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The effectiveness of cyanobacteria nitrogen fixation: Review of bench top and pilot scale nitrogen removal studies and implications for nitrogen removal programs

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1 **The effectiveness of cyanobacteria nitrogen fixation: Review of bench top and pilot scale**
2 **nitrogen removal studies and implications for nitrogen removal programs**

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Abstract: One of the primary goals of eutrophication management of freshwater systems is to lower the risk of cyanobacterial blooms. This is typically accomplished at the watershed scale by placing controls on phosphorus (P) discharge from point sources. However, several researchers have questioned the predominance of the P management paradigm, arguing that dual nitrogen (N) and P controls would be more effective at preventing cyanobacteria blooms than P controls alone. This hypothesis is predicated in part on the hypothesis that if cyanobacteria are starved of N, which is an essential nutrient, cyanobacteria N_2 fixation rates will not be high enough to maintain growth rates and biomass yields at or near previous levels. However, several single species cultures of heterocystous cyanobacteria directly examining the effect of removing N show that, when deprived of ammonium and nitrate, N_2 fixing cyanobacteria compensate biochemically for the high energy cost of fixation when supplied with sufficient nutrients other than N. Biomass and growth rates were only slightly different under N_2 than when grown under ammonium and nitrate, which is consistent with observations from the long-term experimental fertilization of Lake 227. Collectively, these bench top and pilot scale studies suggest that N control programs will not have a major impact on the magnitude of freshwater cyanobacteria blooms, although cyanobacteria species composition and toxin production might be affected.

Keywords: eutrophication, nitrogen fixation, cyanobacteria, nutrient management, proof of concept

Introduction: The link between excessive phosphorus (P) loading to aquatic systems and cyanobacteria blooms, low water clarity and depleted dissolved oxygen in hypolimnetic waters is well documented and widely recognized, providing the basis for generally successful management actions via P controls, primarily from point sources (Dolan and Chapra 2012; Schindler et al. 2016). Indeed, limnologists and managers consider P management to be of paramount importance. However, not all P management programs have successfully mitigated cyanobacterial blooms, at least in the short term (Schindler et al. 2016), and several authors have questioned the predominance of the P management paradigm, arguing that dual nitrogen (N) and P controls (i.e., supplementing P controls with N controls) would be more effective at preventing cyanobacterial blooms than P controls alone (Lewis and Wurtsbaugh 2008; Paerl 2009; Scott and McCarthy 2010; Paerl et al. 2011; Hellweger et al. 2016). In many cases, this means adding N controls to existing point source P control programs.

While external and internal P loads are not supplemented by biological inputs of P from a gas phase, N inputs are supplemented by N_2 fixation if supplies of ammonia and nitrate limit growth. The idea that adding N to existing P control programs will mitigate the severity of blooms is predicated on the argument that if cyanobacteria are starved of N, cyanobacteria N_2 fixation rates will not be high enough to maintain cyanobacteria biomass at or near previous levels (Lewis and Wurtsbaugh 2008; Scott and McCarthy 2010; Paerl et al. 2014). The dual or co-limitation idea has been disputed by Schindler and his colleagues (Schindler et al. 2008; Schindler 2012; Schindler et al. 2012, 2016) who argue that N controls will have little impact, other than to shift the dominant cyanobacteria to N_2 -fixing taxa if the system becomes N-limited.

Despite criticisms of the dual nutrient hypothesis, support for dual nutrient controls has grown (e.g., United States Environmental Protection Agency 2015), an idea which could influence expenditure of public funds.

This brief review examines the claim that N control is an effective method to mitigate cyanobacteria blooms with a focus on adaptation by heterocystous N_2 fixers to N removal in laboratory cultures and a long-term lake fertilization experiment. The potential for N control to affect toxin production by cyanobacteria (Gagnon and Pick 2012; Harke et al. 2015; Gobler et al. 2016; Pick 2016), marine coastal eutrophication (Smith 2003; Scavia and Bricker 2006; Diaz and Rosenberg 2008) and other issues (Schindler et al. 2016) are recognized as topics of concern but are not addressed here.

Proof of concept: Proof of concept, also known as proof of principle, is defined as a small scale demonstration with the aim of determining whether a concept will work as proposed (Dym et al. 2014) - the idea being to test a product before bringing it to market. Since every new concept, or hypothesis, comes with an unknown risk of failure, controlled tests at bench top and larger pilot scales can help determine if the concept has the potential to achieve the desired objective at an industrial scale (or watershed scale in this case), whether the benefits are worth the costs and thus whether the concept should be employed at the watershed scale. Proof of concept is an approach widely used in product development in areas as diverse as engineering (Dym et al. 2014) and drug development (Corr and Williams 2009).

Eutrophication managers will want to know if an investment in N controls will likely result in declines in cyanobacteria bloom magnitude or duration that are large enough to justify the cost. The most valid proof of concept test in this case is direct N removal from experimental systems because it most closely resembles N control programs at a watershed scale. Inferences from relatively indirect evidence not employing N removal, such as descriptive correlations of field observations, N addition bioassays, computational modeling, etc., on the hypothesized effectiveness of N controls (Lewis and Wurtsbaugh 2008; Paerl 2009; Scott and McCarthy 2010; Paerl et al. 2011; Hellweger et al. 2016) can be useful in the early stages of developing an idea, but the risk of misinterpreting indirect evidence is much larger than the risk of misinterpreting direct tests of N removal. Therefore, direct N removal tests should be seen as critical elements of the decision making process because they lower uncertainty.

To what extent do N₂ fixing cyanobacteria adapt to loss of N? One argument for assuming that N₂ fixation rates can be safely ignored by N control programs is based on the high energy cost of N₂ fixation (Stam et al. 1987), a cost high enough that it might be presumed to significantly limit growth rates relative to growth in the presence of ammonium and nitrate if a large fraction of photosynthetic energy is diverted into N₂ fixation at the expense of biosynthesis of other compounds. Total costs stem from the cost of the fixation pathway itself and the cost of minimizing the dissolved oxygen concentration inside the heterocyst. Heterocysts use several passive and active strategies to deal with the latter including a thick impermeable membrane barrier around the cell, not using photosystem II, increased cell size and enhanced respiration rate (Berman-Frank et al. 2007; Flores and Herrero 2010; Inomura et al. 2017). A total cost high

93 enough to lower growth rate could mean a delay in reaching the maximum biomass which is
94 limited by availability of a nutrient other than N₂.

95
96 Cyanobacteria physiologically adapt to the absence of nitrate and ammonium in laboratory
97 cultures. The gross cellular composition of the N₂ fixer *Nostoc paludosum* shifted when the sole
98 source of extracellular N was N₂ (Vargas et al. 1998) with the proportion of N-rich protein
99 decreasing and the proportion of N-poor, energy-rich lipids and carbohydrates increasing (Table
100 1). The shift to less protein when the only N source was N₂ was also observed in *Anabaena*
101 *variabilis* (Sanz et al. 1995) and *Nostoc muscorum* (Bagchi et al. 1985). This strategy could
102 conserve N and provide energy for dark N₂ fixation when photosynthesis-generated ATP is
103 unavailable. Dark fixation appears common in cyanobacteria (Fay 1976 and 1992; Jensen and
104 Cox 1983; Oh et al. 1991; Bebout et al. 1993).

105
106 Several bench top studies have shown that N starvation results in very modest changes in growth
107 rates and biomass yields in single species laboratory cultures of heterocystous N₂ fixing
108 cyanobacteria. Growth rates and yields in nutrient-rich batch and semi-continuous cultures
109 without ammonium and nitrate were only slightly different, about 10%, than in media with
110 ammonium and nitrate (Table 1) (Allen and Arnon 1955; Schlangstedt et al. 1987; Bagchi et al.
111 1985; Vargas et al. 1998). In fact, growth rates and yields were higher in some experiments using
112 only N₂, for example, when measured as dry weight by Allen and Arnon (1955) and cell number
113 by Bagchi et al. (1985). Major declines in growth rate can, however, be achieved by depriving
114 cyanobacteria of cobalt (Holm-Hansen et al. 1954; Reisenauer 1960) and molybdenum (Allen
115 and Arnon 1955), which are essential for fixation. Kratz and Myers (1955) showed that depriving

Nostoc muscorum of cobalt lowered the growth rate in batch culture by 26% when the only N source was N₂. Presumably, growth still occurred because cobalt was added as an impurity in other reagents.

Cylindrospermopsis raciborskii biomass in steady state (flow through) chemostats was 5 to 70% lower under N₂ than when grown with nitrate and ammonium at dilution rates below 0.75 d⁻¹ with negligible biomass at dilution rates ≥ 1.0 d⁻¹ unless supplied with ammonium or nitrate (Kenesi et al. 2009). This study suggests that, when starved of N at high dilution rates, *Cylindrospermopsis raciborskii* diverts a significant fraction of its energy into N₂ fixation instead of organic carbon synthesis. Note that unlike batch cultures, growth rates in chemostats are forced to match the dilution rates making direct comparison between these two methods difficult. These laboratory results suggest that energy compensation mechanisms are efficient and N₂ fixation can supply most, if not all, of the cyanobacteria N requirements in cultures if fixation is not limited by essential micronutrients. N₂ fixation rates may not be able to support very high growth rates (Kenesi et al. 2009), but the batch and semi-continuous culture studies cited herein (Allen and Arnon 1955; Schlangstedt et al. 1987; Bagchi et al. 1985; Vargas et al. 1998) indicate that fixation can probably support lower growth rates more common in eutrophic lakes (e.g., Robarts and Zohary 1987).

These laboratory studies are small scale, having taken place in glassware without sediments, hypoxic bottom waters, algal competitors or grazers over a period of days to a few weeks. While it would not be wise to predict the outcome of long-term, lake-scale N removal programs based

solely on these experiments, they provide a clear answer to a specific physiological issue germane to the debate, demonstrating that N removal does not have a large impact on growth rate and biomass (yield) in single species systems under controlled conditions. Factors operating at the lake scale over longer periods of time may, however, interact to affect N₂ fixation rates necessitating investigation at the whole-lake scale.

Cyanobacteria compensate for the high cost of N₂ fixation in experimentally fertilized Lake 227. This long-term whole-lake fertilization experiment began in 1969 with additions of nitrate and phosphate during the ice-free seasons (Schindler 2008). While phosphate additions have remained constant since 1969, nitrate was lowered in 1975 and then stopped completely in 1990. There were no significant trends in the annual average chlorophyll *a* or phytoplankton biomass over a 41 year period between 1969 and 2009, regardless of the N:P ratio used in loading. There were no significant declines in cyanobacteria biomass over the entire fertilization period or since 1990 (Paterson et al. 2011), although cyanobacteria biomass appears to have been more variable prior to 1990 (Schindler et al. 2008). After 1990, the phytoplankton community was dominated by N₂-fixing *Aphanizomenon* and the apparent annual N₂ fixation rate began to increase based on increasing heterocyst counts (Paterson et al. 2011), presumably in response to gradual depletion of legacy N stored in sediments. As pointed out by Schindler et al. (2016), “nitrogen fixation in 2011 exceeded the sum of fertilizer plus nitrogen fixation in any year before 1990.” These observations clearly show that the amount of N₂ fixed during the bloom period was not impaired and was enough to allow continued cyanobacteria bloom formation.

Conclusions: The efficiency of N₂ fixation has been one of the focal points of argument in the N removal debate. Collectively, the bench top and pilot scale direct proof of concept tests demonstrate that heterocystous N₂ fixers readily adapt to removal of N with at most a small cost to themselves when provided with adequate nutrients to fix N₂. This contrasts with claims based on indirect evidence that cyanobacteria fixation is inefficient and cannot sufficiently compensate for removed N.

If less than 100% of anthropogenic N was removed, a likely scenario for a watershed management program, the predicted impact on cyanobacteria should be less than 10%. In many lakes, biomass decreases of 10% or less would be well within year to year variation, for example, in Lake Erie (Bertani et al. 2016) and, therefore, would be undetectable. Based on direct N removal proof of concept tests, cyanobacteria bloom control does not provide a valid basis for N removal programs unless future N removal studies demonstrate otherwise.

A final point worth mentioning is that the definition of what constitutes an adequate body of evidence varies with the specific issue at hand. In a debate on N controls, single species cultures provide valuable evidence on the efficiency of growth of heterocystous cyanobacteria under N₂ as the sole source of N and should be an important part of the body of evidence under consideration. On the other hand, single species experiments that withhold (or limit) P from culture media would not be as meaningful in a debate on P controls because there are no biological gas phase inputs.

References

- 184 Allen, M.B. and Arnon, D.I. 1955. Studies on nitrogen-fixing blue-green algae. I. Growth and
185 nitrogen fixation by *Anabaena cylindrica* lemm. Plant Physiol. **30**: 366-372.
- 186 Bagchi, S.N., Sharma, R. and Singh, H.N. 1985. Inorganic nitrogen control of growth,
187 chlorophyll, and protein level in cyanobacterium *Nostoc muscorum*. J. Plant Physiol. **121**: 73-
188 81.
- 189 Bebout, B.M., Fitzpatrick, M.W. and Paerl, H.W. 1993. Identification of the sources of energy
190 for nitrogen fixation and physiological characterization of nitrogen-fixing members of a
191 marine microbial mat community. Appl. Environ. Microbiol. **59**: 1495-1503.
- 192 Berman-Frank, I., Quigg, A., Finkel, Z.V., Irwin, A.J. and Haramaty, L. 2007. Nitrogen-fixation
193 strategies and Fe requirements in cyanobacteria. Limnol. Oceanogr. **52**: 2260-2269.
- 194 Bertani, I., Obenour, D.R., Steger, C.E., Stow, C.A., Gronewold, A.D. and Scavia, D. 2016.
195 Probabilistically assessing the role of nutrient loading in harmful algal bloom formation in
196 western Lake Erie. J. Great Lakes Res. <http://dx.doi.org/10.1016/j.jglr.2016.04.002>
- 197 Brauer, V.S., Stomp, M., Rosso, C., van Beusekom, S.A.M., Emmerich, B., Stal, L.J. and
198 Huisman, J. 2013. Low temperature delays timing and enhances the cost of nitrogen fixation
199 in the unicellular cyanobacterium *Cyanothece*. The ISME Journal. **7**: 2105–2115.
- 200 Corr, P. and Williams, D. 2009. E. The Pathway from idea to regulatory approval: Examples for
201 drug development. In Lo, B. and Field, M.J. (editors). Conflict of interest in medical
202 research, education, and practice. Institute of Medicine (US) Committee on Conflict of
203 Interest in Medical Research, Education, and Practice; Washington (DC): National
204 Academies Press (US). Available from: <https://www.ncbi.nlm.nih.gov/books/NBK22930/>
- 205 Diaz, R.J. and Rosenberg, R. 2008. Spreading dead zones and consequences for marine
206 ecosystems. Science **321**: 926-929.

- 207 Dolan, D.M. and Chapra, S.C. 2012. Great Lakes total phosphorus revisited: 1. Loading analysis
208 and update (1994–2008). J. Great Lakes Res. **38**: 730-740.
- 209 Dym, C.L., Little, P. and Orwin, E.J. 2014. Engineering design. 4th edition, John Wiley & Sons,
210 Hoboken, New Jersey.
- 211 Fay, P. 1976. Factors influencing dark nitrogen fixation in a blue-green alga. App. Environ.
212 Microbiol. **31**: 376-379.
- 213 Fay, P. 1992. Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol. Rev. **56**: 340-
214 373.
- 215 Flores, E. and Herrero, A. 2010. Compartmentalized function through cell differentiation in
216 filamentous cyanobacteria. Nature Rev. Microbiol. **8**: 39-50.
- 217 Gagnon, A. and Pick, F.R. 2012. Effect of nitrogen on cellular production and release of the
218 neurotoxin anatoxin-a in a nitrogen-fixing cyanobacterium. Front. Microbiol. June 12.
- 219 Gobler, C.J., Burkholder, J.M., Davis, T.W., Harke, M.J., Johengen, T., Stow, C.A. and Van de
220 Waal, D.B. 2016. The dual role of nitrogen supply in controlling the growth and toxicity of
221 cyanobacterial blooms. Harmful Algae. **54**: 87-97
- 222 Harke, M.J., Davis, T.W., Watson, S.B. and Gobler, C.J. 2015. Nutrient-controlled niche
223 differentiation of western Lake Erie cyanobacterial populations revealed via
224 metatranscriptomic surveys. Environ. Sci. Technol. **15**: 604-614.
- 225 Hellweger, F.L, Fredrick, N.D., McCarthy, M.J., Gardner, W.S., Wilhelm. S.W. and Paerl, H.W.
226 2016. Dynamic, mechanistic, molecular-level modelling of cyanobacteria: *Anabaena* and
227 nitrogen interaction. Environ. Microbiol. **18**: 2721-2731.
- 228 Holm-Hansen, O., Gerloff, G.C. and Skoog, F. 1954. Cobalt as an essential element for blue-
229 green algae. Physiol. Plant. **7**: 665-675.

- 230 Inomura, K., Bragg, J. and Follows, M.J. 2017. A quantitative analysis of the direct and indirect
231 costs of nitrogen fixation: a model based on *Azotobacter vinelandii*. Int. Soc. Microb. Ecol.
232 J. **11**: 166-175.
- 233 Jensen, B.B. and Cox, R.P. 1983. Effect of oxygen concentration on dark fixation and respiration
234 in cyanobacteria. Arch. Microbiol. **135**: 287-292.
- 235 Kenesi, G., Shafik, H.M., Kovacs, A.W., Herodek, S. and Presling, M. 2009. Effect of nitrogen
236 forms on growth, cell composition and N₂ fixation of *Cylindrospermopsis raciborskii* in
237 phosphorus-limited chemostat cultures. Hydrobiol. **623**: 191-202.
- 238 Kranzler, C., Lis, H., Shaked, Y. and Keren, N. 2011. The role of reduction in iron uptake
239 processes in a unicellular, planktonic cyanobacterium. Environ. Microbiol. **13**: 2990-2999.
240 doi:10.1111/j.1462-2920.2011.02572.x.
- 241 Kranzler, C., Rudolf, M., Keren, N., and Schleif, E. 2013. Iron in Cyanobacteria. In Advances in
242 Botanical Research. Elsevier. pp. 57-94.
- 243 Kratz, W.A. and Myers, J. 1955. Nutrition and growth of several blue-green algae. Am. J.
244 Botany. **42**: 282-287.
- 245 Kusta, A., Sañudo-Wilhemmy, S.A. and Carpenter, E.J. 2003. Iron requirements for dinitrogen-
246 and ammonium supported growth in cultures of *Trichodesmium* (IMS 101): comparison with
247 nitrogen fixation rates and iron:carbon ratios of field populations. Limnol. Oceanogr. **48**:
248 1869–1884.
- 249 Lewis, W.M. Jr and Wurtsbaugh, W.A. 2008. Control of lacustrine phytoplankton by nutrients:
250 erosion of the phosphorus paradigm. Internat. Rev. Hydrobiol. **93**: 446-465.

- 251 Lürling, M., Eshetu, F., Faassen, E.J., Kosten, S. and Huszar, V.L.M. 2013. Comparison of
252 cyanobacterial and green algal growth rates at different temperatures. *Freshwat. Biol.* **58**:
253 552-559.
- 254 Oh, H.-M., Maeng, J. and Rhee, G-Yull. 1991. Nitrogen and carbon fixation by *Anabaena* sp.
255 Isolated from a rice paddy and grown under P and light limitations. *J. Appl. Phycol.* **3**: 335-
256 343.
- 257 Paerl, H.W. 2009. Controlling eutrophication along the freshwater—marine continuum: Dual
258 nutrient (N and P) reductions are essential. *Estuar. Coasts.* **32**: 593-601.
- 259 Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y. and Gardner, W.S. 2011.
260 Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China):
261 The need for a dual nutrient (N & P) management strategy. *Wat. Res.* **45**: 1973-1983.
- 262 Paerl, H.W., Xu, H., Hall, N.S., Zhu, G., Qin, B., Wu, Y., Rossignol, K.L., Dong, L., McCarthy,
263 M.J. and Joyner, A.R. 2014. Controlling cyanobacterial blooms in hypertrophic Lake Taihu,
264 China: Will nitrogen reductions cause replacement of non-N₂ fixing by N₂ fixing taxa?
265 PLOSONe. <http://dx.doi.org/10.1371/journal.pone.0113123>
- 266 Paterson, M.J., Schindler, D.W., Hecky, R.E., Findlay, D.L. and Rondeau, K.J. 2011. Comment:
267 Lake 227 shows clearly that controlling inputs of nitrogen will not reduce or prevent
268 eutrophication of lakes. *Limnol. Oceanogr.* **56**: 1545-1547.
- 269 Pick, F.R. 2016. Blooming algae: a Canadian perspective on the rise of toxic cyanobacteria. *Can.*
270 *J. Fish. Aquat. Sci.* **73**: 1149-1158.
- 271 Reisenhauer, H.M. 1960. Cobalt in nitrogen fixation by a legume. *Nature* **186 (4722)**: 375-376.

- 272 Robarts, R.D. and Zohary, T. 1987. Temperature effects on photosynthetic capacity, respiration,
273 and growth rates of bloom-forming cyanobacteria. New Zealand J. Mar. Freshwat. Res. **21**:
274 391-399.
- 275 Sanz, A., Morena-Vivián, C., Maldonado, J.M. and González-Fontes, A. 1995. Effect of a
276 constant supply of different nitrogen sources on protein and carbohydrate content and
277 enzyme activities of *Anabaena variabilis* cells. Physiol. Plant. **95**: 39-44.
- 278 Scavia, D. and Bricker, S.B. 2006. Coastal eutrophication assessment in the United States.
279 Biogeochem. **79**: 187-208.
- 280 Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M., Beaty,
281 K.G., Lyng, M. and Kasian, S E.M. 2008. Eutrophication of lakes cannot be controlled by
282 reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proc. Natl.
283 Acad. Sci. USA. **105**: 11254–11258
- 284 Schindler, D.W. 2012. The dilemma of controlling cultural eutrophication. Proc. Royal Soc. B.
285 **279**: 4322-4333.
- 286 Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty,
287 K.G., Lyng, M. and Kasian, S.E.M. 2012. Eutrophication of lakes cannot be controlled by
288 reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proc. National
289 Acad. Sci. **105**: 11254-11258.
- 290 Schindler, D.W., S.R. Carpenter, S.R., Chapra, S.C., Hecky, R.E. and Orihel, D.M. 2016.
291 Reducing phosphorus to curb lake eutrophication is a success. Environ. Sci. Technol. DOI:
292 10.1021/acs.est.6b02204.
- 293 Schlangstedt, M., Bisen, P.S., Dudel, G. and Kohl, J.-G. 1987. Interaction of combined nitrogen
294 availability and light in the regulation of growth, heterocyst differentiation and dinitrogen

- 295 fixation of the planktic blue-green alga *Anabaena solitaria* Kleb. Arch. Protistenkd. **134**:
296 389-396.
- 297 Scott, J.T. and McCarthy, M.J. 2010. Nitrogen fixation may not balance the nitrogen pool in
298 lakes over timescales relevant to eutrophication management. Limnol. Oceanogr. **55**: 1265-
299 1270.
- 300 Smith, V.H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global
301 problem. Environ. Sci. Pollut. Res. **10**: 126-139.
- 302 Stam, H., Stouthamer, A.H. and van Verseveld, H.W. 1987. Hydrogen metabolism and energy
303 costs of nitrogen fixation. FEMS Microbiology Letters. **46**: 73-92.
- 304 United States Environmental Protection Agency. 2015. Preventing Eutrophication: Scientific
305 Support for Dual Nutrient Criteria. EPA-820-S-15001. Accessed September 29, 2016 from
306 <https://www.epa.gov/sites/production/files/documents/nandpfactsheet.pdf>
- 307 Vargas, M.A., Rodriguez, H., Morena, J., Olivares, H., Del Campo, J.A., Rivas, J. and Guerro,
308 M.G. 1998. Biochemical composition and fatty acid content of filamentous nitrogen-fixing
309 cyanobacteria. J. Phycol. **34**: 812-817.
- 310

Table 1. Effect of nitrogen source on specific growth rate and biochemical composition of the *N₂* fixer *Nostoc paludosum* grown in semi-continuous culture at 1380 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Biochemical composition is expressed as a % of dry weight. Values are means $\pm$ sd; n is shown in parentheses. Data from Vargas et al. (1998).

	Air	Air + nitrate	Air + ammonium
growth rate (d^{-1})	0.69 ± 0.11 (4)	0.76 ± 0.05 (4)	0.74 ± 0.08 (4)
% protein	43.1 ± 2.5 (5)	53.6 ± 0.8 (3)	55.5 ± 2.0 (3)
% carbohydrate	27.1 ± 2.0 (4)	22.5 ± 2.2 (3)	16.9 ± 1.3 (3)
% lipid	8.5 ± 0.4 (4)	6.0 ± 0.7 (3)	4.9 ± 0.7 (3)
% lipid + carbohydrate	35.6 ± 2.0 (4)	28.5 ± 2.3 (3)	21.8 ± 1.5 (3)