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***New Phytologist* Supporting Information Fig. S1, Tables S1–S3 and Methods S1**

Article title: Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: importance of molybdenum availability and implications for current biological nitrogen fixation estimates

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Fig. S1 Unwashed thallus and cephalodia content for V and Mo.

Table S1 Discrimination of lichen compartment with respect to metals

Table S2 Regression parameters for linear regression in Figs 2, 3(e) and 5(b)

Table S3 Literature survey of BNF estimation and measurement conditions for di-nitrogen fixing species in various ecosystems around the world

Methods S1 Contribution of alternative nitrogenase to acetylene reduction and to N_2 fixation.

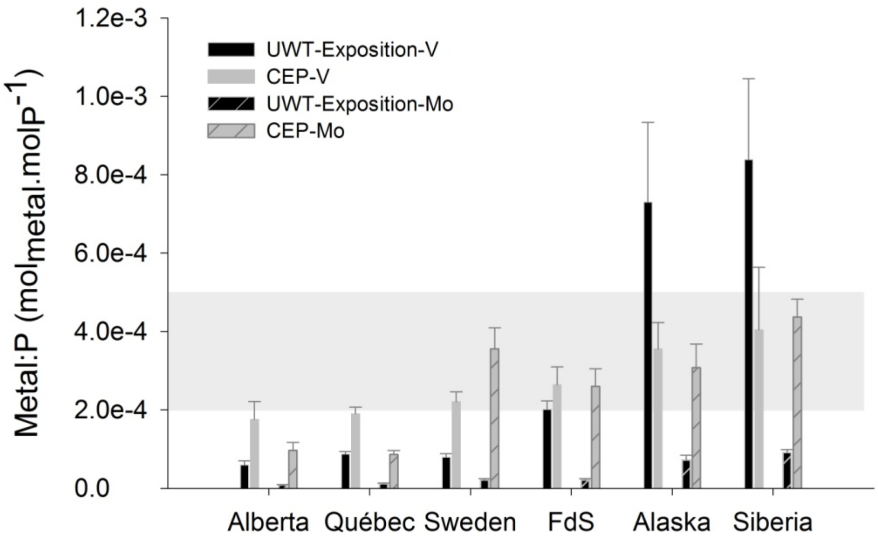
Methods S1 Contribution of alternative nitrogenase to acetylene reduction ($f_{alt\ ara}$) and to N_2 fixation ($f_{alt\ N2}$).

$$f_{alt\ ara} = \frac{(^{13}\varepsilon_{Mo} - {}^{13}\varepsilon_{AR,sample})}{(^{13}\varepsilon_{Mo} - {}^{13}\varepsilon_{alt})}$$

$$f_{alt\ N_2} = \frac{(f_{alt\ ara}/1)}{\left(\frac{f_{alt\ ara}}{1}\right) + (\frac{1 - f_{alt\ ara}}{3})}$$

Contributions were calculated according to Zhang *et al*. (2016): Isozyme specific $^{13}\varepsilon$ (see main text) for Mo and V were determined in deletion mutants CA 1.70 (Mo-Nase only) and CA 11.70 (V-Nase only) of *Azotobacter vinelandii*, in *Rhodopseudomonas palustris* wild type (CGA009) and deletion mutant CGA753 (Mo-Nase only) and CGA766 (V-Nase only), as well as in a wild type strain of *Anabaena variabilis* (ATCC 29413) as $^{13}\varepsilon_{Mo}$ = 13.8 ± 0.3‰ and $^{13}\varepsilon_{V}$ = 7.9 ± 0.2‰. Value of $^{13}\varepsilon_{alt}$ for V was used for all calculations of $f_{alt\ ara}$ in this manuscript.

a



b

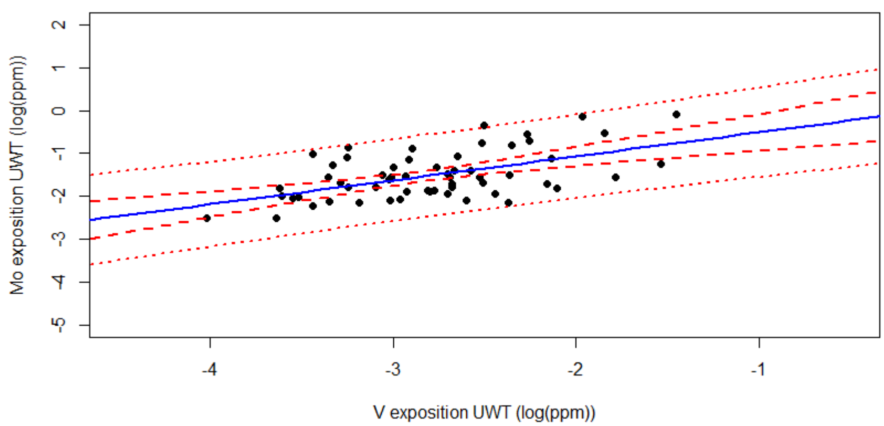


Fig. S1 Unwashed thallus (UWT, i.e. metal exposure) and cephalodia (CEP) content for V and Mo. (a) Metal content of UWT and CEP on sampling sites expressed in $mol_V.mol_P^{-1}$ (modification of Darnajoux *et al.*, 2014) pale background represents optimal V and Mo cellular quota required to sustain diazotrophic growth (more details in Darnajoux *et al.*, 2014). FdS stands for Fjord-du-Saguenay, South Québec. (b) Linear regression (solid line) of Mo exposition ($\mu g_{Mo}.g_{thallus}^{-1}$) and V exposition ($\mu g_{Mo}.g_{thallus}^{-1}$) in lichen thallus in nonheavily contaminated boreal sites (Alberta, Québec, FdS and Sweden). Short dash lines show 95% prediction intervals and long dash lines show 95% confidence intervals. All data were log-transformed to achieve normality of residues and to limit spurious correlation.

Table S1 Discrimination of lichen compartment with respect to metals

Values prediction	n	Recall	Precision	
WT	50	0.9400	0.0408	
UWT	49	0.9592	0.0600	
CEP	49	0.9796	0.0000	
ALG	47	1.0000	0.0208	
Error rate				**0.0308**

Classification functions	WT	UWT	CEP	ALG	*P*-value
Mg	15.16	25.48	7.01	30.10	<0.001
Ti	1.20	0.12	-5.41	1.24	<0.001
V	-48.44	-50.95	-43.32	-49.96	<0.001
Cr	-0.01	-1.80	2.39	1.26	<0.001
Mn	6.20	10.34	4.91	4.90	<0.001
Fe	43.38	41.72	45.95	41.08	0.029
Co	2.53	3.05	-0.94	2.16	<0.001
Ni	-16.64	-15.92	-9.65	-15.73	<0.001
Zn	-1.44	-4.00	-1.24	-5.36	<0.001
Mo	-14.65	-16.11	-8.61	-11.36	<0.001
Cd	-29.02	-28.81	-26.77	-29.45	0.014
Pb	-5.61	-2.30	-9.45	-4.91	<0.001
constant	-470.15	-476.36	-396.87	-454.57	-

Confusion matrix (leave-one-out method) and classification function from linear discriminant analysis of thallus compartment of cyanolichen *P.aphthosa* s.l. (UWT, unwashed thallus (i.e. exposition); WT, oxalate-EDTA washed thallus; ALG, algae; CEP, Cephalodia). Data in metal : P ratio were log-transform to avoid spurious correlation and achieved normality.

Table S2 Regression parameters for linear regression in Figs 2, 3(e) and 5(b)

Regression	Model equation	*n*	*P*-value
<u>Fig. 2 (Sample from Alberta, South Québec (FdS), North Québec and Sweden)</u>			
$\log_{10}(Mo{:}P\ (mol_{Mo}.mol_P^{-1})) = f(\log_{10}(Mo\ UWT\ (\mu g_{Mo}.g_{thallus}^{-1})))$	**0.79 ± 0.14** × $\log_{10}$(Mo UWT) – 2.94 ± 0.16	58	<0.001
$\log_{10}(V{:}P\ (mol_V.mol_P^{-1})) = f(\log_{10}(V\ UWT\ (\mu g_V.g_{thallus}^{-1})))$	0.06 ± 0.10 × $\log_{10}$(V UWT) – **3.71 ± 0.07**	58	0.563
$\log_{10}(V{:}P\ (mol_V.mol_P^{-1})) = f(\log_{10}(Mo{:}P\ (mol_{Mo}.mol_P^{-1})))$	**-0.61 ± 0.17** × $\log_{10}$((Mo : P))² **- 4.7 ± 1.3** × $\log_{10}$(Mo : P) -13 ± 3	58	0.003
<u>Fig. 3e</u>			
$\log_{10}(Mo{:}P\ UWT\ (mol_{Mo}.mol_P^{-1})) = f(\log_{10}(V{:}P\ UWT\ (mol_V.mol_P^{-1})))$	**0.69 ± 0.09** × $\log_{10}$(V:P) – 0.9 ± 0.4	16	<0.001
$\log_{10}(Mo{:}P\ CEP\ (mol_{Mo}.mol_P^{-1})) = f(\log_{10}(V{:}P\ CEP\ (mol_V.mol_P^{-1})))$	**-0.61 ± 0.16** × $\log_{10}$(V:P) – 5.9 ± 0.6	16	0.002
<u>Fig. 5b (Grouped per Time since last fire)</u>			
$\delta 15N\ WT = f(V{:}P\ (mol_V.mol_P^{-1})\ CEP)$	**-0.00009 ± 0.00002 × (V:P) + 0.00027 ± 0.00002**	14	0.023

*Bold figures highlight important parameter of the regression. Uncertainties are ± SE for the regression parameters.

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Table S3 Literature survey of BNF estimates and measurement conditions for di-nitrogen fixing species in various biomes around the world; studies were screened from two review articles on biological nitrogen fixation (Cleveland *et al*., 1999; Elbert *et al*., 2012), with emphasis on mosses and lichens from boreal and arctic area

Geography	Details	Species	BNF estimation (kgN ha^{-1} yr^{-1})	ARA	ARA condition	^{15}N calibration	^{15}N condition	$C_2H_2 : N_2$ (mol mol^{-1})	References
New Mexico	Alpine climate, Douglas fir, spruce fir	*Peltigera* spp.	0.04-3.3	No	---	No	---	Forman 1975 estimations	Forman & Dowden (1977)
West North Carolina	---	*Lobaria pulmonaria*, *Lobaria quercizans*	0.8	Yes	Field (1 h)	No	---	N.C.	Becker (1980)
New Zealand	Urewera National Park,	*Sticta*, *Pseudocyphellaria*	1 - 10	Yes	26°C, 1 h 30–3 h	Yes	26°C, 1 h 30–3 h	$C_2H_2 = 5.6 \times N_2$-6.3	Green *et al.* (1980)
Scotland and South Wales	---	*Peltigera* membranacea, *P. polydactyla*, *Lobaria pulmonaria*	2.4-5.8	Yes	Climatic chamber 2.5–18°C	Yes	Climatic chamber 2.5–18°C	7.8-12.2	Millbank (1981)
Nebraska	Prairies	Cyanolichens, heterotrophs	3.5	Yes	18–22°C	No	---	3	Kapustka & DuBois (1987)
Northwest Pacific	Canopy	*Lobaria oregana*	1.5-16.5	Yes	Field/Lab (0–20°C)	No	---	4	Antoine (2004)
Puerto Rico	Tropical forest	Soil, phyllophyte, moss, lichens	8.4-12.3	Yes	Field	No	---	3	Cusack *et al.* (2009)
South-central Chile	Rain forest chronosequence	Soil, cyanolichens, moss	0-6	Yes	Lab	No	---	3	Pérez *et al.* (2014)
Sweden, Finland, Norway	Boreal forest	*Pleurozium schreberi*	1.5-2	Yes	Field	Yes	Lab	3	DeLuca *et al.* (2002)
Northern Sweden	Boreal forest chronosequence	*Pleurozium schreberi*, *Hylocomnium splendens*	1.6	Yes	---	ND	---	3	Zackrisson *et al.* (2009)
Northern Sweden	Boreal forest chronosequence	*Pleurozium schreberi*	0.2-7	Yes	---	No	---	3	DeLuca *et al.* (2007)
Northern Sweden	Boreal forest chronosequence	*Pleurozium schreberi*	0.5-2	Yes	---	Yes	Lab	3	Zackrisson *et al.* (2004)

Northern Sweden	Boreal forest	*Pleurozium schreberi, Hylocomnium splendens*	0.52-2	Yes	---	Yes (*H.splendens*)	Lab	3	Lagerström *et al.* (2007)
Northern Sweden	Sub-arctic	Moss, Lichens	0.6-2.6	No	---	Yes	Field 7 – 23°C	---	Gavazov *et al.* (2010)
Northern Sweden	Sub-arctic	*Hylocomnium splendens* *Peltigera aphthosa* *Sphagnum*	0.3 0.9 2.6	Yes	Field (10–35°C)	No	---	2.78	Rousk *et al.* (2015)
Northern Sweden	Sub-arctic	*Sphagnum, Drepanocladus*	94	Yes	---	No	---	3	Granhall & Selander (1973)
Northern Sweden	Sub-arctic	*Sphagnum*	32	Yes	---	No	---	3	Basilier & Granhall (1978)
British Colombia	Rain forest	Moss	0.26-0.76	Yes	16°C	No	---	3	Lindo & Whiteley (2011)
Arctic	High Arctic	Soil and cyanobacterial mat	0.6-1	Yes	Field	No	---	3	Henry & Svoboda (1986)
Ohio	Prairies	*Nostoc* sp.	4.6+3.19	Yes	Field (9–30°C)	No	---	3	Dubois & Kapustka (1983)
Inner Mongolia	Steppe	*Nostoc* sp., lichens	0.033-0.087	Yes	25°C	Yes	5°C and 25°C	0.31	Holst *et al.* (2009)
Southern Utah	---	Biocrust	0.02-3.63	Yes	21–22.5°C	No	---	3	Jeffries *et al.* (1992)
Indiana, South Lake Michigan	Sand dune soil	Soilcrust	0.2-8	No	---	No	Mass balance	---	Thiet *et al.* (2005)
China, Loess plateau	Soil, grassland	Biocrust	4-13	Yes	Growth chambers (5–45°C)	No	---	3	Zhao *et al.* (2010)
Southern Utah	---	Biocrust	1.4-13	Yes	26°C	No	---	0.062	Belnap (2002)
Southern Alberta	---	*Nostoc* sp.	0.1-0.773 (31days)	Yes	Field with water bath	No	---	3	Coxson & Kershaw (1983)
Niger	---	Biocrust	3.5	Yes	30°C	No	---	NC	Malam Issa *et al.* (2001)
Northwest China	Desert	Biological soil crust	C2H2 data only	Yes	26°C (24 h)	No	---	3	Wu *et al.* (2009)

Zambia, Botswana	---	Soil crust	0.008-0.044	Yes	Field (48 h)	No	---	3	Aranibar *et al.* (2003)
Costa Rica	Rain forest	*Scytonema* sp.	2-5	Yes	Field	No	---	4	Freiberg (1998)
French Guiana	Tropical forest	Cyanobacteria biofilm	134-233	No	---	No	Value from Freiberg 1998	---	Dojani *et al.* (2007)
North western Ohio	---	Biocrust	1.3	Yes	26°C (4 h)	No	---	3.2	Veluci *et al.* (2006)
Western North Carolina	---	Woody litter, soil, phyllosphere, leaf litter	12.04	Yes	18°C (16–24 h)	No	---	NC	Todd *et al.* (1978)
Papua New Guinea, Australia	---	Phyllosphere	0.5	Yes	Field	No	---	3	Goosem & Lamb (1986)
California	---	Soil	2.1-4.8	Yes	Lab (26°C, 1 h)	Yes	Lab (26°C, 1 h)	3.1-8.4	Steyn & Delwiche (1970)
Central Sweden	Boreal forest	Soil	0.4-1.4	Yes	Lab (20°C)	Yes	Lab (20°C)	1.6-5.6	Nohrstedt (1985)
Ontario	Boreal transition zone	Soil	0.02-0.26	Yes	30°C	No	---	3	Hendrickson (1990)
Hawaii	---	Soil	0.06-1.29	Yes	Lab	Yes	---	3.9	Vitousek & Hobbie (2000)
Oregon	---	Wood log	1.4	Yes	Lab 22°C	Yes	Lab 22°C	3.5	Silvester *et al.* (1982)
Oregon	---	Wood roots	6.3	Yes	Lab 22°C	Yes	Lab 22°C	3.5	Chen & Hicks (2003)
Hawaii	---	Leaf litter, roots, soil	0.1-4.9	Yes	Lab (12–28°C)	Yes	Lab, 25°C	1.07-12.1	Ley & D'Antonio (1998)
Germany (Bavaria)	---	soil	0.2	Yes	Lab (20°C)	Yes	Lab	6-8	Limmer & Drake (1996)
Oregon	Andrew experimental forest	*Lobaria oregana*	3.5	Yes	Field (1 h)	No	---	3	Denison (1979)

Hawaii	Kilauea volcano	*Stereocaulon volcani*, leaf litter, liverworts	0.3-2.8	Yes	Field (24h)	Yes	Lab?	3.1/ 3.8 / 5.4	Vitousek (1994)
New Zealand	60000 yr chronosequence	*Coriaria*/ lichens/moss/ litter	11/0.02-2/0.7-9.6/1.1-1.9	Yes	Field	Yes	Field	1.33/1.58/0.25	Menge & Hedin (2009)

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