

Carbon availability affects nitrogen source utilisation by *Hymenoscyphus ericae*

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Abstract

We compared the ability of five strains of the ericoid mycorrhizal fungus *Hymenoscyphus ericae* to utilise glutamine, ammonium or nitrate at high or low carbon availability. The pattern of intraspecific variation in growth was affected by C availability. When C supply was high, growth differences between strains were explained by the total amount of N taken up, suggesting variation in uptake kinetics. Under C-limiting conditions, strain differences were linked with their nitrogen use efficiency, implying intraspecific differences in N metabolism. The relationship between growth on glutamine and pH shifts in the media indicated that there was intraspecific variation in glutamine transporters. In addition, the correlation between pH changes and the amount of glutamine-N recovered as ammonium in the media indicated that there were intraspecific variations within the enzymatic pathways involved in glutamine metabolism. Our findings, compared with those of a previous study involving the same ericoid strains, draw attention to the temporal variation in nitrogen source utilisation by ericoid mycorrhizal fungi when maintained in axenic culture.

Introduction

Ericoid mycorrhizal (ERM) fungi allow their host plant to access a range of amino acids and complex organic N compounds (Bajwa & Read 1985, 1986, Bending & Read 1996, Kerley & Read 1995, 1997 and 1998). These studies showed unequivocally that ERM fungi, such as *Hymenoscyphus ericae*, have saprophytic capabilities. ERM associations are widespread in harsh habitats both in the northern and southern hemisphere, including environments that are contaminated by toxic metals (Cairney and Meharg, 2003) and acidic soils rich in recalcitrant organic matter where mineralisation is slow and availability of mineral N and P is low (Read 1991). The plant root system and associated fungi are abundant in raw humus layers and have been shown to exclude the roots of competing grass (Genney, Alexander & Hartley 2000, 2002). The saprophytic capabilities of ERM fungi facilitate N mobilisation from litter to the plant, contributing to the fitness and dominance of this type of mycorrhizal symbiosis in such habitats (Read & Perez-Moreno 2003). However, many plant communities dominated by ericaceous plants are now under threat because of increased atmospheric N deposition (Krupa 2003, Lee & Caporn 1998, van der Wal *et al.* 2003) and a shift from organic to mineral N regimes (e.g. Yesmin *et al.* 1995). Ericaceous species are threatened even by low rates of N deposition (e.g. Strengbom *et al.* 2003). In these situations, the relative ability of

ERM fungi to utilise mineral N might affect the fitness and composition of ericaceous populations.

The ability to take up mineral or organic N depends on the presence of transport systems and efficient assimilation of the N form taken up. Readily available carbon, such as hexoses, can alter N uptake kinetics of the transport systems of both plant and fungal species (e.g. Javelle *et al.* 1999, Kubbik-Dobosz, Bakiewicz & Gorska 2001, Logan *et al.* 1997, Persson & Nasholm 2003). Carbon availability has also been shown to influence the production of enzymes involved in the mobilisation of protein N by *H. ericae* (Leake & Read 1991). Turnbull, Goodal & Stewart (1995) suggested that the ability of mycorrhizal fungi to take up amino-N versus mineral N would be affected by the amount of readily-available carbon present in the growth medium. The extent to which C can be mobilised to sustain foraging root / hyphal growth and the energetic requirements of both uptake and assimilation may determine the competitiveness of ERM associations in the utilisation of different forms of N. Any perturbation, spatial or temporal, of plant C allocation below-ground may alter the ability of ericoid mycorrhiza to utilise different forms of N.

Ericaceous root systems host multiple ericoid fungal partners (Bergero *et al.* 2000, Chambers, Liu & Cairney 2000, Johansson 2001, Monreal, Berch & Berbee 1999, Peretto *et al.* 1996, Vralstad, Myhre & Schumacher 2002). *Hymenoscyphus ericae*, a mycobiont frequently isolated from ericaceous roots, is now known to consist of a large genetic complex (Vralstad, Schumacher & Taylor 2002). The inter- and intraspecific diversity of fungal partners present, both at the level of the individual plant or the community, may broaden the range of organic substrates exploitable by ERM associations, assuming that this genetic diversity reflects functional diversity (Perotto, Girlanda & Martino 2002). Different species of ERM fungi differ in their capability to hydrolyse complex substrates (e.g. Leake & Read 1990; Perotto *et al.* 1997, Varma & Bonfante 1994). Furthermore, different isolates of *H. ericae* differ in their abilities to utilise organic and mineral sources of N (Cairney *et al.* 2000). However, the extent to which functional diversity in N nutrition is altered by C availability is unknown.

The aim of this research was to determine whether intraspecific variation in the use of different N sources by *H. ericae* is affected by C availability.

Material and methods

Experimental procedures

Five isolates of *H. ericae* were obtained from root systems of *Calluna vulgaris* at a heathland site at Aylesbeare Common, Devon, U.K. during January 1998. The isolates have been deposited at IMI (accession codes: AC1 = IMI 392566, AC2 = IMI 392567, AC14 = IMI 392569, AC21 = IMI 392568 and AC5 = IMI 392570). The five strains showed the same ITS-RFLP patterns as the *H. ericae* isolate Read 101, using the three restriction endonucleases, *Hinf I*, *Rsa I* and *Hae III* (Sharples *et al.* 2000), with 99% sequence similarity between the ITS region of AC21 and that of isolate read 101 (GenBank nucleotide database, accession code AF069505). All five strains formed typical ericoid mycorrhizal structures in epidermal cells of *Vaccinium macrocarpon* in a dual-culture experiment (Sharples *et al.* 2000). They were maintained on modified Melin Norkrans (MMN) agar medium (Marx & Bryan 1975) at 20°C in the dark, with sub-culturing every 6-8 weeks.

A factorial design was used to grow the 5 strains in liquid media containing ammonium, glutamine or nitrate nitrogen at starting concentration of 7.5 mol m^{-3} with a C:N ratio of either 39:1 or 9:1. Discs of inoculum (3 mm diameter) were cut from the leading edge of three actively growing colonies of the same isolate and three discs, one from each colony, were used to inoculate 20 cm^3 liquid medium in 9 cm diameter Petri dishes. The basal medium for all treatments contained KH_2PO_4 , 2.2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.57; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.22; NaCl , 0.43 mol m^{-3} and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 10.43; Thiamine, 0.3; Ferric EDTA (Na salt), $40.86 \text{ nmol m}^{-3}$ (Sigma ultra grade). Media pH was adjusted to pH 5.25 prior to autoclaving.

Nitrogen was supplied as either $(\text{NH}_4)_2\text{HPO}_4$, glutamine or KNO_3 . Carbon was supplied as glucose adjusted to take account of C in the nitrogen sources. All N sources had their pH adjusted to 5.25 and together with glucose, were filter sterilised ($0.2 \mu\text{m}$) prior to addition to the basal media. Two treatments containing basal medium with no added N, but a similar amount of glucose to that supplied to the ammonium treatments with high and low C:N ratio, were included to account for growth on nitrogen derived from the inoculum discs.

Three replicates dishes for each treatment combination (strain, N source and C:N ratio) were kept in the dark at 20°C for 10, 20 or 30 days. Fungal mycelia were then manually removed from the media, rinsed briefly in distilled water, oven-dried for 48 hr at 80°C and weighed. Growth media were also collected, their pH measured immediately, frozen and kept at -20°C . Dried fungal biomass collected at day 30 was analysed for C and N content using a Carlo Erba NA1500 Elemental Analyser (Italy) and these data were used to compute fungal %N, %C, fungal N and C content in each dish, and fungal C:N ratio. The growth media collected at day 30 were analysed for ammonium and nitrate using a Tecator FIAstar 5010 flow injection analyser (Sweden).

Statistical treatment of results

Biomass data were corrected for growth due to nitrogen contained in inoculum disks by subtracting at each harvest the biomass in basal medium with no added nitrogen.

To find out whether the relative growth of each isolate on a particular N-source was affected by C:N ratio, we calculated two growth indices. First, the relative growth of each isolate on each N source at either the high or the low C:N ratio was expressed as the percentage of the maximum dry weight attained on any N source at that C:N ratio. The relative growth (%) at the low C:N ratio was then divided by the corresponding value at the higher C:N ratio, and this ratio transformed to Log_{10} . A positive or negative value of this first index [Log_{10} (relative growth low C/relative growth high C)] indicated relatively greater or less growth by that isolate on that N source at the lower C:N ratio.

A second growth index was calculated as a measure of the relative growth of each isolate on each N source at either the high or the low C:N ratio, expressed as the percentage of the maximum dry weight attained by any isolate on that N source at that C:N ratio. The relative growth (%) at the low C:N ratio was again divided by the corresponding value at the higher C:N ratio, and this ratio transformed to Log_{10} . A positive or negative value of the index, in this case, indicates more or less growth by that isolate relative to the others on that N source at the lower C:N ratio.

All statistical analyses were performed using Genstat® software (Genstat 6 release 6.1, Lawes agricultural trust, IACR – Rothamsted, 2002). Data were transformed (Log_{10} or square-root) as required to ensure homogeneity of variance and normal distribution of the residuals (tested using a χ^2 goodness of fit test).

The effect of the length of time-course, N source and / or media C:N ratio on biomass data, % N recovered in fungal biomass and relative growth indices were tested using two or three-way analysis of variance in randomised blocks. Significant differences between means were assessed using the Fisher PLSD test. The percentage of variation (%V) explained by each factor or combination of factors (F) was calculated as followed:

$$\%V = \text{sum of squares for F} * 100 / \text{total sum of squares.}$$

Linear regression analyses with or without groups were used to test the relationship between media pH and fungal biomass or the amount of ammonium-N left in the media, respectively. Preliminary to each regression analysis, scatter-plots were checked for any particular distribution patterns. XY dot-plots were also checked for the presence of clusters.

The importance of fungal C and N content and concentration, and fungal C:N ratio, in explaining growth differences between strains at the final harvest was assessed for each level of C:N ratio in the medium using canonical variate analysis (CVA). When strains are considered as the grouping factor, CVA gives the percentage of between-strain variation explained by each canonical variate dimension. The relative size of the loadings within each canonical variate dimension corresponds to the relative weight of each variable in explaining the between-strain variation.

Results

The effect of media C:N ratio on biomass increment

Fungal biomass was significantly ($P < 0.001$) affected by the length of time-course, N source and C:N ratio. All interactions between factors were highly significant, except the interaction between time and strain, implying that growth rates were similar for all strains. The model explained 90% of the total variance, 87 % of which was explained by the effects of time and C:N ratio only, implying that the greatest effect on fungal biomass increment over time was that of C:N ratio.

Biomass production averaged across all strains was greater and lasted longer at the higher C:N ratio (Figure 1). The nitrogen initially provided was not completely incorporated into fungal biomass at either C:N ratio (Table 1). Considering that biomass increment had stopped by day 20 at the low C:N ratio, but continued until day 30 under high C:N ratio, the low and high C:N ratio can be taken to correspond to the presence and absence of C limitation of growth, respectively. Consequently, the two levels of media C:N ratio will hereafter be referred to as high or low C.

The effect of C availability on growth variations between strains

At high C availability, all strains except AC5 grew 1.5 to 2.2 more times on ammonium compared to nitrate (Table 2). The biomass achieved with glutamine was of the same order of magnitude as that produced with nitrate-N for isolates AC14, AC2 and AC21. AC1 grew as well on glutamine as on ammonium, and AC5 grew 1.5 times more on glutamine than on either of the mineral N sources (Table 2).

The values of the two growth indices are presented in figure 2, to assess whether the relative growth of each isolate on a particular N-source (figure 2a) and how the relative growth of the 5 isolates on a particular N source (figure 2b) was affected by the C:N ratio of the medium. AC14, AC2 and AC21 increased their relative ability to

grow on both glutamine and nitrate under low C availability, whilst AC1 increased its relative ability to grow only on nitrate (Figure 2a). The relative growth of AC5 on the three forms of N was similar at both high and low C availabilities (Figure 2a). When N was supplied as ammonium, the relative growth of the five strains relative to each other was similar, irrespective of C availability, except for a marginal increase in the growth of AC14 under low C availability (Figure 2b). When N was supplied as glutamine or nitrate, there was an increase in the relative growth of AC14, AC2 and AC21, and a decrease in the relative growth of AC1 and AC5 (figure 2b).

Relationship between biomass increment and pH

A four-way ANOVA showed that only 2% of the variation in media pH was due to differences in fungal strains, with the remaining 98% being due to the effect of N sources and interaction with time. The pH shifted in 20 days from 5.25 to 2.5 ± 0.1 , 7 ± 0.7 and 7.2 ± 0.7 when N was supplied as ammonium, glutamine and nitrate, respectively. After 20 days, no more significant changes were observed in pH in the growth media, irrespective of N source, C availability or fungal strain. There were marked differences between strains in the final pH of the media when N was supplied as nitrate and glutamine, but not when supplied as ammonium (Table 3). Final pH for each strain was the same at both high and low C when N was supplied as ammonium or nitrate (except for AC5 on nitrate). However, when N was supplied as glutamine, final pH depended on C availability (Table 3). The relationship between final pH and fungal biomass varied depending on N source and C availability (Table 4). However it differed significantly between strains only when N was supplied as glutamine, in which case the relationship between pH and biomass was strong only at low C availability (Table 4). When N was supplied as glutamine, increases in pH were correlated with the amount of ammonium-N recovered in the growth media, but only under low C availability ($P < 0.03$, $R^2 = 0.35$, Figure 3).

Differences in C and N content between strains

Figure 4 shows the loadings for the canonical variate dimension which explained 73 and 68% of the between-strain variation when C availability was high and low, respectively.

Under high C availability, variations between strains were explained by the total amount of N incorporated in the biomass (Nmg), and to a lesser extent, the concentration of N in the biomass (N%) (Figure 4). Therefore under high carbon availability, strain differences were linked to their ability to take up and incorporate nitrogen into their biomass. Under low C availability, strain differences were better explained by the contrast between the total amount of C accumulated (Cmg) and the concentration of N in the biomass (Figure 4), which can be interpreted as variations in N use efficiency (number of unit C fixed in biomass per unit N assimilated).

Discussion

Like Cairney *et al.* (2000), we found that strains of *H. ericae* can grow on glutamine, ammonium and nitrate, but that there are intraspecific differences in their growth. Inter- and intra-specific variations in different N and P substrates have also been shown for some strains of Southern hemisphere ERM fungi (Midgley, Chambers and Cairney, 2004).

On mineral N sources, the pH of the growth media changed to a similar extent whether C availability was low or high, despite biomass increment at high C availability being twice that at low C by day 20. Furthermore, the relationship between pH and biomass did not depend on the strain. Hence the shifts in pH of the media did not reflect the growth differences observed between the five strains. Furthermore, despite the well-known effects of pH shift on the amount and rate of N uptake and fungal growth (e.g. Ek *et al.*, 1994), our results definitively show the existence of intraspecific variations in the uptake and metabolism of mineral N independent of pH changes. When N was supplied as glutamine, we found that final media pH and biomass were significantly correlated, and more importantly, that this relationship differed between the five strains. The implications of this observation are discussed below.

Growth and N uptake at high C availability

When N was supplied as glutamine, the pH of the media increased by ≤ 1.65 units. It is interesting to note that, in contrast, a slight acidification of the growth media was reported for another *H. ericae* isolate grown on glutamine in liquid culture at high C availability (Bajwa & Read 1985). The growth conditions used by Bajwa & Read (1985) were very similar to those used in our experiment, except that the initial pH was lower (4.5 instead of 5.25), and N was added to the medium prior to autoclaving (instead of filter sterilisation after autoclaving). The time-course of growth for the isolate used by Bajwa & Read (1985) however differed, as biomass increase had more or less stopped after 10 days at high C availability, whereas the five isolates in our study were still growing after 30 days.

We also found differences between our five isolates in the magnitude of pH increase and growth on glutamine. The relationship between pH increase and fungal biomass also differed significantly between strains. This suggests that the mechanisms of glutamine uptake may vary between isolates. Uptake of glutamine by ectomycorrhizal (ECM) fungi is thought to involve an H^+ symport mechanism with low pH optimum, suggesting specificity for glutamine in its protonated form (Chalot *et al.* 1995, Chalot & Brun 1998). However, Anderson, Chambers and Cairney (2001a) suggested that glutamine uptake by *Pisolithus* isolates might also involve a transport system that can accept glutamine in its anionic form. In the experiment described here, the pH of the media stabilised at values higher than 5.65 (pHi of glutamine) after 20 days of growth at high C availability. At this pH, the majority of the glutamine species are in anionic form. Nevertheless fungal biomass increased by an average of 40% between day 20 and day 30. Although growth in that period may have been partly sustained by the recycling of glutamine -N that had been previously taken up, (see Chalot & Brun, 1998, Brun *et al.*, 1994), it is reasonable to assume that further glutamine uptake occurred after day 20. These results, together with those reported by Bajwa and Read (1985) suggest that glutamine uptake by *H. ericae* can be mediated via several transport systems, binding with either the protonated or anionic form of glutamine, with or without an H^+ symport mechanism.

Canonical variate analysis suggested that, at high C availability, differences in growth between strains were due to the amount of N taken up, irrespective of N source. At high C availability, therefore, variation in growth reflected intraspecific variations in N uptake kinetics, strengthening our interpretation of the pattern of growth and pH variation with glutamine -N. Inter- and intraspecific differences in K_m or V_{max} for uptake of N by ECM fungi have been reported previously (Anderson *et al.* 2001a, Turnbull *et al.* 1995; Wallenda & Read 1999). Sokolovski, Meharg & Maathuis

(2002) found that both the K_m and V_{max} parameters of amino acid uptake by root cells of *C. vulgaris* were affected by *H. ericae* colonization, K_m and V_{max} values for infected cells being intermediary between the values for non-infected cells and fungal cells. Our data show that intraspecific variations in the kinetics of N uptake may also occur in ERM fungi, the consequences of which remain to be explored at the level of the infected plant cell.

Growth and N uptake at low C availability

Under C limiting conditions, canonical variate analysis suggested that differences in growth between strains were due to both carbon uptake and tissue N concentration, which we interpret as an index of N use efficiency. This implies that isolates differed not only in N uptake kinetics but also in N metabolism, the latter being predominant at low C availability.

The increase in pH associated with glutamine utilisation was significantly higher at low compared to high C availability. The increase in pH was positively correlated with the amount of ammonium measured in the growth media, but only at low C availability. The proportion of N initially supplied as glutamine and recovered as ammonia was highly variable, ranging from less than 1% to up to 22%. Tibbett *et al.* (1998) also reported substantial amounts of ammonium -N in the growth media, when *Hebeloma* species were supplied with glutamic acid at low C availability. Under C limiting conditions, an efflux of ammonium from the fungal cell as a result of the deamination of glutamine leading to oxoglutarate (the entrance point to the tricarboxylic acid cycle) would allow the consumption of glutamine-C as a respiratory substrate. Chalot *et al.* (1994) suggested that the degradation of glutamine by *Paxillus involutus* could follow an alternative pathway to the energy-demanding GOGAT pathway, through the activity of glutamine transaminase and ω -amidase. In canine renal cells, a shift in glutamine metabolism from a predominantly anabolic to a catabolic pathway is associated with cellular acidosis (Coates *et al.* 2002). Similar cellular acidosis would explain the rise in pH observed in the low C media in our experiment. The differences observed in the relative performance of the five strains when N was supplied as glutamine at high or low C availability may be explained by the extent to which glutamine species, once in the fungal cell, are shifted from one enzymatic pathway to the other, to be used as either an amino acid precursor or a respiratory substrate.

C availability and the relative growth of the five strains

Our treatments, set up with two different C:N ratios with a constant level of N, manipulated C availability, without growth being limited by the amount of N supplied. Sangtjean & Schmidt (2002) reported no effect of C:N ratio on the growth of eight different isolates of ECM fungi in four different genera (*Amanita*, *Gymnoboletus*, *Lactarius*, and *Russula*) despite similar C and N concentrations. However in their study, the volume of media supplied was 20 times bigger than in our study. Hence, the total pool of C available limited fungal growth in our study, but probably not in any of their C:N ratio treatments. This highlights the fact that fungal growth may not be sensitive to C:N ratio *per se* but to the amount of C available.

We found that C availability altered the relative performance of five strains of *H. ericae* on three different N sources. The main effect of reducing C availability was to increase the relative ability of most strains to grow on glutamine and nitrate, in comparison to ammonium. This increase was only marginally higher ($p < 0.1$) with glutamine compared to nitrate. This suggests that the cost of assimilating C and N

when supplied as a single reduced molecule was not substantially lower than when N was supplied in an oxidised form and in a separate molecule from C. Eaton & Ayres (2002) also found that carbohydrate availability affected the relative performance of different isolates of ECM fungi (*Amanita*, *Cenococcum*, *Inocybe*, *Lactarius*, *Leccinum*, *Piloderma* and *Scleroderma* species). They also reported that the addition of ammonium nitrate to protein-N enhanced fungal growth only when carbohydrate availability was low. This suggests that uptake and assimilation of protein N was energetically more costly than that of mineral N. Whether this applies to amino N is unclear: our results suggest that it is not the case for *H. ericae*. It is also possible that C limitation in our low C treatment might have been such that new fungal growth and respiration were entirely sustained by the recycling of C from older parts of the colony, in which case differences in N uptake and assimilation between the five strains would have been evened out.

Previous studies have shown that increased growth of some isolates of ERM and ECM fungi on a specific amino acid compared to mineral N can not be generalised to other amino acids (e.g. Whittaker & Cairney 2001, Tibbett *et al.* 1998) nor to more complex organic forms of N such as BSA (e.g. Cairney *et al.* 2000). It would be interesting to assess whether isolates showing a consistent preference for organic N, under both limiting and non-limiting carbohydrate supply, have greater saprophytic abilities than others, as this might affect the functioning of the symbiosis.

Temporal variation in nitrogen source utilisation

Four out of five isolates used in our study were also screened by Cairney *et al.* (2000) using similar experimental conditions. However our results differed in certain respects from those reported by Cairney *et al.* (2000). For example, we found that AC5 grew better with glutamine than with any of the mineral N sources, whereas Cairney *et al.* (2000) reported poorer growth for that strain on glutamine compared to ammonium or nitrate. Nevertheless, the relative growth of the four strains on each N source was broadly similar in the two studies. These discrepancies / similarities suggest some temporal plasticity in the ability of these strains to utilise different forms of N. Such plasticity might be inherent and is likely to arise from the period of maintenance in axenic culture separating the two studies. Although the results reported by both studies demonstrate unequivocally that intraspecific variations exist, the indication is that this variation may not be constant over time. Similarly, Anderson *et al.* (2001b) have shown that the ability of 17 isolates of ECM fungi (all from the genera *Pisolithus* and isolated simultaneously) to utilise a range of amino-acids, decreased after a 3-year maintenance period in axenic culture. On the contrary, most of their isolates showed increased ability to utilise protein-N (BSA). The extent of these decrease / increase varied between isolates and substrates but the patterns of relative growth between and within each isolate on all amino N sources, except BSA, were broadly conserved throughout the maintenance period. Changes in gene expression in ECM fungi during maintenance in axenic culture have already been highlighted by Cairney (1999) as a potential problem in comparative studies involving isolates with different maintenance history. Our study is the first report of such temporal variation in the physiological attributes of ERM fungi.

In comparative physiological studies where mycorrhizal strains have been isolated simultaneously and maintained in similar conditions, the next step is to assess the extent to which physiological variations observed in isolation will reflect variations in the physiological attributes of the mycorrhizal symbioses. Ultimately, this will enable

us to validate the relevance of the numerous screening experiments conducted, to ascertain the functional aspect of genetic diversity.

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Tables and Figures

Table 1. The effect of N source and media C:N ratio on the percentage¹ of N supplied at the start of the experiment recovered in fungal biomass after 30 days of liquid axenic culture.

N source	Media C:N ratio	
	High	Low
Ammonium	93 ^a	62 ^b
Glutamine	91 ^a	72 ^a
Nitrate	58 ^b	28 ^c

¹ Data are means of 15 replicates. Different letters indicate that means differ at $P < 0.05$ within each column, as determined by Fisher PLSD. LSD between means is 4.2 at $P < 0.05$.

Table 2. Maximal biomass¹ of 5 different strains of *H. ericae* at high C availability, with ammonium, glutamine or nitrate as N source.

Strain	Nitrogen source		
	Ammonium	Glutamine	Nitrate
AC1	73 ^a	75 ^a	49 ^b
AC14	69 ^a	53 ^b	46 ^b
AC2	98 ^a	53 ^b	50 ^b
AC21	95 ^a	54 ^b	43 ^c
AC5	69 ^b	90 ^a	62 ^b

¹Data are means of 3 replicates, in mg dry matter. Different letters indicate that means differ at $P < 0.05$ within rows, as determined by Fisher PLSD. LSD between means is 9.1 at $P < 0.05$.

Table 3: pH¹ of the medium after 20 days of liquid axenic culture of five strains of *H. ericae* grown with three different sources of N at high or low C availability.

Strain	C availability	N source		
		Ammonium ²	glutamine	Nitrate
AC1	High	2.5 ^a	5.7 ^a	6.3 ^a
	Low	2.7 ^a	7.8 ^d	6.6 ^a
AC14	High	2.3 ^a	6.4 ^b	8.1 ^d
	Low	2.5 ^a	7.2 ^c	8.1 ^d
AC2	High	2.4 ^a	6.9 ^c	7.7 ^{cd}
	Low	2.5 ^a	7.6 ^d	7.4 ^{bc}
AC21	High	2.4 ^a	6.9 ^c	7.5 ^{bc}
	Low	2.4 ^a	8 ^d	7.7 ^{cd}
AC5	High	2.4 ^a	6.3 ^b	6.2 ^a
	Low	2.6 ^a	7.7 ^d	7.1 ^b

¹ data are means of three replicate.

² mean with different letters within each column differ at $P < 0.05$. Least significant difference between means is 0.468.

Table 4. The effect of strain differences ("Strain") on the relationship between pH media ("pH") and fungal biomass ("DM") during 30 days of liquid axenic culture.

C availability ¹	Nitrogen Source	Correlation index	Level of significance ²		
			pH	Strain	pH x Strain
High	Ammonium	-0.83	***	n.s.	n.s.
Low	Ammonium	-0.75	***	n.s.	n.s.
High	Glutamine	0.32	**	***	n.s.
Low	Glutamine	0.86	***	*	n.s.
High	Nitrate	0.31	*	n.s.	n.s.
Low	Nitrate	0.27	ns	n.s.	n.s.

¹ Linear regression analyses were performed for each carbon and nitrogen treatment to restrict the variation to that existing between strains. Note that estimates of intercept for each strain were all significant at $P > 0.01$ when N was supplied as glutamine.

² Levels of significance (*** for $P < 0.001$, ** for $0.001 < P < 0.01$, * for $0.01 < P < 0.05$ and n.s. for $P > 0.05$) are given for the different explanatory components of the regression $DM = pH + Strain + pH \times Strain$.

Figure 1

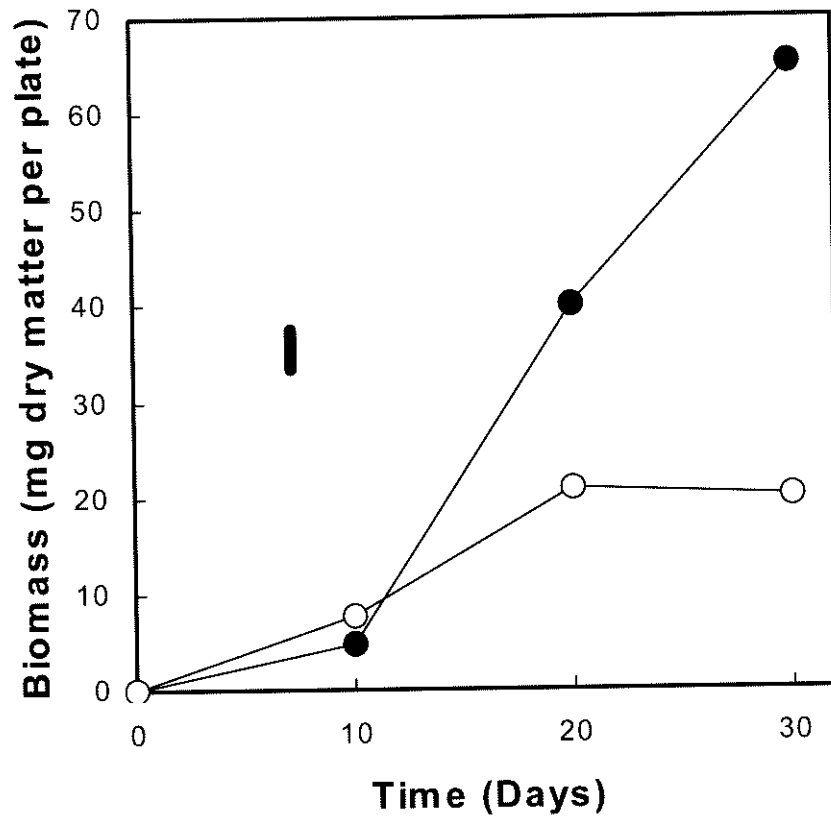


Figure 2

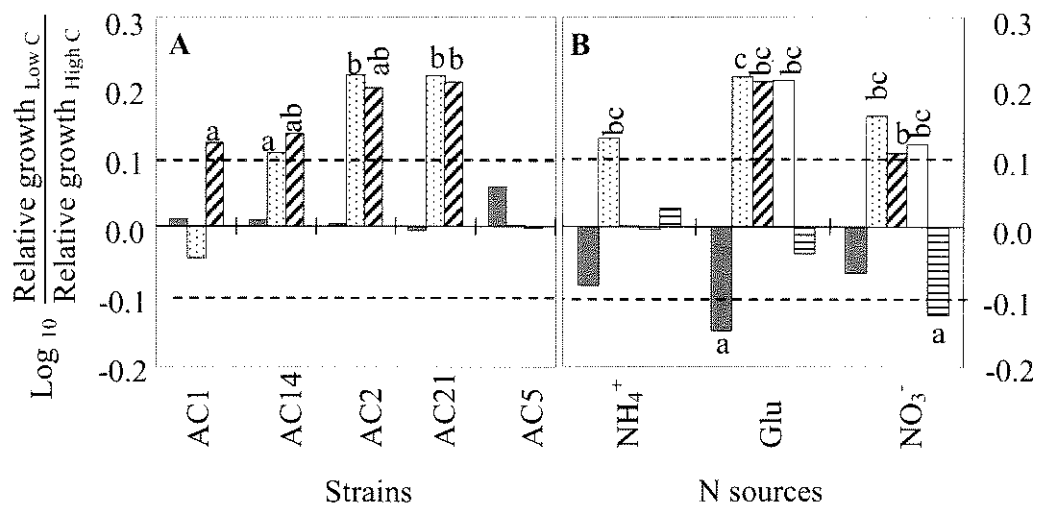


Figure 3

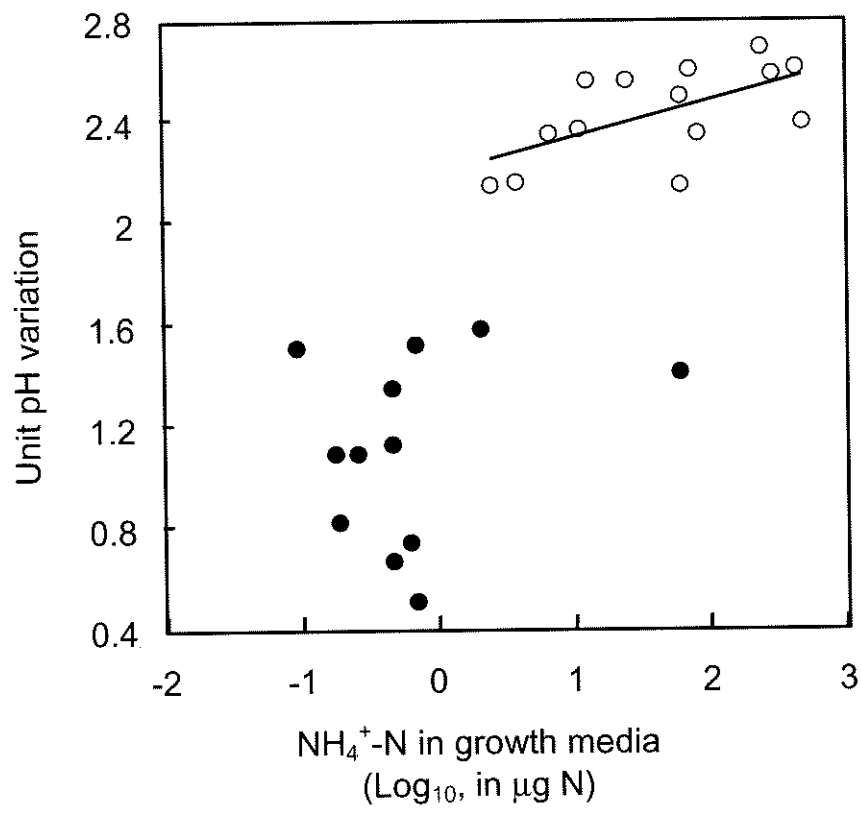


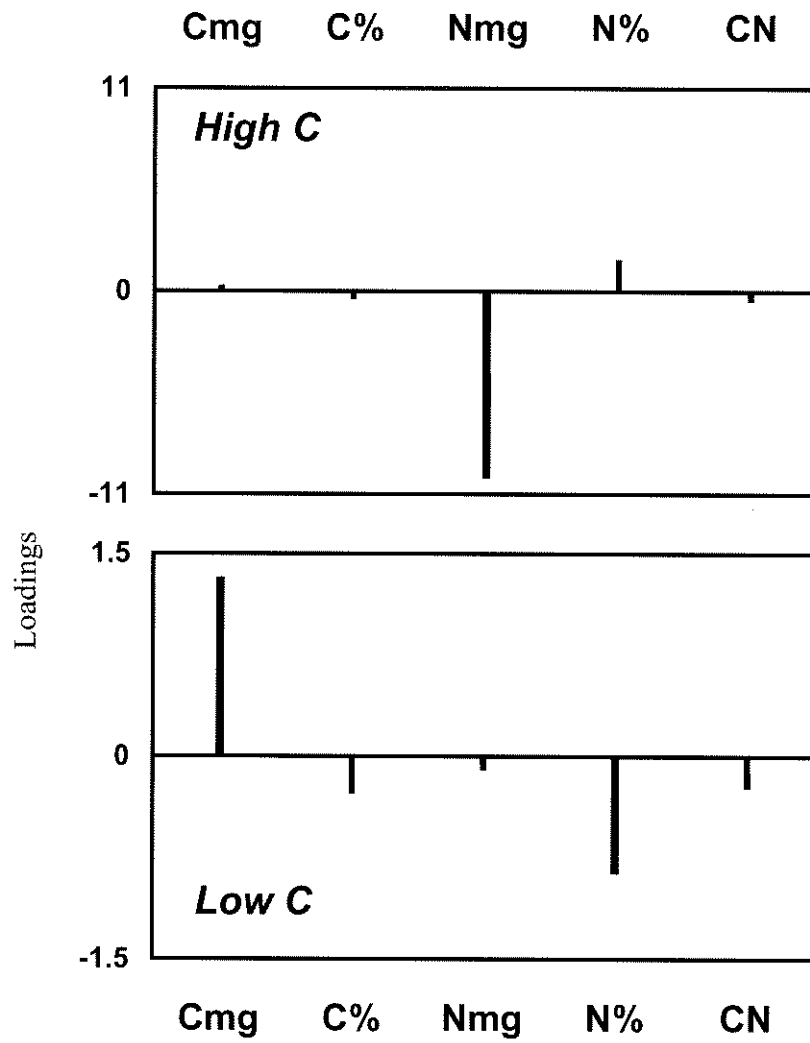
Figure 4

Figure legends

Figure 1: Time course of fungal biomass at high (filled symbols) or low (open symbols) carbon availability, averaged across all strains.

Data are the means of 45 replicates, vertical bar is the least significant difference (LSD) between means ($P < 0.05$). Means and LSD were generated by Analysis of variance using a randomised block design.

Figure 2: Growth Indices showing the extent to which the relative growth of the five isolates is altered by the level of C availability.

Growth indices were calculated to compare the relative growth on Low versus high C availability that each particular strain achieved on either ammonium (plain bars), glutamine (dotted bars) or nitrate (hatched bars)(figure 2a), or that achieved within each particular N source by the five strains AC1, AC14, AC2, AC21 and AC5 (in that order from left to right, figure 2b) .

Data are the means of 3 replicates. A positive, zero or negative value of the growth index indicates that the relative growth is increased, unchanged or decreased under low compared to high C availability. LSD between means is 0.09 at $P < 0.05$. The two dotted horizontal lines set the threshold within which growth index is not significantly ($P < 0.05$) different from zero (i.e. no alteration of relative growth performance by reduced C availability). Means with different letters are different at $P < 0.05$ and different from zero (i.e. correspond to increase or decrease in the relative growth by reduced C availability).

Figure 3: Relationship between media pH change and mass of N recovered as NH_4^+ in the growth media after 30 days of growth under High (●) or Low (○) C availability, with N supplied as Glutamine.

Data are individual measurements. Linear regression between X and Y was significant ($P < 0.03$, $R^2 = 0.35$) only when C availability was low.

Figure 4: Loadings of the canonical variate dimensions explaining the majority of between-strain variation when C availability is high (top chart) or Low (bottom chart).

The relative size of the loadings within each canonical variate dimension corresponds to the relative weight of each variable in explaining the between-strain variation. Variables used in the canonical variate analysis are as followed: Amounts of C and N recovered in fungal biomass (C_{mg} and N_{mg}, respectively) and their ratio (CN) and percentages of C and N in fungal biomass (C% and N%, respectively) measured after 30 days of liquid axenic culture.

