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Inorganic carbon assimilation in the Isoetids, *Isoetes lacustris* L. and *Lobelia dortmanna* L.

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Summary. The inorganic carbon fixation patterns of *Isoetes lacustris* and *Lobelia dortmanna* from an oligotrophic Scottish loch have been examined by following titratable acidity changes in plant sap and light/dark $^{14}CO_2$ incorporation by roots and shoots. The diurnal pattern of titratable acidity changes in *I. lacustris* suggests crassulacean acid metabolism (CAM) while the lack of any change in titratable acidity in *L. dortmanna* suggests C_3 metabolism. Of the carbon fixed by *L. dortmanna*, 99.9% was taken up through the roots and fixation occurred primarily during the day. In *Isoetes*, CO_2 was taken up by both roots and shoots and during both day and night. Regardless of the site of CO_2 uptake, fixation occurred only in the shoots of both plants. Analysis of carbon isotope ratios of plant organic material was used to further investigate the photosynthetic mechanisms of these Isoetids. Considering the absence of a night-time peak in titratable acidity in *L. dortmanna*, the $\Delta^{13}C$ ($\Delta = \delta^{13}C$ plant $- \delta^{13}C$ source) value of the shoots of *L. dortmanna* ($-14.2‰$) is indicative of C_3 photosynthesis limited by the rate of CO_2 diffusion. The less negative Δ of *I. lacustris* ($-6.0‰$) is consistent with both dark acidification of CAM and CO_2 limited C_3 photosynthesis. This is in contrast to the terrestrial *Isoetes durieui* which is shown to have a Δ value which is similar to a terrestrial C_3 plant. The carbon fixation patterns of these Isoetids suggest that the CO_2 concentration in the loch may be growth limiting, and that root uptake and/or dark acidification are means of optimising CO_2 supply. However, in view of the relatively high levels of CO_2 in sediment and bulk water, it is suggested that low levels of nutrients may also limit growth in these plants.

Introduction

The Isoetids are a growth form of freshwater vascular benthic macrophytes. The scheme of den Hartog and Segal (1964) is based on the earlier schemes of Du Rietz (1921, 1930) and defines Isoetids as "rhizophytes with a short stem, a rosette of stiff radial leaves, and with or without stolons". The growth form occurs in species from a wide phylogenetic range of vascular plant families. Thus, the British flora has Isoetids in the Isoetaceae (*Isoetes lacustris* L.), Cruciferae (*Subularia aquatica* L.), Plantaginaceae (*Littorella uniflora* (L) Ascherson), Lobeliaceae (*Lobelia dortmanna* L.) and (?) Eriocaulaceae (*Eriocaulon aquaticum* (Hill) Druce): Clapham et al. (1981) and Haslam et al. (1975).

Isoetids are perennial plants which befits their stress tolerant status with respect to low nutrient supply (Grime 1979). The pools or lakes in which they are found have essentially still water of low pH and low alkalinity and (except in the case of *Littorella uniflora* which grows in waters with a wide range of nutrient concentrations) low nutrient concentration and low organic content (Clapham et al. 1981; Haslam et al. 1975).

Much of the work on the ecophysiology of these plants has concentrated on the supply of exogenous inorganic carbon for their photosynthesis. CO_2 is the predominant C source at the low pH values encountered in oligotrophic lake habitats, with CO_2 levels which may be greater than air equilibration resulting from inputs of allochthonous organic carbon and terrigenous respiratory CO_2 (Wium-Andersen and Andersen 1972). Sediment CO_2 concentrations are often an order of magnitude greater than the overlying water, ranging from 1–5 mol m^{-3} (Raven 1970; Wium-Andersen and Andersen 1972; Sand-Jensen and Søndergaard 1978).

One approach to inorganic carbon supply in Isoetids has involved investigation of the role of the roots in the supply of carbon dioxide to the leaves. The following observations are all consistent with at least some of the carbon dioxide for photosynthesis being derived from the sediment *via* the intercellular gas space system (Gessner 1959; Raven 1970). The root/shoot ratio is large relative to that found in most submerged aquatics; the extensive intercellular airspace system is continuous with root and shoot; and chloroplasts occur in the leaves in cells between air-spaces and the epidermis rather than being concentrated in the epidermis in the "normal" submerged aquatic manner.

This has been tested by Wium-Andersen (1971) and Sand-Jensen and Søndergaard (1978) using $^{14}CO_2$ supplied to the roots and shoots separately; and by studying net oxygen efflux from shoots and roots separately in illuminated plants by Sand-Jensen and Prahl (1982) and by Sand-Jensen et al. (1982). These investigations have shown that 90% of CO_2 uptake or O_2 efflux was mediated via the

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roots in *L. dortmanna*, whereas in *I. lacustris* more than half the O_2 efflux was via the shoots.

The second investigative approach relates to the role of dark carbon dioxide fixation and dark acidification followed by light deacidification and photosynthetic fixation of the carbon dioxide regenerated within the photosynthetic tissue. Keeley (1981a, b, 1982a, b, 1983) and Keeley et al. (1981, 1983) have shown that all submerged *Isoetes* species investigated show this "submerged crassulacean acid metabolism", and up to 100 mol H^+ m^{-3} may be generated overnight. The acidification is related to changes in malic acid content, occurring only in the leaves, and results in *net* carbon dioxide fixation in the dark (Keeley 1981a).

Dark acidification and the use of sediment carbon dioxide represent, respectively, temporal and spatial extensions to the plants' ability to acquire carbon dioxide. We have, therefore, attempted to combine these two approaches in comparing the mechanisms of CO_2 uptake and assimilation in *Isoetes lacustris* and *Lobelia dortmanna* from Loch Brandy near Dundee. The investigation includes measurements of the diurnal course of titratable acidity in a light-dark cycle and of the rate of carbon dioxide uptake in light and in darkness by roots and shoots of the two species incubated at approximately in situ carbon dioxide concentrations. We also extend the investigations of carbon metabolism in Isoetids by measurements of the carbon isotope ratio ($\delta^{13}C$) of the source inorganic carbon from the Loch and of the organic carbon from the plants.

$\delta^{13}C$ analysis can distinguish the contribution of dark fixation of CO_2 by phosphoenolpyruvate carboxylase (PEPc) to plant growth, as well as enabling those processes limiting CO_2 fixation to be elucidated (CO_2 diffusion or carboxylation by Ribulose bisphosphate carboxylase (RUBISCO)) (Farquhar 1980; O'Leary 1981; Farquhar et al. 1982; Raven et al. 1982). Additionally, analysis of plant elemental composition has been made, since carbon supply may not necessarily be the only potentially limiting resource in an oligotropic lake.

Materials and methods

Plants were collected from Loch Brandy, Glen Clova, Angus (Grid Ref. No. 338754) on 5 September 1982, and stored at in situ temperature (7.5–8.0° C) in loch water (pH 4.0) under a 12:12 photoperiod (40 μE m^{-2} s^{-1} from warm white fluorescent tubes) until experimental manipulations were conducted on 7–8 September. Experiments were conducted under the same temperature and light regime.

For ^{14}C incorporation experiments, plants were secured into 25 cm^3 glass test tubes such that the roots were enclosed in the tubes and the shoots were free. A seal comprised of a silicon rubber collar and vaseline enclosed the lower half of the plant in the test tube. The roots were surrounded by 15 cm^3 of loch water with $NaHCO_3$ added (final concentration 4 mol m^{-3}; pH approx. 5.0). Test tubes were immersed in loch water with no added $NaHCO_3$ so that the shoots were fully covered. Our justification for the differing inorganic carbon content of the root and shoot media comes from our own and other workers' records of higher CO_2 concentrations in sediment than in overlying waters in lakes of this type. On this sampling occasion, we determined CO_2 concentration in the sediment interstitial water to be 1.6 mol m^{-3} and that of the overlying water to be 0.43 mol m^{-3}. Determinations were made using a Grubb Parsons 120 infra-red Gas Analyser.

Ten plants of each species were presented with $^{14}CO_2$ (added to the low pH medium as $NaH^{14}CO_3$ from Amersham International) in the root medium. An equal number were exposed to $^{14}CO_2$ in the shoot medium. The specific activity of the labelled carbon was checked and found to be 0.1 μCi/μmol inorganic C in the root medium and 0.07 μCi/μmol inorganic C in the shoot medium. Following a 2-h (1400–1600 hours) exposure to ^{14}C, two plants from each treatment (i.e. root or shoot exposed) and for each species were removed and prepared for liquid scintillation counting and titratable acidity determinations. The remaining plants were washed and resuspended in non-radioactive root and shoot medium. Duplicate plants from each exposure treatment were then harvested at approximately 1, 3, 6 and 10 h after termination of the ^{14}C incubation. The entire procedure was repeated for a dark exposure to ^{14}C (0100–0300 hours).

For liquid scintillation counting, duplicate samples of shoots and roots from each plant were rinsed in loch water, blotted dry with a paper towel, and weighed. The lower part of the *Isoetes* shoots clearly did not contain chlorophyll and shoots of this species were cut so that the radioactivity associated with the green and non-green parts could be determined separately. Plant material was placed in plastic scintillation vials containing acetic acid: methanol (1:4) solution and stored for 24–48 h. Prior to adding scintillant (18 mol m^{-3} PPO in toluene: Triton X; 2:1) the acetic acid:methanol solution was evaporated off. Counting was done in a Packard: TRI-CARB 2660 and appropriate quench corrections were made.

Upon harvesting, plants for titratable acidity determinations were frozen in liquid nitrogen and stored. Determinations were made by taking pressed sap extracts from four replicate leaves using a garlic press. A known volume of sap was titrated against 2.5 mol m^{-3} NaOH using phenolphthalein as an indicator. Volumes of either 0.05 cm^3 or 0.02 cm^3 sap were used, depending on the amount of plant material available.

Initial results suggested that a disproportionate relationship existed between the volume of the sap used and the acidity recorded, such that small volumes of cell sap apparently had higher concentrations of H^+ compared to a larger aliquot *of the same* extracted cell sap sample. This may result from a changing buffering capacity of phenolphthalein, perhaps due to using the same quantity of phenolphthalein in different volumes of sap, and requires further investigation. We decided that the most accurate method of dealing with the data was to use only titratable acidity values obtained from a constant volume of sap, even through this method decreased the apparent number of replicates in our study. Therefore, only results for titratable acidity in which the volume of sap used was 0.05 cm^3 are plotted.

The net weight of leaves, stems and roots was determined by weighing fresh plant tissue which had been lightly blotted with paper towels. Dry weight was determined by drying to constant weight at 80° C. Portions of the dried tissue were heated at 450° C for 15 h in ceramic crucibles to determine ash weight. The difference between ash weight and original dry weight was taken to be the organic weight of the sample.

The carbon and nitrogen content of other portions of

the dried tissue were determined using a Carlo-Erba CHN Analyser. The phosphate content of other portions of the dried tissue was determined on extracts of the tissue (1–4 mg dry weight) in a 5:1 mixture of 72% $HClO_3$:fuming HNO_3, refluxed at 200° C for 30 min (S. Allen, in preparation). After neutralisation, the phosphate content of the extract was determined by the phosphovanadomolybdic acid method (American Public Health 1976).

Samples for mass spectrometric analysis were prepared as in Raven et al. (1982) except that ground *Saccharum* leaves (calibrated at $-13.0^0/_{00}$) were used as a secondary standard; this reduced the variation between sample and standard preparation. CO_2 was precipitated as carbonate from loch water and sediment water in saturated, filtered $Ba(OH)_2$. All samples were analysed on a VG MM 601 single collector mass spectrometer.

Dried specimens of *Isoetes durieui* were kindly supplied by Professor U. Lüttge. At the time of collection, these had been growing in open grassland on Sicily in a position unlikely to have been invaded with surface water. Thus, these plants were unlikely to have been in "vernal pools" (cf. Keeley 1981a, b, 1982a, b) and can be regarded as terrestrial (Tutin et al. 1964).

Results and discussion

The variation in titratable acidity of *I. lacustris* shoots monitored throughout the experimental period (Fig 1) is similar in magnitude to that previously reported for *I. howellii*, *I. orcuttii* and *I. storkii* (Keeley 1981a, b, 1982a; Keeley et al. 1981). A peak of 160 mol m^{-3} H^+ towards the end of the dark period reflects an increase of about 80 mol m^{-3} H^+ over and above the daytime pool organic acids. There was no such increase in titratable acidity of either the corm or roots of *I. lacustris* (data not shown), supporting the observations of Keeley (1981a). The close agreement between those values which overlap at the beginning and end of the experimental period (Fig. 1: 1400 and 1600 hours on day 1; cf. day 2) demonstrates the regularity of the diel pattern.

L. dortmanna demonstrates a virtually constant concentration of H^+ in the cell sap throughout the day of around 20 mol m^{-3} H^+ (Fig. 1). This is 20–30 mol m^{-3} H^+ lower than the lowest values found for *I. lacustris* (Fig. 1) and indicative of obligate C_3 metabolism in *L. dortmanna*. This is despite the findings that many Isoetids and other aquatic macrophytes may show dark CO_2 fixation usually catalysed by PEPc (*Hydrilla verticillata*: Holaday and Bowes 1980; *Scirpus lacustris*: Beer and Wetzel 1981; *Littorella uniflora*: Keeley 1982b; cf. Søndergaard and Sand-Jensen 1979), although such dark fixation often amounts to less than 20% of that observed in *Isoetes* (Keeley 1981a, b, 1982a, b). Figure 1 indicates that there is no evidence that *L. dortmanna* shows any significant acid accumulation during the dark period despite the observation that some carbon was fixed by *L. dortmanna* at this time (see Fig. 2).

Figures 2 and 3 present the results of ^{14}C incubations for *I. lacustris* and *L. dortmanna*. Most striking is the fact that for *L. dortmanna* carbon fixation occurs primarily during the day and almost exclusively utilises CO_2 taken up through the roots. Combining the average values for leaf and root fresh weight per plant (from Table 1) and the ^{14}C incorporation data, we calculate that, for an "average" *Lobelia* plant, 99.9% of carbon fixed in the shoot is initially

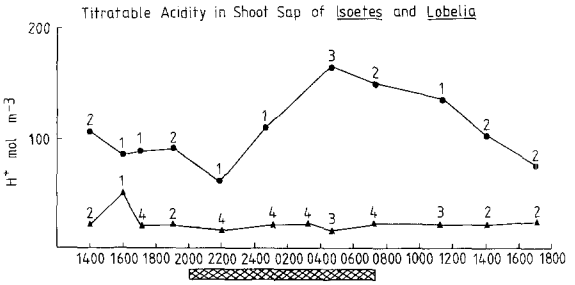

Fig. 1. Titratable acidity (mol H^+ m^{-3}) of pressed shoot sap of *Isoetes lacustris* (*upper line*) and *Lobelia dortmanna* (*lower line*) during the diel experimental period. Only data from samples of 0.05 cm^3 are shown (see Materials and Methods) with the number of replicates indicated at each sampling point

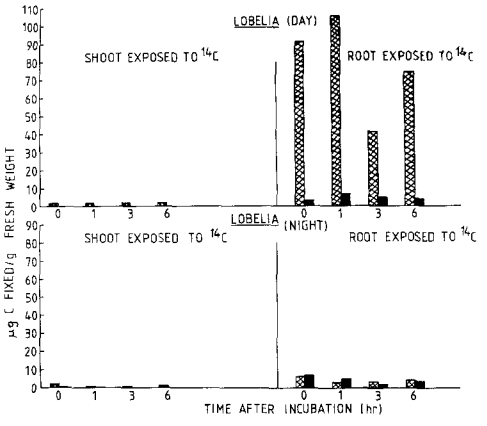

Fig. 2. Distribution of ^{14}C (µg C fixed/g fresh wt) in *Lobelia dortmanna* for 6 h following a 2-h pulse with $^{14}CO_2$ to shoots or roots during day (1400 hours) and night (0100 hours): ^{14}C in organic carbon of shoot (▨) and root (■)

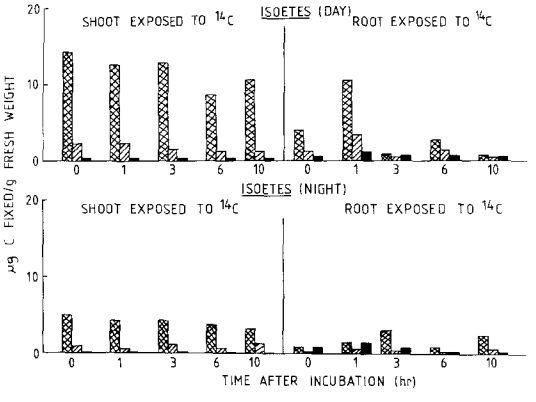

Fig. 3. Distribution of ^{14}C (µg C fixed/g fresh wt) in *Isoetes lacustris* for 6 h following a 2-h pulse with $^{14}CO_2$ to shoots or roots during day (1400 hours) and night (0100 hours): ^{14}C in organic carbon of green shoot (▨), white portion of shoot (▨) and root (■)

taken up through the roots and 82% of the carbon fixation is carried out during the day. This is in close agreement with the findings of Wium-Andersen (1971), Sand-Jensen and Søndergaard (1978), Sand-Jensen and Prahl (1982) and Sand-Jensen et al. (1982).

In *I. lacustris*, both the roots and the shoots were involved in carbon uptake (Fig. 3). In an "average" plant,

Table 1. Number per plant, fresh weight, dry weight, and carbon, nitrogen, phosphorus, ash, and ash alkalinity content, of leaves, stems and roots of *Isoetes lacustris* and *Lobelia dortmanna*, and nitrogen and phosphorus content of (leaves + stems) and of roots of *Isoetes durieui*

	Isoetes lacustirs	*Lobelia dortmanna*	*Isoetes durieui*
Leaf (>10 mm) number per plant	9.86±1.08 (7)[a]	5.14±0.51 (7)	N.D.[b]
Root (>10 mm) number per plant	22.4±84 (7)	47.0±8.0 (7)	N.D.
Leaf fresh weight per plant/mg	467.7±79.6 (7)	340.0±90.0 (7)	N.D.
Stem fresh weight per plant/mg	207.3±19.3 (7)	264.0±50.3	N.D.
Root fresh weight per plant/mg	1,121.2±146.5 (7)	869.7±165.4 (7)	N.D.
Leaf dry weight per plant/mg	33.9±7.0	25.4±3.2 (7)	N.D.
Stem dry weight per plant/mg	25.4±3.5 (7)	20.7±4.5 (7)	N.D.
Root dry weight per plant/mg	33.0±5.1 (7)	47.0±10.7 (7)	N.D.
Leaf length per plant/mm	408.0±60.8 (7)	201.4±19.9 (7)	N.D.
Root length per plant/mm	2,355.0±145 (7)	2,658.0±528 (7)	N.D.
Leaf carbon/μmol g dry wt.$^{-1}$	29,320, 29,710	34,350, 34,310	29,460, 30,000
Stem carbon/μmol g dry wt.$^{-1}$	34,780, 34,630	32,990, 33,780	N.D.
Root carbon/μmol g dry wt.$^{-1}$	31,940, 31,810	34,490, 34,190	32,280
Leaf nitrogen/μmol g dry wt.$^{-1}$	1,293, 1,257	1,871, 1,829	1,993, 2,207
Stem nitrogen/μmol g dry wt.$^{-1}$	1,200, 1,229	1,529, 1,638	N.D.
Root nitrogen/μmol g dry wt.$^{-1}$	1,271, 1,243	1,600, 1,600	900
Leaf phosphorus/μmol g dry wt.$^{-1}$	7.39±1.36 (3)	25.6±1.6 (3)	N.D.
Stem phosphorus/μmol g dry wt.$^{-1}$	9.86±0.53	7.12, 12.20	N.D.
Root phosphorus/μmol g dry wt.$^{-1}$	7.07±1.55 (3)	7.92±4.8 (3)	N.D.
Leaf ash/mg g dry wt.$^{-1}$	185, 192	104, 106	N.D.
Stem ash/mg g dry wt.$^{-1}$	104, 108	111	N.D.
Root ash/mg g dry wt.$^{-1}$	150, 182	105, 111	N.D.

[a] Errors quoted are standard errors of the mean, with number of samples in parentheses; where less than three replicates were used, individual values are given

[b] Not Determined

only 39% of the total carbon fixed in the shoot during the two incubations was taken up through the roots. Of the total carbon fixed, 72% was taken in during the daytime incubation. These values fit remarkably well with the O_2 release data for these two species presented by Sand-Jensen et al. (1982) despite possible damage to the root systems during our collection. Nevertheless, we note that these other workers record root releases of O_2 as 39% of the total release in *I. lacustris* and as 100% of the total release in *L. dortmanna*. Thus, there is good agreement between the ratio of gas exchange by roots to that of the total plant reported for these species and the ratio of carbon fixed throught the roots to total carbon fixed by the plants in our study.

If we compare *absolute* rates of gas exchange reported for the species and those we calculated from ^{14}C incorporation, we find our specimens of *L. dortmanna* took up an average of 61.3 μmol CO_2 (g dry weight)$^{-1}$ h^{-1} in light at 7.5–8.0° C (Fig. 2; Table 1). Sand-Jensen et al. (1982) reported 97.5 μmol O_2 (g dry weight)$^{-1}$ h^{-1} at 15° C for this species. For *I. lacustris* we find 17.9 μmol CO_2 (g dry weight)$^{-1}$ h^{-1} (Fig. 3: Table 1) compared to the 56 μmol O_2 (g dry weight)$^{-1}$ h^{-1} at 15° C reported by Sand-Jensen et al. (1982).

The substantial dark fixation in *I. lacustris* (some 6.16 μmol CO_2 (g dry weight)$^{-1}$ h^{-1}) may be compared with the net dark acidification presented in Fig. 1. Over the 8.5-h dark period, some 70 μmol titratable acid (g fresh weight of leaf)$^{-1}$ accumulates. This corresponds to an accumulation of 20.9 μmol CO_2 (g dry weight)$^{-1}$ h^{-1} (Table 1) in the dark. At least a part of this discrepancy may be attributed to the dilution of the supplied radioactive CO_2 by unlabelled, respiratory CO_2 within the tissue. We also noted some considerable variation between the absolute amount of carbon fixed on a fresh weight basis by different leaves in the same *Isoetes* plant. This may have been due to the relative ages of the different leaves. We used different parts of the shoot for carbon uptake determinations and for titratable acidity measurements and this, too, may account for some of the discrepancy noted.

Table 2. $\delta^{13}C$ for organic C from *Isoetes lacustris* and *Lobelia dortmanna* from Loch Brandy and for inorganic C from the loch water/sediment interstitial water; $\delta^{13}C$ for *Isoetes durieui*, a terrestrial plant from Sicily, with likely δ value of atmospheric CO_2 at the time of sampling. δ values also corrected for source CO_2 ($\Delta\delta^{13}C$); number of replicates in parentheses; data±standard error of the mean

	$\delta^{13}C$ (‰)	$\Delta\delta^{13}C$ (‰)[a]
Isoetes lacustris		
Shoots ($n=4$)	−23.5±1.2	− 6.0
Roots ($n=4$)	−23.1±0.9	− 5.6
Lobelia dortmanna		
Shoots ($n=4$)	−31.7±0.4	−14.2
Roots ($n=$)	−30.0±0.6	−12.5
Sediment/water CO_2	−17.5	
Isoetes durieui		
Shoots ($n=1$)	−26.5	−19.5
Roots ($n=1$)	−26.6	−19.6
Atmospheric CO_2 (1950s)	− 7.0	

[a] $\Delta\delta^{13}C(‰) = \delta$ plant material $- \delta$ source carbon

Analysis of carbon isotope ratio (δ) in aquatic plants has, until recently, been fraught with problems of interpretation. It is now appreciated that the δ value in such plants reflects the source CO_2 δ value, the influence of CO_2 diffu-

sion resistance pathways (e.g. boundary layers), carboxylation discrimination and the C species utilised (CO_2/HCO_3^-); see Raven (1970), Smith and Walker (1980), Osmond et al. (1981), and Raven et al. (1982). The low pH value of the loch and sediment interstitial water in our study (approx. pH 4.0) would preclude HCO_3^- formation (Mook et al. 1974) and thus simplify the interpretation of the δ values.

There have been no previous reports which integrate the photosynthetic metabolism and δ in Isoetids. The values presented in Table 2 show that the δ value for *I. lacustris* is less negative than that for *L. dortmanna*, with no significant differences occurring between plant parts. The δ value of CO_2 dissolved in the loch water ($-17‰$) is consistent with the input of allochtonous carbon from sediment organic material (Wium-Andersen and Andersen 1972; cf. Vogel 1980; Osmond et al. 1981; Raven et al. 1982) since, at the temperature and pH encountered at Loch Brandy, air equilibrated CO_2 should have a δ value of $-9.1‰$ (see Mook et al., 1974). This is further supported by the observation that the CO_2 concentration in the water was higher than that expected from air equilibration.

Once corrected for source CO_2 ($\Delta = \delta$ plant $- \delta$ source), Δ values (Table 2) can be used to interpret carboxylation reactions and CO_2 limitation in photosynthesis (Farquhar 1980; Farquhar et al. 1982). *L. dortmanna*, already predicted to have a C_3 type metabolism, has a Δ value ($-13‰$) which is less negative than for terrestial C_3 plants (about $-20‰$). This is indicative of a "transport resistance" which is of similar magnitude to the "carboxylation resistance". Consideration of the anatomy of *L. dortmanna* (Table 1 and discussion thereof; Sand-Jensen and Prahl 1982) suggests that the area of root surface through which CO_2 is taken up exceeds by about 100-fold the cross-sectional area of the intercellular air spaces by which gaseous CO_2 moves to the leaf plastids. Despite the considerable length of the gaseous diffusion path (several tens of mm: Table 2), the 10^4-fold higher diffusion coefficient of CO_2 in air than in water means that an unstirred aqueous-phase diffusion path of only a few mm would make this the major component of the 'transport resistance' to CO_2.

Interpretation of the *I. lacustris* data is complicated by the fixation of CO_2 by PEPc. In terrestrial CAM plants, this process may lead to a δ of newly fixed carbon of $-7‰$ when CO_2 fixation is limited equally by both diffusion and carboxylation (O'Leary and Osmond 1980; Holtum et al. 1982). Of CO_2 fixation by the experimental plants 70% occurred during the light period (Fig. 3). Thus, the δ value for *I. lacustris*, less negative than for *L. dortmanna*, reflects both night time CO_2 fixation by PEPc in the CAM pathway and daytime fixation by RUBISCO which is limited by the rate of CO_2 diffusion (O'Leary and Osmond 1980; cf. Farquhar et al. 1982).

We note that the rates of dark CO_2 fixation and acidification may well exceed the rate of dark respiration, thus giving a net CO_2 fixation in the dark (Sand-Jensen 1978; Sand-Jensen et al. 1982; Keeley 1981a), involving exogenous CO_2 ($\delta = -17.5‰$ as well as endogenous, respiratory CO_2 ($\delta = -23.5‰$) in the PEPc reaction. Further, in the light, in order that the net exogenous CO_2 assimilation as well as the assimilation of CO_2 produced by malic acid deacidification shall occur, the steady-state CO_2 concentration in an illuminated *I. lacustris* plant must be lower than in the medium. A higher longitudinal gas-phase diffusion resistance may be expected in *I. lacustris* than in *L. dortmanna* in view of the presence of transverse septa in the gas spaces of the former but not of the latter (Haslam et al. 1975).

The terrestrial *Isoetes durieui* has a δ value which is characteristic of a C_3 plant. It is interesting to note that *Isoetes* leaves which are partially exposed in vernal pools show considerably reduced fluctuations in titratable acidity (Keeley 1981b; Keeley et al. 1983) and may represent the transition from facultative CAM to C_3 metabolism. These plants also develop functional stomata (Keeley et al. 1983). Submerged CAM is therefore analagous to CAM in astomatous roots of epiphytic orchids (Winter et al. 1983; Benzing and Pridgeon 1983).

The dry weight results presented in Table 1 show that there is no difference in total plant dry weight between *I. lacustris* and *L. dortmanna*. The large fraction of the plant dry weight which is found in the roots is typical of Isoetids (Westlake 1965; Moeller 1978; Sand-Jensen et al. 1982). The larger fraction of dry weight in the root fraction of *L. dortmanna* than of *I. lacustris* is also in good quantitative agreement with previous estimates (Sand-Jensen et al. 1982). There is no significant difference in root *length* per plant (Table 1) between the two species so that, granted the overlapping ranges of root diameters for the two plants (0.65–0.95 mm for *L. dortmanna* and 0.7–1.0 mm for *I. lacustris*), the root surface area will not be significantly different for the two species. The area of *I. lacustris* roots is increased by root hairs and that of *L. dortmanna* by mycorrhizas (Søndergaard and Laegaard 1977).

The ratio of fresh weight to dry weight for the plant parts is within the range expected for submerged aquatics (Westlake 1965). The high ratio of fresh to dry weight for *I. lacustris* roots may be related to the partial infiltration of the intercellular gas spaces with water which was observed. We note that the volume (mm^3) of the roots (and other organs) exceeds that expected for their freshweight (mg) which is consistent with the substantial fraction of gas space noted in sections of the leaf and root tissues. The large root area may be, in part, related to anchoring these positively buoyant plants to the sediment. The greater infiltration of *I. lacustris* roots than *L. dortmanna* roots may be related to the large single gas space in *Isoetes* roots as compared to the multiple cortical air channels in *Lobelia*.

The elemental composition data in Table 1 show rather lower C/dry weight in *I. lacustris* leaves than in *L. dortmanna* leaves, a difference which is largely attributable to the greater ash content of the *I. lacustris* leaves. A similar argument applies to *I. lacustris* roots compared to *L. dortmanna* roots. The nitrogen content of leaves of *I. lacustris* is substantially less than that of either *L. dortmanna* or *I. durieui*, although nitrogen values for *L. dortmanna* leaves may be lower (Moeller 1978).

A possible explanation for the lower nitrogen content of leaves of *I. lacustris* relative to *L. dortmanna* and *I. durieui* relates to the partially CAM nature of the photosynthetic metabolism in the former plants compared to the C_3 metabolism of the the two latter plants. By analogy with C_4 metabolism, (Brown 1978; cf. Öztürk et al. 1981) it is possible that CAM plants can have lower RUBISCO contents and still achieve the same carbon fixation rate as C_3 plants if the high CO_2 concentration resulting from CAM deacidification accelerates carboxylase and slows oxygenase reaction of RUBISCO. Since RUBISCO is such

a large fraction of the total protein in many plant leaves, a lower N content of the leaves could result from a decreased RUBISCO content. A subsidiary hypothesis which could explain low N/dry weight values for CAM plants relates to the requirements for a large pool of 'catalytic carbon' in the leaves of CAM plants which is converted from starch (or soluble sugar) to organic acid during acidification, and reforms carbohydrate in the light deacidification period (see Black et al. 1982). The need for 'catalytic carbon' might increase the C/N ratio in CAM leaves.

Phosphorus content is much higher in the leaves of *L. dortmanna* than in the stems or roots of this plant or than in any of the tissues of *I. lacustris* (Table 1). The P/dry weight values of Moeller (1978) for North American *L. dortmanna* in September are higher for leaves and much higher for stems plus roots than are the values reported in Table 1. The low P/dry weight content values we report here have precedents among aquatic plants (see Raven 1976, 1981; Hutchinson 1975) but they are clearly at the lower end of the range reported for macrophytes. The higher P content of the leaves of *L. dortmanna* than those of *I. lacustris* may be related to the mycorrhizality of the former plant (Søndergaard and Laegaard 1977; Smith 1980). Mycorrhizality might improve access to P in the aerobic sediments inhabited by Isoetids in which the concentration and diffusion coefficient of phosphate is probably very low (Krom and Berner 1980a, b; Nye and Tinker 1977; Sand-Jensen et al. 1982).

Conclusions

Net inorganic carbon assimilation by the two Isoetids investigated involves CAM (*Isoetes lacustris*) and root uptake of carbon (*Lobelia dortmanna* > *Isoetes lacustris*), as means of temporally and spatially optimising CO_2 supply in an oligotrophic environment. Capitalizing on the higher CO_2 concentrations in the sediments and CO_2 uptake at night as well as during the day can be interpreted as implicating CO_2 as a limiting resource for growth. Analyses of carbon isotope ratios suggest that CO_2 diffusion may indeed limit photosynthesis in both *I. lacustris* and *L. dortmanna*. However, despite the use of mycorrhizas (*L. dortmanna*) and root hairs (*I. lacustris*) in order to enhance nutrient acquisition, plant nitrogen and phosphorus levels were generally low and thus may also be a limiting factor in the growth of Isoetids. Future investigations into the ecophysiology of Isoetids should therefore attempt to relate both inorganic carbon supply and nutrient supply and utilisation.

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