Mycorrhiza (1998) 7:255–260

ORIGINAL PAPER

J. M. Sharples · J. W. G. Cairney

Assimilation of inorganic nitrogen by a mycobiont isolated from *Pisonia grandis* R. Br. (Nyctaginaceae) mycorrhiza

Accepted: 16 October 1997

Abstract Single isolates of a mycobiont isolated from *Pisonia grandis* R. Br., *Pisolithus tinctorius* (Pers.) Coker & Couch and *Tylospora fibrillosa* (Burt.) Donk were compared with regard to their relative abilities to produce key enzymes of inorganic nitrogen assimilation. Nitrate reductase (NR) activities in the *P. grandis* mycobiont and *T. fibrillosa* were significantly lower than in *P. tinctorius*. While specific activities for glutamate dehydrogenase (GDH) were higher in *P. tinctorius* than the other two fungi following NH_4^+ pre-treatment, glutamine synthetase (GS) activity did not differ significantly between the three fungi. In all three fungi, specific activities for GS were significantly higher than for GDH. NR activity was expressed in all three fungi regardless of the nitrogen source in the medium, but in *P. tinctorius* diminished following continued exposure to either NO_3^-, NH_4^+, glutamine or NO_3^- + glutamine. The data are discussed in relation to nitrogen utilisation by the *P. grandis* mycobiont.

Key words Nitrate reductase · Glutamate dehydrogenase · Glutamine synthetase · Ectomycorrhiza · Pisoniod mycorrhiza

Introduction

Pisonia grandis R. Br. (Nyctaginaceae) is a widespread tree species on coral cays throughout the Western Indian and Eastern Pacific oceans (St John 1951; Airy Shaw 1952; Stemmerick 1964; Hunt 1967). Short lateral roots of *P. grandis* support a distinctive mycorrhizal association wherein the fungus surrounds the root forming a mycelial sheath, but only occasionally penetrates between root cortical cells to form a Hartig net (Ashford and Allaway 1982, 1985; Allaway et al. 1985; Ashford et al. 1988). Rather, the symbiosis is characterised by transfer-cell-like ingrowths on the host epidermal cell walls that abut the fungus. The wall ingrowths are regarded as increasing the surface area for nutrient exchange between the partners, so constituting an alternative to the Hartig net formation characteristic of ectomycorrhizal (ECM) associations (Ashford and Allaway 1982, 1985).

The existence of a sheathing mycorrhizal association on roots of *P. grandis* in natural stands represents something of a paradox. Since *P. grandis* is the preferred nesting and roosting site for large colonies of seabirds, nutrient inputs to soil under *P. grandis* in the form of bird guano can be high (of the order of 103 g m^{-2} $year^{-1}$ for nitrogen and 22 g m^{-2} $year^{-1}$ for phosphorus) (Allaway and Ashford 1984). Such high nutrient inputs appear to run contrary to accepted wisdom that mycorrhizal associations are of primary importance under conditions of low nitrogen and phosphorus availability (eg. Read 1991). While much of the deposited phosphorus appears to be retained in the *Pisonia* forest soils in the form of calcium phosphate (Fosberg 1957; Allaway and Ashford 1984), most of the nitrogen is likely to be rapidly leached from the freely draining soil by the high annual rainfall characteristic of coral cay environments (Heatwole et al. 1981; Ashford and Allaway 1982; Allaway and Ashford 1984). It has thus been proposed that the *Pisonia* mycorrhizal association benefits the host by rapidly absorbing nitrogen, along with other mobile elements such as potassium, from soil prior to their leaching to the ocean (Ashford and Allaway 1982, 1985; Allaway and Ashford 1984).

Most of the nitrogen deposited in avian guano is in the form of uric acid, but ammonia and urea can be present in significant quantities (Schmidt-Nielsen 1972). A recent study by Sharples and Cairney (1997) indicates that a mycobiont isolated from a *P. grandis* mycorrhiza (Cairney et al. 1994), while unable to utilise uric acid for growth, can readily utilise products of uric acid degradation, such as allantoin and urea. It has thus

J. M. Sharples · J. W. G. Cairney (✉)
Mycorrhiza Research Group, School of Science,
University of Western Sydney (Nepean), P.O. Box 10,
Kingswood, NSW 2747, Australia
Fax: +61-2-9685-9516; e-mail: j.cairney@nepean.uws.edu.au

been suggested that the *P. grandis* mycobiont is important in scavenging the transiently available products of uric acid breakdown by the soil microflora prior to their leaching from coral cay soils (Sharples and Cairney 1997). The ability of the *P. grandis* mycobiont to utilise inorganic nitrogen in the form of either NH_4^+ or NO_3^- was also shown to be poor relative to its ability to utilise a range of organic nitrogen sources (Sharples and Cairney 1997). This may reflect either a deficiency in the ability to absorb or to metabolise inorganic nitrogen compounds. In this paper, we report the results of a comparative investigation of the production of the key enzymes of inorganic nitrogen metabolism by the *P. grandis* mycobiont relative and two ECM fungi during in vitro growth on a variety of nitrogen sources.

Materials and methods

Culture maintenance and growth

The unidentified mycobiont isolated from *P. grandis* R. Br. roots (Cairney et al. 1994) is held by the following culture collections: University of New South Wales (UNSW 014), University of Western Sydney, Nepean (UWSN PIG), American Type Culture Collection (ATCC 200485), Virginia Tech. (VT 3327). It is routinely maintained on half-strength potato dextrose agar (Oxoid). Single isolates of *Pisolithus tinctorius* (Pers.) Coker & Couch and *Tylospora fibrillosa* (Burt.) Donk were maintained on modified Melin-Norkrans agar medium (Marx and Bryan 1975). For all experiments, two circular plugs of the fungi (6.0 mm diameter) were cut from the leading edge of colonies on agar medium and inoculated into 9-cm Petri dishes containing 25 ml liquid medium. The liquid medium for all experiments was the basal medium described by Sharples and Cairney (1997) with 1.0 mM urea as the nitrogen source.

Nitrate reductase assays

Nitrate reductase (NR) (EC 6.3.1.2) activities were measured using a modification of the method of Ho (1987). Following incubation in the various nitrogen sources, mycelial mats were washed sequentially in distilled H_2O and 80 mM potassium phosphate buffer (pH 7.5) and transferred to tubes containing the same buffer, along with 1.0 M KNO_3 and 1.0 ml 0.1 M succinic acid (pH adjusted to 7.0 using 0.5 M NaOH). Tubes containing mycelia were infiltrated under vacuum at 30 °C for 4 h, after which mycelia were removed by filtration and fungal biomass determined gravimetrically following drying at 80 °C. The concentration of NO_2^- in filtrates was determined by measuring absorbance at 540 nm (Shimadzu UV-1201 spectrophotometer) following the addition of 2.5 ml 1% (w/v) sulfanilamide in 3.0 M HCl and 2.5 ml 0.02% (w/v) N-(1-napthyl)-ethylene diamine dihydrochloride, with an incubation period of 20 min at ambient temperature. The influence of external nitrogen source on NR activity was determined by transferring mycelia from liquid media to basal medium without nitrogen for 24 h, followed by 1–48 h incubation in basal medium containing either 5.0 mM $(NH_4)_2HPO_4$, 6.0 mM KNO_3, 5.0 mM glutamine or 6.0 mM KNO_3 and 5.0 mM glutamine. All treatments were replicated in triplicate for each fungus.

Glutamate dehydrogenase and glutamine synthetase assays

For NADP-specific glutamate dehydrogenase (GDH) (EC 1.4.1.4) and glutamine synthetase (GS) (EC 6.3.1.2) assays, mycelia were ground with cold 0.1 M Tris-HCl (pH 8.0), containing 5.0 mM $MgCl_2$, 10% (v/v) glycerol, 2.0% (w/v) polyvinylpyrrolidone, 5.0 mM Na-EDTA and 14 mM 2-mercaptoethanol. Debris was removed by centrifugation at 40 000 *g* for 30 min and supernatants used as a crude extract for enzyme assays. GDH activity was estimated by reductive amination of 2-oxoglutarate (following oxidation of NADPH at 340 nm in a Shimadzu UV-1201 spectrophotometer) as described by Chalot et al. (1991). Specific activities were calculated using the molar extinction coefficient of NADPH (6.2×10^3 mole^{-1}). GS activity was determined as hydroxamate formation (Shapiro and Stadtman 1970) using the protocol outlined by Chalot et al. (1991) in a Shimadzu UV-1201 spectrophotometer at 540 nm. Total protein contents of the crude extracts used for GDH/GS assays were estimated using the Bradford (1976) assay. To determine the influence of external nitrogen source on GDH and GS activities, mycelia were transferred from liquid medium to basal medium without nitrogen for 24 h, followed by 48 h incubation in basal medium containing 25 mM $(NH_4)_2HPO_4$ or KNO_3. All treatments and assays were replicated in triplicate for each fungus.

Data analysis

Data for enzyme assays were analysed by one-way ANOVA, with significant differences determined by Fisher's PLSD test using Statview software (Abacus Concepts).

Results

NR assays

NR activity in the *P. grandis* mycobiont and *T. fibrillosa* was low (< 0.1 and < 0.45 mmol NO_2^- released g^{-1} dry wt. h^{-1}, respectively) and, while mean values for *P. grandis* were consistently lower that for *T. fibrillosa*, in no case were they significantly different at the 95% level (Fig. 1). Neither nitrogen source nor duration of pre-incubation had a significant influence on NR activity in the *P. grandis* mycobiont or *T. fibrillosa*. Following $<$ 24-h pre-treatment, NR activities in *P. tinctorius* were significantly higher than for the other two fungi, with the highest activity (5.3 mmol NO_2^- released g^{-1} dry wt. h^{-1}) recorded after a 2-h incubation in NO_3^- + glutamine (Fig. 1). In all nitrogen pre-treatments, there was a progressive decline in NR activity in *P. tinctorius* with increasing length of pre-treatment. After a 48-h pre-treatment, NR activity for *P. tinctorius* was very low, there being no significant difference between the three fungi regardless of the nitrogen pre-treatment (Fig. 1).

GDH and GS assays

GDH activity was detected in the *P. grandis* mycobiont and both ECM fungi following incubation in NO_3^- medium for 48 h. The specific activity for GDH following NO_3^- treatment was significantly higher in *P. tinctorius* than in the *P. grandis* mycobiont or *T. fibrillosa* at all concentrations (Fig. 2). Pre-treatment with NH_4^+ repressed GDH activity in the *P. grandis* mycobiont and *P. tinctorius*, but did not significantly influence the spe-

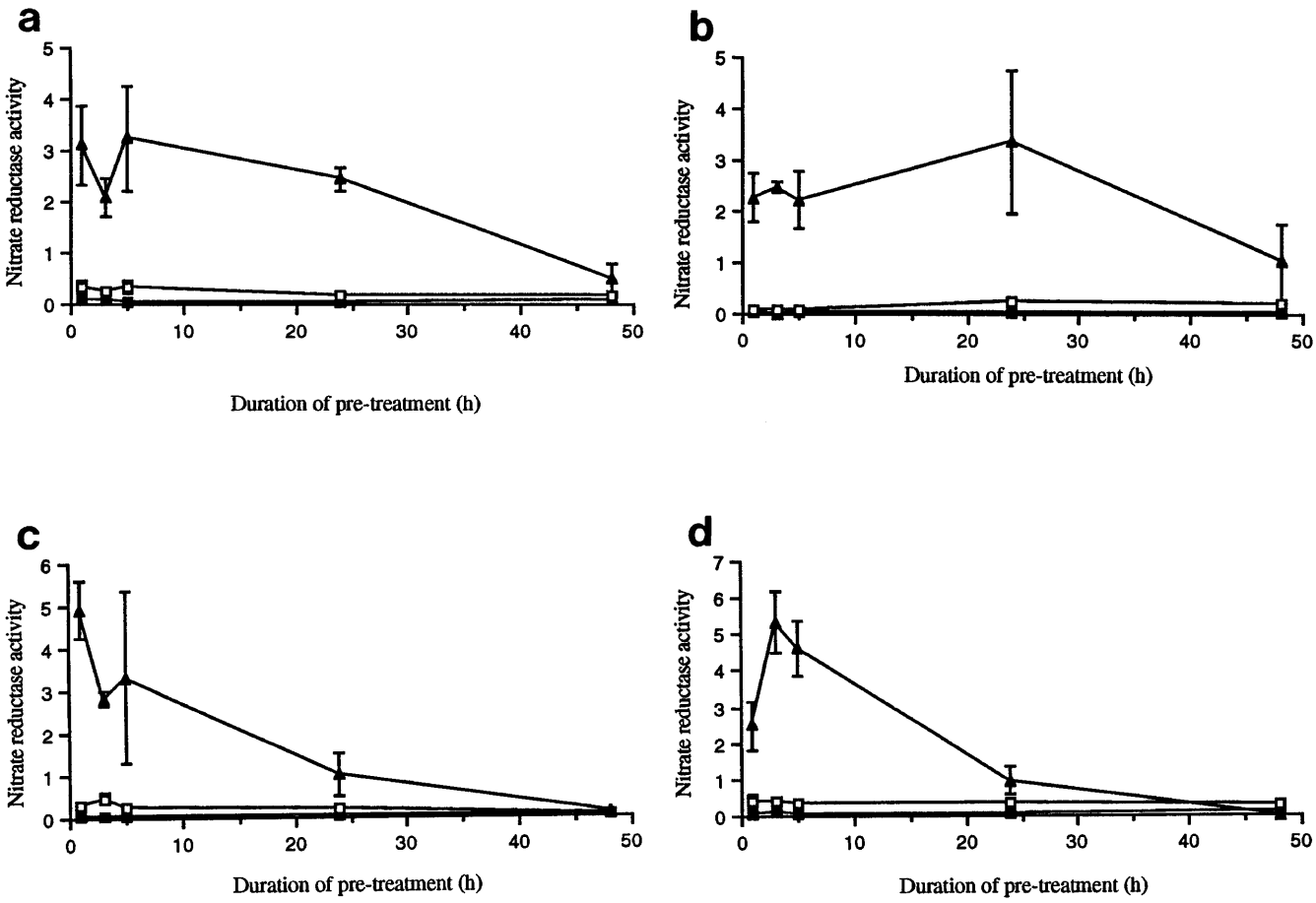

Fig. 1 Mean nitrate reductase activities (μmol NO_2^- released g^{-1} dry wt. h^{-1}) for a *P. grandis* mycobiont (■), *P. tinctorius* (▲) and *T. fibrillosa* (□) following pre-incubation in **a** NO_3^-, **b** NH_4^+, **c** glutamine, **d** NO_3^- + glutamine; bars ± se

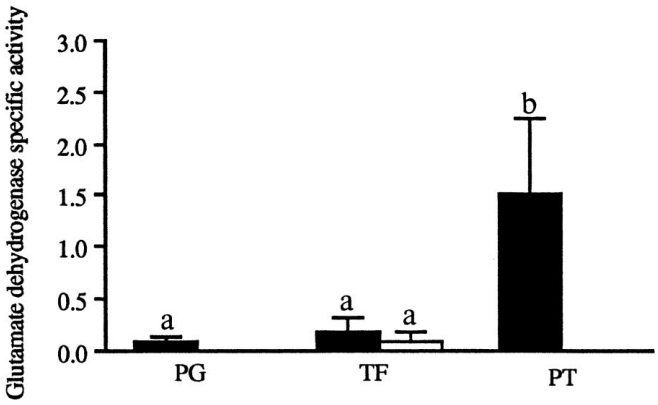

Fig. 2 Mean specific activities for glutamate dehydrogenase (nkat mg $protein^{-1}$ ± se in a *P. grandis* mycobiont (*PG*), *P. tinctorius* (*PT*) and *T. fibrillosa* (*TF*) following pre-incubation in NO_3^- (■) or NH_4^+ (□). Different letters above bars indicate significant differences ($P < 0.05$) between specific activities

cific activity in *T. fibrillosa* relative to NO_3^- pre-treatment (Fig. 2). Specific activities for GDH did not differ significantly between *P. grandis* and *T. fibrillosa* or following NO_3^- or NH_4^+ pre-treatment.

The *P. grandis* mycobiont and both ECM fungi had measurable GS activity following incubation in either NO_3^- or NH_4^+ medium for 48 h. In NO_3^- treated mycelia, specific activities for GS did not differ significantly between *P. grandis*, *P. tinctorius* or *T. fibrillosa* (Fig. 3). NH_4^+ pre-treatment significantly reduced GS activity in the *P. grandis* mycobiont, but resulted in a significant increase in *P. tinctorius* relative to mycelia pre-treated with NO_3^-. GS activity was not significantly influenced by nitrogen pre-treatment in *T. fibrillosa* (Fig. 3). Specific activities for GS were considerably higher than for GDH in the *P. grandis* mycobiont and the two ECM fungi (Figs. 2, 3).

Discussion

Investigations of the utilisation of inorganic nitrogen for growth by ECM fungi indicate marked interspecific variation. While some ECM fungi show a preference for growth on NH_4^+, some grow equally well on both NH_4^+ and NO_3^-, and others produce significantly greater yields on NO_3^- (France and Reid 1984; Finlay et al. 1992). Although there appears to be a general correlation between the preferred source of inorganic nitrogen and the predicted degree of nitrification in the

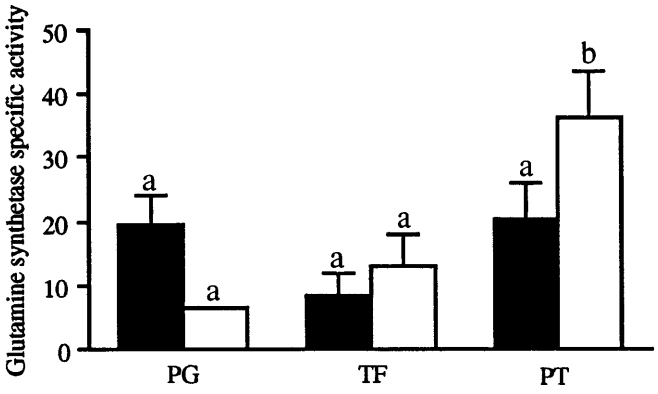

Fig. 3 Mean specific activities for glutamine synthetase (nkat mg protein^{-1} ± se in a *P. grandis* mycobiont (*PG*), *P. tinctorius* (*PT*) and *T. fibrillosa* (*TF*) following pre-incubation in NO_3^- (■) or NH_4^+ (□). Different letters above bars indicate significant differences ($P < 0.05$) between specific activities

soils from which particular ECM fungi originate, it is blurred by the considerable intraspecific variation observed in some instances (see Botton and Chalot 1995). Sharples and Cairney (1997) reported that the mycobiont isolated from *P. grandis* utilises inorganic nitrogen relatively poorly; however, it was not clear to what extent this reflected an inability to absorb or assimilate NO_3^- and NH_4^+.

NH_4^+ absorption by fungi is thought to occur via a carrier-mediated uniport mechanism with NO_3^- transport probably operating via an electrogenic NO_3^-/H^+ symport (Jennings 1995). The relatively low rates of growth of the *P. grandis* mycobiont on inorganic nitrogen sources might reflect a deficiency in its ability to synthesise the trans-membrane carriers, but may equally reflect feedback effects from deficiencies in assimilation of absorbed nitrogen. In the latter context, it has been shown, for example, that intracellular NR activity is positively linked to regulation of NO_3^- transporter genes in some fungi and green algae (Fu and Marzluf 1990; Galvan et al. 1992). In the case of NH_4^+, deficiencies in organic acid synthesis may limit the availability of α-ketoglutarate as a substrate to GDH (Scheromm et al. 1990a), preventing NH_4^+ incorporation and reducing absorption (Plassard et al. 1991). NR activities reported here for *P. tinctorius* are of a similar order to those reported previously for different isolates of the fungus (Ho 1987). Activities for *T. fibrillosa* and, in particular, the *P. grandis* mycobiont were somewhat lower than NR activities reported by several workers for a range of ECM fungi (Ho and Trappe 1980, 1987; Sarjala 1990; Plassard et al. 1991), suggesting that both fungi are relatively deficient in their ability to produce the enzyme. NR in ECM fungi appears to be constitutive in most basidiomycetes (Sarjala 1990; Scheromm et al. 1990b), but requires de-repression in the ECM ascomycete *Wilcoxina mikolae* (Prabhu et al. 1996). Given that NR activity was measurable in all three fungi (albeit at very low activity in the *P. grandis* mycobiont and *T. fibrillosa*) in the present study, regardless of the nitrogen pre-treatment, the activity is presumed to be constitutive. In this respect, the *P. grandis* mycobiont appears similar to other ECM basidiomycetes.

In general, growth rates of ECM fungi in media containing NO_3^- are closely related to the NR activities and NR production is frequently cited as the major rate-limiting step in NO_3^- utilisation (Botton and Chalot 1995). This is in keeping with the relationship between NR activity and NO_3^- transporter genes already demonstrated in some non-mycorrhizal fungi (see above). Repression of NR activity in *P. tinctorius* with time following exposure to all four nitrogen pre-treatments is at odds with the situation in the ECM basidiomycete *Hebeloma cylindrosporum*, wherein no repression was observed during prolonged exposure to either NO_3^- or NH_4^+ (Scheromm et al. 1990b). In non-mycorrhizal and ECM ascomycetes, however, there is good evidence that glutamine (the end-product of NH_4^+ assimilation) represses NR activity (Premakumar et al. 1979; Prabhu et al. 1996). The data presented here suggest a similar pattern of NR repression in *P. tinctorius*. Indeed, the isolate of *P. tinctorius* used in the present work utilises NO_3^- poorly relative to NH_4^+ (JM Sharples and JWG Cairney, unpublished data).

NH_4^+ assimilation in ECM fungi occurs via the GDH pathway or the GS/glutamate synthase (GOGAT) pathways (Botton and Chalot 1995). The presence of both GDH and GS in mycelium of the *P. grandis* mycobiont indicates an ability to assimilate NH_4^+. While we have yet to investigate the ability of the *P. grandis* mycobiont to produce GOGAT, this suggests that the low ability of the fungus to utilise NH_4^+ for growth (Sharples and Cairney 1997) results from an inability of the fungus to transport the ion across the plasmamembrane. It cannot be discounted at this stage, however, that deficiencies in organic acid synthesis also limit substrate availability for GDH, preventing NH_4^+ incorporation assimilation via this pathway and so reducing absorption (see above). Some ECM fungi can utilise either the GDH or the GS/GOGAT pathway for NH_4^+ assimilation (e.g. Ahmad et al. 1990; Chalot et al. 1991; Brun et al. 1992); however, their relative contributions and regulation remain the subject of debate. For some ECM basidiomycetes, GDH may be important in the initial assimilation of NH_4^+ (Chalot et al. 1991), while in others, including *P. tinctorius*, there is strong evidence for the GS/GOGAT cycle being the major route of NH_4^+ assimilation (Kershaw and Stewart 1992; Martin et al. 1994; Turnbull et al. 1996). The higher specific activities of GS relative to GDH reported in the present study appear to confirm previous findings for *P. tinctorius* and argue for the importance of the GS/GOGAT cycle in both the *P. grandis* mycobiont and *T. fibrillosa*. When mycelia were pre-treated with NO_3^-, there was no significant difference between specific activities of GS, indicating that the *P. grandis* mycobiont has the same ability to produce this enzyme as the two ECM fungi.

For all three fungi, there appeared to be a derepression of GDH activity when NO_3^- (as opposed to NH_4^+) was the nitrogen source in the external medium, although the effect was only significant at the 0.05 level in *P. tinctorius*. This supports the hypothesis for down-regulation of GDH in ECM fungi at high concentrations of exogenous NH_4^+ (Martin et al. 1994; Lorillou et al. 1996). Likewise, the decrease in GS activity in the *P. grandis* mycobiont in the presence of exogenous NH_4^+ parallels the decrease observed in the ECM basidiomycetes *Laccaria bicolor* and *Hebeloma cylindrosporum* under similar conditions (Ahmad et al. 1990; Chalot et al. 1991). By contrast, exogenous NH_4^+ stimulated GS activity in *P. tinctorius*, a phenomenon previously reported in an ECM ascomycete (Martin et al. 1988).

Only single isolates of each fungus were compared in the present study and intraspecific differences are known to occur in patterns of inorganic nitrogen utilisation and assimilation (France and Reid 1984; Botton and Chalot 1995). To date, however, only one isolate of the *P. grandis* mycobiont has been successfully isolated (Cairney et al. 1994), precluding the use of multiple isolates in current investigations. Nonetheless, our data suggest that the relatively poor utilisation of NO_3^- by the *P. grandis* mycobiont (Sharples and Cairney 1997) reflects an inability of the fungus to produce NR. Poor utilisation of NH_4^+, however, does not appear to be correlated with a deficiency in the enzymes required for initial NH_4^+ assimilation. Furthermore, the patterns of assimilation of both NO_3^- and NH_4^+ were broadly similar in *T. fibrillosa*. Both the *P. grandis* mycobiont and *T. fibrillosa* occur naturally in soils which, for different reasons, contain little nitrogen in the form of either NO_3^- or NH_4^+. Large inputs of organic nitrogen coupled with high rates of leaching from the coral cay soils inhabited by *P. grandis* (Sharples and Cairney 1997) and low rates of ammonification and nitrification in upland peat soils where *T. fibrillosa* is often the dominant ECM symbiont (Taylor and Alexander 1991; Erland et al. 1994), ensure that nitrogen mainly exists as organic forms in both environments. Our data thus suggest adaptation of the *P. grandis* mycobiont to nitrogen acquisition in such environments and support the notion of Sharples and Cairney (1997) that the *P. grandis* mycobiont plays a major role in the cycling of organic nitrogen in coral cay soils.

References

Ahmad I, Carleton TJ, Malloch DW, Hellebust JA (1990) Nitrogen metabolism in the ectomycorrhizal fungus *Laccaria bicolor* (R. Mre.) Orton. New Phytol 116:431–441

Airy Shaw HK (1952) On the distribution of *Pisonia grandis* R. Br. (Nyctaginaceae) with special reference to Malaysia. Kew Bull 7:87–97

Allaway WG, Ashford AE (1984) Nutrient input by seabirds to the forest on a coral island of the Great Barrier Reef. Mar Ecol Prog Ser 19:297–298

Allaway WG, Carpenter JL, Ashford AE (1985) Amplification of inter-symbiont surface by epidermal transfer cells in the *Pisonia* mycorrhiza. Protoplasma 128:227–231

Ashford AE, Allaway WG (1982) A sheathing mycorrhiza on *Pisonia grandis* R. Br. (Nyctaginaceae) with development of transfer cells rather than a Hartig net. New Phytol 90:511–519

Ashford AE, Allaway WG (1985) Transfer cells and Hartig net in the root epidermis of the sheathing mycorrhiza of *Pisonia grandis* R. Br. from Seychelles. New Phytol 100:595–612

Ashford AE, Peterson CA, Carpenter JL, Cairney JWG, Allaway WG (1988) Structure and permeability of the fungal sheath in the *Pisonia* mycorrhiza. Protoplasma 147:149–153

Botton B, Chalot M (1995) Nitrogen assimilation: enzymology in ectomycorrhizas. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin, pp 325–363

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal Biochem 72:248–254

Brun A, Chalot M, Botton B, Martin F (1992) Purification and characterisation of glutamine synthetase and NADP-glutamate dehydrogenase from the ectomycorrhizal fungus *Laccaria laccata*. Plant Physiol 99:938–944

Cairney JWG, Rees BJ, Allaway WG, Ashford AE (1994) A basidiomycete isolated from a *Pisonia* mycorrhiza forms sheathing mycorrhizas with transfer cells on *Pisonia grandis* R. Br. New Phytol 126:91–98

Chalot M, Brun A, Debaud JC, Botton B (1991) Ammonium-assimilating enzymes and their regulation in wild and NADP-glutamate-dehydrogenase-deficient strains of the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Physiol Plant 83:122–128

Erland S, Henrion B, Martin F, Glover LA, Alexander IJ (1994) Identification of the ectomycorrhizal basidiomycete *Tylospora fibrillosa* Donk by RFLP analysis of the PCR-amplified ITS and IGS regions of ribosomal DNA. New Phytol 126:525–532

Finlay RD, Frostegård Å, Sonnerfeldt A-M (1992) Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud. New Phytol 120:105–115

Fosberg FR (1957) Description and occurrence of atoll phosphate rock in Micronesia. Am J Sci 255:584–592

France RC, Reid CPP (1984) Pure culture growth of ectomycorrhizal fungi on inorganic nitrogen sources. Microbiol Ecol 10:187–195

Fu Y, Marzluf GA (1990) *nit2*, the major positive-reacting regulatory gene of *Neurospora crassa*, encodes a sequence-specific DNA-binding protein. Proc Natl Acad Sci USA 87:5331–5335

Galvan A, Cardenas J, Fernandez E (1992) Nitrate reductase regulates expression of nitrate uptake and nitrite reductase activities in *Chlamydomonas reinhardtii*. Plant Physiol 98:422–426

Heatwole H, Done T, Cameron E (1981) Community ecology of a coral cay. Junk, The Hague

Ho I (1987) Comparison of eight *Pisolithus tinctorius* isolates for growth rate, enzyme activity, and phytohormone production. Can J For Res 17:31–35

Ho I, Trappe JM (1980) Nitrate reductase activity of nonmycorrhizal Douglas-fir rootlets and of some associated mycorrhizal fungi. Plant Soil 54:395–398

Ho I, Trappe JM (1987) Enzymes and growth substances of *Rhizopogon* species in relation to mycorrhizal hosts and infrageneric taxonomy. Mycologia 79:553–558

Hunt DR (1967) Nyctaginaceae. Kew Bull 21:251

Jennings DH (1995) The physiology of fungal nutrition. Cambridge University Press, Cambridge

Kershaw JL, Stewart GR (1992) Metabolism of ^{15}N-labelled ammonium by the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker & Couch. Mycorrhiza 1:71–77

Lorillou S, Botton B, Martin F (1996) Nitrogen source regulates the biosynthesis of NADP-glutamate dehydrogenase in the ectomycorrhizal basidiomycete *Laccaria bicolor*. New Phytol 132:289–296

Martin F, Stewart GR, Genetet I, Mourot B (1988) The involvement of glutamate dehydrogenase and glutamine synthetase in ammonia assimilation by the rapidly growing ectomycorrhizal ascomycete *Cenococcum geophilum* Fr. New Phytol 110:541–550

Martin F, Côté R, Canet D (1994) NH_4^+ assimilation in the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) Orton, a ^{15}N-NMR study. New Phytol 128:479–485

Marx DH, Bryan WC (1975) Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. For Sci 21:245–254

Plassard C, Scheromm P, Mousain D, Salsac L (1991) Assimilation of mineral nitrogen and ion balance in the two partners of ectomycorrhizal symbiosis: data and hypothesis. Experientia 47:340–349

Prabhu V, Wilcox HE, Boyer GL (1996) Regulation of nitrate reductase in the mycorrhizal ascomycete *Wilcoxina mikolae* var. *mikolae*. Mycol Res 100:333–336

Premakumar R, Sorger GJ, Gooden D (1979) Nitrogen metabolite repression of nitrate reductase in *Neurospora crassa*. J Bacteriol 137:1119–1126

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–390

Sarjala T (1990) Effect of nitrate and ammonium concentration on nitrate reductase activity in five species of mycorrhizal fungi. Physiol Plant 79:65–70

Scheromm P, Plassard C, Salsac L (1990a) Effect of nitrate and ammonium nutrition on the metabolism of the ectomycorrhizal basidiomycete *Hebeloma cylindrosporum* Romagn. New Phytol 114:227–234

Scheromm P, Plassard C, Salsac L (1990b) Regulation of nitrate reductase in the ectomycorrhizal basidiomycete *Hebeloma cylindrosporum* Romagn. cultured on nitrate or ammonium. New Phytol 114:441–447

Schmidt-Nielsen B (1972). Mechanisms of urea excretion by the vertebrate kidney. In: Campbell JW, Goldstein L (eds) Nitrogen metabolism and the environment. Academic, London, pp 79–103

Shapiro BM, Stadtman ER (1970) Glutamine synthetase (*Escherichia coli*). In: Tabord H, White C (eds) Methods in enzymology, vol 17A. Academic, London, pp 910–922

Sharples JM, Cairney JWG (1997) Organic nitrogen utilization by an unidentified mycobiont isolated from mycorrhizas of *Pisonia grandis*. Mycol Res 101:315–318

St John H (1951) The distribution of *Pisonia grandis* (Nyctaginaceae). Pacific plant studies No. 10. Webbia 8:225–228

Stemmerick JF (1964) Florae Malesianae praecursores XXXVIII. Notes on *Pisonia* L. in the old world (Nyctaginaceae). Blumea 12:275–284

Taylor AFS, Alexander IJ (1991) Ectomycorrhizal synthesis with *Tylospora fibrillosa*, a member of the Corticiacea. Mycol Res 95:381–384

Turnbull MH, Goodall R, Stewart GR (1996) Evaluating the contribution of glutamate dehydrogenase and the glutamate synthase cycle to ammonia assimilation by four ectomycorrhizal fungal isolates. Aust J Plant Physiol 23:151–159