Supporting Information for the article, "A long-term copper exposure on freshwater ecosystem using lotic mesocosms: Invertebrate community responses"

by Sandrine Joachim, Hélène Roussel, Jean-Marc Bonzom, Eric Thybaud, Christopher A. Mebane, Paul Van den Brink and Laury Gauthier

This file consists of photographs of key features of the experimental stream design and tables detailing the sampling designs

Figure S1 : Overview of channels during initial establishment of stream communities, prior to extensive macrophyte growth.

Figure S2 : Coarse grain and fine sediment substrate setup in one mesocosm

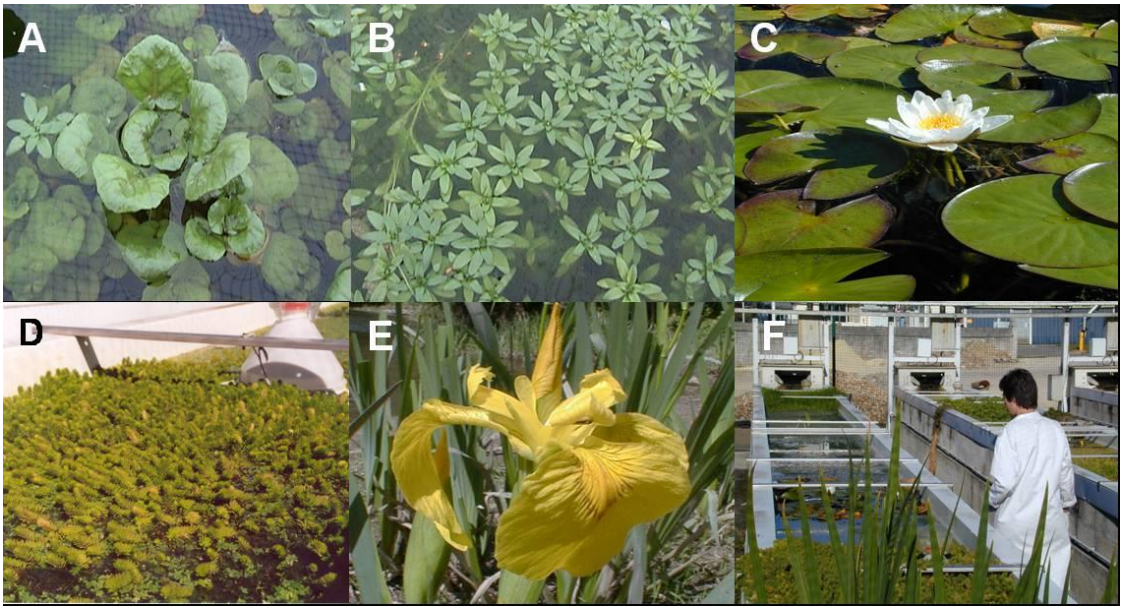

Figure S3 : Different macrophyte species introduced in each channel. (A) *Nasturtium officinale*, (B) *Callitriche platycarpa*, (C) *Nymphea alba*, (D) *Myriophyllum verticillatum*, (E) *Iris pseudacorus*, (F) General view of the macrophytes in one channel in September 2003.

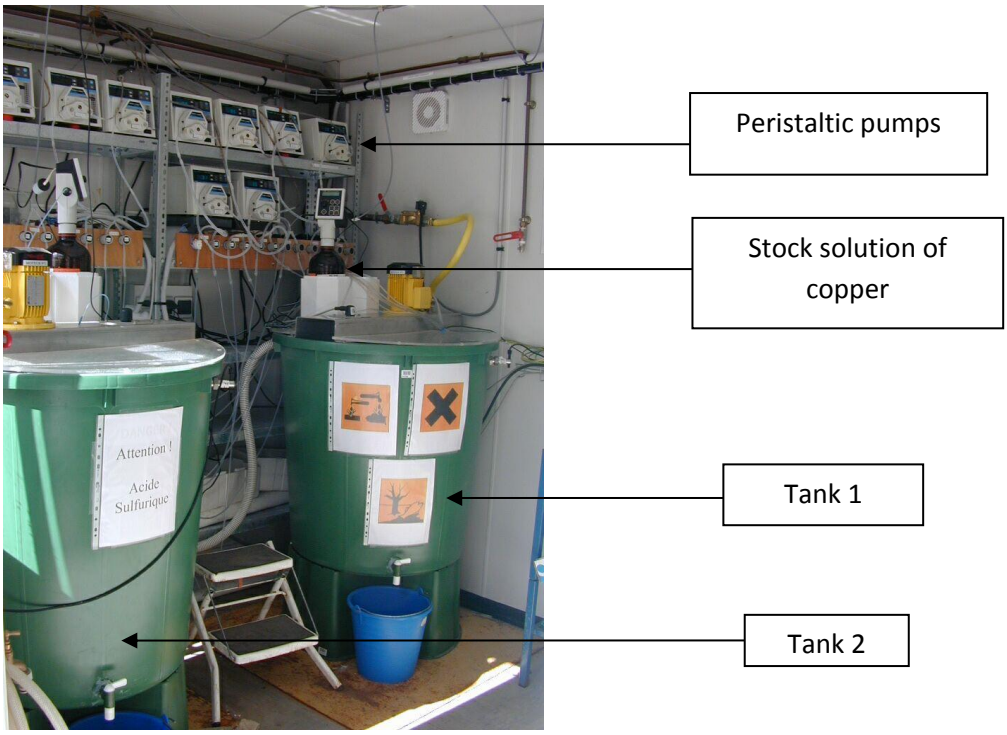

Figure S4 : Automatic dosing system

Two stock solutions composed of a technical grade of copper sulphate (MilliQ water and 15 % H_2SO_4) were placed onto two separate 500 liter tanks supplied with tap water (Tank 1 and Tank 2). Injection of the stock solution into Tank 1 was operated automatically. After a mixing period of 30 minutes, peristaltic pumps connected to the Tank 1 and the different mesocosms then delivered copper sulfate at the appropriate concentrations (the flow rates are adapted). The injection of the stock solutions, the water levels of the two tanks and the peristaltic pumps were controlled by an automatic system. When the water level of the Tank 1 was half empty, the automatic system commanded the injection in Tank 2. When Tank 1 was empty, the peristaltic pumps transferred to Tank 2. This operation was repeated every 8h to ensure a continuous contamination of the mesocosms. The stock solutions of copper sulphate were changed every 7 days.

Figure S5 : Overview of the 12 mesocom channels

TableS1 : Mesocosm dosing lay-out

Mesocosm	Concentration (µg/L)
1	25
2	75
3	5
4	0
5	25
6	75
7	5
8	0
9	25
10	75
11	5
12	0

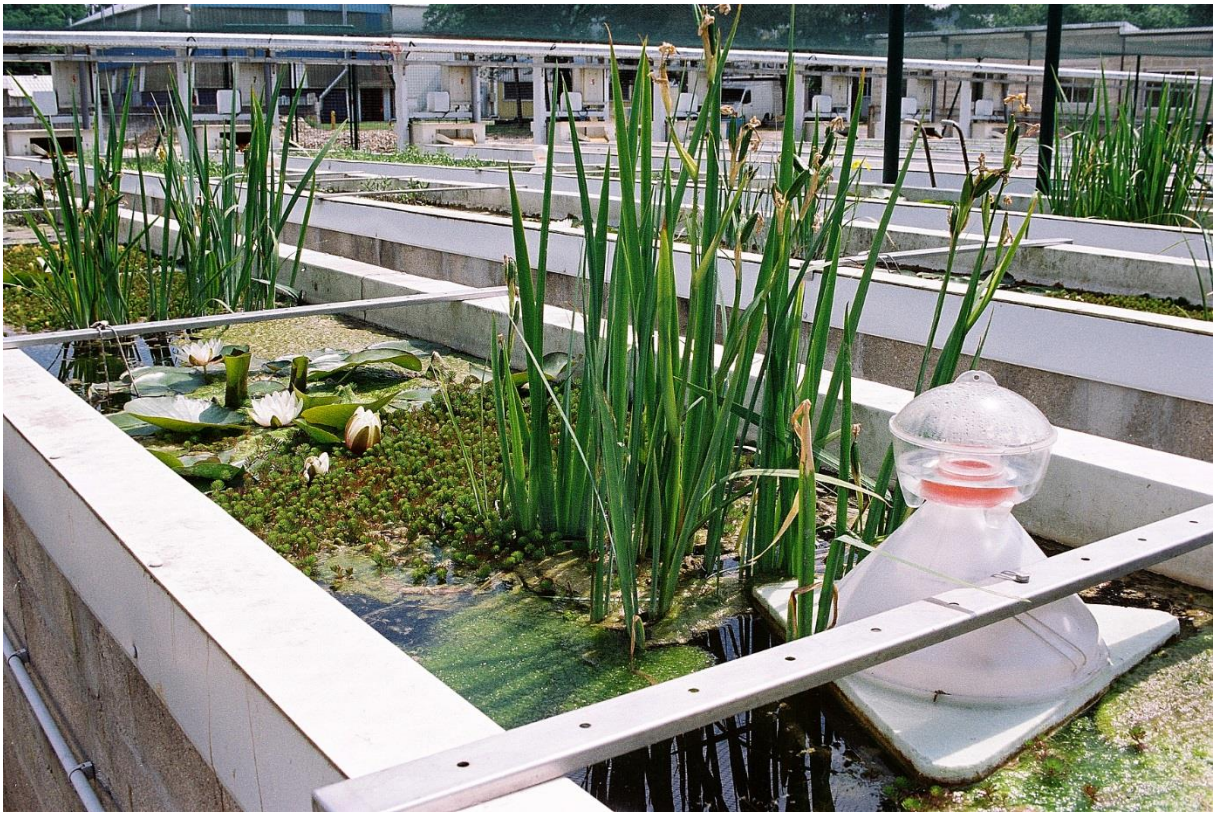

Figure S6 : Emergence trap in one mesocosm

Figure S7 : Example of late summer, dense macrophyte growth in the deeper, low-velocity sections of a channel.

Table S2 : Overview of all of compartments, populations and communities sampled throughout the experiment.

Organism/ Compartment	Endpoint	Unités	Frequency (in weeks)	References
Water	pH, Temperature, dissolved oxygen, conductivity	pH, °Celsius, mg/L, µmho/cm	-2, -1, 0, 1, 2, 3…	
	Dissolved and labile copper	µg/L	-4,0, 2, 4, 6, 8…	Labile copper, dissolved copper and total copper NF EN ISO 11885
	Cl, NO_3, SO_4	mg/L	-4, 0, 4, 8, 9, 10…	NF EN ISO 11885 émission spectrometry
	PO_4	mg/L	-4, 0, 12, 24…	NF EN ISO 10304 : ion exchange chromatography
	Ca, Na, Mg, K, Al, Fe, Si, DOC, TOC, SM, carbonates, NH_4,	mg/L	-4, 0, 4, 8…	NF EN 1484, NF EN ISO 9963, NF EN 872
Sediments	TOC, Carbone, Hydrogen, nitroge, phosphorus, sulfide, Fe, Mn	mg/L	-4, 0, 12, 24…	NF EN ISO 11885
	Total copper	µg/L	-4, 0, 12, 24…	NF EN ISO 11885
Periphyton	Bioaccumulation	µg copper/mg dry weight	-3, 0, 3, 6, 9…	NF EN 11885
Macrophytes (*Callitriche fluitans* et *Nasturtium officinale*),			-4, 0, 4, 8,…	
Lymnaeidae			-4, 0, 4, 8,…	
Gammaridae			-4, 0, 4, 8,…	

Organism/ Compartment	Endpoint	Unités	Frequency (in weeks)	References
Poisson (foie)		µg copper/ kg dry liverc	0, 2, 18, 34,…	
Fish (*Gasterosteus aculeatus)*	Glutathion	µmol/g prot	0, 2, 18, 34,…	Vandeputte *et al.* 1994
	Glutathion Reductase	µmol/g prot	0, 2, 18, 34,…	Carlberg, 1985
	SOD, CAT, GPx	U/g prot	0, 2, 18, 34,…	Paglia and Valentine 1967; Paoletti *et al.* 1986; Babo and Vasseur 1992
	TAS	Trolox equivalent (mmol/g prot)	0, 2, 18, 34,…	Miller *et al.* 1993
	Lipoperoxydation	Nmol TABRS / g prot	0, 2, 18, 34,…	Armstrong, 1998
	GST	U/g prot	0, 2, 18, 34,…	Habig *et al.* 1974
	EROD	pmol/min/mg prot	0, 2, 18, 34,..	Flammarion 1997
	Stress proteins	ng/mg prot	0, 2, 18, 34,…	Lewis *et al.* 1999
Phytoplankton and zooplankton	Abundance and Diversity	Number/L Diversity index	-2, -1, 0, 1, 2, 3…	Van den Brink, 1999
Periphyton	Abundance and Diversity	Number/L	-3, 0, 3, 6, 9…	Van den Brink, 1999
	Biomass	mg/cm²	-3, 0, 3, 6, 9…	Van den Brink, 1999

Organism/ Compartment	Endpoint	Unités	Frequency (in weeks)	References
	Chlorophylle-a	µg/cm	-3, 0, 3, 6, 9…	Norme « essais des eaux » T90-117, 1984
	Chlorophylle-a (on macrophyte)	µg/cm²	-3, 0, 3, 6, 9…	Van de Brink 1999
Macrophytes	Coverage	m²	-8, 0, 8, 30, 42,..	Van de Brink 1999
	Biomass	g de dry weight	-8, 0, 8, 30, 42,..	Van de Brink 1999
	Diversity	Number of species	-8, 0, 8, 30, 42,..	OECD, 2001
Macroinvertebrates	Abundance and Diversity	Number/L Diversity index	-4, 0, 4, 8,…	OECD, 2001
Macroinvertebrates in the sediment	Abundance and Diversity	Number/L Diversity index	-4, 0, 4, 8,…	Van den Brink, 1999
Emerging insects	Abundance and Diversity	Number/L Diversity index	-1, 0, 1,2,….33 50, 51,52,…	Caquet, 2000
Fish	Weight	g	0, 2, 18, 34,…26, 76	Bonzom and Poulsen, 2000
	Abundance	Number/mesocosm	26, 76	Bonzom and Poulsen, 2000
	Length	cm	0, 2, 18, 34,…28, 76	Bonzom and Poulsen, 2000

Organism/ Compartment	Endpoint	Unités	Frequency (in weeks)	References
Aquatic Hyphomycetes	Abundance and Diversity	Number/L	-5, -3, -1, 1…	Baldy and Gessner, 1997
	Biomass			
Macroinvertebrate decomposers	Abundance and Diversity	Number/L Diversity index		
Litter	Mass loss	g		

…., indicates that samples were taken regularly during this time lapse
0 = first date of exposure. -3 = 3 weeks before exposure etc.

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