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Lena Dahlman · Jörgen Persson · Kristin Palmqvist
Torgny Näsholm

Organic and inorganic nitrogen uptake in lichens

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Abstract In order to learn more about nitrogen (N) acquisition in lichens, and to see whether different lichens differ in their affinity to various N sources, N uptake was measured in 14 various lichen associations (''species''). These species represented various morphologies (fruticose or foliose), contrasting microhabitat preferences (epiphytic or terricolous), and had green algal, cyanobacterial or both forms of photobionts. N was supplied under non-limiting conditions as an amino acid mixture, ammonium, or nitrate, using ^{15}N to quantify uptake. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was used to separate active and passive uptake. Thallus N, amino acids, soluble polyol concentrations, and the biont-specific markers chlorophyll *a* and ergosterol were quantified, aiming to test if these metabolites or markers were correlated with N uptake capacity. Ammonium uptake was significantly greater and to a higher extent passive, relative to the other two N sources. Nitrate uptake differed among lichen photobiont groups, cyanobacterial lichens having a lower uptake rate. All lichens had the capacity to assimilate amino acids, in many species at rates equal to nitrate uptake or even higher, suggesting that organic N compounds could potentially have an important role in the N nutrition of these organisms. There were no clear correlations between N uptake rates and any of the measured metabolites or markers. The relative uptake rates of ammonium, nitrate and amino acids were not related to morphology or microhabitat.

L. Dahlman (✉) · K. Palmqvist
Department of Ecology and Environmental Science,
Umeå University, 901 87 Umeå, Sweden
E-mail: lena.dahlman@eg.umu.se
Tel.: +46-90-7866712
Fax: +46-90-7866705

J. Persson · T. Näsholm
Umeå Plant Science Center,
Department of Forest Genetics and Plant Physiology,
SLU, 901 83 Umeå, Sweden

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Abbreviations CCCP: Carbonyl cyanide *m*-chlorophenylhydrazone · Chl: Chlorophyll · N: Nitrogen

Introduction

In spite of the fact that nitrogen (N) might be limiting for growth and distribution of lichens (Nash 1996), we lack knowledge about available N sources and N acquisition rates for lichens in their natural habitat. Moreover, the question of whether different lichens differ in their capacities to absorb various N compounds has been poorly addressed.

Lichens are taxonomically diverse (Tehler 1996). They appear in different anatomical and morphological forms (Honegger 1991; Richardson 1999), and they occupy different substrata whereby some are predominantly epiphytic on trees whereas others are terricolous (Hale 1983; Nash 1996). It seems likely that both morphology and substratum preferences should influence the composition of N compounds available for uptake. Such variations in the availability of various N compounds could possibly also be mirrored in varying capacities to absorb these different N forms.

N availability in lichen habitats has been poorly studied, and we know little about the preferences for various N forms in different types of lichens. There are indications that the inorganic N forms, ammonium and nitrate, are common ingredients in rainwater as well as in canopy through-fall, and in stem-flow water (Carlisle et al. 1966, 1967; Butler and Likens 1995, Cornell et al. 2003). It also appears that rainwater that has been filtered through the canopy is further enriched in amino acids (Carlisle et al. 1966, 1967), indicating that epiphytic lichens might experience a higher availability of organic N relative to terricolous lichens. Several investigators have shown that intact lichens can take up

ammonium and nitrate (Smith 1960a; Lang et al. 1976; Shapiro 1984; Crittenden 1996, 1998), while few have studied their capacities to take up organic N (Smith 1960b; Kielland 1997). This is in spite of the fact that utilization of organic N compounds is a well-known feature of free-living algae, cyanobacteria, and lichen mycobionts when grown in isolated cultures (Ahmadjian 1977; Cho et al. 1981; Rai 1988; Grossman and Takahasi 2001; Kinoshita et al. 2001; Bhattacharya et al. 2002).

The mode of N acquisition in lichens might also vary depending on the associated photobiont. Lichens with green algae (bi-partite green algal lichens) are solely dependent on direct N deposition on the thallus surface, a trait that is commonly linked to low thallus N concentrations (Honegger 1991; Crittenden et al. 1994; Palmqvist et al. 2002). In contrast, mycobionts associated with cyanobacteria (bi-partite cyanobacterial lichens and tripartite lichens) have the additional access to N_2 from the atmosphere via the cyanobacterial biont. Cyanobacterial lichens are therefore generally characterized by high thallus N concentrations (Rai 1988; Palmqvist et al. 2002). Due to the differences in N acquisition modes depending on the photobiont partner we might expect qualitative, as well as quantitative differences in N preference and N uptake capacity, where lichens associated with a cyanobacterial partner might not be as dependent on exogenous N sources compared to lichens associated with a green algal photobiont.

Exogenous N sources and other elements that are deposited on the lichen surface are transferred upon rehydration of the thallus via the mycobiont-derived outermost hyphal cortex and into a fungal apoplastic continuum that encloses the photobiont cells (Honegger 1993). All nutrients are therefore primarily dissolved in this fungal apoplastic continuum before being absorbed by the different lichen symbionts. No studies have been conducted on the individual biont's assimilation rates from the apoplast, but it is generally assumed that the mycobiont absorbs the major part of the acquired nutrients.

In order to investigate the capacities for N uptake in the form of amino acids, ammonium, and nitrate we measured short-term N uptake under non-limiting conditions in 14 lichens with different photobionts (green algal, tripartite, or cyanobacterial), different morphologies (foliose or fruticose) and different substratum preferences (epiphytic or terricolous). The lichens were incubated in ^{15}N-enriched solutions of the various N forms, and tracer levels in the thalli were subsequently analyzed by mass spectrometry. In addition to measuring thallus N, amino acid and soluble carbohydrate concentrations, the biont-specific markers chlorophyll (Chl) *a* (photosynthetic capacity) and ergosterol (active mycobiont tissue) were also measured, aiming to test whether any of these metabolites or markers might be correlated with the rate of short-term N uptake.

Materials and methods

Lichen material

The lichens were collected on two occasions: thalli for the amino acid uptake experiment in mid May 2002, and thalli for the nitrate and ammonium experiments in mid September 2002. The thalli were collected from the same site and populations for both sets of experiments. All lichens were collected in their natural habitats within a 70-km radius from Umeå, Västerbotten, Sweden. *Hypogymnia physodes* (L.) Nyl., *Platismatia glauca* (L.) W. Culb. *Nephroma bellum* (Sprengel) Tuck., and *Peltigera membranacea* (Ach.) Nyl. were collected in a mature and dense *Picea abies* (Norway spruce) dominated forest at Omagaliden in Vindeln. *Leptogium saturninum* (Dickson) Nyl. was collected in a *Salix caprea* stand outside Brännland. *Lobaria pulmonaria* (L.) Hoffman., *Bryoria capillaries* (Ach.) Brodo & D. Hawksw., *Bryoria fuscescens* (Gyelnik) Brodo & D. Hawksw., and *Alectoria sarmentosa* (Ach.) Ach. were collected in an old Norway spruce forest at Buberget, Vindeln. *Cetraria islandica* (L.) Ach., was collected on a *Pinus sylvestris* (pine) heath outside Vindeln. *Peltigera malacea* (Ach.) Funck and *Peltigera aphthosa* (L.) Willd. were collected on bare soil (sand) on a south-facing slope above Vindelälven. *Nephroma arcticum* (L.) Torss. and *Cladina stellaris* (Opiz) Pouzar & Vezda, were collected in an open forest area at Kulbäcksliden, Vindeln.

All lichen thalli were rinsed free of debris and epiphytic algae immediately after collection, and were thereafter stored dry in darkness at 15°C until they were re-activated for experimental use (see later), within one month after collection. Each lichen association (''species'') was represented by four samples in the amino acid experiment and by three samples each in the ammonium and nitrate experiments

Uptake studies

The N uptake measurements were performed according to Persson and Näsholm (2002) after modifying the incubation procedure to lichens. The lichen thalli were first sprayed with water and allowed to re-activate and stabilize their metabolism in a controlled-climate chamber (Palmqvist 1993) at 15°C, 95% relative humidity, 4 h dark:12–17 h light; 50 μmol m^{-2} s^{-1}. The lichens were thereafter transferred from the climate chamber to 30 ml of an incubation solution, containing labelled $(^{15}NH_4)_2SO_4$, or $K^{15}NO_3$, or a mixture of 16 universally (^{13}C, ^{15}N) labelled amino acids, composed as: arginine (Arg, 7.0%), alanine (Ala, 7.0%), glycine (Gly, 6.0%), isoleucine (Ile, 4.0%), leucine (Leu, 10.0%), lysine (Lys, 14.0%), phenylalanine (Phe, 4.0%), proline (Pro, 7.0%), serine (Ser, 4.0%), threonine (Thr, 5.0%), and

valine (Val, 5.0%), aspartic acid (Asp, 10.0%), glutamic acid (Glu, 10.0%), histidine (His, 2.0%), methionine (Met, 1.0%), tyrosine (Tyr, 4.0%). Asp, Glu, His, Met and Tyr were not analyzed and the proportions of uptake of individual amino acids reported below will therefore refer to the 11 amino acids analyzed. All uptake solutions had a total N concentration of 1.0 mM (U-^{13}C, $^{15}N > 98\%$), and the labelled compounds were obtained from Cambridge Isotope Laboratories, MA, USA. The incubation solution contained additional nutrients and the pH was adjusted to 5.0 (Persson and Näsholm 2002).

The uptake experiments were performed in darkness at 15°C and lasted for 30 min. The lichens were completely submerged in the incubation solution, which was heavily bubbled with air to avoid anaerobic conditions. After the 30-min incubation the lichens were directly washed in a non-labelled solution with a 10 mM concentration of the corresponding N source that had been used in the experiment, and thereafter washed twice in a 1 mM $CaCl_2$ solution in order to wash off labelled substrate passively attached to the cell walls. Following the washing procedure, thalli were immediately frozen in liquid N_2 and stored in a deep freezer (−80°C).

In a parallel experiment performed exactly as outlined above an additional thallus of each species was incubated in each of the three N solutions together with 100 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which specifically inhibits ATP-dependent transport across cellular plasma membranes (cf. Martin et al. 1991; Persson and Näsholm 2002). This experiment was performed in order to separate passive and active uptake of the various N sources in these lichens.

Analyses

The thalli were freeze-dried (Lyovac GT 2; Steris, Hürth, Germany) after the uptake experiments, and subsequently milled to a homogenous powder in liquid N_2 using a ball mill. The powder of each sample was divided into aliquots for different assays, stored dry at −80°C, and analyzed within 1 week. Amino acid concentrations and uptake were analyzed by gas chromatography–mass spectrometry (GC–MS) as described in Persson and Näsholm (2001). The NH_4^+ and NO_3^- uptake, respectively, were analyzed on a carbon–nitrogen analyzer (ANCA-NT system, solid/liquids preparation module; Europa Scientific, Crewe, Cheshire, UK) coupled to a Europa 20–20 Isotopic Ratio Mass Spectrometer (IRMS; Europa Scientific), according to Olsson and Wallmark (1999). Total N concentrations were not measured in the thalli used in the amino acid uptake experiment. Chlorophyll was quantified after extraction in $MgCO_3$-saturated dimethyl sulfoxide (DMSO; 60°C for 40 min; Palmqvist and Sundberg 2001), and the fungal component ergosterol was measured on an HPLC system (Waters, Milford, MA, USA) according to Dahlman et al. (2001) modified from Ekblad et al. (1998). The water-extracted carbohydrates were analyzed according to Dahlman et al. (2003) on a Varian 3800 gas chromatograph connected to a Varian Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA, USA).

Statistics

The lichens were grouped according to photobiont, morphology, and substratum preference (Table 1), and

Table 1 Inhibition of N uptake by CCCP. Values are the percent reduction of N uptake by lichens when CCCP (100 μM) is added to the N uptake solutions

Lichen type	Species	Reduction of N uptake (%)		
		Amino acid mixture	NH_4^+	NO_3^-
Green algal lichens				
Fruticose				
Terricolous	*Cetraria islandica*	84	74	83
	Cladina stellaris	87	64	77
Epiphytic	*Hypogymnia physodes*	78	51	75
	Bryoria capillaris	79	84	94
	Bryoria fuscescens	82	81	96
	Alectoria sarmentosa	71	61	74
Foliose				
Epiphytic	*Platismatia glauca*	83	83	92
Triparite lichens				
Foliose				
Terricolous	*Nephroma arcticum*	87	53	54
	Peltigera aphthosa	17	52	56
Epiphytic	*Lobaria pulmonaria*	87	68	78
Cyanobacterial lichens				
Foliose				
Terricolous	*Peltigera malacea*	76	50	79
	Peltigera membranacea	84	40	95
Epiphytic	*Leptogium saternium*	79	35	79
	Nephroma bellum	90	59	54

uptake rates of the different groups were tested within a one-way ANOVA using a statistical package from SPSS (Chicago, IL, USA). All correlation regressions were performed using the same statistical package. All values reported refer to mean ± standard error.

Results

All lichen species had the capacity to take up amino acids, although at widely varying rates (Fig. 1). On average, the tripartite lichens had a significantly lower amino acid uptake rate than green algal and cyanobacterial lichens $P < 0.05$ and $P < 0.01$, respectively (Fig. 2a). Relative uptake rates of the 11 amino acids varied inter-specifically, also generally differing from the relative abundance of the respective amino acid in the incubation solution (Fig. 3). It was not possible to distinguish any general trends in the relative uptake of individual amino acids among the lichens. There was, however, a notable intra-specific variation in the lichen amino acid preferences (Fig. 3).

With the exception of *Nephroma bellum*, ammonium uptake rates were generally significantly higher than the amino acid or nitrate uptake rates (Fig. 1). In contrast to the amino acid uptake, the tripartite lichens had significantly higher rates of ammonium uptake as compared to the green algal ($P < 0.01$) and the cyanobacterial lichens ($P < 0.001$). On average the ammonium uptake rate was 33.40 ± 1.33 for the tripartite lichens, 22.35 ± 2.34 for the green algal lichens, and 16.92 ± 2.26 μmol g^{-1} DW $h^{-1}$for the cyanobacterial lichens (Fig. 2a).

The nitrate uptake rate was low in cyanobacterial lichens (0.33 ± 0.06 μmol g^{-1} DW h^{-1}), intermediate in the tripartite (5.43 ± 0.34 μmol g^{-1} DW h^{-1}), and highest in the green algal lichens (10.07 ± 1.18 μmol g^{-1} DW h^{-1}); $P < 0.01$ in all comparisons (Fig. 2a).

With the exception of the amino acid uptake in *Peltigera aphthosa*, CCCP treatment resulted in a severe inhibition of the uptake of all three N sources in all the lichens (Table 1). When excluding *P. aphthosa*, the average CCCP inhibition of amino acid uptake was 83%. The average inhibition of the nitrate uptake was 75% when all species were included, although being slightly lower (54%) for *N. arcticum*, *N. bellum* and *P. aphthosa* (Table 1). The CCCP treatment inhibited ammonium uptake to a somewhat lower extent, on average being 46% in the cyanobacterial lichens, 58% in the tripartite lichens, and 71% in the green algal lichens (Table 1).

Compared to the terricolous lichens, the epiphytic lichens had significantly higher rates of both amino acid ($P < 0.05$) and nitrate uptake ($P < 0.01$), while there was no significant difference in their ammonium uptake rates (Fig. 2b). Amino acid uptake rates were 7.02 ± 0.80 for epiphytic lichens and 4.40 ± 0.61 μmol g^{-1} DW h^{-1} for terricolous lichens, while the nitrate uptake rates were 8.23 ± 1.34 and 3.70 ± 0.62 μmol g^{-1} DW h^{-1}, respectively. Compared to the fruticose lichens, the foliose lichens had significantly lower rates of nitrate uptake ($P < 0.0001$), while the ammonium and the amino acid uptake rates were similar in the two groups (Fig. 2b). In agreement with previous studies, thallus N concentrations varied depending on photobiont group, with the three groups being significantly different from each other for $P < 0.0001$. On average, the cyanobacterial lichens had the highest N concentrations (40.28 ± 0.9 mg g^{-1} DW), followed by the tripartite lichens (25.96 ± 1.86 mg g^{-1} DW) with the lowest concentrations in the green algal lichens (6.53 ± 0.37 mg g^{-1} DW). Further, metabolite concentrations, and the biont marker concentrations also varied depending on the asso-

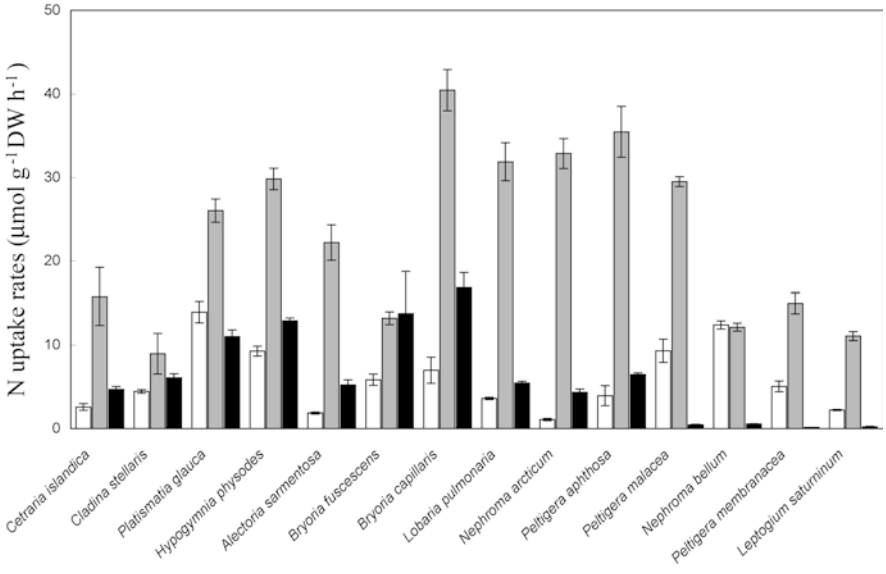

Fig. 1 N uptake by lichens supplied with various N sources. *White bars* represent total amino acid uptake of a mixture containing 11 amino acids ($n = 4$), *gray bars* represent NH_4^+ uptake rates ($n = 3$), and *black bars* NO_3^- uptake rates ($n = 3$). Data are means ± SE

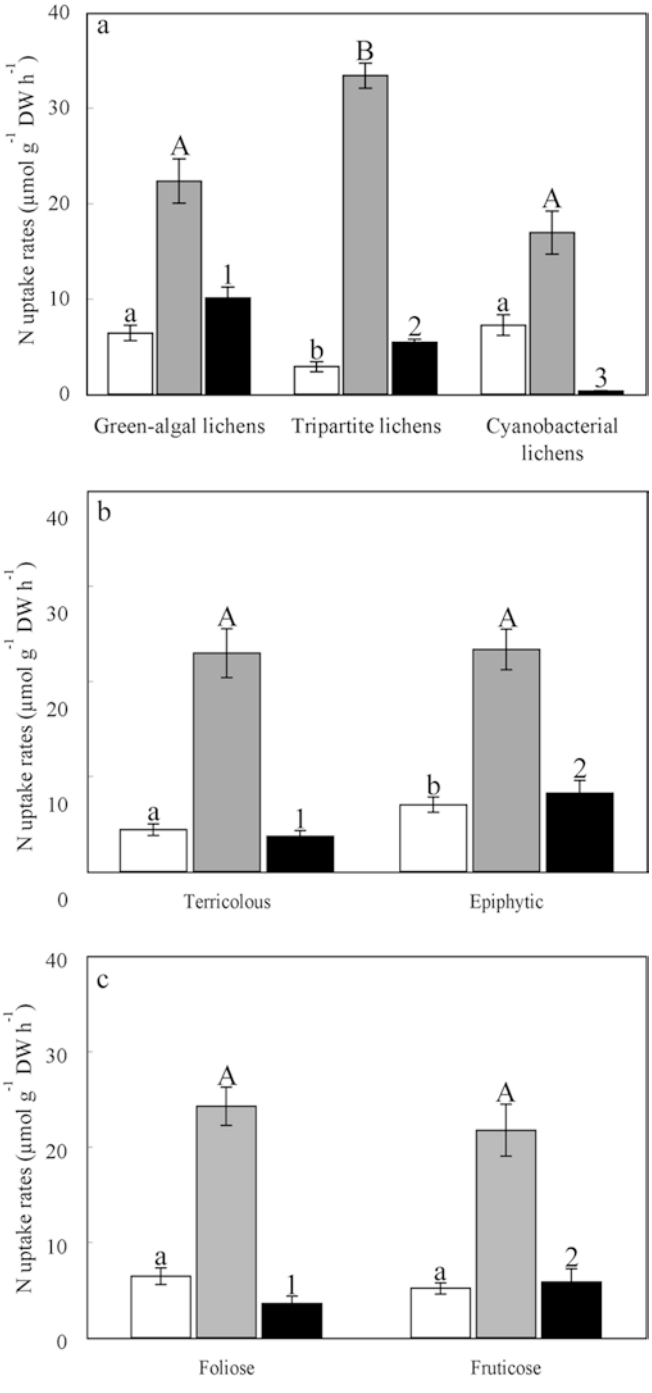

Fig. 2a–c Mean N uptake by lichens supplied with various N sources. **a** Lichens according to photobionts: green algal lichens ($n = 21$, 28), tripartite lichens ($n = 9$, 12), and cyanobacterial lichens ($n = 12$, 16). **b** Lichens with different life-styles: terricolous ($n = 18$, 24) and epiphytic ($n = 24$, 32). **c** Lichens with different morphologies: foliose ($n = 24$, 32) and fruticose ($n = 18$, 24). *White bars* represent total amino acid uptake of a mixture containing 11 amino acids ($n = 4$), *gray bars* represent NH_4^+ uptake rates ($n = 3$), and *black bars* NO_3^- uptake rates ($n = 3$). Data are means ± SE

ciated photobiont (Table 2). The cyanobacterial and the tripartite lichens generally had higher concentrations of all these components in comparison to the green algal lichens; however, with the exception of the two polyols ribitol and arabitol which were highest in the bipartite green algal lichens (Table 2), this is agreement with previous studies (Palmqvist et al. 2002; Dahlman et al. 2003).

Discussion

As described in the Introduction, the mode of nutrient acquisition differs among lichens, and is often linked to the associated photobiont. The most obvious example of this is that lichens with an associated cyanobacterium can assimilate atmospheric N_2 while bipartite green algal lichens are solely dependent on depositions of combined N onto their thalli. The most striking differences in rates of uptake of the various N compounds recorded in the present study can also be correlated with the associated photobiont.

The inhibition of the N uptake in the lichens by CCCP (Table 1) indicates that the uptake of N compounds is an active, ATP- and proton-gradient-dependent process in lichens, thus being similar to uptake systems characterized for other organisms (e.g. Grossman and Takahasi 2001; Williams and Miller 2001). CCCP inhibited nitrate and amino acid uptake to a similar extent, while ammonium uptake was less sensitive (Table 1). This emphasizes that ammonium absorption is to a larger extent passive, relative to amino acid and nitrate absorption. The larger uptake rates of ammonium could then be partly explained by a larger adhesion of this cation to the negatively charged cell walls (Brown et al. 1994).

All 14 lichens displayed high rates of ammonium uptake, although the tripartite lichens had significantly higher uptake rates relative to the two forms of bipartite lichens (Figs. 1, 2a). The reason for the differences in ammonium uptake rates among lichen groups can only be speculated upon and further studies are needed to address this question.

The capacity to take up amino acids was found in all 14 lichen species, although tripartite lichens again differed significantly from the general pattern with a lower amino acid uptake capacity (Fig. 2a). Using a mix of amino acids, instead of a single amino acid when testing the uptake, offered the possibility to compare relative uptake rates of each specific amino acid. The inhibition of this uptake in the presence of CCCP and the interspecific variation in the acquisition of individual amino acids (Table 1; Fig. 3), gives further support that the amino acid uptake was active.

The rates of nitrate uptake varied significantly among lichen species and were primarily linked to the photobiont (Fig. 2a), bipartite green algal lichens having the highest uptake rates whilst bipartite cyanobacterial lichens only absorbed low or minute amounts of nitrate. Due to the higher endogenous N concentrations, a lower nitrate uptake might be expected in cyanobacterial lichens since nitrate uptake is generally down-regulated by high endogenous N concentrations, and is induced by

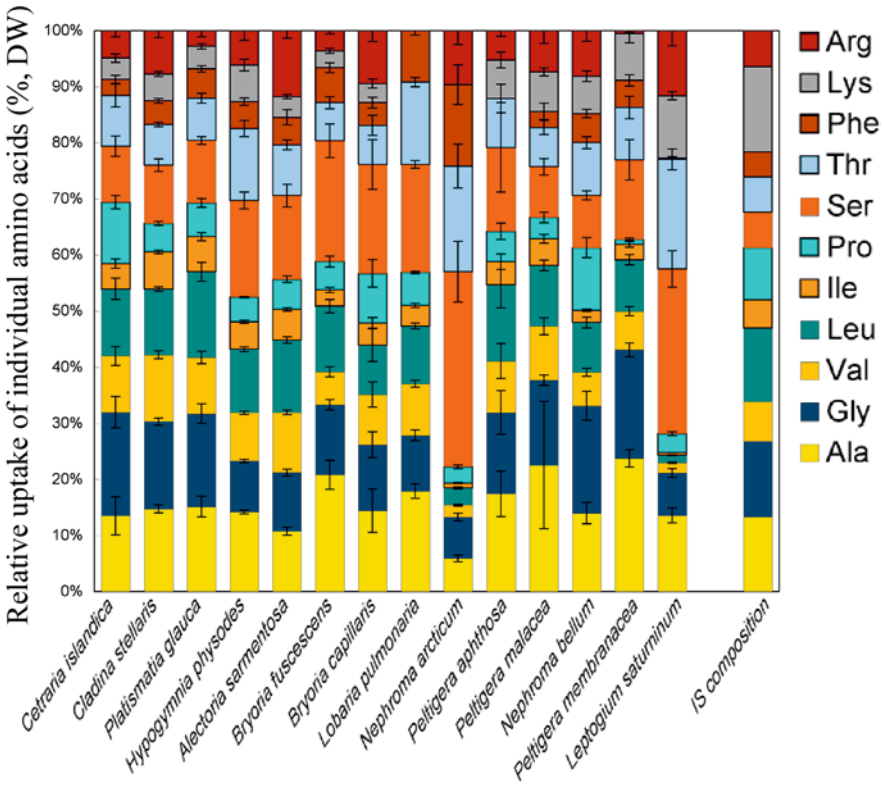

Fig. 3 Relative uptake of individual amino acids. An entire bar indicates the total uptake, while colored fields within a bar indicate the relative contribution to the total uptake of each individual amino acid. Individual fields indicate percent mean uptake rates (μmol g^{-1} DW h^{-1}), while error bars displays SE ($n = 4$)

low cellular N concentrations (Lejay et al. 1999; Table 1). There was, however, no significant correlation between nitrate uptake rate and thallus N concentration in the investigated lichens (not shown).

The general concept of nutrient uptake in lichens states that the mycobiont should absorb the major part of N deposited on the thallus. However, even though the absorbed nitrogen is primarily located in the hyphal apoplastic continuum of the thallus, this does not rule out the possibility that the photobiont might be able to compete for this N with the mycobiont. We might further speculate that in the particular case of nitrate, a green algal photobiont might be better at utilizing this N source, relative to the mycobiont, due to its direct access to reductive power through photosynthetic electron transport (Grossman and Takahasi 2001). This speculation is in part supported by the low nitrate uptake rates in bipartite cyanobacterial lichens, indicating that both the mycobiont and the cyanobacteria of these lichens absorb nitrate only at very low rates (Figs. 1, 2a). However, for the tripartite lichens with similar nitrogen content as bipartite cyanobacterial lichens there was a significant nitrate assimilation that might have primarily been conducted by the green algal partner. However, there are also reports that lichen mycobionts, as well as free-living fungi in general, have the capacity to assimilate and reduce nitrate (Marzluf 1981; Rai 1988), emphasizing that further studies are needed to address to what extent lichen photobionts are able to compete with the mycobiont for nitrogenous compounds.

The current study provides information regarding the capacities of different lichens to absorb various N forms from the environment. Under field conditions, uptake rates will, however, most probably to a large extent depend on the availability of different N sources in the habitats of the different lichens. Albeit for cyanobacterial lichens that have the intrinsic capacity for N_2 fixation, N availability in the habitat might be of lesser importance relative to that for green algal lichens. Thus, in order to pinpoint the relative importance of different N compounds to lichen N nutrition in the field, it is imperative to have reliable data on N availability in these habitats. Variation in N uptake capacity amongst lichens could thus be dependent on either a difference in N acquisition strategies and/or possibly mirror N availability in the habitats of these lichens (Lang et al. 1976; Shapiro 1984; Valladares and Sancho 2000). There were indeed differences in N uptake rates between terricolous and epiphytic lichens, and between fruticose and foliose morphologies (Fig. 2b,c), suggesting that both life-style and morphology can affect N uptake rates. Such speculations should, however, be treated with the utmost caution, as our results only represent a snapshot of lichen uptake at a single (relatively high) substrate concentration under very specific environmental conditions. Moreover, the recorded difference in N uptake rates between both fruticose and foliose lichens, and terricolous and epiphytic life-styles (Fig. 2b,c) could, alternatively, be due to the fact that the different photobiont associations were not evenly distributed in the different lichen groups (cf. Table 1).

Table 2 Enodgenous concentrations of measured metabolic compounds in the different lichens. Minor amino acids (*aa*) and carbohydrates were pooled (leucine, valine, isoleucine, threonine, phenylalanine, ornithine, asparagine, lysine, tyrosine, proline, serine; sucrose, fructose) and are not shown individually. Values presented are means ± SE ($n=4$). *GABA*γ-Amino-butyric acid

Lichen type	Species	Alanine	Glycine	GABA	Aspartic acid	Glutamic acid	Glutamine	Arginine	Total aa pool	Total N	Ergosterol	Chl *a*	Ribitol	Mannitol	Arabitol	Glucose	Total soluble carbohydrate pool
		(μmol g^{-1} DW)	(μmol g^{-1} DW)	(μmol g^{-1} DW)	(μmol g^{-1} DW)	(μmol g^{-1} DW)	(μmol g^{-1} DW)	μmol g^{-1} DW)	(μmol g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)
Green algal lichens																	
Fruticose																	
Terricolous	*Cetraria islandica*	2.00 ± 0.40	1.19 ± 0.18	2.43 ± 0.21	0.55 ± 0.08	10.82 ± 4.24	7.63 ± 5.09	3.04 ± 0.71	32.39 ± 5.95	7.62 ± 0.98	0.65 ± 0.06	0.99 ± 0.05	2.61 ± 0.34	6.21 ± 0.57	18.55 ± 2.55	2.18 ± 0.47	30.86 ± 3.73
	Cladina stellaris	1.60 ± 0.08	1.20 ± 0.09	1.34 ± 0.17	0.39 ± 0.03	1.68 ± 0.27	0.88 ± 0.14	1.91 ± 0.31	10.92 ± 0.7	4.63 ± 0.38	0.63 ± 0.05	0.35 ± 0.03	0.81 ± 0.11	0.61 ± 0.15	6.35 ± 0.51	0.09 ± 0.04	7.93 ± 0.82
Epiphytic	*Hypogymnia physodes*	1.79 ± 0.04	0.94 ± 0.01	0.35 ± 0.05	0.85 ± 0.10	14.61 ± 1.50	0.38 ± 0.22	3.24 ± 0.35	26.06 ± 0.75	6.45 ± 0.42	0.73 ± 0.01	0.45 ± 0.03	2.75 ± 0.24	2.41 ± 0.08	22.40 ± 0.85	0.83 ± 0.08	28.81 ± 1.06
	Bryória capilláris	2.43 ± 0.40	1.91 ± 0.48	4.52 ± 1.24	0.63 ± 0.13	10.85 ± 2.70	0.58 ± 0.20	1.60 ± 0.07	27.22 ± 2.26	7.82 ± 0.79	0.92 ± 0.07	1.31 ± 0.14	8.57 ± 0.76	2.61 ± 0.20	32.01 ± 2.62	1.45 ± 0.53	46.03 ± 4.10
	Bryória fuscéscens	1.89 ± 0.04	1.23 ± 0.05	8.80 ± 0.66	0.41 ± 0.05	6.16 ± 1.82	0.27 ± 0.05	2.34 ± 0.31	27.03 ± 1.09	9.00 ± 0.67	0.70 ± 0.04	1.16 ± 0.07	7.12 ± 1.83	3.45 ± 0.57	35.46 ± 5.32	2.82 ± 0.55	50.18 ± 8.38
	Alectória sarmentósa	1.28 ± 0.06	0.52 ± 0.08	3.50 ± 0.62	0.20 ± 0.01	2.45 ± 0.08	0.45 ± 0.17	4.98 ± 0.58	16.66 ± 1.04	5.83 ± 0.22	0.42 ± 0.09	0.50 ± 0.04	4.56 ± 0.38	1.64 ± 0.10	18.19 ± 1.34	0.50 ± 0.07	25.60 ± 1.68
Foliose																	
Epiphytic	*Platismatia glauca*	3.08 ± 0.26	2.74 ± 0.23	7.64 ± 0.69	0.55 ± 0.06	3.70 ± 0.22	1.03 ± 0.09	1.51 ± 0.42	25.41 ± 2.30	4.56 ± 0.25	0.79 ± 0.04	0.57 ± 0.03	2.27 ± 0.47	1.92 ± 0.39	12.50 ± 2.15	0.78 ± 0.03	17.72 ± 3.34
Triparite lichens																	
Foliose																	
Terricolous	*Nephroma arcticum*	0.73 ± 0.06	0.25 ± 0.02	1.76 ± 0.16	0.63 ± 0.11	26.23 ± 6.94	0.66 ± 0.18	0.70 ± 0.19	33.11 ± 7.91	23.37 ± 0.84	1.80 ± 0.06	0.95 ± 0.03	0.73 ± 0.51	25.01 ± 6.08	37.61 ± 10.35	0.09 ± 0.10	65.18 ± 17.25
	Peltigera aphthosa	15.01 ± 2.61	3.14 ± 0.58	9.16 ± 1.52	1.43 ± 0.40	42.21 ± 2.45	1.10 ± 0.28	1.52 ± 0.25	67.20 ± 5.55	31.65 ± 0.10	1.62 ± 0.07	1.54 ± 0.15	0.14 ± 0.06	13.75 ± 1.68	13.96 ± 2.14	2.72 ± 0.23	32.30 ± 4.22
Epiphytic	*Lobaria pulmonaria*	4.69 ± 0.05	0.72 ± 0.09	2.02 ± 0.72	2.13 ± 0.19	86.74 ± 5.41	1.40 ± 0.17	0.40 ± 0.05	98.80 ± 4.88	21.11 ± 0.45	1.91 ± 0.10	1.79 ± 0.09	4.40 ± 0.74	4.38 ± 0.60	32.40 ± 4.74	0.38 ± 0.08	41.56 ± 6.11
Cyanobacterial lichens																	
Foliose																	
Terricolous	*Peltigera malacea*	10.05 ± 0.40	3.68 ± 0.26	9.21 ± 4.89	3.43 ± 0.71	16.71 ± 2.34	0 ± 00	2.24 ± 0.04	48.76 ± 6.66	36.22 ± 0.16	1.47 ± 0.03	1.03 ± 0.05	0 ± 00	29.01 ± 8.11	0 ± 00	0 ± 00	30.75 ± 7.14
	Peltigera membranacea	15.99 ± 1.72	4.61 ± 0.45	0.84 ± 0.09	6.29 ± 1.29	47.29 ± 8.27	13.39 ± 2.42	0.79 ± 0.22	88.59 ± 10.97	44.25 ± 0.42	2.37 ± 0.09	1.35 ± 0.05	0.09 ± 0.04	37.52 ± 3.33	0 ± 00	1.94 ± 0.37	41.22 ± 3.61
Epiphytic	*Leptogium saternium*	1.44 ± 0.12	0.74 ± 0.07	0.16 ± 0.01	8.30 ± 0.29	28.09 ± 5.25	2.40 ± 0.28	6.32 ± 0.57	55.38 ± 5.49	42.67 ± 0.31	1.11 ± 0.02	2.03 ± 0.08	0.01 ± 0.00	16.76 ± 1.38	0 ± 00	2.02 ± 0.07	19.11 ± 1.78
	Nephroma bellum	9.64 ± 1.30	5.13 ± 0.79	10.02 ± 1.61	2.49 ± 0.29	81.42 ± 9.21	1.50 ± 0.32	3.42 ± 0.83	116.37 ± 8.44	39.20 ± 0.42	2.76 ± 0.03	1.85 ± 0.04	2.11 ± 1.02	18.51 ± 3.64	47.16 ± 6.46	0 ± 00	69.70 ± 7.67

Conclusion

Our results suggest that organic N, in addition to ammonium, nitrate and N_2 fixation, may be a significant N source for a wide range of lichens, regardless of morphology, associated bionts or life-style. Moreover, the present study draws the attention to the lack of proper data on N cycling and dynamics in the lichen symbiosis, and illustrates a need for further studies in these areas. It also emphasizes the need to study N uptake by individual lichen associations, as well as within groups of lichens associated with similar photobionts, in order to elucidate relative biont contributions to total uptake under varying situations.

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