Molecular clues on eco-physiological response of lichens to nitrogen

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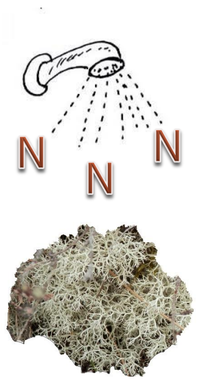
At Whim, an unmanaged ombrothrophic bog …

… where N manipulation experiments have been carried on since 2001,…

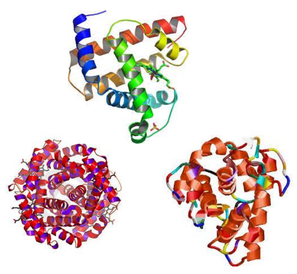
… thalli of Cladonia portentosa *were transplanted and exposed to nitrogen treatments for 6 months…*

…under local climatic conditions.



- 2 forms: NH_4^+ and NO_3^-
- 3 doses: 16, 32 and 64 kg N ha^{-1} yr^{-1}
- Cumulative exposure of 6 months (short-term) and 11 years (long-term)
- Addition/no addition of phosphorous and potassium: added as K_2HPO_4 at a 1:14 PN ratio to give 16 kg N + 0.57 kg P $ha^{-1}yr^{-1}$ and 64 kg N + 4 kg P $ha^{-1}yr^{-1}$

p r o t e o m e

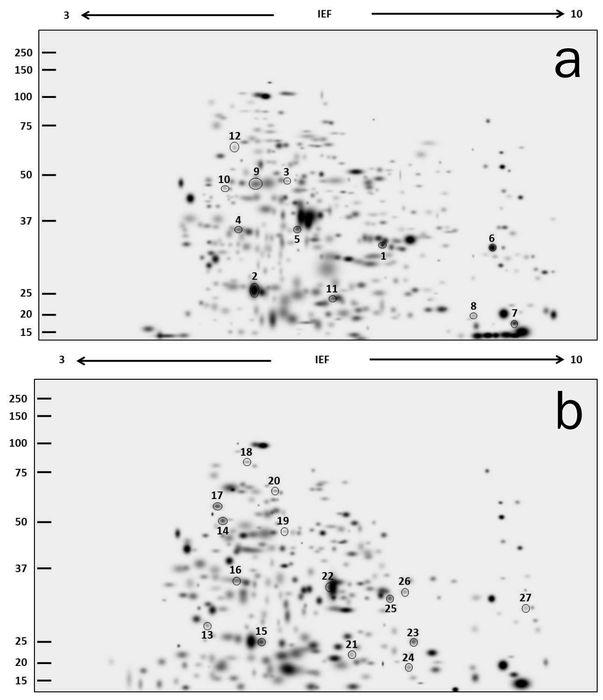


M&M

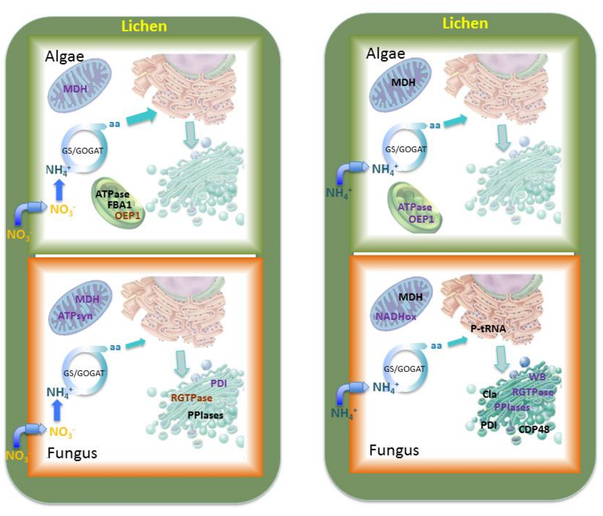
The proteome of *C. portentosa* was extracted and analyzed following Nicolardi et al., 2012 (Environmental Pollution 160, 1-10).

Significantly different spots between treatments were selected when comparing: 6-months treatments with different doses of NH_4^+ and with and without PK; 6-months treatments with different doses of NO_3^- and with and without PK.

Results



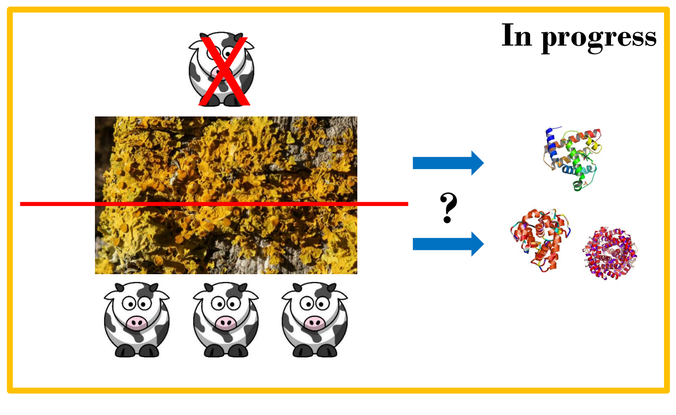
Master (virtual) gel obtained from the PDQuest-based comparison of 2-D electrophoresis gels of a) NO_3 treated samples and b) NH_4^+ treated samples. Spots analysed by MALDI-TOF are circled and numbered.



Summary of changes in protein expression (black=upregulation, brown=downregulation, violet=no defined pattern). Abbreviations: aa=aminoacids, GS/GOGAT=glutamate synthase/glutamine oxoglutarate aminotransferase; see table for proteins.

Summary of changes in protein expression caused by addition of nitrogen

Spot n.	Protein (abbreviation)	Function	Organism	N form	Response to N dose	Threshold	Response to PK
3	ATP synthase subunit beta (ATPase)	Photosynthesis	algae	NO_3^-	upregulated	yes	downregulated
2	Oxygen-evolving enhancer protein 1 (OEP1)	Photosynthesis	algae	NO_3^-	downregulated at 64	yes	downregulated
5	Fructose-bisphosphate aldolase (FBA)	Photosynthesis	algae	NO_3^-	upregulated	yes	downregulated
19	ATP synthase subunit beta (ATPase)	Photosynthesis	algae	NH_4^+	unclear	yes	unclear
15	Oxygen-evolving enhancer protein 1 (OEP1)	Photosynthesis	algae	NH_4^+	unclear	no	downregulated
22	Prolyl-tRNA synthetase	Protein synthesis	bacteria	NH_4^+	upregulated	no	downregulated
12	Protein disulfide-isomerase (PDI)	Folding	fungus	NO_3^-	no	no	upregulated at 64 kg
14-17	Protein disulfide-isomerase (PDI)	Folding	fungus	NH_4^+	upregulated	yes	unclear
24	Peptidyl-prolyl cis-trans isomerase (PPIases)	Folding	fungus	NH_4^+	unclear	no	upregulated at t64 kg
4-10	Ran-specific GTPase-activating protein 1	Regulation	fungus	NO_3^-	downregulated	yes	downregulated at 64 kg
6	Hypothetical protein AN4402.2	Regulation	fungus	NO_3^-	slightly upregulated	yes	downregulated
7	Peptidyl-prolyl cis-trans isomerase (PPIases)	Regulation	fungus	NO_3^-	upregulated	yes	downregulated at 64 kg
27	Putative prohibitin	Regulation	fungus	NH_4^+	downregulated	no	upregulated at 64 kg
13	Clathrin light chain (CLA)	Secretion	fungus	NH_4^+	upregulated	no	downregulated
16	Ran-specific GTPase-activating protein 1	Secretion	fungus	NH_4^+	unclear	yes	unclear
20	Outer membrane autotransporter putative	Secretion	amoeba	NH_4^+	unclear	yes	downregulated
18	Cell division control protein 48 (CDP48)	Protein degradation	fungus	NH_4^+	upregulated	yes	unclear
23	Woronin body major protein (WB)	Stress response	fungus	NH_4^+	unclear	unclear	unclear
11	Thiol-specific antioxidant protein 3	Stress response	fungus	NO_3^-	no	no	upregulated
8	Hypothetical protein DSC_01120	Stress response	bacteria	NO_3^-	upregulated	yes	downregulated
1	Malate dehydrogenase (MDH)	Respiration	algae, fungus	NO_3^-	unclear	unclear	unclear
9	ATP synthase subunit beta (ATPase)	Respiration	fungus	NO_3^-	no	yes	downregulated at 64 kg
25-26	Malate dehydrogenase (MDH)	Respiration	algae, fungus	NH_4^+	upregulated	yes	upregulated at 64 kg
21	NADH-quinone oxidoreductase (NADHox)	Respiration	fungus	NH_4^+	unclear	unclear	unclear



MOLECULAR RESPONSE

The photobiont showed upregulation of energetic metabolism and photosynthesis → Analysis of chlorophyll *a* fluorescence parameters showed no impairment of algal functionality

The majority of the under/over expressed proteins belong to the fungus and are involved in protein transportation and regulation (specific tolerance mechanisms?) → Lichen species with the same photobiont can have different tolerance degrees to N

Several protein showed a bell-shaped curve with highest expression at the intermediate concentration (existence of a threshold for N) → Fertilizing effect of a small amount of traffic related N; Repeated exposures can exacerbate the toxic effects of N

Upregulation of enzymes involved in protein degradation in case of NH_4^+ but not NO_3^- (futile cycle like in higher plants?) → NH_4^+ is more harmful to lichens than NO_3^-

ECO-PHYSIOLOGICAL OBSERVATIONS