usage.

Plants of the F and NF groups were further split into three subgroups (20 plants each) for the soil fertilization treatments: three times per week for the duration of the experiment, plants in each subgroup received 50 mL of 1/10 strength nutrient solution (H treatment), 50 mL of 1/20 strength nutrient solution (L treatment) or 50 mL of distilled water (O treatment). We used a balanced nutrient mixture (Hoagland's No. 2 Basal Salt Mixture; Sigma-Aldrich, St Louis, MO, USA) to avoid

potential deficiencies of some nutrients caused by abundance of another. Similar dilutions have been used in feeding experiments for other carnivorous plant species (e.g. Butler and Ellison, 2007). Plants in the NF–O treatment combination, receiving neither flies nor soil nutrients, were considered as control. Each time before treatment application, pots were haphazardly shuffled on the greenhouse bench to avoid a location effect.

In order to ensure that the amount of nutrients provided to plants via flies or soil solution did not differ substantially, we determined the amount of nitrogen available to plants from either source. The amount of nitrogen in flies was measured as described for plant samples in the section $\delta^{15}N$ analysis below. Throughout the nutrient-addition experiment, plants in the corresponding treatment groups were supplied weekly with ~3·1 mg (H treatment) and 1·05 mg (L treatment) of N through the soil. The fine texture of the moss peat in the soil medium aided in retaining the nutrient solution and water. Plants in the F–O treatment received a total of ~2·1 mg of N from insects, which corresponded to 60 % (range 52–69 %) of their total N pool. We assumed that the relative concentrations of other nutrients to N were similar between flies and fertilizer.

To track the above-ground growth of plants under different treatment combinations, we counted the number of fully developed leaves and measured the length (cm) of the longest leaf on each plant at the beginning of the experiment (d 14 after emergence) and every week or second week until the end of the experiment (11 weeks later; d 91 after emergence). We then defined size as the number of leaves × length of longest leaf (cm). This size measure is biologically significant as it approximates the available leaf area for prey capture and has been used in other studies of this species (Paniw *et al.*, 2016). Once the experiment was terminated, we removed plants from the pots, washed them in distilled water to remove fruit flies from leaves and soil from roots, separated above-ground (shoot) and below-ground (root) material of each plant, and oven-dried them for 72 h at 65 °C to constant weight. We then weighed the shoot and root dry biomass of each plant to the nearest 0·01 mg.

$\delta^{15}N$ analysis

Previous analyses found an average $\delta^{15}N$ signature of 18·6 ‰ (range 18·0–19·0 ‰) in the Hoagland's nutrient solution used in this study (Hoagland's No. 2 Basal Salt Mixture), an anomalously high value for standard synthetic fertilizers ($\delta^{15}N = -0·2 \pm 2·1$ ‰, mean ± s.d.; Bateman and Kelly, 2007), and much higher than the $\delta^{15}N$ signature detected in *Drosophila virilis* flies (range 2·8–3·0 ‰). This highly $\delta^{15}N$-enriched nutrient solution provided an excellent means to explore whether *Drosophyllum* plants were able to take up and assimilate soil nutrients through the roots. After being weighed, shoot and root dry biomass samples of all *Drosophyllum* plants from the nutrient addition experiment were separately placed into plastic vials (up to three samples per plant part if enough biomass was produced), ground to powder using stainless steel beads with a Mixer Mill MM400 cell disrupter (Retsch, Llanera, Spain), and analysed for percentages of N and $\delta^{15}N$ using combustion in a Flash EA1112 elemental analyser interfaced with Finnigan Tracer Mass Isotope Ratio Mass

Spectrometer. Analyses were performed at the Analytical Service Laboratory of the University of A Coruña (Spain). The $\delta^{15}N$ results are expressed in parts per thousand (‰), where $\delta = [(^{15}N/^{14}N) - 1] \times 1000$. All $\delta^{15}N$ values had a precision of 0·3 ‰.

Statistical analysis

The overall effects of insect feeding (F, NF), soil fertilization (H, L, O) and their interaction on above-ground size changes over time were determined by means of a two-way repeated-measures ANOVA. The plant size variable was log-transformed prior to analysis to meet the homoscedasticity assumption. We also explored the effects of insect feeding (F, NF) and soil fertilization (H, L, O) on the final dry biomass (g) of the above-ground (shoot) and below-ground (root) portions of the plants by performing a two-way ANOVA. Shoot and root dry biomass variables were previously log-transformed to ensure the homoscedasticity assumption of multivariate ANOVA (MANOVA). *Post hoc* comparisons using Tukey's honestly significant difference (HSD) tests to search for pairwise differences between the six treatment combinations were implemented separately for shoot and root dry biomass variables. An equivalent analysis for whole-plant biomass can be found in Supplementary Data Appendix S1.

In order to explore whether plants provided with soil nutrients changed their root:shoot allocation patterns, we calculated the percentage contribution of roots to the total plant dry biomass, and tested significant differences between the six treatments by using a non-parametric Kruskal–Wallis rank test. Finally, we also used the Kruskal–Wallis rank test to search for differences in the $\delta^{15}N$ signature of the above-ground (shoot) and below-ground (root) tissue of plants between the six treatment combinations. As the Kruskal–Wallis rank test corresponds to a non-parametric one-way ANOVA, subsequent *post hoc* pairwise comparisons between treatment combinations were done using Bonferroni-corrected Mann–Whitney *U*-tests. All statistical analyses were performed in R (R Core Team, 2015).

RESULTS

Insect-fed plants grew >4-fold as much as non-insect-fed plants during the experiment (Fig. 1) and produced a >5-fold higher dry biomass, both above and below ground (Fig. 2), regardless of soil fertility conditions.

The two-way repeated-measures ANOVA detected significant effects of the two factors, insect feeding and soil fertilization, on relative plant growth (Table 1; Fig. 1). In addition, plant size changed significantly with time (days after sowing), with plants growing significantly faster when fed with flies compared with unfed plants (Table 1; Fig. 1). Correspondingly, the two-way MANOVA showed significant effects of both factors on the final dry biomass of above-ground (shoot) and below-ground (root) portions of plants, and a significant interaction between the two factors (Table 2; Fig. 2). The significant interaction effect stemmed from soil fertilization having a slight but significant effect on final dry biomass only when plants were not supplied with fruit flies (Table 2). No significant
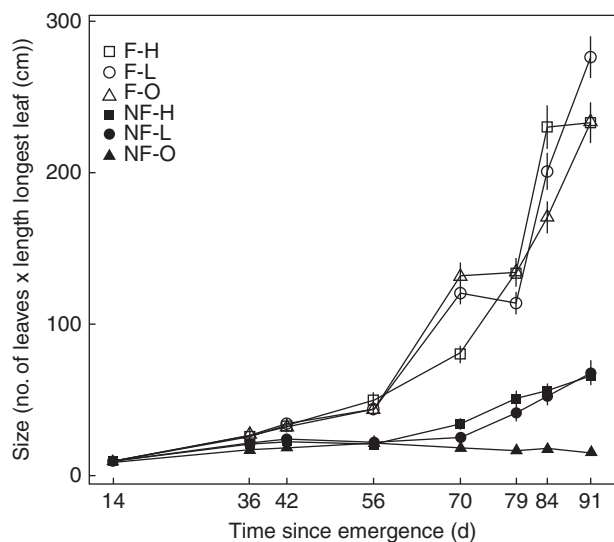
FIG. 1. Changes in size, defined as number of leaves × length of longest leaf (cm), of *Drosophyllum* plants through time (d) as a function of two treatments, insect feeding [feeding (F)/no feeding (NF)] and soil fertilization [addition of high-strength (H), low-strength (L) nutrient solution or distilled water (O)] and their interaction, resulting in six treatment groups.
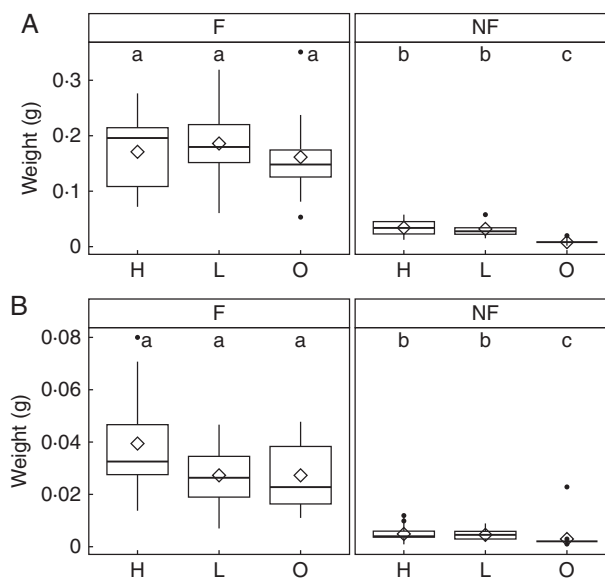

FIG. 2. Box-plot of dry biomass of (A) above-ground (shoot) and (B) below-ground (root) parts of *Drosophyllum* plants measured at the end of the nutrient addition experiment as a function of two main treatments and their interactions: feeding with flies (F) or no feeding (NF) and addition of high-strength (H), low-strength (L) nutrient solution or distilled water (O). The combinations of the treatment levels resulted in six treatment groups. Different letters represent significant pairwise differences (Tukey's HSD, $P < 0.05$) in group means (diamonds) between the six treatment groups. Note that the statistical comparisons were performed on the log-transformed biomass measure to ensure variance homoscedasticity.

differences in final dry biomass were detected between the H and L soil fertilization levels (Fig. 2). Insect-fed plants grew much larger, both above and below ground, than soil-fed plants, and no additive effects of soil fertilization on them were detected (Fig. 2).

On average, the root *Drosophyllum* plants made up 14·7 % ($\pm$ 0·06 s.d.) of the total plant dry biomass. This result was similar to previous investigations of root:shoot ratios in *Drosophyllum* (Adamec, 2009). No significant differences in the relative contribution of roots to total plant biomass were found between the six treatment combinations (Kruskal–Wallis $\chi^2 = 9.6$, d.f. $= 5$, $P = 0.1$).

Soil-fertilized plants presented significantly higher $\delta^{15}N$ signatures in both shoot and root tissues than non-fertilized plants, regardless of whether or not they were supplied with fruit flies on the leaves (Fig. 3; Kruskal–Wallis $\chi^2 = 70.0$, d.f. $= 5$, $P < 0.05$). Taking into account the high $\delta^{15}N$ values of the nutrient solution used for soil fertilization (see Materials and methods), this result indicates that *Drosophyllum* plants are able to take up and assimilate dissolved soil nutrients through the roots. It should be noted that higher $\delta^{15}N$ signatures were detected in H-fertilized than in L-fertilized plants (Fig. 3), although higher fertilization strength did not cause an increase in plant growth (Figs 1 and 2).

DISCUSSION

Carnivorous plants are predicted to benefit from prey capture under a specific set of environmental conditions, i.e. nutrient-poor, wet soils and open habitats, which offset the cost of producing trapping structures (Givnish *et al.*, 1984; Benzing, 1987, 2000). However, our nutrient addition experiment provides the first evidence that a strong carnivorous syndrome may evolve in dry environments. *Drosophyllum* plants invest resources in

TABLE 1. *Two-way repeated-measures ANOVA of the effects of insect feeding, soil fertilization, and their interaction on changes in above-ground size through time (days since emergence) of* Drosophyllum *plants*

	d.f.	Mean square	F ratio	P value
Between-group effect: error (plant ID)				
Insect feeding	1	1.4×10^6	483·0	<0·01
Soil fertilization	2	1.3×10^4	4·5	0·01
Insect feeding × soil fertilization	2	6.5×10^3	2·2	0·1
Residuals	113	2.9×10^3		
Within-subject effect				
Days	6	2.8×10^5	668·7	<0·01
Insect feeding × days	6	1.6×10^5	395·3	<0·01
Soil fertilization × days	12	6.9×10^3	16·3	<0·01
Insect feeding × soil fertilization × days	12	3.4×10^3	8·1	<0·01
Residuals	678	419		

carnivorous structures as well as in well-developed, deep roots (Adlassnig 2005, 2006) that, however, seem to play only a minor role in nutrient acquisition. Plants fed with insects in the greenhouse acquired on average >5-fold as much biomass as soil-fertilized plants, with root nutrient uptake showing no additive benefits in plant growth (Fig. 2). These results support the hypothesis that root functionality other than nutrient acquisition (e.g. securing water availability) may be a key factor determining the distribution of carnivorous plants with respect to soil

TABLE 2. *Two-way MANOVA, using the Pillai test statistic, of the effects of insect feeding and soil fertilization factors and their interaction on above-ground (shoot) and below-ground (root) dry biomass (g)*

	Pillai	Approx *F*	Num d.f.	Demom d.f.	*P*-value
Shoot and root dry biomass (g)					
Insect feeding	0·865	170·172	2	53	<0·01
Soil fertilization	0·352	5·767	4	108	<0·01
Insect feeding × soil fertilization	0·369	6·099	4	108	<0·01

Num d.f. and demon d.f. are the numerator and denominator degrees of freedom, respectively, of the *F* ratio corresponding to the Pillai test.
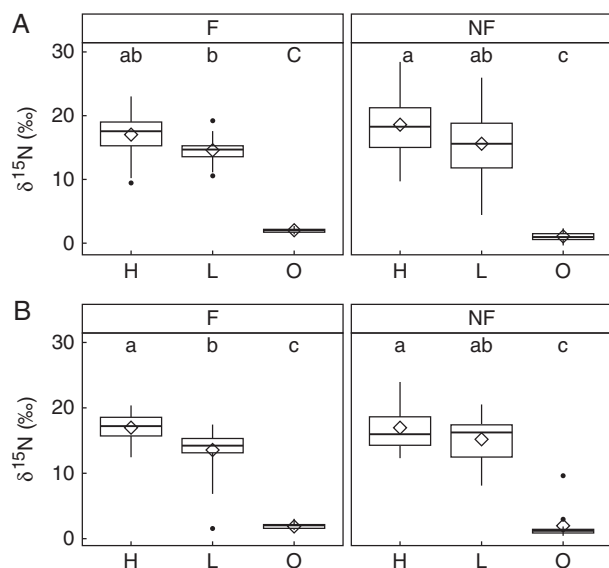

FIG. 3. Box-plot of $\delta^{15}N$ in (A) shoot and (B) root tissues of *Drosophyllum* plants measured at the end of the nutrient addition experiment as a function of two main treatments and their interactions: feeding with flies (F) or no feeding (NF) and addition of high-strength (H) or low-strength (L) nutrient solution or distilled water (O). Different letters represent significant pairwise differences (Kruskal–Wallis $\chi^2 = 70\cdot0$, d.f. = 5, $P < 0\cdot05$) in group means (diamonds) between the six treatment groups.

moisture (Brewer *et al.*, 2011). Indeed, unlike most other carnivorous plant species, *Drosophyllum* produces large, xeromorphic roots most likely as an adaptation to water uptake in non-waterlogged soils under a Mediterranean climate (Carlquist and Wilson, 1995; Adlassnig *et al.*, 2005). Both the xeromorphic root features (for soil water acquisition) and carnivory (for nutrients) may allow this species to persist in nutrient-poor, dry Mediterranean heathlands.

The strong reliance of *Drosophyllum* on prey-derived nutrients for growth highlighted by our greenhouse study is corroborated by field observations and field experiments showing great efficiency of plants of this species in attracting prey (Darwin, 1875; Bertol *et al.*, 2015). Individuals produce complex, mucilaginous stalked glands, multicellular and vascularized with both xylem and phloem vessels (Renner and Specht, 2011). It should be emphasized that this species is, together with the part-time carnivorous *Triphyophyllum peltatum*, the only fly-paper carnivorous species whose glandular trichomes have phloem vessels (Renner and Specht, 2011). This would allow *Drosophyllum* plants to add phloem sap exudates, including carbohydrates and volatile organic compounds, to the mucilage droplets, increasing their viscosity and hygroscopicity (carbohydrates; Adlassnig *et al.*, 2006) as well as their efficiency in insect attraction (volatile organic compounds; Jürgens *et al.*, 2009). As a result, even juvenile *Drosophyllum* individuals, consisting of one rosette with ten leaves, may contain >100 prey insects (Bertol *et al.*, 2015). The strong carnivorous character of *Drosophyllum* stands out compared with *Byblis lamellata* (Byblidaceae), the only morphologically and ecologically similar carnivorous species, found in non-waterlogged, seasonally dry, siliceous sands (Conran *et al.*, 2002). Unlike *Drosophyllum*, *B. lamellata* has simple trapping structures and does not have sessile, proteolytic enzyme-producing glands to directly digest prey insects, but may use insect mutualists that feed on trapped prey to gain nutrients by digesting their faeces (Hartmeyer, 1998).

Despite the strong reliance on prey for plant growth, our results indicate that *Drosophyllum* is able to take up soil nutrients from the roots, when available, and assimilate them in both root and leaf tissue (Fig. 3), although growth is far from optimal in the absence of insect prey (Figs 1 and 2). *Drosophyllum* is a post-fire-dwelling species (Paniw *et al.*, 2015) with life-history adaptations to recurrent fires, which include mass post-fire recruitment from a persistent soil seed-bank (Müller and Deil, 2001). Fires release a flush of mineral nutrients to soil, including N and P, which are quickly (within 1 year) leached away (Certini, 2005; Dijkstra and Adams, 2015). By being able to assimilate nutrients from the roots, *Drosophyllum* plants might benefit from that transient post-fire flush in their early seedling stages, when insect capture is unlikely, due to small size. They might hence use it to assist plant growth to prey-capture levels. Similar results have been found for another fire-adapted carnivorous plant, *Dionaea muscipula*, and may also indicate adaptations to post-fire nutrient fluctuations (Gao *et al.*, 2015). As lateral roots appear to be lost in mature *Drosophyllum* plants (Adamec, 2009), nutrient uptake via roots is likely limited to the seedling and juvenile plant stages, but future studies must determine whether mature *Drosophyllum* individuals can also potentially assimilate nutrients from the soil.

In practice, nutrient absorption via roots in adult *Drosophyllum* plants is likely limited as roots lack adaptations, such as microsymbiont associations or cluster roots, for nutrient scavenging in low-fertility soils (Carlquist and Wilson, 1995; Adlassnig *et al.*, 2005, 2006). On the other hand, virtually all non-carnivorous plant species in heathland habitats show root adaptations for nutrient scavenging (Lambers *et al.*, 2006). Carnivory in *Drosophyllum* may therefore be seen as an alternative strategy to acquire nutrients in nutrient-poor, Mediterranean heathlands, with high specialization for leaf prey capture and digestion to compensate for the lack of root

adaptations. Such a trade-off or constraint-avoidance solution has been shown in wetland soils, where carnivorous genera produce shallow, low-porosity roots to prevent hypoxia, obtaining nutrients from prey instead (Karlsson and Pate, 1992; Brewer *at al.*, 2011; Gao *et al.*, 2015).

Despite showing little efficiency in nutrient acquisition, roots may be critical in allowing *Drosophyllum* to persist in dry habitats. In many *Drosophyllum* populations, plants consume prey insects throughout the year, even in the dry summer months (Adlassnig *et al.*, 2006; M. Paniw and F. Ojeda, pers. comm.). It has been suggested that plants satisfy a large part of their water demand through the highly hygroscopic mucilage of leaf glands that capture water from air moisture (Adlassnig *et al.*, 2006; Adamec, 2009). However, it is unlikely that hygroscopic mucilage is sufficient to maintain the water balance in *Drosophyllum* individuals, particularly in the dry summers, where average air humidity does not exceed $66 \cdot 5$ % ($\pm$ $9 \cdot 0$ s.e.) (Supplementary Data Appendix S2). The xeromorphic features and relatively large size of tap roots in this species, typical of plants adapted to water-limited soils (Carlquist and Wilson, 1995), indicate that, apart from their anchoring role, roots would play an important role in maintaining the water balance in *Drosophyllum* plants.

CONCLUSIONS

Contrary to the prediction of the cost–benefit analysis of the evolution of plant carnivory, we provide evidence that carnivory may evolve in non-waterlogged, dry soils. Therefore, roots, decoupled from nutrient-acquisition functions, may be critical in determining the distribution of carnivorous genera in response to soil moisture. Previous investigation on the nutrition of carnivorous plants has largely focused on a few genera, all found in boggy or waterlogged soils, where the ecological conditions have favoured a reduction of the root system (Brewer, 2003; Brewer *et al.*, 2011) coupled with the maintenance of flexible nutrient acquisition strategies (e.g. Ellison and Gotelli, 2002; Millett *et al.*, 2012), or even the ability to switch off carnivory under increasing soil nutrients (Ellison *et al.*, 2003). Although it is certainly true that a majority of carnivorous plants are found in waterlogged soils and have reduced, shallow roots (Adlassnig *et al.*, 2005; Brewer *et al.*, 2011), a full understanding of the carnivorous syndrome can only be gained by considering species that have adapted to extremely low soil fertility conditions with no association with boggy habitats. Our study species, *D. lusitanicum*, has complex, sticky glands on their flypaper-trap leaves and is very effective in attracting prey insects (Bertol *et al.*, 2015). At the same time, the species is also very effective at avoiding water stress, allowing it to persist on dry soils (Adlassnig *et al.*, 2006). Using a unique system, our study supports the hypothesis that root functionality coupled with carnivory may explain the distribution of carnivorous plants better than photosynthetic cost and benefits *per se*. We therefore urge that more studies should be undertaken on underrepresented carnivorous taxa from non-waterlogged habitats, such as *Byblis* spp. in Australia or epiphytes such as *Catopsis berteroniana* (Adamec, 2010), to gain a more complete picture of the link between soil properties and the evolution of plant carnivory beyond bogs.

REFERENCES

Abbott MJ, Brewer JS. 2016. Competition does not explain the absence of a carnivorous pitcher plant from a nutrient-rich marsh. *Plant and Soil* 1–10.

Adamec L. 1997. Mineral nutrition of carnivorous plants: a review. *Botanical Review* **63**: 273–299.

Adamec L. 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. *New Phytologist* **155**: 89–100.

Adamec L. 2009. Ecophysiological investigation on *Drosophyllum lusitanicum*: why doesn't the plant dry out? *Carnivorous Plant Newsletter* **38**: 71–74.

Adamec L. 2010. Carnivorous plants: ecophysiological look at plant carnivory. Why are plants carnivorous? In: Seckbach J, Dubinski Z, eds. *All flesh is grass. Plant-animal interrelationships. Cellular origin, life in extreme habitats and astrobiology.* Dordrecht: Springer Science + Business Media, 457–492.

Adlassnig W, Peroutka M, Lambers H, Lichtscheidl IK. 2005. The roots of carnivorous plants. *Plant and Soil* **274**: 127–140.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**: 255–262.

Bateman AS, Kelly SD. 2007. Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies* **43**: 237–247.

Benzing DH. 1987. The origin and rarity of botanical carnivory. *Trends in Ecology and Evolution* **2**: 364–369.

Benzing DH. 2000. *Bromeliaceae: profile of an adaptive radiation*. Cambridge, UK: Cambridge University Press.

Bertol N, Paniw M, Ojeda F. 2015. Dynamics of prey capture for a flypaper-trap carnivorous plant: luring insects versus passive trap. *American Journal of Botany* **102**: 1–6.

Brewer JS. 2003. Why don't carnivorous pitcher plants compete with non-carnivorous plants for nutrients? *Ecology* **84**: 451–462.

Brewer JS, Baker DJ, Nero AS, *et al.* 2011. Carnivory in plants as a beneficial trait in wetlands. *Aquatic Botany* **94**: 62–70.

Butler JL, Ellison AM. 2007. Nitrogen cycling dynamics in the carnivorous northern pitcher plant, *Sarracenia purpurea*. *Functional Ecology* **21**: 835–843.

Carlquist S, Wilson EJ. 1995. Wood anatomy of *Drosophyllum* (Droseraceae): ecological and phylogenetic considerations. *Bulletin of the Torrey Botanical Club* **122**: 185–189.

Certini G. 2005. Effects of fire on properties of forest soils: a review. *Oecologia* **143**: 1–10.

Conran JG, Lowrie A, Moyle-Croft J. 2002. A revision of *Byblis* (Byblidaceae) in southwestern Australia. *Nuytsia* **15**: 11–19.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnean Society* **140**: 383–390.

Darwin C. 1875. *Insectivorous plants*. London: John Murray.

Darwin F. 1878. Experiments on the nutrition of *Drosera rotundifolia*. *Journal of the Linnean Society, Botany (London)* **17**: 17–23.

Dijkstra FA, Adams MA. 2015. Fire eases imbalances of nitrogen and phosphorus in woody plants. *Ecosystems* **18**: 769–779.

Ellison AM. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* **8**: 740–747.

Ellison AM, Gotelli NJ. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology & Evolution* **16**: 623–629.

Ellison AM, Gotelli NJ. 2002. Nitrogen availability alters the expression of carnivory in the northern pitcher plant *Sarracenia purpurea*. *Proceedings of the National Academy of Sciences of the USA* **99**: 4409–4412.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants—Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* **60**: 19–42.

Ellison AM, Gotelli NJ, Brewer JS, *et al.* 2003. The evolutionary ecology of carnivorous plants. *Advances in Ecological Research* **33**: 1–74.

Farnsworth EJ, Ellison AM. 2008. Prey availability directly affects physiology, growth, nutrient allocation and scaling relationships among leaf traits in 10 carnivorous plant species. *Journal of Ecology* **96**: 213–221.

Gao P, Loeffler TS, Honsel A, *et al.* 2015. Integration of trap-and root-derived nitrogen nutrition of carnivorous *Dionaea muscipula*. *New Phytologist* **205**: 1320–1329.

Garrido B, Hampe A, Maranon T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**: 335–350.

Givnish TJ. 2015. New evidence on the origin of carnivorous plants. *Proceedings of the National Academy of Sciences of the USA* **112**: 10–11.

Givnish TJ, Burkhardt EL, Happel RE, Weintraub JD. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist* **124**: 479–497.

Gotelli NJ, Ellison AM. 2002. Nitrogen deposition and extinction risk in the northern pitcher plant, *Sarracenia purpurea*. *Ecology* **83**: 2758–2765.

Hanslin HM, Karlsson PS. 1996. Nitrogen uptake from prey and substrate as affected by prey capture level and plant reproductive status in four carnivorous plant species. *Oecologia* **106**: 370–375.

Hartmeyer S. 1998. Carnivory in *Byblis* revisited II: the phenomenon of symbiosis on insect trapping plants. *Carnivorous Plant Newsletter* **27**: 110–113.

Heubl G, Bringmann G, Meimberg H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales—revisited. *Plant Biology* **8**: 821–830.

Jürgens A, El-Sayed AM, Suckling DM 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology* **23**: 875–887.

Karlsson PS, Carlsson B. 1984. Why does *Pinguicula vulgaris* L. trap insects? *New Phytologist* **97**: 25–30.

Karlsson PS, Pate JS. 1992. Contrasting effects of supplementary feeding of insects or mineral nutrients on the growth and nitrogen and phosphorous economy of pygmy species of *Drosera*. *Oecologia* **92**: 8–13.

Karlsson PS, Nordell KO, Carlsson BÅ, Svensson BM. 1991. The effect of soil nutrient status on prey utilization in four carnivorous plants. *Oecologia* **86**: 1–7.

Król E, Płachno BJ, Adamec L, Stolarz M, Dziubińska H, Trębacz K. 2012. Quite a few reasons for calling carnivores 'the most wonderful plants in the world'. *Annals of Botany* **109**: 47–64.

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**: 693–713.

Millett J, Svensson BM, Newton J, Rydin H. 2012. Reliance on prey-derived nitrogen by the carnivorous plant *Drosera rotundifolia* decreases with increasing nitrogen deposition. *New Phytologist* **195**: 182–188.

Moran JA, Merbach MA, Livingston NJ, Clarke CM, Booth WE. 2001. Termite prey specialization in the pitcher plant *Nepenthes albomarginata*—evidence from stable isotope analysis. *Annals of Botany* **88**: 307–311.

Müller J, Deil U. 2001. Ecology and structure of *Drosophyllum lusitanicum* (L.) Link populations in the south-west of the Iberian Peninsula. *Acta Botanica Malacitana* **26**: 47–68.

Ojeda F, Pausas JG, Verdú M. 2010. Soil shapes community structure through fire. *Oecologia* **163**: 729–735.

Paniw M, Quintana-Ascencio PF, Ojeda F, Salguero-Gómez R 2016. Accounting for uncertainty in dormant life stages in stochastic demographic models. *Oikos*, doi:10.1111/oik.03696.

Paniw M, Salguero-Gómez R, Ojeda F. 2015. Local-scale disturbances can benefit an endangered, fire-adapted plant species in Western Mediterranean heathlands in the absence of fire. *Biological Conservation* **187**: 74–81.

Pavlovič A, Saganová M. 2015. A novel insight into the cost–benefit model for the evolution of botanical carnivory. *Annals of Botany* **115**: 1075–1092.

R Core Team. 2015. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. http://www.R-project.org/.

Renner T, Specht CD. 2011. A sticky situation: assessing adaptations for plant carnivory in the Caryophyllales by means of stochastic character mapping. *International Journal of Plant Sciences* **172**: 889–901.

Schulze W, Schulze ED, Pate JS, Gillison AN. 1997. The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californica*. *Oecologia* **112**: 464–471.

Schulze W, Schulze ED, Schulze I, Oren R. 2001. Quantification of insect nitrogen utilization by the venus fly trap *Dionaea muscipula* catching prey with highly variable isotope signatures. *Journal of Experimental Botany* **52**: 1041–1049.

Thorén LM, Tuomi J, Kämäräinen T, Laine K. 2003. Resource availability affects investment in carnivory in *Drosera rotundifolia*. *New Phytologist* **159**: 507–511.