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Rapid report

Is vanadium a biometal for boreal cyanolichens?

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Summary

- Molybdenum (Mo) nitrogenase has long been considered the predominant isoenzyme responsible for dinitrogen fixation worldwide. Recent findings have challenged the paradigm of Mo hegemony, and highlighted the role of alternative nitrogenases, such as the vanadium-nitrogenase.
- Here, we first characterized homeostasis of vanadium (V) along with other metals *in situ* in the dinitrogen fixing cyanolichen *Peltigera aphthosa*. These lichens were sampled in natural sites exposed to various levels of atmospheric metal deposition. These results were compared with laboratory experiments where *Anabaena variabilis*, which is also hosting the V-nitrogenase, and a relatively close relative of the lichen cyanobiont *Nostoc*, was subjected to various levels of V.
- We report here that V is preferentially allocated to cephalodia, specialized structures where dinitrogen fixation occurs in tri-membered lichens. This specific allocation is biologically controlled and tightly regulated. Vanadium homeostasis in lichen cephalodia exposed to various V concentrations is comparable to the one observed in *Anabaena variabilis* and other dinitrogen fixing organisms using V-nitrogenase.
- Overall, our findings support current hypotheses that V could be a more important factor in mediating nitrogen input in high latitude ecosystems than previously recognized. They invite the reassessment of current theoretical models linking metal dynamics and dinitrogen fixation in boreal and subarctic ecosystems.

Introduction

Biological nitrogen fixation (BNF) is the main source of new nitrogen (N) for the biosphere, accounting for up to 97% of N input in unmanaged terrestrial ecosystems (Vitousek *et al.*, 2002; Galloway *et al.*, 2004; Houlton *et al.*, 2008; Wang *et al.*, 2010). This reaction is catalysed by the enzyme nitrogenase (Nase) in few prokaryotes. Three nitrogenases have been identified so far (Reed *et al.*, 2011). In N_2 fixers associated with higher plants, only the molybdenum (Mo) dependent nitrogenase (Mo-Nase) has been identified. However, in many other N_2 fixers two additional isoenzymes have been reported (Rehder, 2000; Boyd *et al.*, 2011; Reed *et al.*, 2011); the vanadium (V) dependent nitrogenase (V-Nase) and iron-only dependent nitrogenase (Fe-Nase). The role of these alternative nitrogenases (V-Nase and Fe-Nase) in natural habitats has been mostly overlooked, because they are found in communities that were not considered major contributors to N inputs. In recent years, N_2 fixation associated with mosses and lichens has captured the interest of the scientific community for its importance toward global N input in high latitude ecosystems (Crittenden & Kershaw, 1978; DeLuca *et al.*, 2002; Reed *et al.*, 2011; Elbert *et al.*, 2012). Within this context, it is imperative to characterize the role of alternative nitrogenases in these biomes. Various observations suggest that alternative nitrogenases could play an important role in N_2 fixation in these cold environments. Molybdenum limitation on free-living and symbiotic N_2 fixers is now well documented (Horstmann *et al.*, 1982; Silvester, 1989; Barron *et al.*, 2009), and the genes coding for the alternative nitrogenases have been found in most ecosystems (Betancourt *et al.*, 2008). More recently, V-Nase has been found in cyanolichens (Hodkinson *et al.*, 2014). These findings challenge the traditional view of Mo hegemony on N input and justify a re-evaluation of the importance of alternative nitrogenases in natural habitats (Reed *et al.*, 2011). If alternative means of N_2 fixation are more common and active than recognized so far, it would deeply affect our conceptual models relating to N_2 fixation and trace metal dynamics in the environment (Reed *et al.*, 2011). Not only would this affect our fundamental understanding of N_2 fixation and N cycling, it would also impact our ability to predict ecosystems response to global climate change.

Approaches to assess the importance of V for N_2 fixation in natural habitats, include monitoring V-Nase activity *in situ* by gas chromatography (Dilworth *et al.*, 1987) or quantifying the expression of genes coding for the V-Nase. None of these approaches are trivial. For instance, the low N_2 fixation activity

by most N_2 fixers jeopardizes the detection of V-Nase activity through the monitoring of ethylene and ethane production during acetylene reduction assays (Dilworth *et al.*, 1987). But more importantly, both approaches might be compromised by the versatility in the use of the nitrogenases; many biotic and abiotic parameters influence the expression, efficiency and use of the various nitrogenases. Thus, the odds of detecting V-Nase activity in field samples are spatiotemporally dependent. This might explain why attempts to provide direct evidences for V-mediated N_2 fixation in natural habitats have failed so far.

Here, we opted for an alternative approach relying on the principles of metal homeostasis in order to overcome these challenges. The purpose of homeostasis is to maintain internal conditions independently of variations of the external environment. Internal concentrations of essential biometals are often tightly regulated, and maintained close to metabolic optima. Unlike gene expression or metalloenzyme activity, which can be influenced by quickly changing external parameters (i.e. temperature), homeostasis of many biometals is fairly stable. Thus, for alternative nitrogenases, assessing the biological importance of a metal by characterizing its homeostasis is justifiable compare to detecting the activity of the enzymes directly.

We studied V homeostasis in the tri-membered lichen *Peltigera aphthosa*, a ubiquitous cyanolichen in boreal regions. Our choice was motivated by the following reasons. First, the homeostasis-based approach is particularly relevant for V. Other than its role in N_2 fixation, V has no significant biological purposes in terrestrial organisms and can easily become toxic (Crans *et al.*, 2004). Expectations about V homeostasis are thus very different depending on whether or not V is used for N_2 fixation. For instance, homeostasis has been shown to be more tightly controlled for V than Mo in the free-living N_2 fixer *Azotobacter vinelandii* (Bellenger *et al.*, 2011) owning the three Nases. Second, *P. aphthosa* offers a particularly suitable model for the study of metal homeostasis. This lichen is formed by the symbiotic association of: a fungus (*P. aphthosa,* mycobiont); a carbon (C) fixing unicellular green alga (*Coccomyxa* sp., phycobiont) located in an algal layer; and a N_2 fixing cyanobacterium (*Nostoc* sp., cyanobiont) restricted to small specialized structures called cephalodia (Supporting Information Fig. S1). While this question has been poorly addressed so far for lichens (Goyal & Seaward, 1981), the allocation and concentration of metals within the lichen thallus are likely to be tightly regulated, reflecting the specific metal requirements of the green alga and cyanobacterium located in specific compartments. Finally, from a theoretical perspective, the alternative V-Nase presents several advantages over the Mo-Nase for lichens in boreal and subarctic areas. At low temperatures (< 15°C), the repression of alternative Nase genes by Mo stops (Walmsley & Kennedy, 1991) and the activity of V-Nase is higher than Mo-Nase (Miller & Eady, 1988). It is also worth reminding that Mo is the least abundant biometal in the earth's crust (Wedepohl, 1995) and that Mo has been reported to be limiting N_2 fixation by free living N_2 fixers and lichens (Horstmann *et al.*, 1982; Silvester *et al.*, 1982; Barron *et al.*, 2009; Jean *et al.*, 2013). Vanadium is 50–200 times more abundant in the earth's crust than Mo (Wedepohl, 1995). Thus, the V-Nase could be particularly relevant for species that live in high latitude ecosystems and rely on atmospheric depositions for their mineral nutrition such as *P. aphthosa* and other cyanolichens. The study of V homeostasis in thalli of the tri-membered cyanolichen *P. aphthosa* collected in high latitude habitats enhances our ability to unveil the biological importance of V in N_2 fixation.

Materials and Methods

Samples collection

Specimens of *Peltigera aphthosa* (L) Willd. were collected in northern Québec, Canada (August 2011, $n = 29$, SN6-SN9 and E0-E200), in southern Québec, Canada (Fjord du Saguenay (FdS), June 2013, $n = 9$), in Alaska, USA (Fairbanks, August 2011, $n = 6$, 64°50.37′N 147°43.23′W) and Krasnoyarsk territory, Russia (Stolby Reserve, June 2012, $n = 5$, 55°53.15′N 92°46.22′E) (Supporting Information Fig. S1). All thalli were manually cleaned for foreign materials (bryophytes and leaves) with forceps and then kept dry in opaque paper bags at room temperature until further procedures.

Thallus washing procedure

Lichens were washed to remove metals present at the surface of the thallus (nonspecific absorption sites and particles). Then, 0.1–0.2 g of each sample of lichen were washed twice for 30 min with 20 ml of an oxalate/EDTA (0.1 M/0.05 M) solution (Tang & Morel, 2006) and once with 5 ml of MilliQ® (MQ) water. Samples were allowed to dry in an oven at 70°C before weighting. The use of nickel chloride ($NiCl_2$) wash (a very common washing technique) was avoided to prevent metal contamination of the sample. High concentration of nickel (Ni) and chlorine (Cl) is particularly problematic for inductively coupled plasma mass spectrometry (ICP-MS) analysis as it can interfere with the quantification of trace metals such as Mo and V. Oxalate/EDTA solutions are commonly used in our laboratory to wash various biological materials before elemental analysis. It has proven efficiency on bacteria, algae, and plants. EDTA wash are also commonly used with lichens (Branquinho, 1994). Thus, oxalate/EDTA wash was also selected for consistency purposes.

Separation of the symbionts

Cephalodia were manually dissected under a binocular microscope using razor blades and forceps. All cephalodia were freed of algal layer and fungal hypha debris after excision. Each sample was washed twice with an oxalate/EDTA (Tang & Morel, 2006) solution (0.1/0.05 M), then once with MQ (grade I) water. Clean cephalodia were allowed to dry at 70°C in an oven before weighting. Algae from Québec and Alaska were separated using a density separation on Percoll® gradient method modified from Gasulla *et al.* (2010); no Tween 20® was used to prevent metal contaminations and three additional washes (oxalate/EDTA (0.1 M/0.05 M)) were performed at the end of the procedure. Isolated algae were dried and kept at −80°C until further manipulations.

Elemental analysis

Unwashed and washed thalli were digested using a microwaved assisted procedure. Briefly, 200–500 mg of each sample was transferred into a Teflon tube to which 10–12 ml of nitric acid (70%, trace metal grade, Fisher Scientific, Ottawa, Canada) were added. The samples were then digested at 170°C for 50 min on a MarsXpress microwave digester (CEM, Buckingham, UK). Cephalodia and algae were digested in 1.5–2 ml of concentrate nitric acid (70%, trace metal grade, Fisher scientific) at 60°C for 48 h. Samples were diluted with MilliQ® water, spiked with internal standards (40 ppb Rh, 2 ppb In) and analysed for their concentrations of aluminium (Al), titanium (Ti), phosphorus (P), Mo, V, magnesium (Mg) and lead (Pb) by ICP-MS (XSeries® II, Thermofisher). Metal contents are reported in $mol_{metal}\ mol_P^{-1}$ (Figs 1, 2) and in $\mu g_{metal}\ g_{dryweight}^{-1}$ (Supporting Information Fig. S2). Both ratios provide similar conclusions. Metal contents in the alga are reported only in $mol_{metal}.mol_P^{-1}$, because the low amount of extracted algae did not allow an accurate measurement of the dry mass.

Vanadium addition experiments

Four lichen thalli from the same site in northern Québec (0.1 g) were incubated each in 20 ml of a different solution of Na_2VO_4/Na_2MoO_4 with concentration of V ranging from 10^{-7} to 10^{-4} M. Incubation lasted 20 min with agitation and light, then thalli were rinsed with MQ water before being left to dry. The addition was repeated twice within 2 wk and in duplicate with lichens from two different sites (SN7 and SN9; Supporting Information Fig. S1). Lichens were kept dry in the dark for six months, then cephalodia were excised and thalli and cephalodia were washed with ox/EDTA before elemental analysis (see earlier). A second experiment was conducted to refine data in the range of 10^{-8} to 10^{-6} M V exposure using thalli (triplicate from one site) from a location exposed to higher metal deposition (southern Québec, FdS; Supporting Information Fig. S1). Controls are corresponding values from the same sampling site without any addition.

Vanadium homeostasis of *Anabaena variabilis*

Anabaena variabilis (strain ATCC 29413) was cultured in different concentration of V (10^{-8}–10^{-4} M) at 15°C and 20°C in an eight-fold dilution of Allen and Arnon medium (Allen & Arnon, 1955) under a 12 : 12 h, night/day cycle in Infors HT Multitron shaker. Then, 10 ml of culture (OD_{730} *c.* 0.6) were centrifuged and washed three times with oxalate/EDTA (0.1 M/0.05 M) before digestion (see earlier). Results from different temperature were combined together.

Results and Discussion

Vanadium distribution within the lichen thallus

In order to determine whether or not V homeostasis is characteristic of a biometal essential for N_2 fixation in *Peltigera aphthosa*, we first examined the distribution of metals within the lichen thallus. Our hypothesis was that the repartition of elements within the different compartments of the thallus (i.e. cephalodia containing *Nostoc* vs the remaining part of the thallus containing the algal layer) will be characteristic of their metabolic function. We measured metal contents in the whole thallus and in each symbiont (green alga and cyanobacterium) separately. We selected sites in north-eastern Canada (Supporting Information Fig. S1) that were as far as possible from active mining sites to avoid metal contaminations that can interfere with homeostasis of trace metals. In these localities, metal concentrations in lichens are among the lowest reported on earth. Magnesium, which is associated with ATP in all living cells, is consistent with the

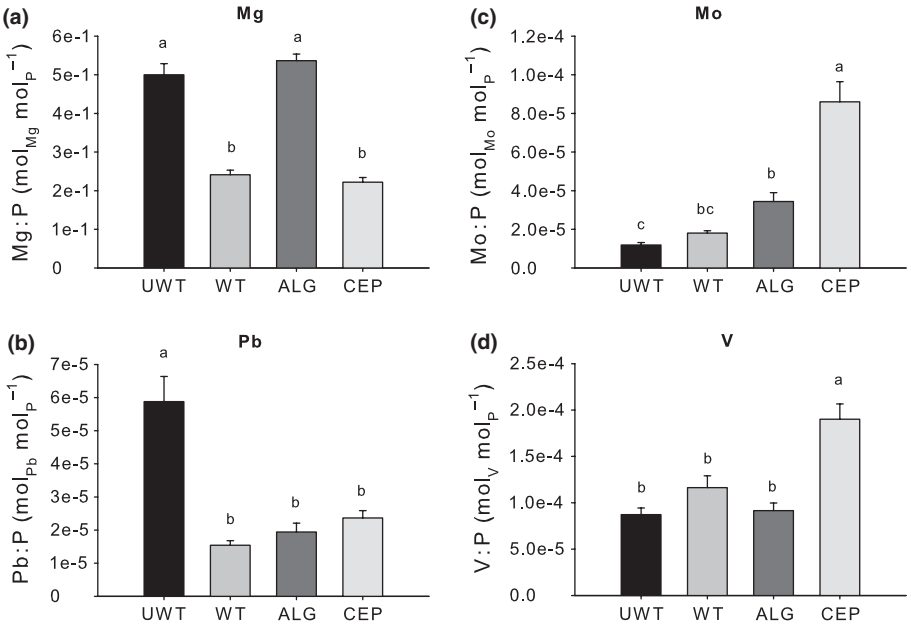

Fig. 1 Distribution of metals within *Peltigera aphthosa* thalli from Québec, Canada. Metal contents (a) magnesium (Mg), (b) lead (Pb), (c) molybdenum (Mo), and (d) vanadium (V), are reported in metal to phosphorus (P) molar ratios ($mol_{metal}\ mol_P^{-1}$). UWT, unwashed thallus (i.e. exposition); WT, ox/EDTA-washed thallus; ALG, alga; CEP, cephalodia. Error bars represent standard errors (n = 28, 29, 26 and 27 for UWT, WT, ALG and CEP, respectively). Identical letters above bars indicate no significant difference according to a one-way analysis of variance (ANOVA), Holm-Sidak (Supporting Information Table S1). Data in $g_{metal}\ g_{dryweight}^{-1}$ are available in Supporting Information Fig. S2.

distribution of an essential biometal; it is present in washed thalli, alga and cephalodia. The higher Mg content in the alga, compared with cephalodia and washed thalli, could be related to the role of Mg in C fixation (the core metal in chlorophyll) (Fig. 1a). Looking at Pb, a metal with no biological purpose and potentially toxic, we observed that it is mostly present at the surface of the thallus (easily washed, Fig. 1b). Within the thallus, Pb is equally distributed among partners; no specific translocation of the metal occurs. This likely reflects protective mechanism that prevents this nonessential and toxic metal to enter the thallus. On the contrary, we observed that Mo is highly concentrated in cephalodia, and to a lesser extent in the alga (Fig. 1c). This distribution of Mo likely reflects the importance of Mo for N_2 fixation by the cyanobacterium *Nostoc* and nitrate reduction by the alga *Coccomyxa* (Williams & Frausto da Silva, 2002). As for Mo, we found that cephalodia are preferentially enriched in V, where it is 2–3 times more concentrated than in the alga and the whole thallus (Fig. 1d). Vanadium is highly toxic when not required to sustain N_2 fixation. In such situation, it is fair to assume that the distribution of V should be similar to the one of Pb. The preferential allocation of V to the cephalodia, comparable to Mo, suggests its biological importance for N_2 fixation in *P. aphthosa*.

Vanadium to titanium ratios

We then evaluated whether or not this preferential allocation of V to cephalodia is a biologically driven process. A simple way to address this question is to measure the relative abundance of titanium (Ti) and V in the different compartments of the lichen thallus. The main source of V for *Peltigera aphthosa* is through atmospheric deposition of particles. These deposits have characteristic V : Ti ratios, which are often used to characterize their origin in biomonitoring studies (Addison & Puckett, 1980). High correlation between terrigenous metals, such as Ti and V, also indicates the particulate origin of the metals. Here we compared the correlation between V and Ti abundances in the unwashed thallus (representative of metal exposure), in the washed thallus (mostly representative of the fungus which represent > 90% of the biomass), in the alga and in cephalodia (Table 1). We observed that Ti and V are correlated in the unwashed thallus, washed thallus and alga (r^2 *c.* 0.48, 0.92 and 0.64, respectively). This suggests that the concentration of Ti and V in washed thallus and alga is still representative of particle deposition (unwashed thallus). However, the lower V : Ti ratio in the alga and washed thalli compared with unwashed thalli shows that V is less accumulated than Ti in these compartments (Table 1). This result is consistent with the high toxicity of V and the necessity to limit its accumulation in living cells.

Contrary to the alga, washed and unwashed thalli, no correlation was detected between Ti and V in the cephalodia (Table 1). Therefore, the relative content of V and Ti is not representative of particle deposition in the cephalodia, but rather reflects the preferential allocation of V compared with Ti in this compartment (Table 1). While the concentrations of Ti in the washed and unwashed thalli are similar to the concentrations in the cephalodia, the concentration of V is significantly higher in the

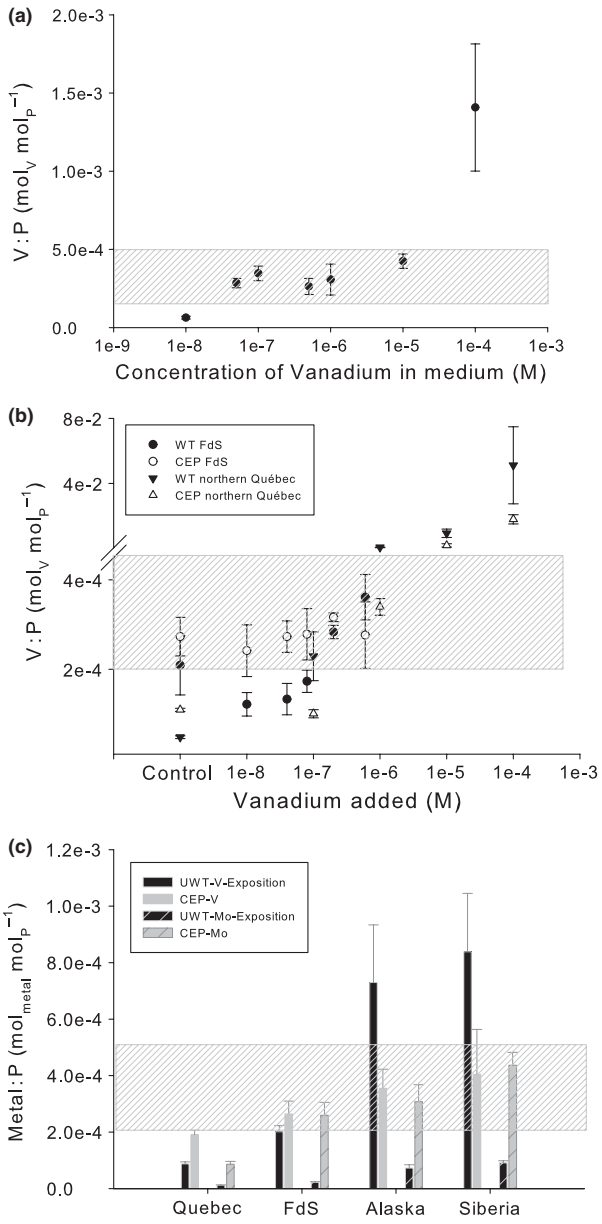

Fig. 2 Cellular response of *Anabaena variabilis* to increasing extracellular vanadium (V) concentration. (a) Effects of increasing V concentration on V cellular concentrations in *Anabaena variabilis* growing diazotrophically (in absence of molybdenum (Mo)). Error bars are standard errors $n = 2$. (b) Effects of artificial V exposures on *Peltigera aphthosa* from pristine location (northern Québec, Canada; triangles) and Fjord of Saguenay (FdS, Canada; circles). Vanadium contents are reported in metal to phosphorus (P) molar ratios ($mol_{metal}\ mol_P^{-1}$). Washed thalli (WT; close symbols) and cephalodia (CEP; open symbols). The grey box represents the range of optimal intracellular V : P ratio from (a). Error bars are standard errors ($n = 2$ for northern Québec, $n = 3$ for FdS). (c) Comparison of V and Mo contents in specimens of *P. aphthosa* from pristine (northern Québec) and metal exposed locations (Fairbanks, Alaska, USA; FdS, Québec, Canada; and Stolby Reserve, Siberia, Russia). Unwashed thallus (UWT; black bars) and cephalodia (CEP; grey bars). The grey box represents the range of intracellular V optima from (a). V, solid bars; Mo, hatched bars. Error bars are standard errors; $n = 28$, and 27 for UWT and CEP in Québec, $n = 6$ for Alaska, $n = 9$ for FdS and $n = 5$ for Russia). Grey shaded boxes in (a–c) represent the range of optimal intracellular V:P ratio ($mol_V\ mol_P^{-1}$) for the diazotrophic growth of *Anabaena variabilis* (a) and other N_2 fixers (i.e. *Azotobacter vinelandii* in Bellenger *et al.*, 2011).

Table 1 Content and ratio of vanadium (V) and titanium (Ti) for different compartments of *Peltigera aphthosa*

		Cephalodia		Alga		Washed thalli		Unwashed thalli	
		Ti	V	Ti	V	Ti	V	Ti	V
Metal content (ppm)	Content	2.57[a]	0.41[A]	–	–	3.31[a]	0.15[B]	2.65[a]	0.21[B]
	SE	0.46	0.04	–	–	0.40	0.01	0.54	0.02
Paired-ratio (mol:mol) $n = 23$	Ratio V:Ti	2.74E-01[a]		4.36E-02[b]		5.51E-02[b]		1.26E-01[c]	
	SE	4.42E-02		5.27E-03		4.80E-03		2.13E-02	
Linear regression	r^2	<0.001		0.64		0.92		0.48	
	n	27		26		29		28	

V and Ti contents are expressed in $\mu g_{element}\ g_{ODWlichen}^{-1}$ (letters represent results of analysis of variance (ANOVA) Holm-Sidak $P = 0.05$), V:Ti (mol:mol) ratios from paired samples (only thalli with all compartments represented were kept, letters represent results of paired t-test between compartment, $n = 23$) and correlation factor (r^2) from linear regression of Ti:phosphorus (P) (mol:mol) ratio and V:P (mol:mol) ratio.

cephalodia (> two fold). Similar conclusions can be made if considering another terrigenous element as reference, such as Al (Supporting Information Table S2). The mechanisms involved in the redistribution of V and its selective recruitment by cephalodia remains unclear, however this phenomenon is clearly biologically driven. Recently, lichen substances have been shown to be involved in the homeostasis of several metals in *Hypogymnia physodes* (Hauck, 2008). Analogous to vanadophores and molybdophores, that is, low molecular weight ligands specialized in the recruitment and homeostasis of Mo and V, found in N_2 fixing bacteria (Bellenger *et al.*, 2008), some lichen substances could be involved in the homeostasis of Mo and V.

Natural and artificial vanadium exposures

We further examined V homeostasis by characterizing the effect of increasing V exposure on intracellular V concentrations in pure culture of *Anabaena variabilis*, a cyanobacterium closely related to *Nostoc*. *Anabaena variabilis* was grown under diazotrophic conditions in presence of V and in absence of Mo (Fig. 2a). Intracellular V concentrations are maintained in a narrow range ($2–5 \times 10^{-4}$ $mol_V\ mol_P^{-1}$) over a large array of external V concentrations (from 5×10^{-8} to 10^{-5} M V). These results are comparable to those reported in other N_2 fixing organisms, such as *Azotobacter vinelandii* in which V is limiting at $[V]_{medium} < 10^{-7}$ M, is toxic at $[V]_{medium} > 10^{-5}$ M, and is optimal for N_2 fixation at *c.* 5×10^{-4} mol mol_P^{-1} (Bellenger *et al.*, 2011). Then, we measured the effect of V exposure on V concentration in cephalodia of *Peltigera aphthosa* in a laboratory setting. Our goal was to determine whether or not V homeostasis in *P. aphthosa* is similar to *A. variabilis*. Below 10^{-6} M V, V concentrations in cephalodia are maintained in a narrow range ($1–3 \times 10^{-4}$ $mol_V\ mol_P^{-1}$), close to the V optimum (Fig. 2b; Bellenger *et al.*, 2011), independently of the external conditions (V concentration). Above 10^{-5} M V, V concentrations in cephalodia significantly increase with increasing V exposure. Finally, we compared specimens from our pristine locations in Québec with specimens collected in areas affected by human activity (southern Québec, Alaska and Siberia), that is, with significantly higher total metal concentrations (unwashed thalli). This set of samples represents a field gradient in metal exposure. While metal exposure cannot be tightly controlled as in the laboratory experiment, this set of samples is more representative of the response of *P. aphthosa* to long-term metal exposure in natural habitats. Both Mo and V increase with total exposure in the field (Fig. 2c). While total Mo and V increase by a factor of eight to ten, Mo content in cephalodia increases by a factor of five but V only by a factor of *c.* two. It is worth noting that V concentrations in cephalodia are higher than V exposures (unwashed thallus) in our pristine specimens. Whereas, in samples collected in contaminated areas (especially Alaska and Siberia), V concentrations in cephalodia are lower than V exposures. This rules-out the passive allocation of V to cephalodia in response to V exposure. More interestingly, both Mo and V reach comparable concentrations in the cephalodium (*c.* 4×10^{-4} mol mol_P^{-1}) under high metal exposure conditions (Fig. 2c). This concentration is similar to the optimum V (and Mo) concentration required for the diazotrophic growth of *A. variabilis* (Fig. 2a) and other cyanobacteria (Glass *et al.*, 2010) and soil N_2 fixers (Bellenger *et al.*, 2011). Overall, the results from Fig. 2 show that V homeostasis in cephalodia of *P. aphthosa* is characteristic of an essential biometal for N_2 fixation, that is, V concentrations are maintained close to the biological optimum for N_2 fixation under a wide array of external conditions. As a final note we want to highlight that in samples collected in pristine locations (northern Québec), Mo concentrations in cephalodia are below the optimum Mo requirement to sustain N_2 fixation (Bellenger *et al.*, 2011). Whereas, V concentrations are close to the optimal concentrations observed in this study (Fig. 2a,b) and by Bellenger *et al.* (2011). This illustrates that V could be an important nutrient for N_2-fixing cyanobacteria and cyanolichens in northern regions.

Conclusions

To the best of our knowledge, this is the first attempt to characterize metal homeostasis in lichen thalli containing a cyanobacterium, collected in their natural habitats. We show here for the first time that V homeostasis in cyanolichens present the characteristic of an essential biometal for N_2 fixation. More specifically, our results demonstrates that: V is preferentially allocated to cephalodia, the structure specialized in hosting *Nostoc*, and where N_2 fixation takes place; this allocation is biologically driven; and V content achieved under various metal exposures (replete conditions) are comparable to the V optimum observed in pure culture

of cyanobacteria and in other N_2 fixers using V-Nase. Our results highlight the biological importance of V in cyanolichens. They strongly suggest that not only *Peltigera* and other cyanolichens own the V-Nase (Hodkinson *et al.*, 2014), but that V very likely plays an active role in N_2 fixation in boreal ecosystems. The exact conditions at which V comes to play, and the relative contribution of the V-Nase, compared with Mo-Nase, remains to be elucidated. Further research is required to determine the importance of V in other cyanolichen species and other climatic regions. Overall, our results incite a reconsideration of the role V plays in N_2 fixation by cyanolichens in warm, and cold, ecosystems, and encourage a reassessment of current theoretical models linking metal dynamics and N_2 fixation.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Localization of the sampling sites in Québec, Canada, Alaska, USA, and Siberia, Russia; as well as a picture of the tri-membered lichen *Peltigera aphthosa* taken in the field.

Fig. S2 Comparison of metals content in ppm (μg_{metal} $g_{dryweight}^{-1}$) between unwashed lichens, (i.e. exposition) (UWT), ox/EDTA-washed lichen thalli and cephalodia.

Table S1 *P*-values of one-way analysis of variance (ANOVA) Holm-Sidak from Fig. 1

Table S2 Content and ratio of vanadium (V) and aluminium (Al) for different compartments of *Peltigera aphthosa*

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