

## Endomycorrhizae of isoetids along a biogeochemical gradient

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### Abstract

Endomycorrhizae of aquatic plants may be important in phosphorus uptake and carbon exchange in lakes, but the environmental controls on mycorrhizal distribution are not known. We examined biogeochemical variables that were correlated with aquatic endomycorrhizae of isoetid-type macrophytes in an oligotrophic, softwater lake. Endomycorrhizal infection was greatest in the shallow stations with high sediment redox potential and lowest in the deeper stations where there was low redox potential and high sediment organic content and porewater P levels. There was a significant ( $r = +0.93$ ,  $P < 0.05$ ) positive correlation between percentage of hyphal infection of the roots and a root ergosterol (a specific fungal sterol) index determined per root mass. Fungal vesicle infection was also positively correlated with the root ergosterol index, although not significantly ( $r = +0.76$ ,  $P = 0.14$ ). Furthermore, the root ergosterol index was significantly correlated with plant rosette density ( $r = +0.97$ ,  $P < 0.05$ ). An increase in rosette density of isoetids increases the number of lacunae transporting oxygen to the roots, perhaps increasing fungal infection. The root ergosterol index was also significantly ( $P < 0.05$ ) correlated with sediment redox status, porewater phosphate, solid phase iron-bound P, exchangeable inorganic P, and inorganic adsorbed P. There were no significant correlations between above- or belowground biomass or porewater  $\text{NH}_4^+$  with the root ergosterol index, and porewater DOC was weakly correlated ( $P = 0.08$ ) with the ergosterol index. Isoetid-type plants are common in oligotrophic softwater lakes, and knowledge of environmental variables that are associated with endomycorrhizae will facilitate in management and restoration of these types of submersed vegetation.

The first report of mycorrhizae (fungal-root symbioses) of submersed aquatic vegetation was by Søndergaard and Laegaard (1977) in Danish lakes. Prior to this, mycorrhizal fungi were assumed not to exist in waterlogged or submersed environments (Harley and Smith 1983). Subsequently, the occurrence of endomycorrhizal fungi in roots of submersed macrophytes has been documented frequently (Bagyaraj et al. 1979; Chaubal et al. 1982; Clayton and Bagyaraj 1984; Farmer 1985; Wigand and Stevenson 1994). Recently, it has been suggested that aquatic mycorrhizae may help promote restoration of macrophytes to disturbed sites (Cooke and Lefor 1990) and facilitate phosphorus uptake in at least one submersed plant (Wigand and Stevenson 1997). However, the environmental variables that promote or inhibit aquatic mycorrhizae, as well as the role of this mutualism in nutrient cycling and responding to intermittent disturbances in aquatic systems, are not well studied.

Aquatic endomycorrhizal fungi are thought to have an oxygen requirement (Harley and Smith 1983; Tanner and Clayton 1985); therefore, fungal infection would more likely oc-

cur in the wave-wash zone with low-sediment organic matter (Clayton and Bagyaraj 1984; Tanner and Clayton 1985). In addition, plant characteristics (e.g. high rosette density, extensive aerenchyma) that allow for increased channeling of oxygen to the root zone would also promote endomycorrhizae (Cooke et al. 1993; Wigand and Stevenson 1994). An increase in rosette density of isoetids increases the number of lacunae (oxygen channels) transporting oxygen to mycorrhizal fungi of roots. In contrast, sediment of high organic content may inhibit aquatic mycorrhizae because of high reductant levels and oxygen demand associated with decomposition processes (Tanner and Clayton 1985; Khan 1993). Furthermore, sediments of high organic content often have elevated porewater P, and aquatic mycorrhizae are reported from only P-poor systems (e.g. Søndergaard and Laegaard 1977).

Farmer (1985) sampled isoetids in a number of Scotland lochs at varying depths (0.1–2.0 m) and found little or no difference in the presence of mycorrhizae at different depths. However, Clayton and Bagyaraj (1984) observed a general decline in endomycorrhizae with increasing water depths in *Lagarosiphon major*. Furthermore, in a recent study examining endomycorrhizal infection in wetland-adapted trees, Khan (1993) found a significant relationship between endomycorrhizal infection and oxidation–reduction status, suggesting that more oxygenated sediments supported higher fungal biomass and more active endomycorrhizae. We examined endomycorrhizal fungi (biomass, morphology) along a geochemical gradient from shallow depths (0.6 m) to deeper water (4.6 m) in the oligotrophic Lake Kalgaard (Denmark) vegetated with isoetids (*Littorella uniflora*, *Isoetes lacustris*, *Lobelia dortmanna*).

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Isoetids are a group of submersed macrophytes with a well-developed root system by which nutrients and inorganic carbon are assimilated (Wium-Andersen 1971; Christiansen et al. 1985) and an extensive lacunal system by which oxygen is laterally released into the rhizosphere (Sand-Jensen et al. 1982; Pedersen et al. 1995). This lateral oxygen release by isoetids can oxygenate the entire rhizosphere in sediment of low organic content (Christensen and Andersen 1996) and forms a horizon of reduced iron below the oxidized root zone in some lakes (Tessenow and Baynes 1975, 1978; Christensen et al. 1997). In the oxidized rhizosphere, dissolved inorganic P levels are lowered due to formation of insoluble ferric phosphate and ferric hydroxides that adsorb phosphate (Wium-Andersen and Andersen 1972; Tessenow and Baynes 1975; Jaynes and Carpenter 1986; Andersen and Olsen 1994).

In this study we cannot discern whether certain sediment characteristics promote mycorrhizal plants or if the mycorrhizal plants alter sediment chemistry; however, we can determine which variables are correlated with aquatic mycorrhizae. We hypothesized that plant characteristics and geochemical variables that affect sediment oxidation-reduction status, sediment organic content, or plant nutrient availability would be significantly correlated with endomycorrhizal infection. We investigated if edaphic and plant characteristics were correlated with endomycorrhizal infection along the vegetated transect.

## Methods

The study site was Lake Kalgaard (*see* Sand-Jensen and Søndergaard [1978, 1979] for a detailed description), a shallow (10.5 ha; mean depth of 4.65 m) oligotrophic softwater lake in central Jutland, Denmark (56°1'N, 9°27'E). The transect was placed in the northeastern part of the lake with stations at depths of 0.6, 1.0, 1.8, 2.8, and 4.6 m that extended from shore to 30 m across the lake. The first four stations were each ~5 m apart (5, 10, 15, and 20 m from shore) and the last station was ~30 m from shore and marked the farthest extent of vegetation in the lake.

Geochemical variables, including sediment redox potential, solid-phase P fractions, dissolved inorganic phosphorus (DIP), ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), and dissolved organic carbon (DOC), were measured at each of five stations. For the present study only the root zone means (integrated 0–6.5 cm) for geochemical variables are reported. Sediment cores (5.2-cm i.d.) were collected by divers using scuba, and oxidation-reduction status (four cores collected per station) was measured in the field using a platinum electrode with a calomel electrode as a reference (Hargrave 1972). Additional cores (two each for Sta. 1–3 and three each for Sta. 4 and 5) were brought back to the laboratory to measure sediment organic content (% of DW) in the root zone (0–6 cm) for each station. Dried (105°C, 24 h) and homogenized sediment samples were combusted at 520°C for 2 h to estimate total organic matter content from loss of mass on ignition.

Sediment porewater DIP, DOC,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were sampled using equilibrators (two per station; Hesslein 1976; Bottomley and Bayley 1984) with Spectra/Por-regenerated cel-

lulose membranes (MW cutoff of 6,000–8,000 Da) fastened on both sides of a PVC stake drilled with 2.5-cm holes set at ~2.5-cm intervals to allow for diffusion of pore water into the equilibrators over a 1-week sampling period at each station. Prior to affixing the membranes to the equilibrators, each was rinsed three times with deionized water for 3 h. In addition, residual sulfur was removed from the membrane using the Spectrum sulfide and heavy metal removal solution. The root zone pore water was sampled by four holes on the equilibrator from just below the sediment surface to 6.5 cm, and the mean is reported for each equilibrator. Pore water was sampled in the field and placed in acidified polyethylene vials for DIP, frozen for nitrogen measurements, and placed into precombusted glass vials for DOC measurements. DIP was measured by spectrophotometry as molybdate-reactive P. Porewater  $\text{NO}_3^-$  was measured on a flow-injection analyzer (Tecator FIAstar 5010) using the sulphanimide-naphthylene-diamine method for the  $\text{NO}_3^-$  measurements after reduction of  $\text{NO}_3^-$  to  $\text{NO}_2$  by a cadmium column.  $\text{NH}_4^+$  was measured by the modified salicylate-hypochlorite method (Bower and Holm-Hansen 1980) on a microplate-reader at 640 nm (Microwell EL301). DOC was measured with a total organic carbon analyzer (Shimadzu TOC-5000) on acidified samples.

For analysis of the various fractions of solid phase P, three cores (5.2-cm i.d.) were sampled by scuba from each station along the transect, and a sequential extraction scheme with wet sediment was used to quantify exchangeable inorganic P, inorganic P bound to oxidized forms of iron and manganese, inorganic P adsorbed onto clay minerals and aluminum oxides, inorganic P bound in humic acids, calcium-bound inorganic P, and organic P (Jensen and Thamdrup 1993; Paludan and Jensen 1995). More details on the extraction procedure and the calculation of P pools are given in Christensen et al. (1997b).

While the geochemical variables at each station were measured, above- and belowground biomass and rosette density were sampled by hand using a 510-cm<sup>2</sup> quadrat. At each station three quadrats of plants were randomly sampled and stored on ice until processed at the laboratory within 48 h. A subsample of roots from each quadrat was randomly selected and preserved in a Formalin-aceto-alcohol (FAA) reagent (5:5:90 vol/vol/vol) for histological examination of the presence or absence of fungal structures (vesicles, arbuscules, hyphae). Roots from each quadrat were cut into ~1-cm sections and stained using standard methods of KOH treatment and Trypan Blue staining (Phillips and Hayman 1970). We observed endomycorrhizal infection at 200× with a compound microscope and reported infection as percentages of vesicles, arbuscules, or hyphal infection for the three replicates each of ~10 1-cm root segments.

A second subsample (4–8 g WW; roots of at least 50 rosettes when possible) was refrigerated in 10 ml of methanol for high-performance liquid chromatography (HPLC) analysis of ergosterol, a specific fungal sterol, using standard methods (Newell et al. 1988). To extract ergosterol, samples were transferred to 250 ml distillation flasks with a total of 50 ml of methanol and refluxed for 2 h. After removing undigested roots by filtering, the extracts were saponified by addition of 5 ml of 4% KOH in 95% ethanol and refluxed

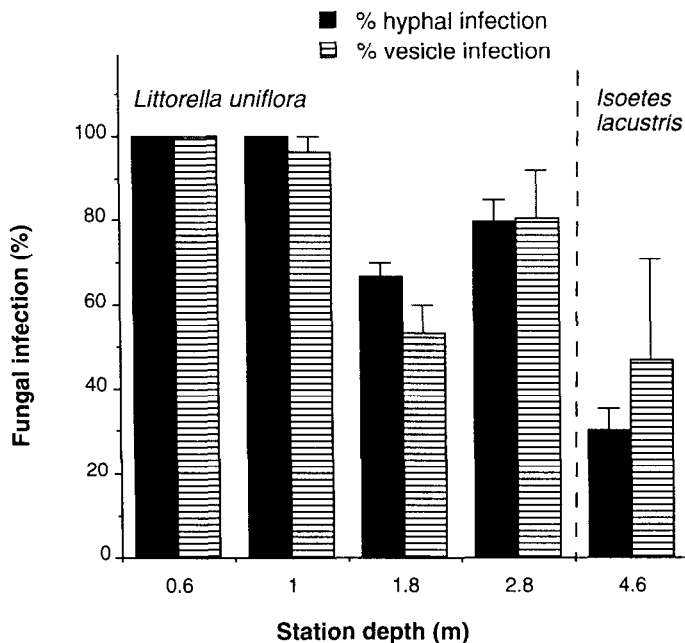


Fig. 1. Hyphal and vesicle infection of isoitid roots along a transect from shallow (0.6 m) to deeper waters (4.6 m). Means and SE are shown for three replicates of 10 root segments each. The first four stations (0.6–2.8 m) were vegetated with *L. uniflora* and the fifth station (4.6 m) with *I. lacustris*.

for an additional 30 min. After cooling, the extracts were transferred to 125-ml separatory funnels containing 10 ml of water. Each sample was extracted three times with pentane (10, 10, 5 ml), and the pentane extracts were combined and evaporated in a fume hood. The residue was redissolved in 1.0 ml of methanol and ergosterol quantified using the following configuration on a Waters 991 photodiode array detector (solvent, methanol; flow rate, 2 ml min<sup>-1</sup>; sample size, 100  $\mu$ l; column, C-18, 250  $\times$  4.6-mm cartridge with guard column; detection absorbance at 282 nm; ergosterol retention time, 6.5 min). The detection limit for ergosterol for the system was 0.02  $\mu$ g ml<sup>-1</sup>. Ergosterol was reported as  $\mu$ g ergosterol g (DW) root<sup>-1</sup>. Root wet weight estimates were converted to dry weights by regression analysis of root wet and dry weight subsamples for each plant species. For all five stations, we correlated root ergosterol with microscopic observations of root fungal structures (i.e. vesicles and hyphae) to determine if ergosterol measurements could be used as a surrogate for endomycorrhizal infection in isoitids.

Subsequent to our ergosterol analyses for this study, we improved methods for ergosterol extraction for *Littorella uniflora* roots by over 10-fold (Wigand unpubl. data). We were unable to make additional ergosterol measurements on the original root samples, and therefore the ergosterol measurements for this study represent only an ergosterol index.

We first examine the relationships between fungal infection and the root ergosterol index for the five stations and then correlate plant and edaphic characteristics with the ergosterol index. For plant-specific characteristics (e.g. rosette density) we use only the first four stations vegetated with *L. uniflora* in the regression analyses. However, in the analyses



Fig. 2. Squash preparation of endomycorrhizae of *L. uniflora* collected from Lake Kalgaard. Vesicles can be over 100  $\mu$ m in length.

between the log-ergosterol index and edaphic characteristics we include all five stations regardless of the plant species composition. A logarithmic transformation was performed to normalize the distributions and provide for homogeneity of variances when necessary in the regression analyses. One-way ANOVA and multiple-range analyses were used to examine for differences in fungal infection, plant biomass, rosette density, and sediment redox potential among the five stations.

## Results

*Isoetes lacustris* roots at the deepest station were on average 47% infected with small vesicles (10–20  $\mu$ m) unattached to hyphae (Fig. 1). For comparison, *L. uniflora* at the shallower stations showed 80–100% infection (Fig. 1), with larger vesicles (30–130  $\mu$ m) attached to hyphae (Fig. 2). Along the transect, there was significantly ( $P < 0.05$ ) greater hyphal infection at shallow stations (100%), intermediate levels (73%) in middle stations, and lowest infection (30%) at the deepest station (Fig. 1). Similarly, there was significantly ( $P < 0.05$ ) greater vesicle infection in the shallow stations and lower infection in the deeper stations (Fig. 1). No arbuscules were observed in the root samples of *I. lacustris* at the deepest station. Arbuscules and remnants of arbuscules were present in some root samples at all four *L. uniflora* stations, but because the arbuscules were not often distinct we used percentages of fungal vesicle and hyphal infection in the statistical analyses.

The four shallowest stations were vegetated with *L. uniflora* and the deepest station with *I. lacustris*. Sparse stands of *Lobelia dortmanna* were only found at depths of 1.5 m or less along the transect. The above- and belowground biomass along the transect showed highest values at intermediate depths (Fig. 3). At the 1.8 m station the aboveground biomass (107 g (DW) m<sup>-2</sup>) of *L. uniflora* was significantly ( $P < 0.05$ ) greater than the other stations. There were significant ( $P < 0.05$ ) differences among stations for both rosette density and sediment redox potential, with highest val-

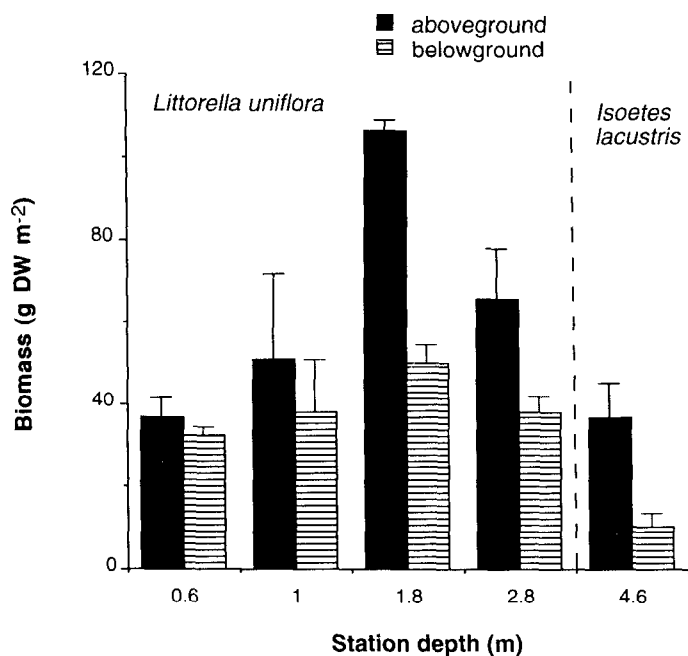


Fig. 3. Above- and belowground biomass ( $n = 3$  with SE for each) of *L. uniflora* at the first four stations (0.6–2.8 m) and *I. lacustris* at the deepest station (4.6 m) in Lake Kalgaard.

ues at the 1.0-m depth station (3,059 rosettes  $m^{-2}$ , Fig. 4A; 472 mV, Fig. 4B). Rosette density of *I. lacustris* (280  $m^{-2}$ ) was an order of magnitude lower than *L. uniflora*, and it grew only at the deepest station (4.6-m depth) with the lowest redox potential and the highest sediment organic content (Table 1).

There was a significant and positive correlation ( $P < 0.05$ ,  $r = +0.93$ ) between percentage of hyphal infection and the log-ergosterol index (Fig. 5A), and a positive correlation ( $r = +0.76$ ) for percentage of vesicle infection and the log-ergosterol index, although not significant ( $P = 0.14$ ) (Fig. 5B). We observed a significant positive relationship between the log-ergosterol index and *L. uniflora* log-rosette density

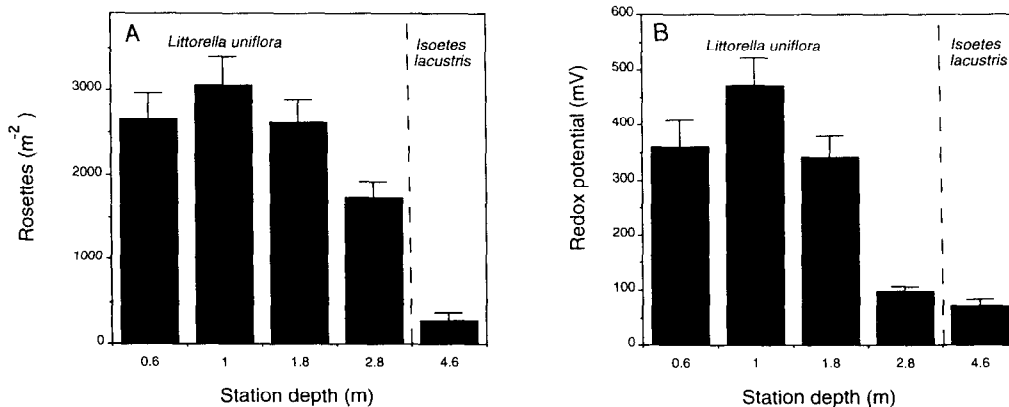


Fig. 4. A. Rosette density ( $n = 3$  with SE) of isoetids in Lake Kalgaard. B. Sediment redox potential ( $n = 4$  with SE) along a transect from shallow to deeper water in Lake Kalgaard. The first four stations (0.6–2.8 m) were vegetated with *L. uniflora* and the fifth station (4.6 m) with *I. lacustris*.

Table 1. Sediment organic content ( $n = 2$  or 3) and rhizosphere porewater variables ( $n = 2$ ) at isoetid stations (values are  $\pm$ SE).

Station depth (m)	Organic content (% of DW)	Pore water ( $\mu$ M)		
		PO <sub>4</sub> <sup>3-</sup>	NH <sub>4</sub> <sup>+</sup>	DOC
0.6	0.93 $\pm$ 0.22	0.39 $\pm$ 0.11	5.28 $\pm$ 1.22	5.14 $\pm$ 0.53
1.0	2.25 $\pm$ 0.16	0.25 $\pm$ 0.02	2.67 $\pm$ 0.88	2.86 $\pm$ 0.86
1.8	2.47 $\pm$ 0.01	0.27 $\pm$ 0.07	7.16 $\pm$ 2.73	1.86 $\pm$ 1.21
2.8	20.78 $\pm$ 0.86	0.70 $\pm$ 0.15	3.28 $\pm$ 0.04	0.43 $\pm$ 0.07
4.6	23.51 $\pm$ 1.45	1.00 $\pm$ 0.09	7.36 $\pm$ 1.29	0.18 $\pm$ 0.05

(Fig. 6A). We only analyzed rosette density for the four *L. uniflora* stations because of possible plant-interspecific differences; however, correlation analysis between rosette density at all five stations and root ergosterol also results in a significant positive relationship ( $P < 0.05$ ). In addition, there is a significant positive relationship ( $P < 0.05$ ) between the log-ergosterol index and the log-redox potential for all five stations (Fig. 6B). Furthermore, there was a negative relationship ( $r = -0.84$ ;  $P = 0.07$ ) between the log-transformed sediment organic content (see Table 1 for values) and the log-ergosterol index, although it was not statistically significant. There was no significant correlation between above- or belowground biomass with the ergosterol index.

Rhizosphere porewater variables for each station are reported in Table 1, and root ergosterol was significantly ( $P < 0.05$ ) correlated with porewater phosphate ( $r = -0.93$ , Table 2). Porewater DOC (Table 2) showed a positive correlation with the ergosterol index ( $r = +0.84$ ), but this association was only significant at  $P = 0.08$ . Porewater NH<sub>4</sub><sup>+</sup> showed no relationship with the ergosterol index (Table 2), and NO<sub>3</sub><sup>-</sup> concentrations were below detection ( $<0.7 \mu$ M).

The concentrations of iron-bound phosphate, exchangeable inorganic P, and inorganic P adsorbed onto clay minerals and aluminum oxides were significantly ( $P < 0.05$ ) and negatively correlated with the ergosterol index (Table 2, Fig. 7), but there were no relationships of organic or calcium-bound P fractions. The humic acid phosphorus ( $r = -0.82$ )

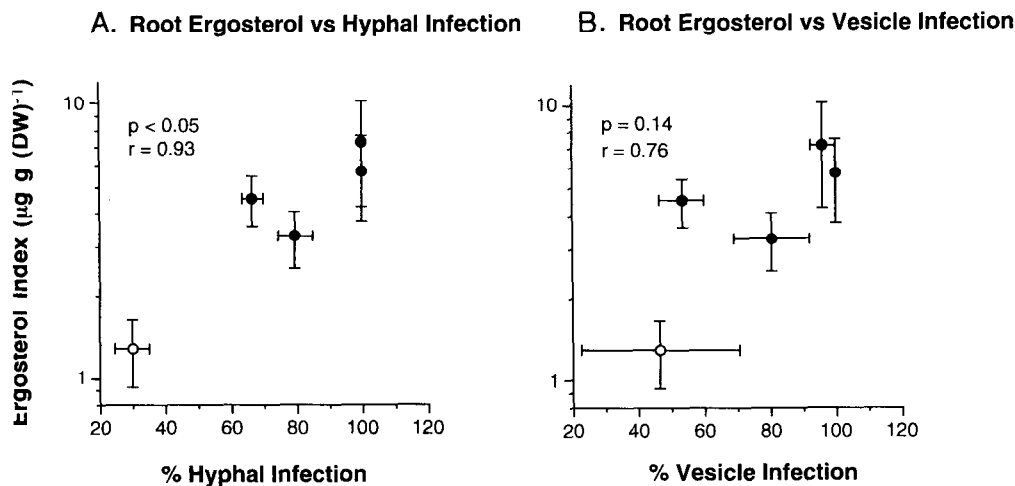


Fig. 5. Relationship of the percentage of hyphal infection (A) and percentage of vesicle infection of isoetids (B) with the root ergosterol index (log-transformed). Each point is an average of three with SE. ●, *L. uniflora*; ○, *I. lacustris*.

and total P ( $r = -0.81$ ) were negatively correlated with ergosterol, but the relationships were not significant ( $P = 0.09$ ; Table 2).

## Discussion

High redox status, low organic content, and low P content are characteristics of sediment that correlate with high abundance of aquatic endomycorrhizae of isoetids. Other researchers have proposed that aquatic endomycorrhizae have an oxygen requirement (Harley and Smith 1983; Tanner and Clayton 1985), and the positive and significant correlation between the root ergosterol index and redox potential in this study supports this hypothesis. In oligotrophic lakes vegetated with isoetids, the oxidation–reduction status reflects

root oxygen release and sediment characteristics (Wium-Andersen and Andersen 1972; Andersen and Olsen 1994). Elevated redox potential associated with the broad and extensive root systems associated with isoetids is common for sediment of low organic content (Wium-Andersen and Andersen 1972; Christensen and Andersen 1996). In contrast, sediment with high organic content consumes oxygen, lowers the oxidation–reduction status, and exhibits elevated porewater phosphate levels. Under these environmental conditions mycorrhizal infection is low.

Our findings from the deepest station (4.6 m) vegetated with *I. lacustris* suggest that mycorrhizal associations may be disrupted by reduced, organic-rich sediments, resulting in small vesicles unattached to hyphae or in the disappearance of root fungi. In addition, at the deepest station plants may

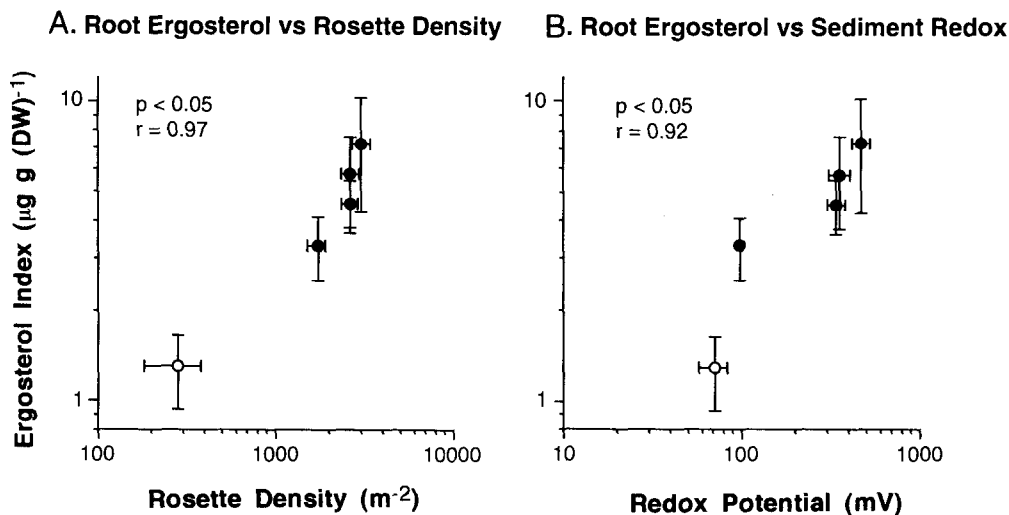


Fig. 6. The relationship of log-ergosterol index ( $n = 3$  with SE) with (A) the log-rosette density ( $n = 3$  with SE) for *L. uniflora* at four stations and (B) log-redox potential ( $n = 4$  with SE) for all five stations. In panel A, *I. lacustris* was added to the figure as a reference point. ●, *L. uniflora*; ○, *I. lacustris*.

Table 2. Correlations between the log-ergosterol index, log-porewater variables, and solid-phase phosphorus for isoetid stations ( $n = 5$ ) (IP, inorganic phosphorus).

Relationship with (log) ergosterol	$r$	Probability
Porewater (log)		
Phosphate	-0.93	*
Ammonium	-0.59	0.30
DOC	+0.84	0.08
Solid-phase		
Exchangeable IP	-0.88	*
IP bound to oxidized forms of iron and manganese	-0.92	*
Organic P	-0.47	0.43
Calcium-bound IP	+0.29	0.64
IP adsorbed onto clay minerals and aluminum oxides	-0.91	*
IP bound in humic acids	-0.82	0.09
Total P	-0.81	0.09

\*  $P < 0.05$ .

be light limited, resulting in reduced photosynthesis and oxygen translocation. Søndergaard and Laegaard (1977) found no infection in *I. lacustris* during their survey of Lake Kalgaard in the mid-1970s, which is in contrast to our findings, although we only found low infection rates in this species. In *L. uniflora* we observed a general decline in root fungal infection with depth, as did Clayton and Bagyaraj (1984). In addition, our results suggest that there may be interspecific differences in fungal infection rates between isoetid species. At depths of 2.8 and 4.6 m, sediment redox status and organic content were similar, but *I. lacustris* showed less infection than did *L. uniflora*. However, the two species did not coexist at the stations. Miller and Sharitz (1996) reported interspecific differences in mycorrhizal infection rates when examining wetland species under similar sediment conditions.

Among the mechanisms suggested for P acquisition by submersed macrophytes, fungal mediation is often overlooked and is not well studied (Barko et al. 1991). Because of high mycorrhizal infection levels in isoetids of oligotrophic lakes with low P concentrations, fungal mediation may be a major mechanism of phosphate assimilation (Søndergaard and Laegaard 1977; Pedersen et al. 1995). We estimated that *L. uniflora* in Lake Kalgaard could turnover the available P in the pore water in a few days (Christensen et al. 1998); however, there was an abundant P resource in the solid-phase fractions. Mycorrhizae of isoetids may acquire P from solid-phase fractions usually unavailable to the plant roots. It has been suggested in terrestrial studies that one mechanism by which mycorrhizal fungi improve P availability to plant roots is by solubilizing inorganic forms of P by release of organic acids (Bolan 1991). This may be one of the reasons for the significant negative correlations between inorganic P bound to oxidized form of iron and manganese, exchangeable inorganic P, inorganic P adsorbed onto clay and aluminum oxides, and root ergosterol in this study. The submersed macrophyte *Vallisneria americana* has high mycorrhizal infection that appears to increase P uptake by

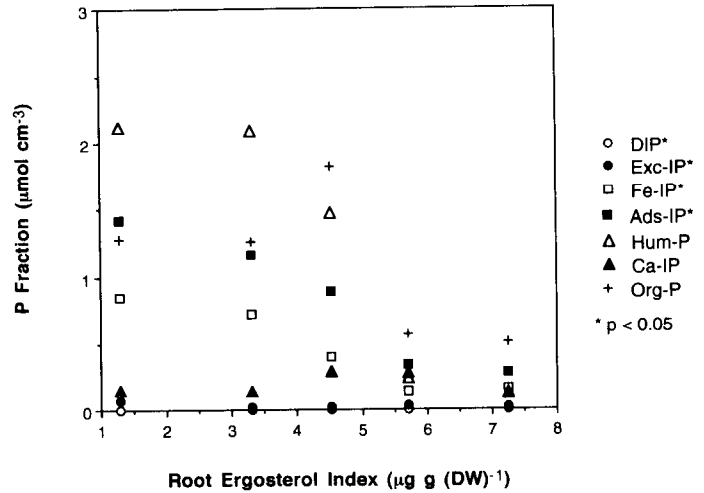


Fig. 7. The relationship of the ergosterol index ( $n = 3$  with SE) with the sediment DIP and solid-phase phosphorus pools for all five stations. Correlation analyses are shown in Table 2.

over 75% in the upper Chesapeake Bay where porewater DIP can be limiting (Wigand and Stevenson 1997). In contrast, in the upper Chesapeake Bay there appears to be no significant mycorrhizal effect on  $\text{NH}_4^+$  uptake (Wigand and Stevenson 1997), and in Lake Kalgaard, we found no significant relationship between mycorrhizae and  $\text{NH}_4^+$ .

Besides promoting a hospitable oxygenated environment in the roots, the isoetids most likely transfer carbon to endomycorrhizal fungi. Although our correlation analyses show only a trend ( $P = 0.08$ ) between DOC and the ergosterol index, we suggest that DOC release by mycorrhizal isoetids may be an important mechanism for supporting fungal symbionts. At the three shallower stations acetate was a common constituent (48, 77, and 54%, respectively) of the DOC pool, whereas at the two deeper stations acetate was not detectable (Holmer unpubl. data). Research with aquatic rooted macrophytes showed release of ethanol from roots into the rhizosphere (Smith et al. 1988; Smits et al. 1990), and metabolic pathways of some aquatic plants may convert ethanol into organic acids such as acetate before release into the rhizosphere. Alternatively, DOC (e.g. organic acids) may also be released from the mycorrhizal fungi to the sediment (Bolan 1991). The fact that the highest DOC concentration was found at the stations with the lowest content of organic matter supports the hypothesis that the DOC was released either from the mycorrhizal fungi or the plant roots. However, sulphate reduction rates at the deeper stations were significantly greater than at the shallow stations and may also account for low DOC concentrations at the deeper stations (Holmer unpubl. data). Therefore, further studies to evaluate carbon cycling in endomycorrhizal associations of isoetids are necessary.

Because the ergosterol index significantly correlated with hyphal infection of isoetids, this biochemical method, which is easy to conduct, should facilitate surveys of endomycorrhizae in oligotrophic lakes. In addition, the ergosterol assay may be a better indicator of living fungal biomass than total fungal hyphal length (Stahl and Parkin 1996), and may

therefore provide a better correlate with environmental variables in the system. Although we found low levels of ergosterol for mycorrhizal isoetids using standard methods, the ergosterol concentrations were not unlike the levels reported for some terrestrial vesicular-arbuscular mycorrhizae using similar methods ( $11 \mu\text{g g (DW)}^{-1}$  for Red Clover; Frey et al. 1994). We are refining the ergosterol-extraction method for submersed macrophytes to increase extraction efficiency by modifying or eliminating saponification and secondary pentane extraction.

In terrestrial systems, it is uncertain what edaphic characteristics promote endomycorrhizal fungi, and research is often concerned with reporting plant parameters and not with characterizing the soil (Sylvia and Williams 1992). Similarly, little is known about the sediment characteristics promoting endomycorrhizal fungi in aquatic environments (Khan and Belik 1995), although in this study we show sediment redox status, sediment organic matter, and P content to be correlated with fungal infection in isoetids. The interaction of fungi-vegetation-sediment in aquatic environments is a fertile area for research, and the mechanisms driving these relationships are important for management and restoration of isoetids in lakes. In terrestrial systems mycorrhizae sustain species diversity, facilitate nutrient cycling, promote seedling success, and reduce adverse effects of toxicants (Allen 1991); we propose that endomycorrhizae of submersed macrophytes may provide similar functions in aquatic systems as they do in terrestrial ones.

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