

Supplement of Biogeosciences, 14, 1527–1539, 2017
<http://www.biogeosciences.net/14/1527/2017/>
doi:10.5194/bg-14-1527-2017-supplement
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Supplement of

New insights on resource stoichiometry: assessing availability of carbon, nitrogen, and phosphorus to bacterioplankton

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Table 1. Schematic representation of the standard growth curves design

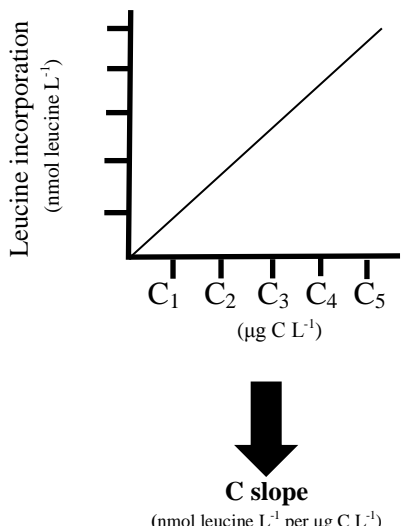
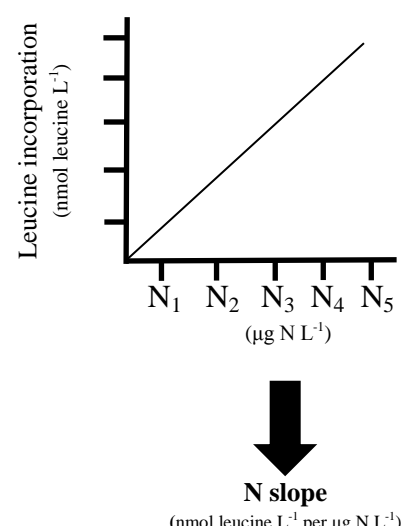
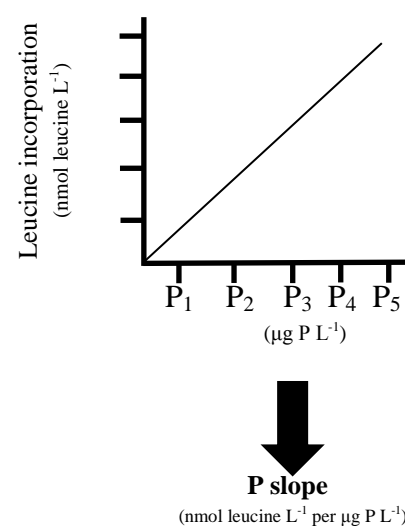
Experiment Step	Standard C curve	Standard N curve	Standard P curve
Sampling, pre-filtration, storage	Water sampled September 2012 0.7 µm filtration, storage at 1 °C		
Sample division	Sub-volume 1	Sub-volume 2	Sub-volume 3
Filtration and Inoculation, addition of "L16"	0.2 µm filtration 2 % (v/v) standard inoculum 5 % (v/v) "L16" bacterial growth medium	0.2 µm filtration 2 % (v/v) standard inoculum 5 % (v/v) "L16" bacterial growth medium	0.2 µm filtration 2 % (v/v) standard inoculum 5 % (v/v) "L16" bacterial growth medium
Induction of element limitation	C-limitation induced +2000 µg N L ⁻¹ as NH ₄ NO ₃ +200 µg P L ⁻¹ as Na ₂ HPO ₄	N-limitation induced +20000 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ +200 µg P L ⁻¹ as Na ₂ HPO ₄	P-limitation induced +20000 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ +2000 µg N L ⁻¹ as NH ₄ NO ₃
Subsample division	sub-volume 1.1, 1.2, 1.3, 1.4, 1.5	sub-volume 2.1, 2.2, 2.3, 2.4, 2.5	sub-volume 3.1, 3.2, 3.3, 3.4, 3.5
Bioassay "spiking" with known element concentration	+330 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ (C ₁) +660 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ (C ₂) +1000 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ (C ₃) +1330 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ (C ₄) +1500 µg C L ⁻¹ as C ₆ H ₁₂ O ₆ (C ₅)	+105 µg N L ⁻¹ as NH ₄ NO ₃ (N ₁) +133 µg N L ⁻¹ as NH ₄ NO ₃ (N ₂) + 205 µg N L ⁻¹ as NH ₄ NO ₃ (N ₃) +305 µg N L ⁻¹ as NH ₄ NO ₃ (N ₄) +405 µg N L ⁻¹ as NH ₄ NO ₃ (N ₅)	+15.5 µg P L ⁻¹ as Na ₂ HPO ₄ (P ₁) +18.8 µg P L ⁻¹ as Na ₂ HPO ₄ (P ₂) +20.5 µg P L ⁻¹ as Na ₂ HPO ₄ (P ₃) +30.5 µg P L ⁻¹ as Na ₂ HPO ₄ (P ₄) +40.5 µg P L ⁻¹ as Na ₂ HPO ₄ (P ₅)
Bioassay incubation	7-day incubation at 20 °C in Eppendorf tubes		7-day incubation at 20 °C in Eppendorf tubes
Determination and integration of leucine uptake	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days
Standard growth curves	 <p>Leucine incorporation (nmol leucine L⁻¹)</p> <p>C₁ C₂ C₃ C₄ C₅ (µg C L⁻¹)</p> <p>C slope (nmol leucine L⁻¹ per µg C L⁻¹)</p>	 <p>Leucine incorporation (nmol leucine L⁻¹)</p> <p>N₁ N₂ N₃ N₄ N₅ (µg N L⁻¹)</p> <p>N slope (nmol leucine L⁻¹ per µg N L⁻¹)</p>	 <p>Leucine incorporation (nmol leucine L⁻¹)</p> <p>P₁ P₂ P₃ P₄ P₅ (µg P L⁻¹)</p> <p>P slope (nmol leucine L⁻¹ per µg P L⁻¹)</p>

Table 2. Schematic representation of experimental controls design

Experiment step	C control	N control	P control
Inoculation	2 % (v/v) standard inoculum	2 % (v/v) standard inoculum	2 % (v/v) standard inoculum
Addition of "L16"	5 % (v/v) "L16" bacterial growth medium	5 % (v/v) "L16" bacterial growth medium	5 % (v/v) "L16" bacterial growth medium
Induction of element limitation	C-limitation induced +2000 $\mu\text{g N L}^{-1}$ as NH_4NO_3 +200 $\mu\text{g P L}^{-1}$ as Na_2HPO_4	N-limitation induced +20000 $\mu\text{g C L}^{-1}$ as $\text{C}_6\text{H}_{12}\text{O}_6$ +200 $\mu\text{g P L}^{-1}$ as Na_2HPO_4	P-limitation induced +20000 $\mu\text{g C L}^{-1}$ as $\text{C}_6\text{H}_{12}\text{O}_6$ +2000 $\mu\text{g N L}^{-1}$ as NH_4NO_3
Bioassay incubation	7-day incubation at 20 °C in Eppendorf tubes (5 replicates)	7-day incubation at 20 °C in Eppendorf tubes (5 replicates)	7-day incubation at 20 °C in Eppendorf tubes (5 replicates)
Determination and integration of leucine uptake	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days

Table 3. Schematic representation of determination of bioavailable C, N and P

Experiment step	Bioavailable C	Bioavailable N	Bioavailable P
Sampling, pre-filtration, storage	Water sample, 0.7 μm filtration 1 °C storage		
Sample division	Sub-volume 1	Sub-volume 2	Sub-volume 3
Filtration and inoculation, addition of "L16"	0.2 μm filtration 2 % (v/v) standard inoculum 5 % (v/v) "L16" bacterial growth medium	0.2 μm filtration 2 % (v/v) standard inoculum 5 % (v/v) "L16" bacterial growth medium	0.2 μm filtration 2 % (v/v) standard inoculum 5 % (v/v) "L16" bacterial growth medium
Induction of element limitation	C-limitation induced +2000 $\mu\text{g N L}^{-1}$ as NH_4NO_3 +200 $\mu\text{g P L}^{-1}$ as Na_2HPO_4	N-limitation induced +20000 $\mu\text{g C L}^{-1}$ as $\text{C}_6\text{H}_{12}\text{O}_6$ +200 $\mu\text{g P L}^{-1}$ as Na_2HPO_4	P-limitation induced +20000 $\mu\text{g C L}^{-1}$ as $\text{C}_6\text{H}_{12}\text{O}_6$ +2000 $\mu\text{g N L}^{-1}$ as NH_4NO_3
Bioassay incubation	7-day incubation at 20 °C in Eppendorf tubes (5 replicates)	7-day incubation at 20 °C in Eppendorf tubes (5 replicates)	7-day incubation at 20 °C in Eppendorf tubes (5 replicates)
Determination and integration of leucine uptake	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days	Measurements of leucine incorporation at discrete time points (t1, t2, t3, t4, t7) and integration for 7 days
Correction for blanks	Corrected leucine incorporation = average measurements of leucine incorporation (n = 5) – average leucine incorporation from experimental controls (n = 5)	Corrected leucine incorporation = average measurements of leucine incorporation (n = 5) – average leucine incorporation from experimental controls (n = 5)	Corrected leucine incorporation = average measurements of leucine incorporation (n = 5) – average leucine incorporation from experimental controls (n = 5)
Determination of amounts of bioavailable element taken up during 7 days	Bioavailable C taken up ($\mu\text{g C L}^{-1}$) = Corrected leucine incorporation (nmol of leucine L^{-1}) \div C slope (nmol leucine L^{-1} per $\mu\text{g C L}^{-1}$)	Bioavailable N taken up ($\mu\text{g N L}^{-1}$) = Corrected leucine incorporation (nmol of leucine L^{-1}) \div N slope (nmol leucine L^{-1} per $\mu\text{g N L}^{-1}$)	Bioavailable P taken up ($\mu\text{g P L}^{-1}$) = Corrected leucine incorporation (nmol of leucine L^{-1}) \div P slope (nmol leucine L^{-1} per $\mu\text{g P L}^{-1}$)