Novel soil/water quality monitoring tools

Paul L.E. Bodelier

Netherlands Institute of Ecology, Wageningen, Netherlands

Assessing impacts of land use for biobased economy on soil water and sediment biodiversity, ecosystem processes and services

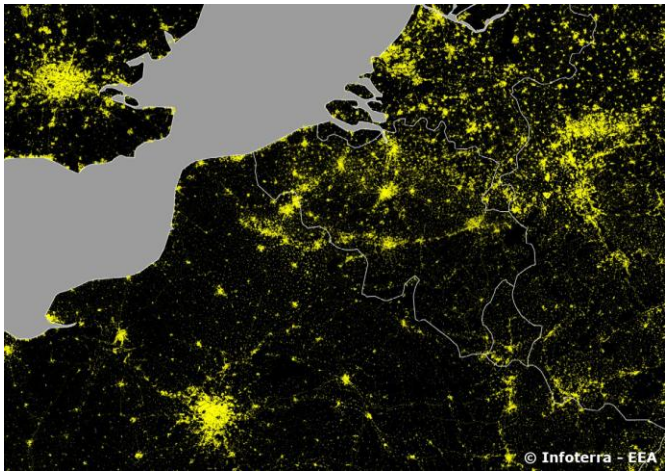
Wim van der Putten, Paul Bodelier, Riks Laanbroek, Manuela di Lorenzo, Bas W. Ibelings, NIOO-KNAW, Wageningen

BE-Basic

Netherlands Institute of Ecology, Wageningen

The Netherlands Institute of Ecology (NIOO-KNAW) conducts marine, terrestrial and freshwater ecological research, with the aim of elucidating how living organisms interact with each other and with their surroundings.
The Netherlands Institute of Ecology (NIOO) is a top research institute of the Royal Netherlands Academy of Arts and Sciences (KNAW).

Bio-based economy, Biodiversity and Ecosystem functioning



NIOO

BE-Basic

Bio-based economy, Biodiversity and Ecosystem functioning

Jeremy Woods, BBEST august 15: "…we have to embrace complexity.."

Soil, sediment, water biota are the engines of our ecosystems…complexity is inherent to ecosystems and the result of billions of years of evolution …we have to deal with it.

Effects of Bio-based economy:
Loss of Biodiversity per se not interesting.
Traits of species and interactions matter….
Disruption of Ecological networks!!!

NIOO

BE-Basic

BE-Basic: Bio-Based Ecologically Balanced Sustainable Industrial Chemistry

BE-Basic is a leading international public-private composed of over 25 Parties, running and R&D program of about 120 million Euro in the period 2010-2015 developing new sustainable biobased technologies for the chemical and materials industry, energy supply as well as for monitoring, controlling and improving the environment and quality of life.

Mission :“To develop industrial bio-based solutions for a sustainable society”.

The ambition is to build an international top-level public-private consortium on bio-based solutions and to implement these in the Dutch industry and society and to provide a global outreach of ecologically-balanced scientific-technological knowledge and knowhow, through a network of international collaborations.

BE-Basic: Bio-Based Ecologically Balanced Sustainable Industrial Chemistry

Flagship 8: Safety and Environmental Impact of Chemicals, Biobased Molecules and Processes.

Objective: To develop novel and efficient methods for the evaluation and improvement of chemical safety in the bio-based economy.

Ecogenomics-based approaches to develop comprehensive animal friendly methods for the evaluation of human- and ecological safety of chemicals, biomolecules and waste streams.

NIOO

BE-Basic

Assessing impacts of land use for biobased economy on soil water and sediment biodiversity, ecosystem processes and services

Monitoring tools at various levels of biological organization (ecosystem-community-individual-molecular).

Central role for microbes supporting all life on earth.

Flow scheme of project

Task 8.2.4.1
Remote sensing

Task 8.2.4.1
Plant chemsitry
HPLC, LC-MS, NMR

Biorefinery

Waste
(C,N,P,
pollutants)

Task 8.2.4.4
Cyanobacterial
transcriptome analyses

GHG emission (CH_4/N_2O)

Run-off
(C,N,P,
pollutants)

Waterquality

Blooms,
toxin production

Diversity
cycles

Diversity
cycles
Rates

Task 8.2.4.1
NGS, micro arrays
Physico-chemistry

Task 8.2.4.3
In situ Biomonitor
usign Nitrosomonas
genome array .

Task 8.2.4.2
Functional Micro arrays,
Biogeochemical Rates,
Physico-chemistry

NIOO

BE-Basic

Aims subproject 1: Plants, soil quality and reflectance spectra

Prof. Wim van der Putten (Department of Terrestrial Ecology)

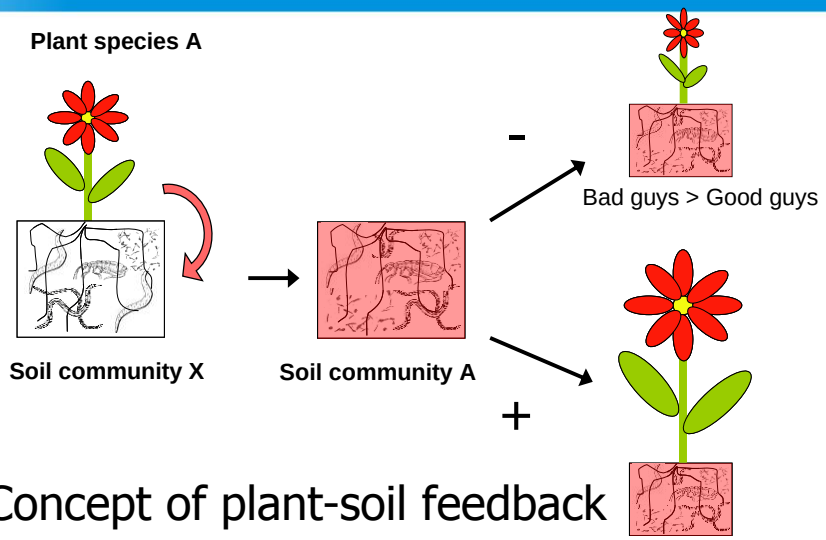
Aim a.
To test how plant/crop species and growth conditions influence soil community composition, biodiversity, soil ecosystem processes and services.

Aim b. To test applications of the combined plant-soil interaction and remote sensing approach to plant-soil systems with plant species to be grown as bio-based economy crops.

NIOO

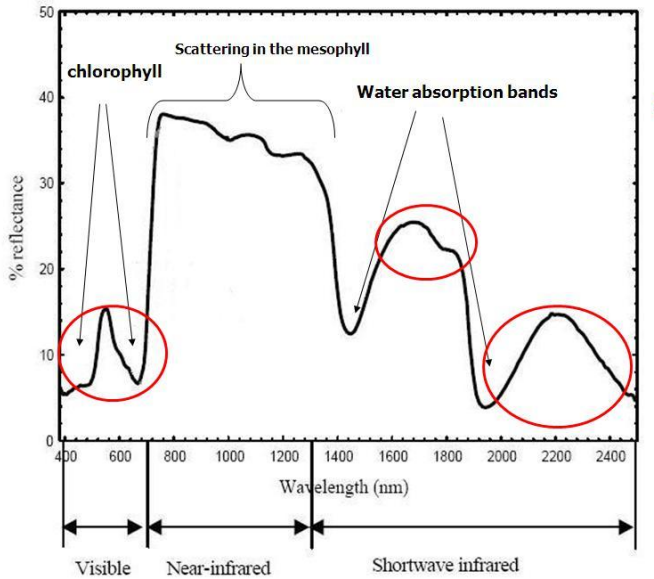
BE-Basic

subproject 1: Plants, soil quality and reflectance spectra

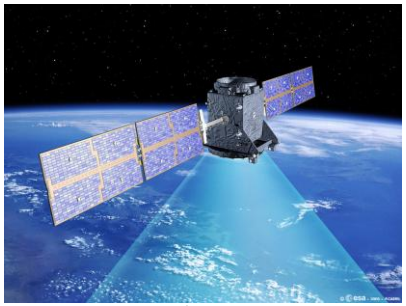


Concept of plant-soil feedback

spectrum of green vegetation & it's properties



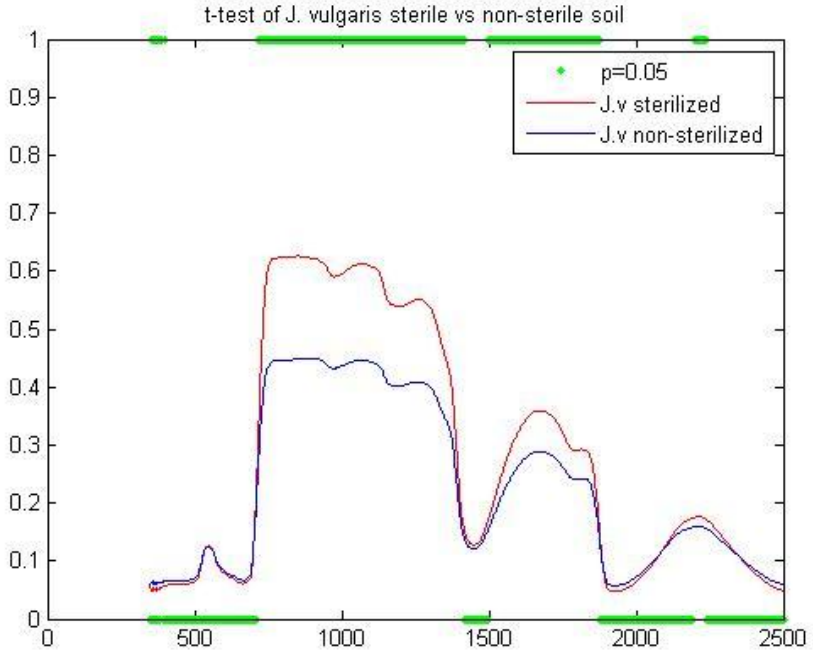
- Molecules chemical bonds !
  - C-H
  - C=H
  - N-H
  - C-N
  - O-H
  - Etc.

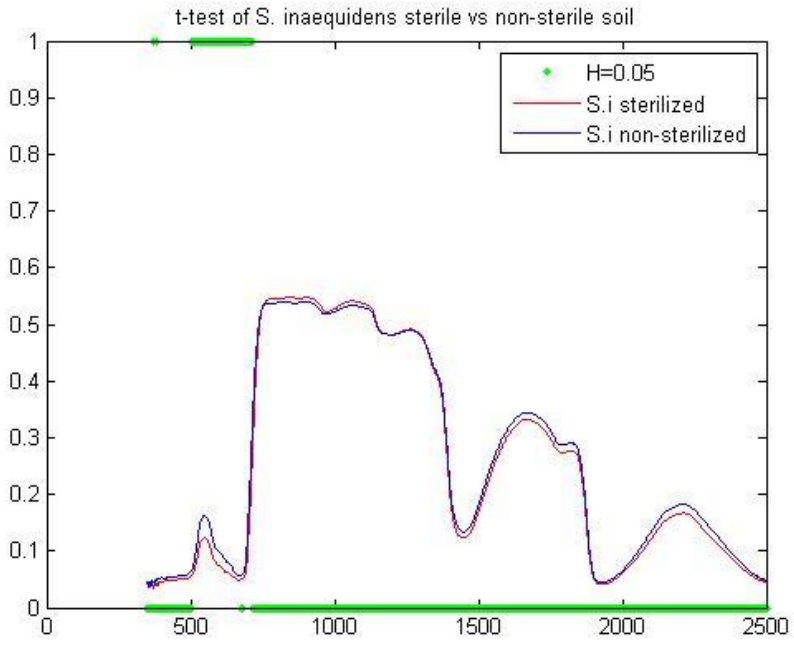


Sabrina Carvalho
(NIOO-ITC)

BE-Basic

Exotics: no soil pathogens





Senecio jacobaea
(native)

Senecio inaequidens
(exotic invader)

BE-Basic

Approach subproject 1

Compare intensive/extensive soil management strategies and fast growing crop plants *vs* plants with novel chemistry in mesocosms. Soils collected from extensively and intensively farmed soils (Rutgers et al. 2009; experimental sites of BLGG AgroXpertus).
Microbial diversity assessed using NGS sequencing.

Aims subproject 2: Functional Biogeochemical indicators

Dr. Paul L.E. Bodelier (Department of Microbial Ecology)

Aim a.
Inventory of microbial activity, diversity and gene expression in wetland systems with varying trophic status and degree of pollution.

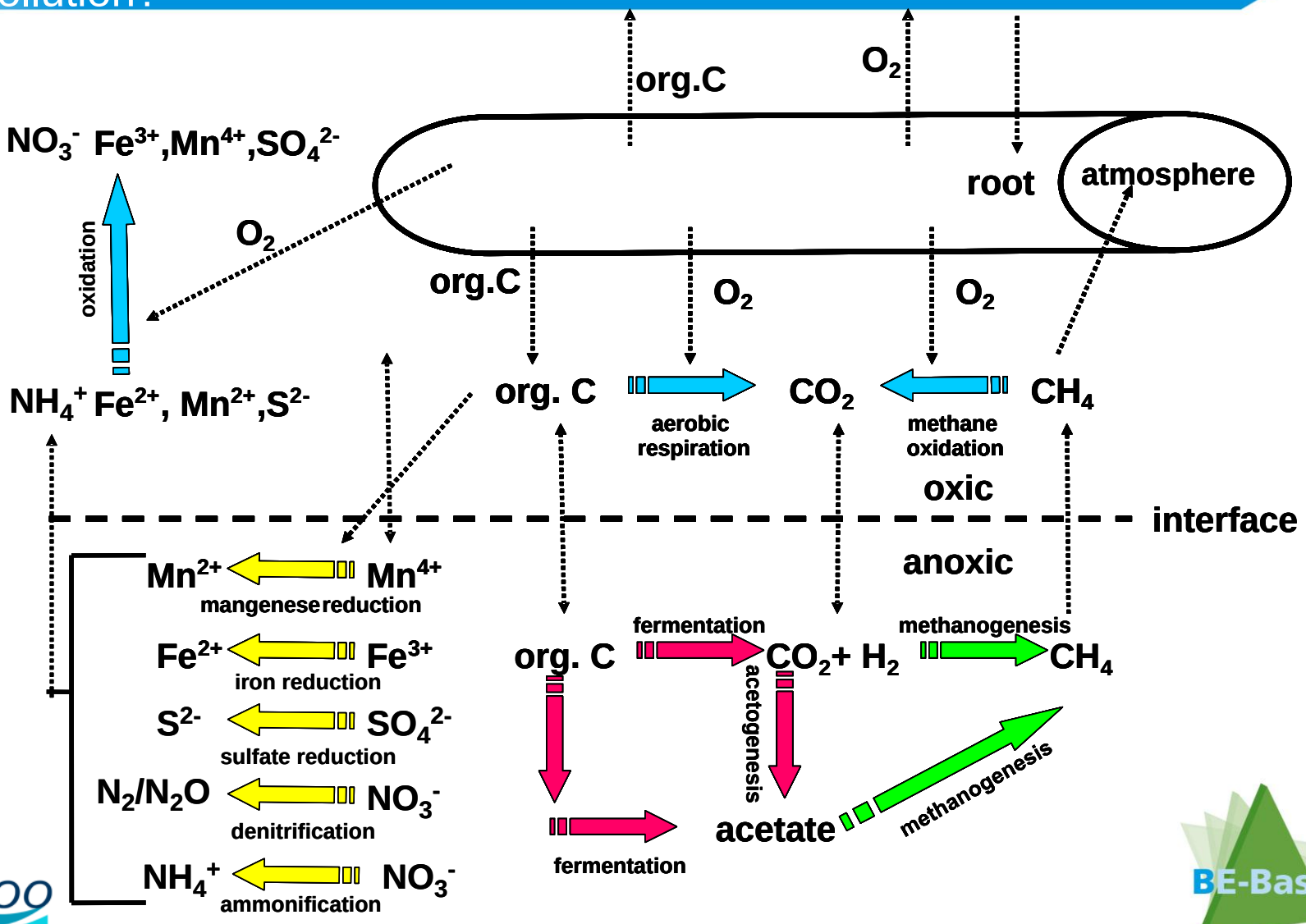
Aim b.
Couple gene expression and abundance to selected measurable biogeochemical functions (cycling of methane, nitrogen, iron, sulphur) to calibrate the coupling between gene abundance and transcription to biogeochemical functioning.

Aim c.
Select and test a subset of “indicator” genes for assessing effects of biobased production on important biogeochemical reactions and greenhouse gas emission from wetland ecosystems.

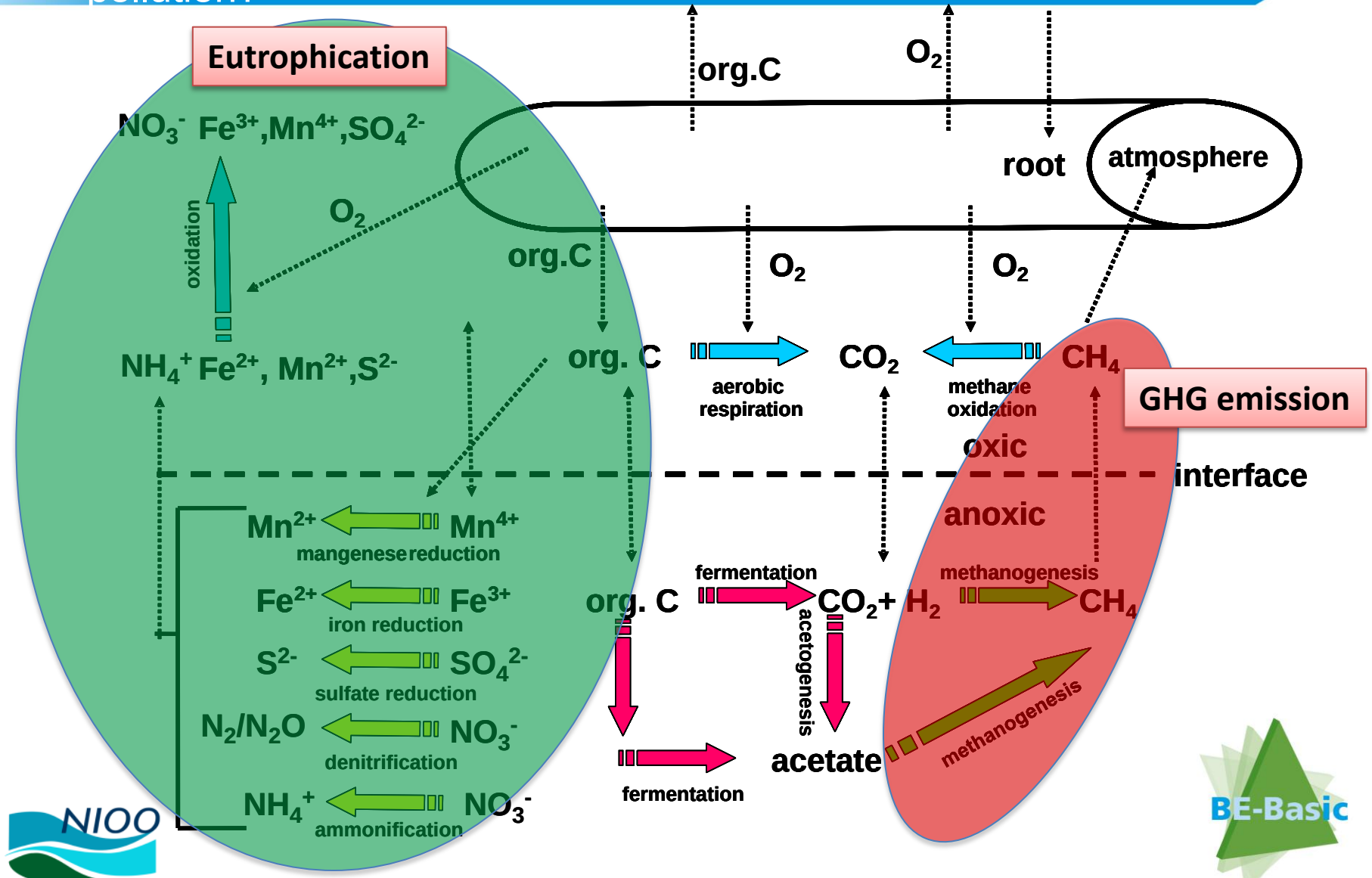
NIOO

BE-Basic

Wetland Biogeochemical networks linked to trophic status and pollution?



Wetland Biogeochemical networks linked to trophic status and pollution?

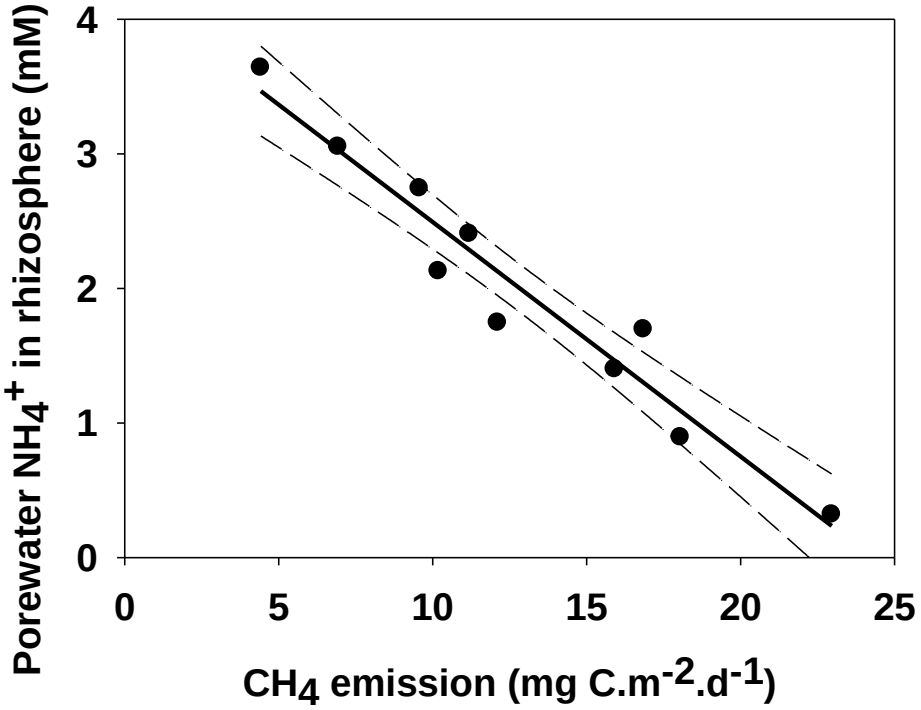


NIOO

BE-Basic

Direct link between Methane emission and N-status (trophic status)

Methane consumption and emission is controlled by N-status of wetlands.

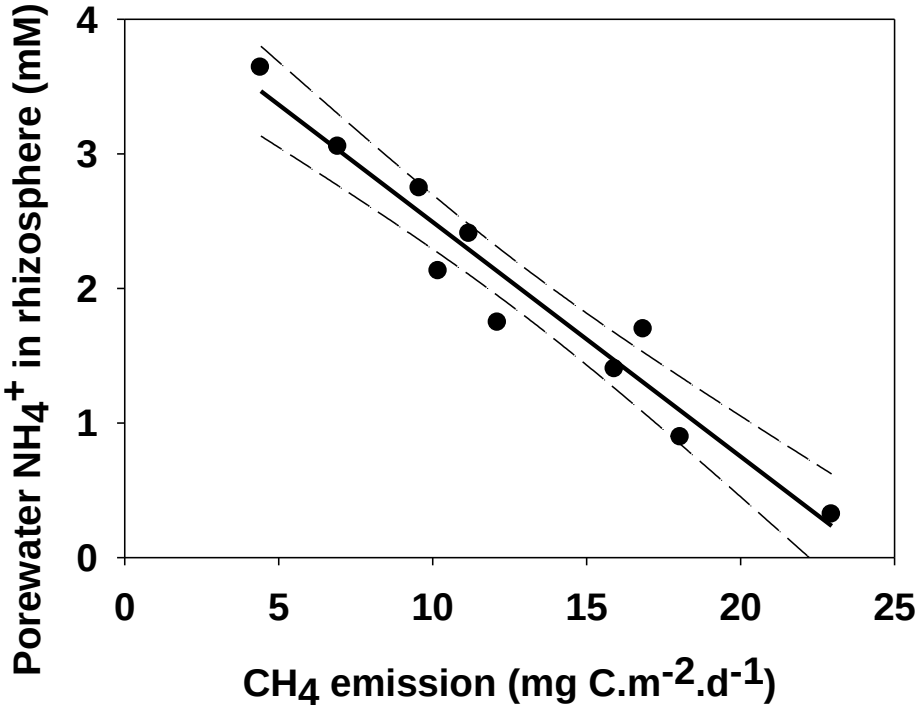


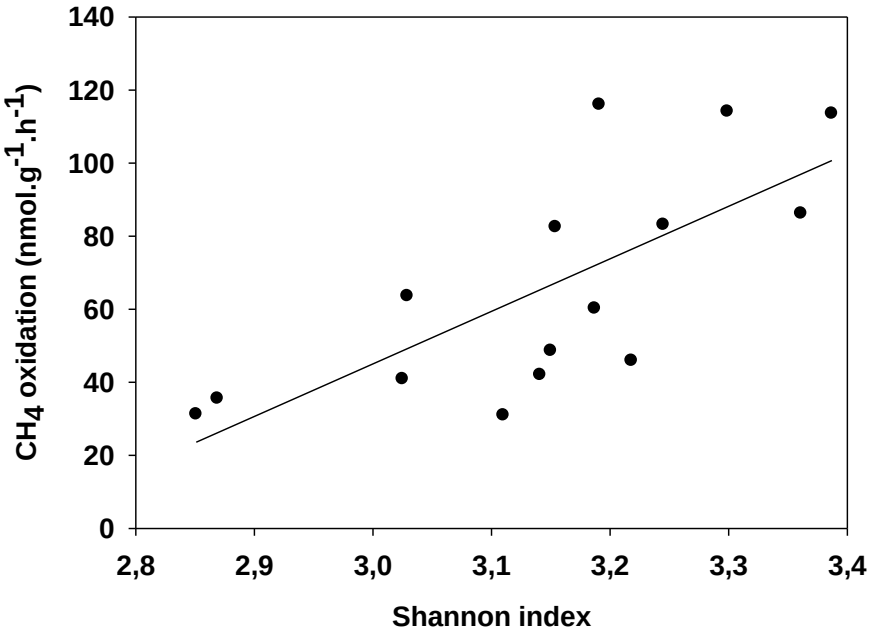
Bodelier et al 2000. Nature 403:421-424

Direct link between Methane emission and N-status

Methane consumption and emission is controlled by N-status of wetlands.

Methane consumption linked to species composition on functional gene basis.





Bodelier et al 2000. Nature 403:421-424

Bodelier et al. in prep

Approach subproject 2: Screening using GeoChip 3.0

57000 gene-probes covering C,N,Fe,P cycles, heavy metals, organic contaminants, antibiotic resistance.

The ISME Journal (2010) 4, 1167–1179

www.nature.com/ismej

ORIGINAL ARTICLE

GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity

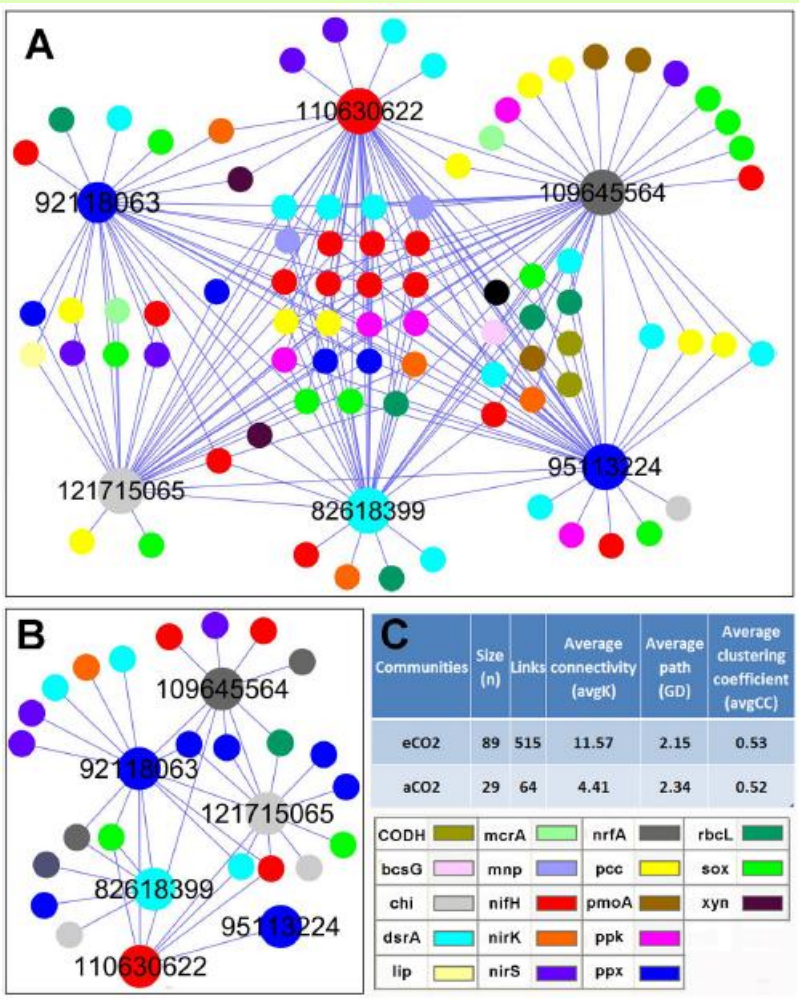
Zhili He[1,8,9], Ye Deng[1,8,9], Joy D Van Nostrand[1,8], Qichao Tu[1,8], Meiying Xu[1,2], Christopher L Hemme[1,8], Xingyuan Li[3], Liyou Wu[1,8], Terry J Gentry[4], Yifeng Yin[5], Jost Liebich[6], Terry C Hazen[7,8] and Jizhong Zhou[1,7,8]

Approach subproject 2: Screening using GeoChip 3.0

Detection of Functional Microbial Networks (FMN) as proxy for ecological interactions.

Elevated CO_2

Ambient CO_2



Zhou et al 2010. mBio 1(4) e00169-10.

BE-Basic

Approach subproject 2: Screening using GeoChip 3.0

Compare wetland sediments (± 100 sites) covering a gradient from pristine to hypereutrophic (N,P), and polluted selected from existing research and monitoring networks in Europe, China, (Brasil)?

Approach subproject 2: Screening using GeoChip 3.0

Compare wetland sediments (± 100 sites) covering a gradient from pristine to hypereutrophic (N,P), and polluted selected from existing research and monitoring networks in Europe, as well as China where physicochemical data of the respective sediments are available.

Validate mRNA based GeoChip data with traditional techniques; CLPP (Biolog), methane oxidation and production, nitrification and denitrification, sulfate reduction.

NIOO

BE-Basic

Approach subproject 2: Screening using GeoChip 3.0

Compare wetland sediments (± 100 sites) covering a gradient from pristine to hypereutrophic (N,P), and polluted selected from existing research and monitoring networks in Europe, as well as China where physicochemical data of the respective sediments are available.

Validate mRNA based GeoChip data with traditional techniques; CLPP (Biolog), methane oxidation and production, nitrification and denitrification, sulfate reduction.

Select indicator genes (central Hubs in FMN) for Biogeochemical Diagnostic QPCR assays.

NIOO

BE-Basic

Approach subproject 2: Screening using GeoChip 3.0

Compare wetland sediments (± 100 sites) covering a gradient from pristine to hypereutrophic (N,P), and polluted selected from existing research and monitoring networks in Europe, as well as China where physicochemical data of the respective sediments are available.

Validate mRNA based GeoChip data with traditional techniques; CLPP (Biolog), methane oxidation and production, nitrification and denitrification, sulfate reduction.

Select indicator genes for Biogeochemical Diagnostic QPCR assays.

Bio-based case study. International cooperation?

NIOO

BE-Basic

Approach subproject 2: Screening using GeoChip 3.0

Compare wetland sediments (± 100 sites) covering a gradient from pristine to hypereutrophic (N,P), and polluted selected from existing research and monitoring networks in Europe, as well as China where physicochemical data of the respective sediments are available.

Validate mRNA based GeoChip data with traditional techniques; CLPP (Biolog), methane oxidation and production, nitrification and denitrification, sulfate reduction.

Select indicator genes for Biogeochemical Diagnostic QPCR assays.

Bio-based case study. International cooperation?

Private partners: BioClear, Deltares.

BE-Basic

Aims subproject 3: Nitrifiers as bio-indicators

Prof. Riks L. Laanbroek (Department of Microbial Ecology)

Aim a.
Draw genetic stress response maps of the nitrifying bacterium *Nitrosomonas europaea* and link the mounting of a stress response to inhibition of nitrification.

Aim b.
Apply the stress response maps developed in model system to screen for the presence of stress factors in bio-based crop fields and their catchment water bodies.

NIOO

BE-Basic

Nitrifiers as bio-indicators

Ecological Indicators 9 (2009) 1212–1221

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development

Karl Ritz [a,*], Helaina I.J. Black [b,c], Colin D. Campbell [b], Jim A. Harris [a], Claire Wood [c]

Indicator	Indicator descriptor	F_A	Sub-cat. 1	Sub-cat. 2	Ref #
(a) Deployment status = 2. Cutoff point $F_A > 100$					
TRFLP—ammonia oxidisers/denitrifiers	Genetic profile—specific group	769	Genotype	Nucleic acid	115
PLFA profiles	Composition—total community	615	Phenotype	Biomarker	18
TRFLP—ITS fungal	Genetic profile—specific group	437	Genotype	Nucleic acid	118
Multiple substrate-induced respiration (MSIR) GC	Activity capability profile—total community	311	Function	Activity	158
Nematode Baermann extraction procedure	Numbers, composition and size of nematode community	302	Phenotype	Fauna	52
TRFLP—bacteria	Genetic profile—specific group	295	Genotype	Nucleic acid	117
Microarthropods Tullgren dry extraction	Numbers, composition and size of invertebrates community within soil	188	Phenotype	Fauna	50
On-site visual recording—flora and fauna	Numbers estimate of animals	173	Phenotype	Other	162
Microplate fluorometric assay—multi-enzyme	Enzyme potential activity—wide range	172	Function	Enzyme	30
TRFLP—Archaea	Genetic profile—specific group	146	Genotype	Nucleic acid	116
TRFLP—methanogens/methanotrophs	Genetic profile—specific group	123	Genotype	Nucleic acid	122
Invertebrates pitfall traps	Numbers, composition and size of invertebrates motile aboveground	123	Phenotype	Fauna	46
TRFLP—actinomycetes	Genetic profile—specific group	121	Genotype	Nucleic acid	113
(b) Deployment status = 1. Cutoff point $F_A > 100$					
TRFLP—nematodes	Genetic profile	437	Genotype	Nucleic acid	119
Multiple substrate induced respiration (MSIR) MicroResp	Activity capability profile	313	Function	Activity	160
TRFLP—protozoa	Genetic profile	291	Genotype	Nucleic acid	120
qPCR AM Fungi	Genetic profile	111	Genotype	Nucleic acid	92
(c) Deployment status = 0. Cutoff $F_A > 50$					
Functional gene arrays	Genetic profile	788	Genotype	Nucleic acid	84
Phylogentic gene arrays	Genetic profile	511	Genotype	Nucleic acid	91
FISH—keystone species	Genetic profile	138	Genotype	Nucleic acid	83
Soil proteomics	Phenotypic profile	51	Phenotype	Other	108

Deployment status defined, as at mid-2005, as follows: 2 = fully deployable; 1 = likely to be ready for deployment in the short-term; 0 = not ready, some years development still needed.

NIOO

BE-Basic

Nitrifiers and (Bio-based) environment

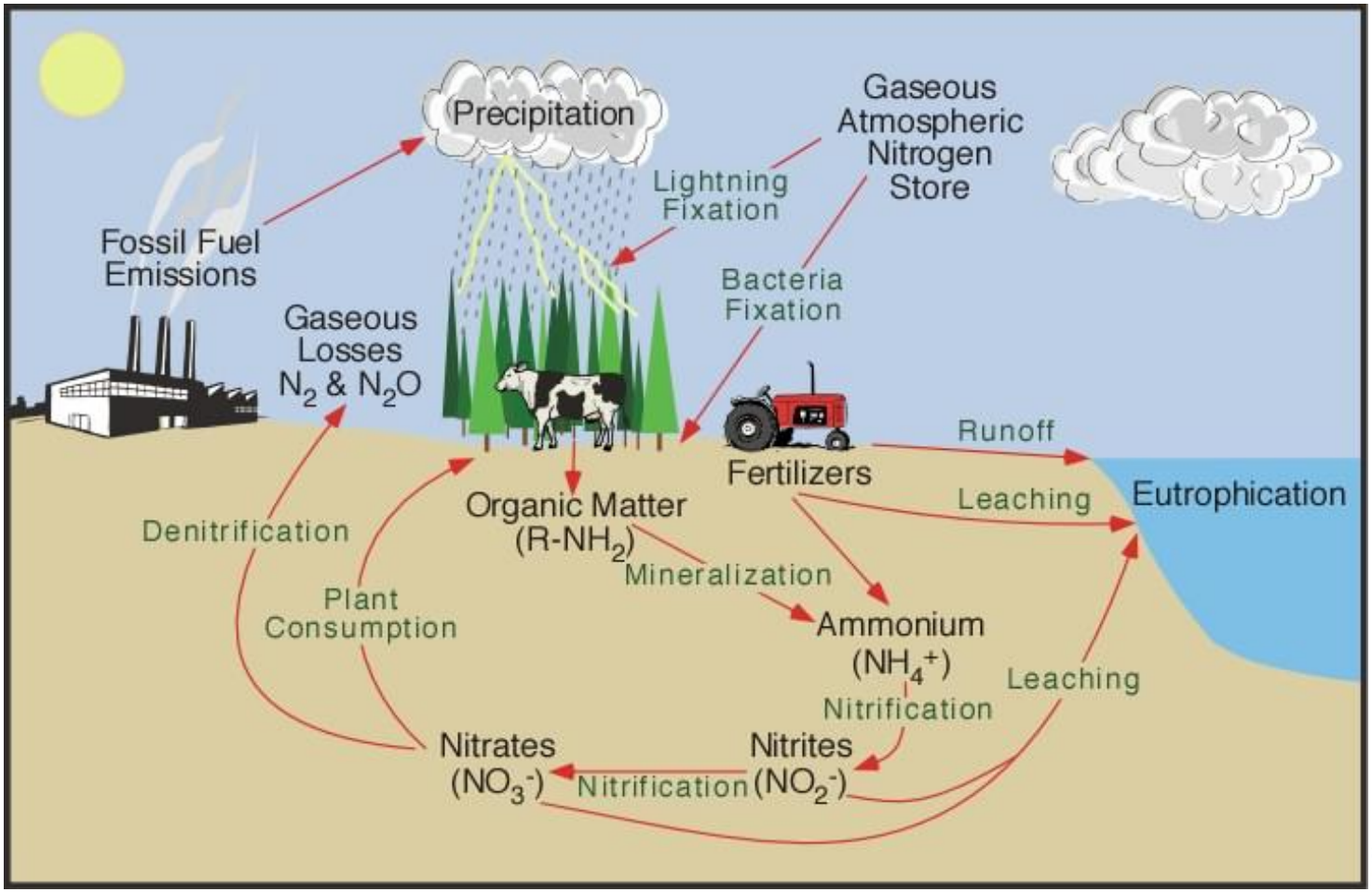
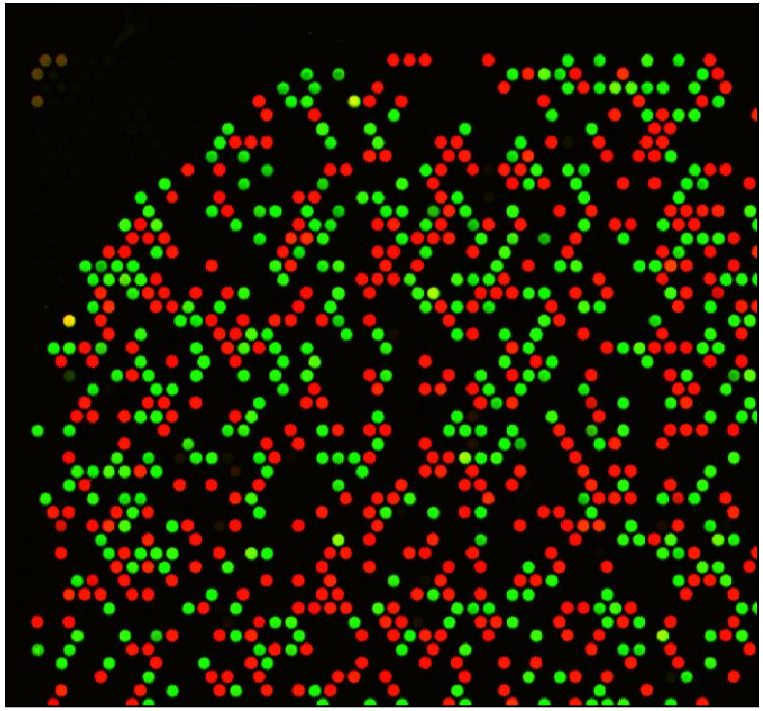


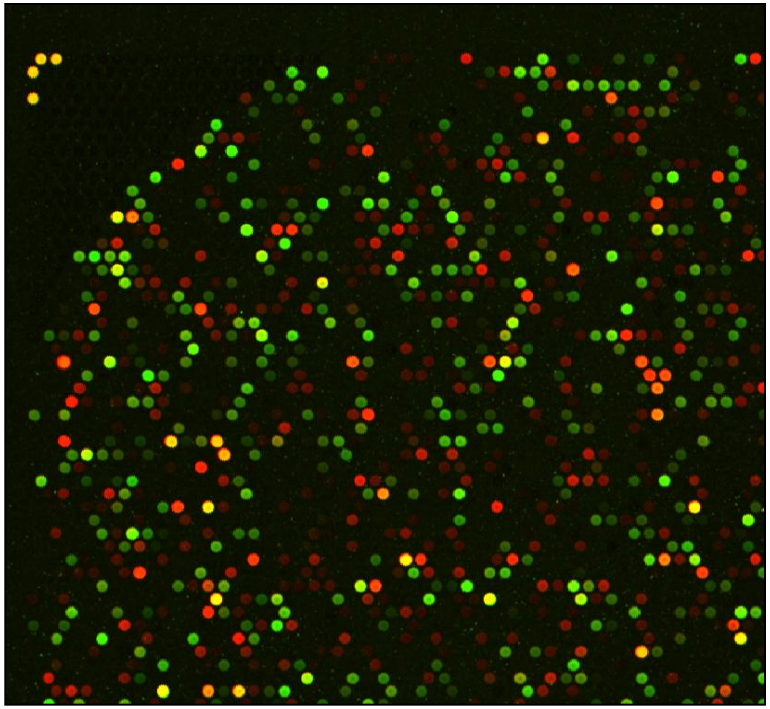
Figure created by Michael Pidwirny at Okanagan University College

Approach subproject 3: Stress response by genome analysis



N. europaea Cy3-gDNA

N. winogradskyi Cy5-gDNA



N. europaea Cy3-aRNA

N. winogradskyi Cy5-aRNA

Di Lorenzo and Laanbroek, in prep.

BE-Basic

Approach subproject 3: Stress response maps and sentinel genes

Stress response maps under controlled stress in laboratory cultures (e.g. metals, bio-based chemicals). Select “stress response genes” as sentinel genes.

Apply the stress response maps developed in model system to screen for the presence of stress factors in bio-based crop fields and their catchment water bodies.

Deployment of cultures in situ in diffusion chambers.

Aim subproject 4: Cyanobacterial transcriptomics as early warning systems

Dr. Bas W. Ibelings (Department of Aquatic Ecology)

Aim a:
Build a genome wide transcriptome database of the toxic cyanobacterium *Microcystis* with emphasis on genes involved in microcystine production.

Aim b: To develop a new, fast and convenient tool to assess the growth and microcystine production potential of cyanobacteria at early, pre-bloom stages.

NIOO

BE-Basic

Cyanobacteria and (Bio-based) environment

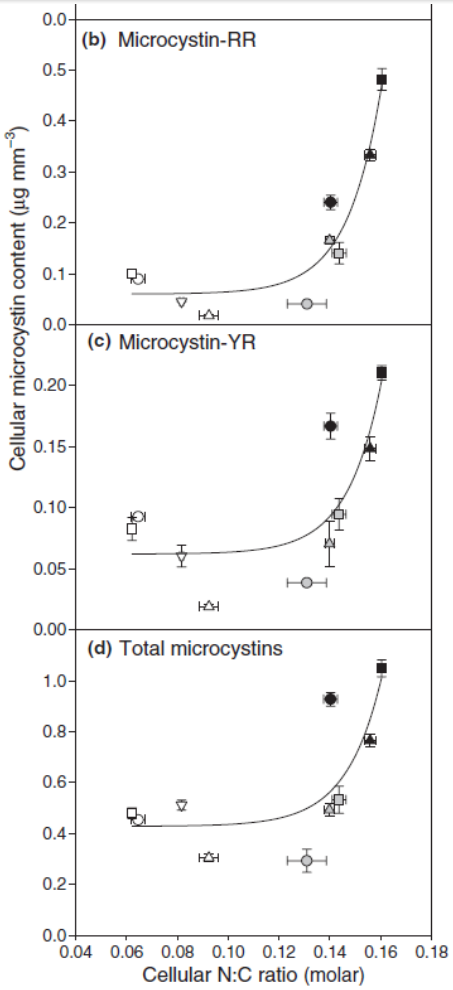
Fertilization leads to blooms of toxic algae with large consequences for quality of surface waters.

NIOO

BE-Basic

Toxin production linked to N/P status of surface water

Fertilization leads to blooms of toxic algae with large consequences for quality of surface waters.



ECOLOGY LETTERS

Ecology Letters, (2009) **12**: 1326–1335 doi: 10.1111/j.1461-0248.2009.01383.x

LETTER

The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis

Dedmer B. Van de Waal,[1,2] Jolanda M. H. Verspagen,[1] Miquel Lürling,[3] Ellen Van Donk,[2] Petra M. Visser[1] and Jef Huisman[1]*

[1]Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands

[2]Department of Aquatic Ecology, Netherlands Institute of Ecology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands

[3]Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands

**Correspondence: E-mail: j.huisman@uva.nl*

Abstract

The elemental composition of primary producers reflects the availability of light, carbon and nutrients in their environment. According to the carbon-nutrient balance hypothesis, this has implications for the production of secondary metabolites. To test this hypothesis, we investigated a family of toxins, known as microcystins, produced by harmful cyanobacteria. The strain *Microcystis aeruginosa* HUB 5-2-4, which produces several microcystin variants of different N:C stoichiometry, was cultured in chemostats supplied with various combinations of nitrate and CO_2. Excess supply of both nitrogen and carbon yielded high cellular N:C ratios accompanied by high cellular contents of total microcystin and the nitrogen-rich variant microcystin-RR. Comparable patterns were found in *Microcystis*-dominated lakes, where the relative microcystin-RR content increased with the seston N:C ratio. In total, our results are largely consistent with the carbon-nutrient balance hypothesis, and warn that a combination of rising CO_2 and nitrogen enrichment will affect the microcystin composition of harmful cyanobacteria.

Keywords

Carbon dioxide, carbon-nutrient balance hypothesis, climate change, C : N ratio, ecological stoichiometry, eutrophication, harmful algal blooms, microcystins, *Microcystis aeruginosa*, secondary metabolites.

Basic

Approach subproject 4: Cyanobacterial transcriptomics as early warning systems

Selection of platform for experiments (oligonucleotide arrays, tilling arrays, RNA seq.)

Concerted Changes in Gene Expression and Cell Physiology of the Cyanobacterium *Synechocystis* sp. Strain PCC 6803 during Transitions between Nitrogen and Light-Limited Growth[1][W][OA]

Eneas Aguirre von Wobeser[2,3], Bas W. Ibelings[2], Jasper Bok[4], Vladimir Krasikov, Jef Huisman, and Hans C.P. Matthijs*

Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 1098 XH Amsterdam, The Netherlands (E.A.v.W., V.K., J.H., H.C.P.M.); and Department of Aquatic Ecology, Netherlands Institute of Ecology, 3631 AC Nieuwersluis, The Netherlands (B.W.I., J.B.)

Plant Physiology, March 2011, Vol. 155, pp. 1445–1457

Gene expression studies in laboratory exposing *Microcystis* to a range of environmental variables selected from a literature study.

Selection of subset of transcripts to construct a condensed information chip (CICHIP) for use by watermanagers to detect presence of stressors (e.g. derived from Bio-based production/agriculture) and its impact on toxin production.

NIOO

BE-Basic

Envisaged results overall project

Tools to assess the environmental consequences of a bio-based economy in a cost-effective way.

Assessment tools minimise negative and maximize positive effects on the crop production system including the soil and the adjacent aquatic ecosystems.

Improve the knowledge of how biobased economy influences soil/sediment/water biodiversity and how that relates to sustainable delivery of ecosystem goods and services.

Project team

Prof. Wim H. van der Putten	NIOO-KNAW	PI, PI Task 1
Dr. Paul L.E. Bodelier	NIOO-KNAW	PI Task 2
Prof. H.J. (Riks) Laanbroek	NIOO-KNAW	PI Task 3
Dr. Manuela Di Lorenzo	NIOO-KNAW	PI Task 3
Dr. A.J. Termorshuizen	BLGG AgroXpertus	Private partner Task 1
Dr. I.Dinkla	Bioclear bv, Groningen	Private partner Task 2
Drs. B. Geurkink	Bioclear bv., Groningen	Private partner Task 2
Ir. G. Faber	Bioclear bv., Groningen	Private partner Task 2
S. Doddema	Bioclear bv. Groningen	Private partner Task 2
MSc M. Wagelmans	Bioclear bv, Groningen	Private partner Taks 2
Dr. A. Langenhoff	Deltares	Private partner Task 2
Dr. B. van der Zaan	Deltares	Private partner Taks 2
H. Mizab	Deltares	Private partner Taks 2
Dr. Gerlinde B. De Deyn	NIOO-KNAW/EU-MC	Task 1
Mrs. Tanja Bakx	NIOO-KNAW	Task 1
Mrs Ciska Raaijmakers	NIOO-KNAW	Task 1
Dr. W.H. Gera Hol	NIOO-KNAW	Task 1
Dr. Sascha Krause	NIOO-KNAW	Task 2
Ing. Marion Meima-Franke	NIOO-KNAW	Task 2
Dr. Kees Hordijk	NIOO-KNAW	Task 2
R. M. Keijzer MSc	NIOO-KNAW	Task 3
Dr. Bas W. Ibelings	NIOO-KNAW	PI, Task 4
Prof. Dr. Ellen van Donk	NIOO-KNAW	Task 4
Dr. Steven Declerck	NIOO-KNAW	Task 4
Dr Liesbeth E.S. Bakker	NIOO-KNAW	Task 4
Ing. Nico R. Helmsing	NIOO-KNAW	Task 4
MSc. Suzanne Wiezer	NIOO-KNAW	Task 4
Dr. Miquel Lürling	NIOO-KNAW / WUR	Task4
Dr. Hans C.P. Matthijs	UvA-IBED	Task 4
Dr. Miguel Dioniso Pires	Deltares	Private Partner Task 4
Dr. Edwin Kardinaal	KWR	Private Partner Task 4

BE-Basic

Challenges in environmental impact assessment

Use of (microbial) Bioindicators:
Focusing on effects on single species will not give us information on ecosystem functioning. Ecosystems are about Ecological interactions and networks and it will be a challenge to create a “simple” costs effective assay to monitor effects on interactions.

Functional Biodiversity:
BEF research is moving on from species richness to traits and functional relationships. For microbial communities in soil/sediment/water assigning “traits” and functional relationships to “species” and groups of species is still digging in a black box. Omics makes a lot possible. The biggest challenge will be to standardise omics techniques in a way that one habitat or sample can be reliably (unbiased) compared to another. This “robustness” is a prerequisite before these tools can be incorporated in sustainability analyses.

BE-Basic