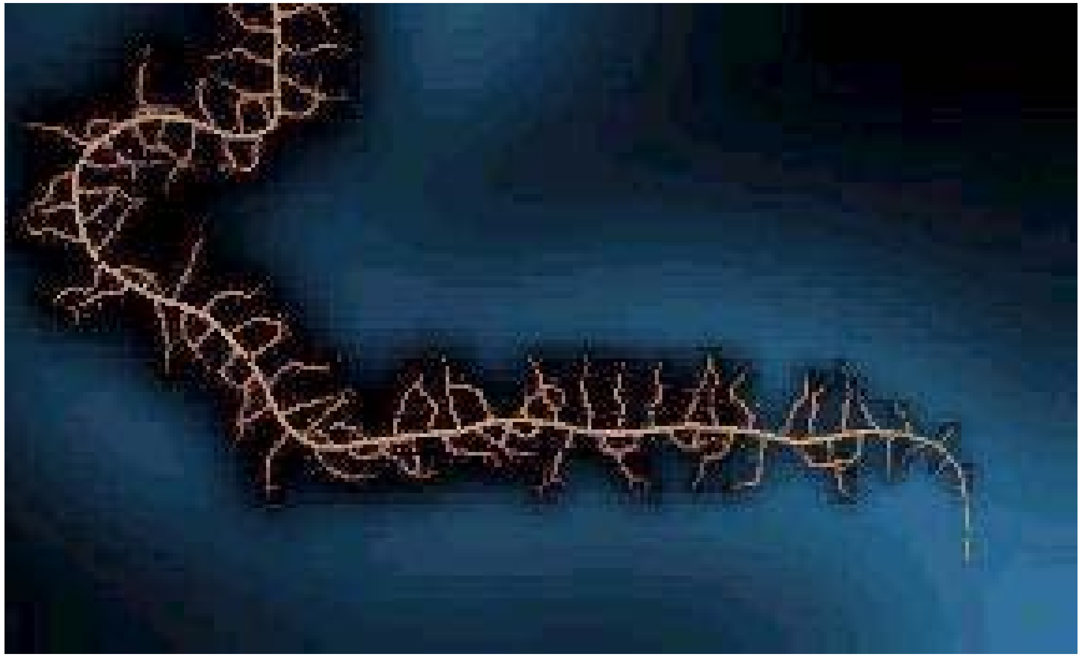
Rhizosphere processes involved in phytoremediation of contaminated soils

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

Markus Puschenreiter
Walter W. Wenzel

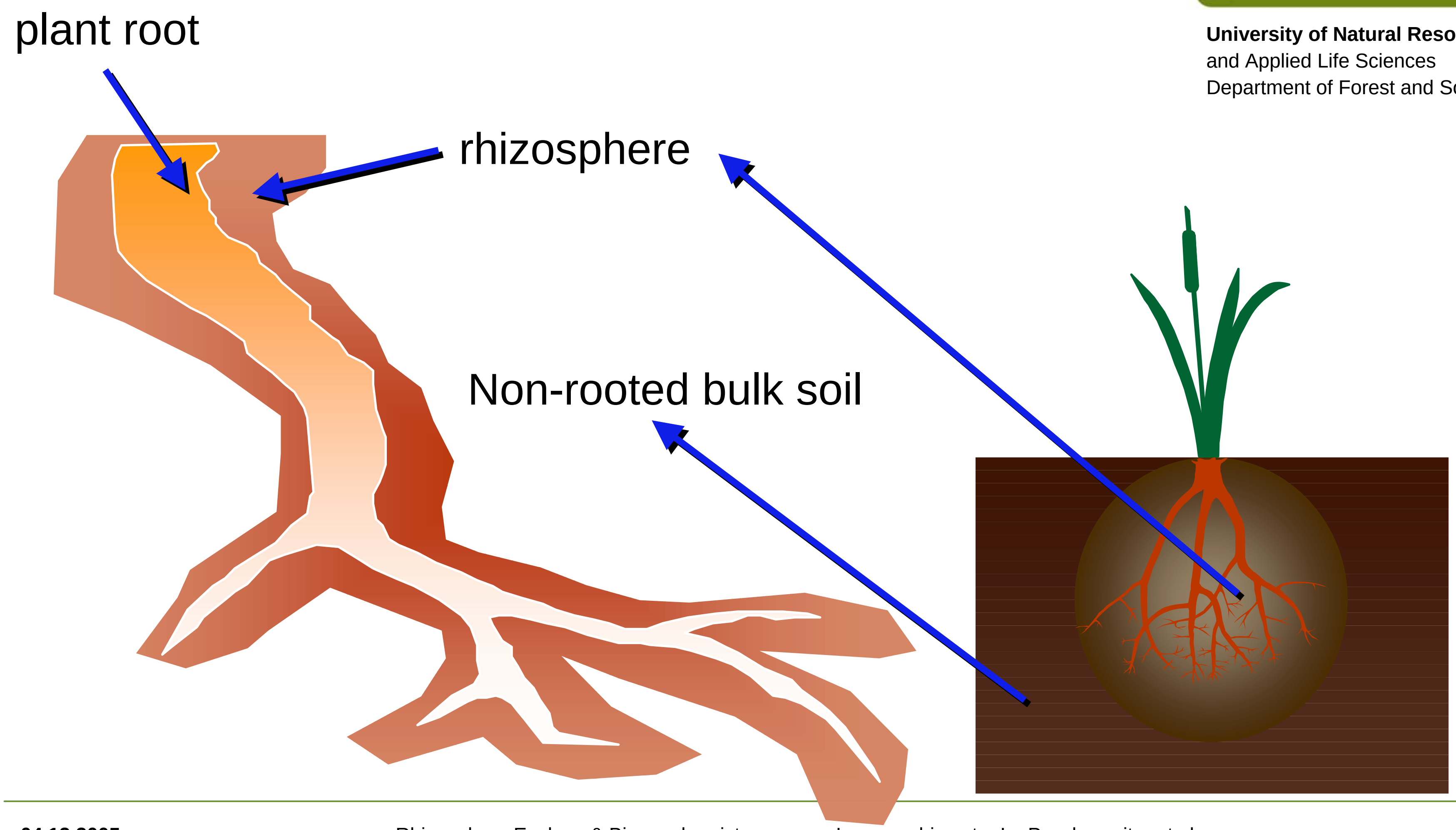
Rhizosphere Ecology &
Biogeochemistry Group
www.rhizo.at
www.boku.ac.at

The rhizosphere



- rhizosphere – soil influenced by plant roots
- different characterisitics compared to bulk soil
- enhanced microbial activity
- „biochemical reactor“

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences



Changes of rhizosphere characteristics

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- pH
- Redox potential
- Dissolved organic carbon
- Bioavailability
  * Conc. in soil solution
  * Exchangeable fraction
- Microbial activity

Wenzel et al. (2004)

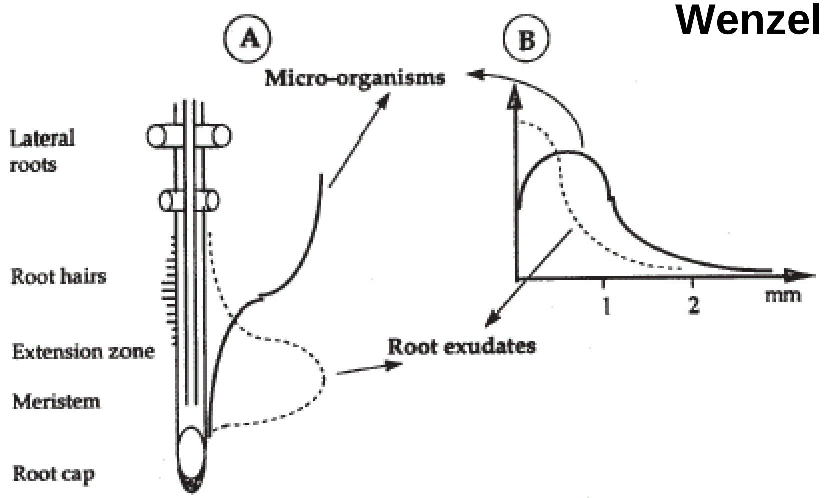


Fig. 13.2 Distribution patterns of microbial populations and root exudates in the rhizosphere *A* along the rhizoplane and *B* perpendicular to the rhizoplane (compiled from Römheld 1991 and Marschner 1995)

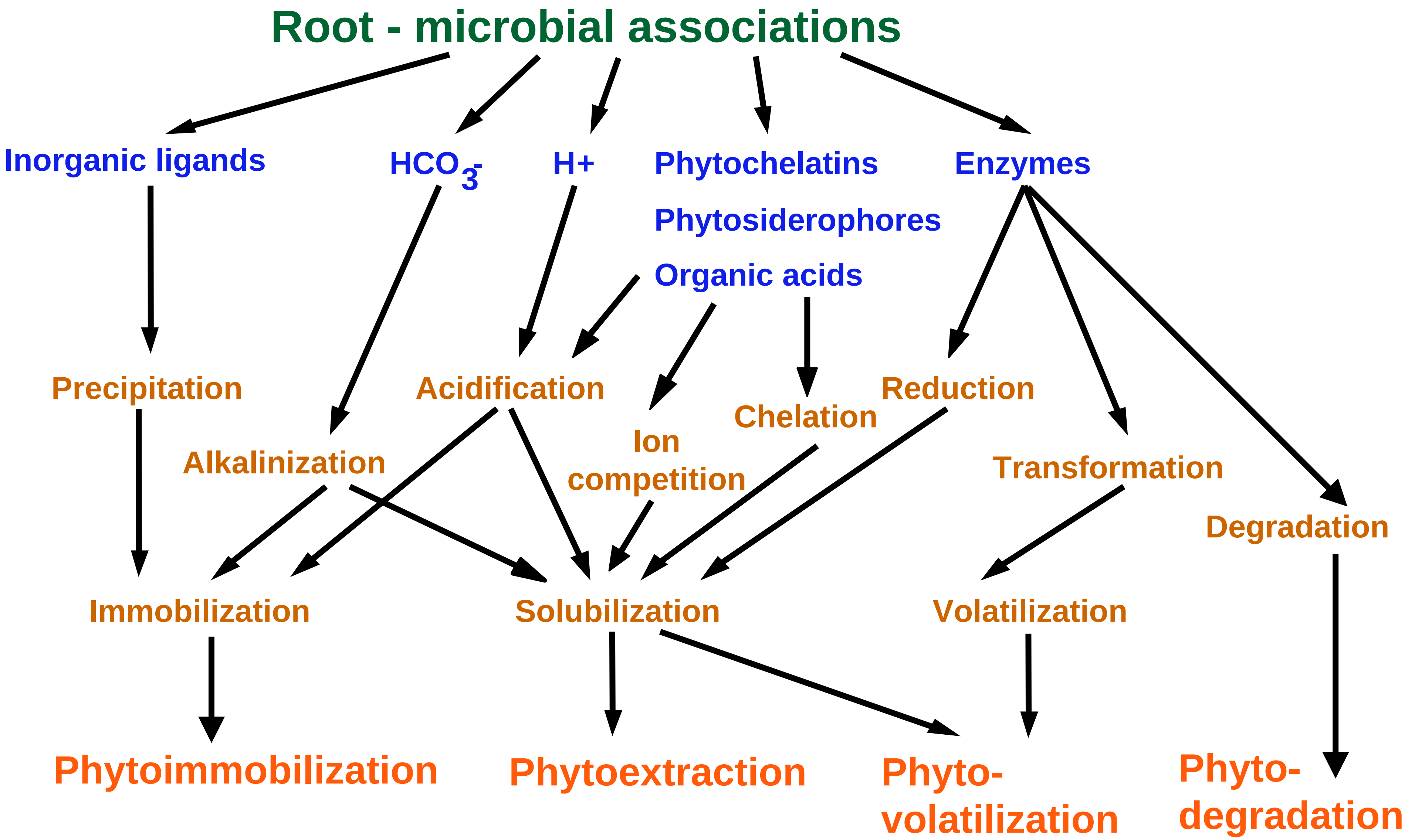
University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

Rhizosphere processes involved in Phytoremediation

- **Root exudation** → **mobilisation of heavy metals** (protons, organic acids)
  → **enhancing microbial activity** (any carbohydrates)
  → **degradation of hydrocarbons** (enzymes)

- **Microbial exudates** → **mobilisation of heavy metals** (protons, organic acids, sidero-phores)
  → **degradation of hydrocarbons** (enzymes)

Rhizosphere processes in phytoremediation



Wenzel et al. (1999)

Rhizosphere management to increase Phytoremediation efficiency

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

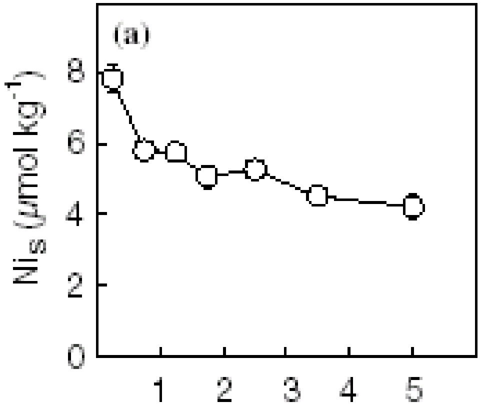
- Inoculation of specific microbes (bacteria, mycorrhiza)
- Selection of cultivars / clones → specific root exudation pattern
- Intercropping – mix the rhizosphere of different plant species

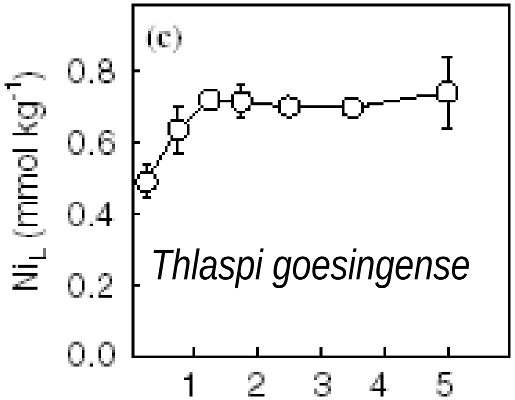
Phytoextraction of heavy metals:

Studies on hyperaccumulators

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

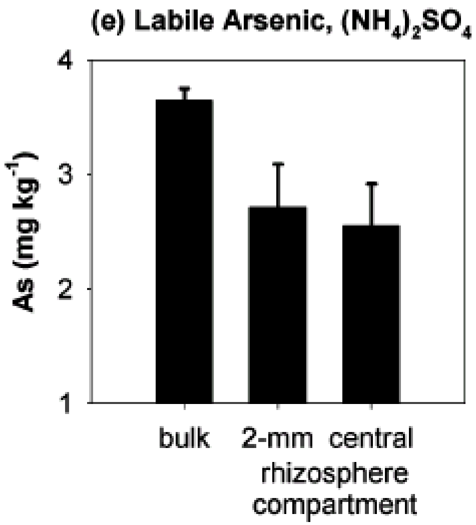
Puschenreiter et al. 2005; PLSO 271





Fitz et al. 2003; ES&T 37

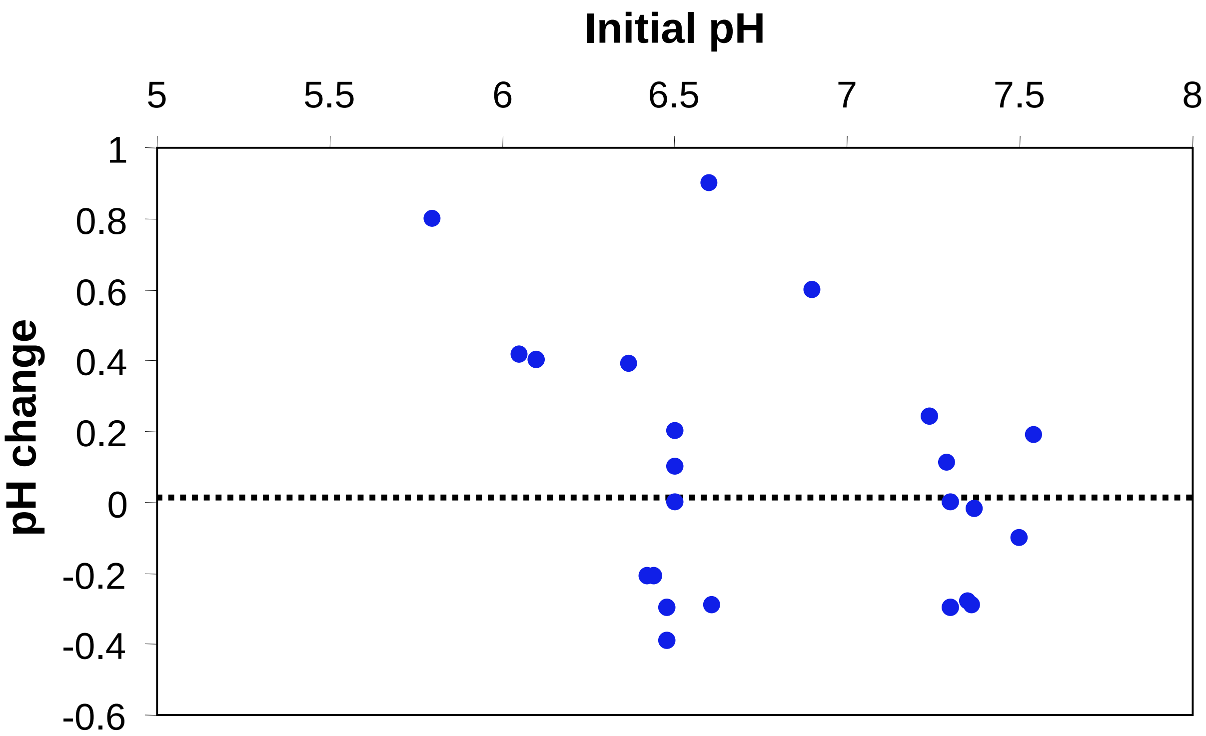
Pteris cretica



Phytoextraction of heavy metals:

Studies on hyperaccumulators

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences



Alyssum murale
Pteris vittata
Pteris cretica
Thlaspi caerulescens
Thlaspi goesingense

Bernal et al. (1994)
Fitz et al.
Knight et al. (1997)
Luo et al. (2000)
McGrath et al. (1997)
Puschenreiter et al. (2001)
Puschenreiter et al. (2005)
Wenzel et al. (2003)
Whiting et al. (2001)

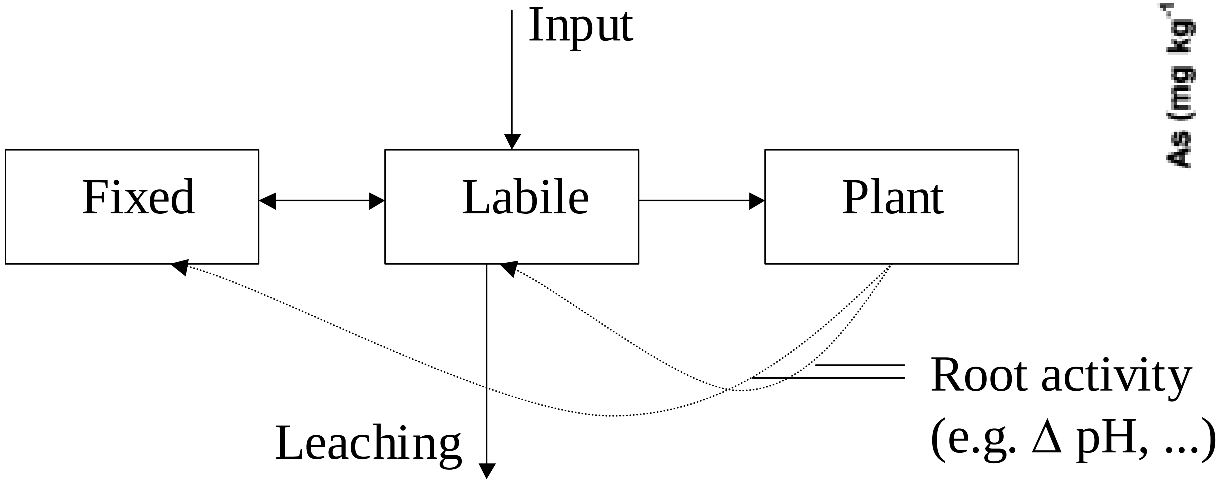
Phytoextraction of heavy metals:

Studies on hyperaccumulators

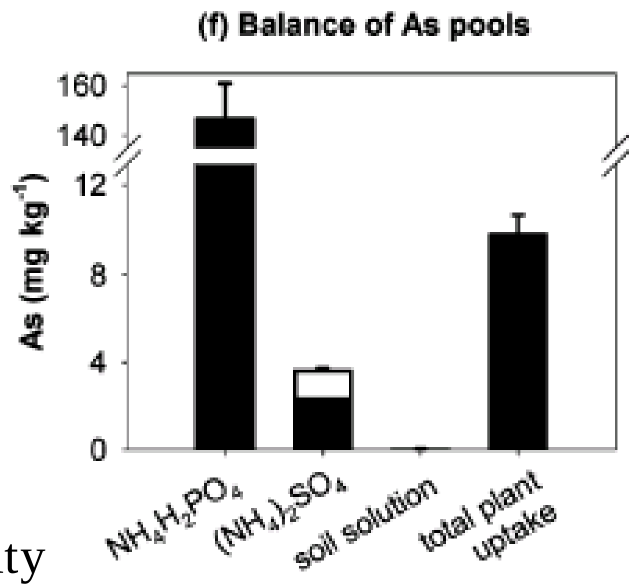
University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Changes of labile pools cannot explain plant uptake
- Mobilisation of heavy metals from less soluble fractions by root/microbial exudates?

Puschenreiter et al. 2003; JPNSS 166



Fitz et al. 2003; ES&T 37

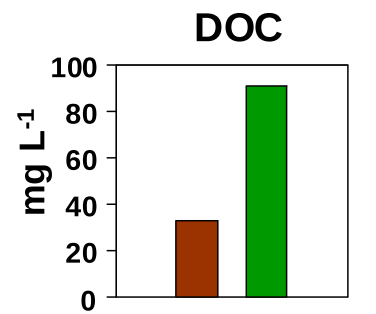


Phytoextraction of heavy metals:

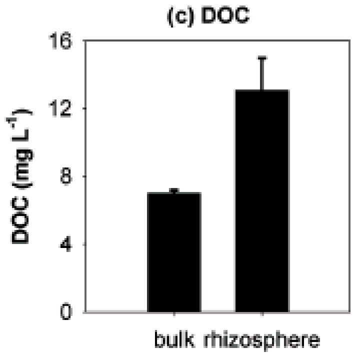
Studies on hyperaccumulators

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Root exudates involved in mobilisation of heavy metals?
- Increase of DOC was measured in the rhizosphere
- Compounds of root exudation not determined yet
  (in soil environment)

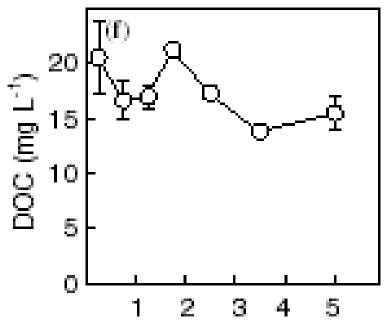


Fitz et al. 2003; ES&T 37

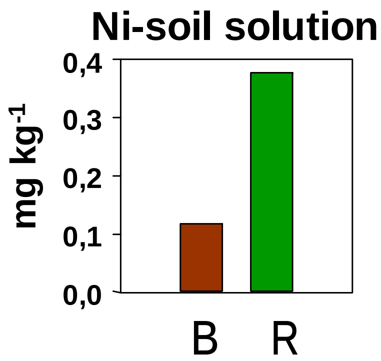


Pteris cretica

Puschenreiter et al. 2005; PLSO 271



Thlaspi goesingense



Wenzel et al. 2003

Phytoextraction of heavy metals:

Studies on hyperaccumulators

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

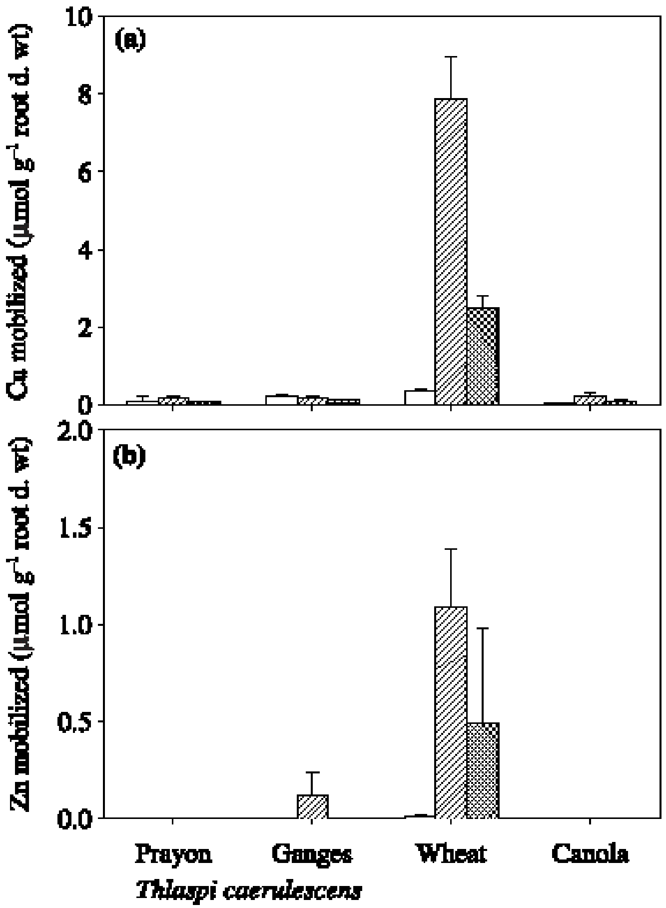


Fig. 2 Mobilization of Cu (a) and Zn (b) from Cu- or Zn-loaded resin by root exudates collected from *Thlaspi caerulescens*, wheat and canola. Error bars indicate SEs. Control, open columns; –Fe, hatched columns; –Zn, dotted columns.

Zhao et al. 2001; New Phytol 151

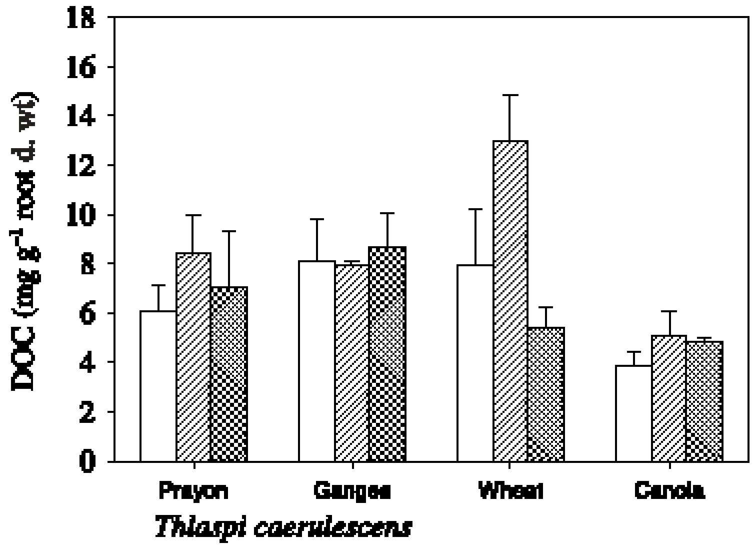


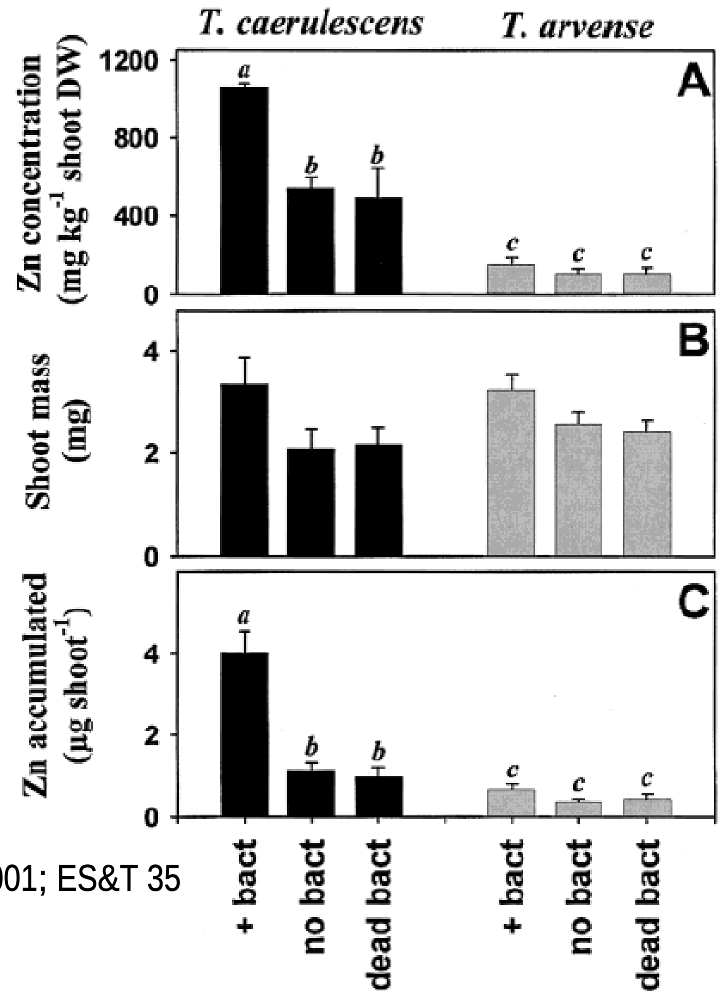
Fig. 1 Amounts of dissolved organic C in the root exudates collected from *Thlaspi caerulescens*, wheat and canola. Error bars indicate SEs. Control, open columns; –Fe, hatched columns; –Zn, dotted columns.

Phytoextraction of heavy metals:

Studies on hyperaccumulators

- Rhizosphere bacteria may significantly contribute to heavy metal uptake

- Bacterial exudates? Siderophores? ...



Whiting et al. 2001; ES&T 35

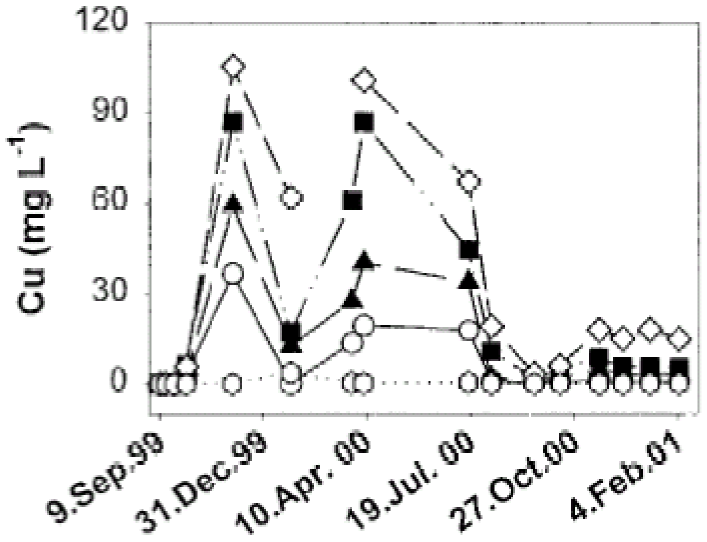
Phytoextraction of heavy metals:

Rhizosphere manipulation

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Induced phytoextraction: addition of synthetic chelates to enhance the bioavailability of metals
- problem: persistance of chelates in soil (slow microbial degradation) → leaching to groundwater!

Wenzel et al. 2003



Phytoextraction of heavy metals:

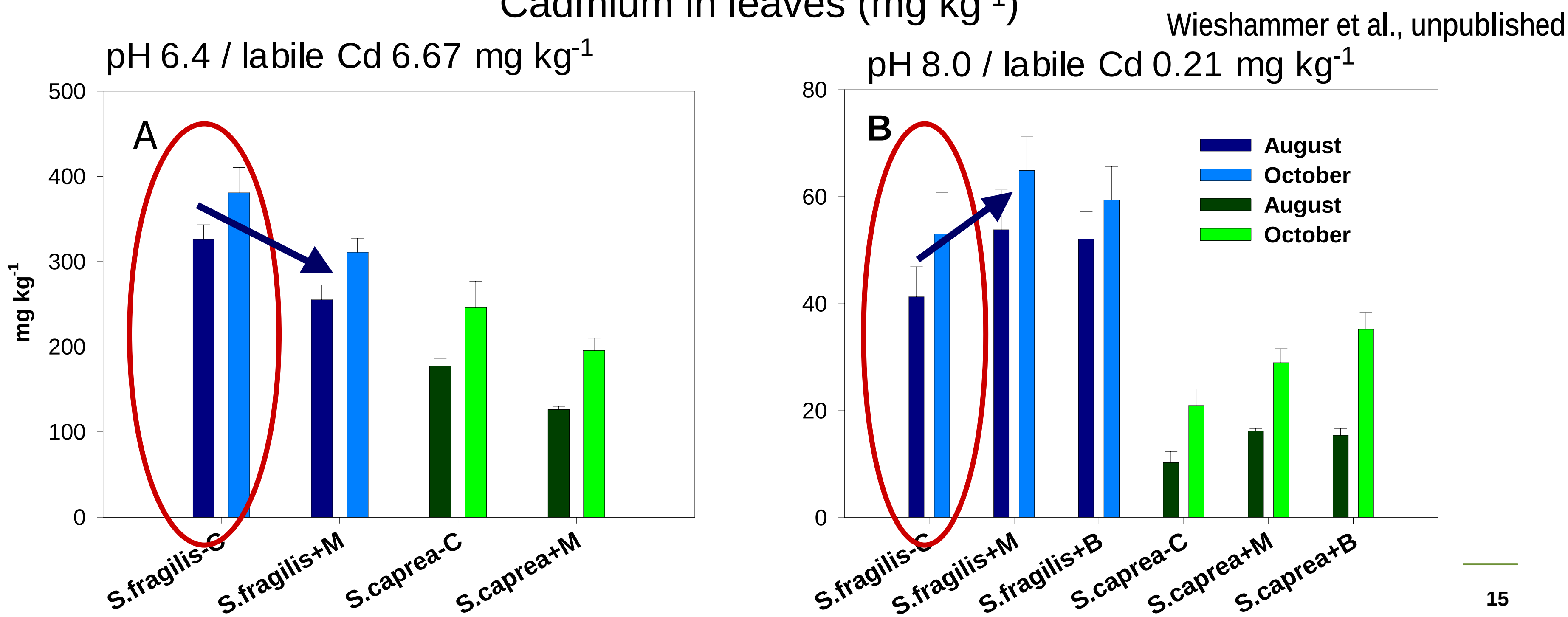
Studies on metal-accumulating willows

+ M: Mycor™ *Pisolithus tinctorius* and different rhizobacteria (*Bacillus* sp.)

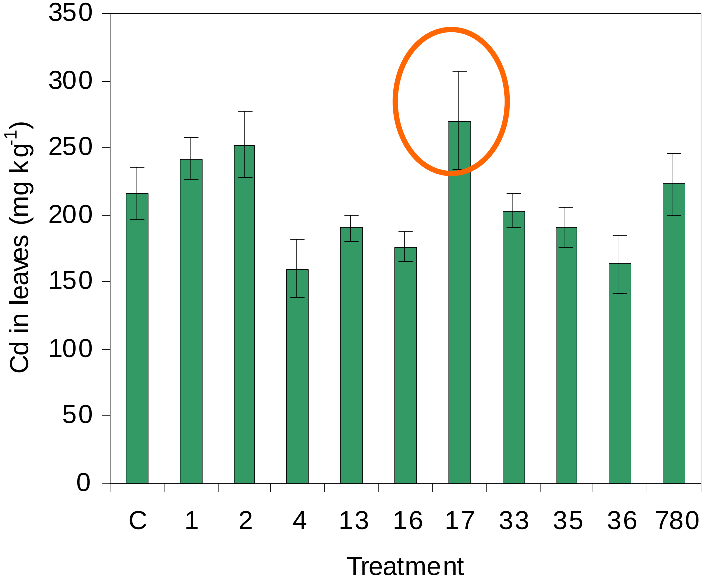
+ B: Rhizosphere bacteria isolated from *Salix caprea* rhizosphere grown on contaminated site Arnoldstein (Austria)

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

Cadmium in leaves (mg kg^{-1})



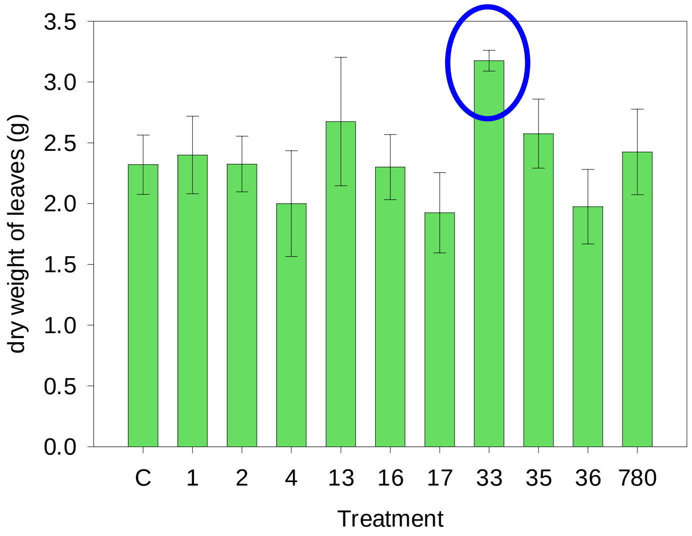
Puschenreiter et al., unpublished

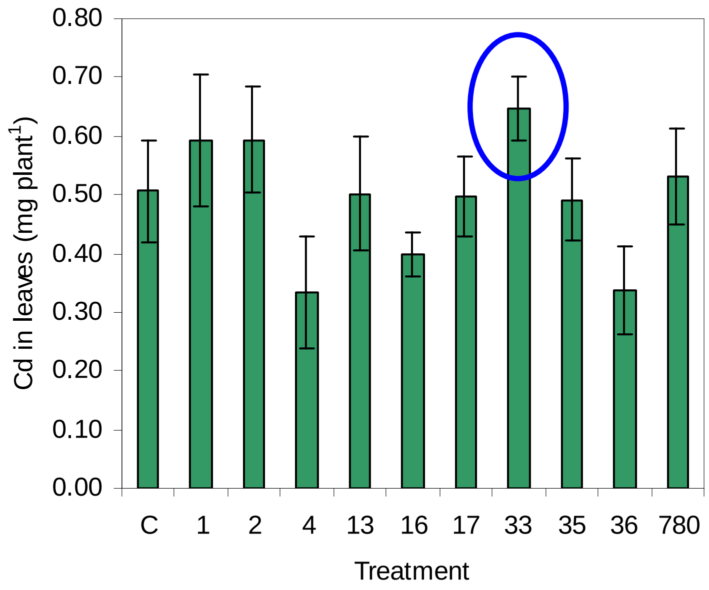


Phytoextraction of heavy metals:

Studies on metal-accumulating willows

- Influence of rhizosphere microbes on Zn/Cd accumulation by *Salix caprea*
- 17: *Streptomyces sp.*
  33: *Agromyces ramosus*



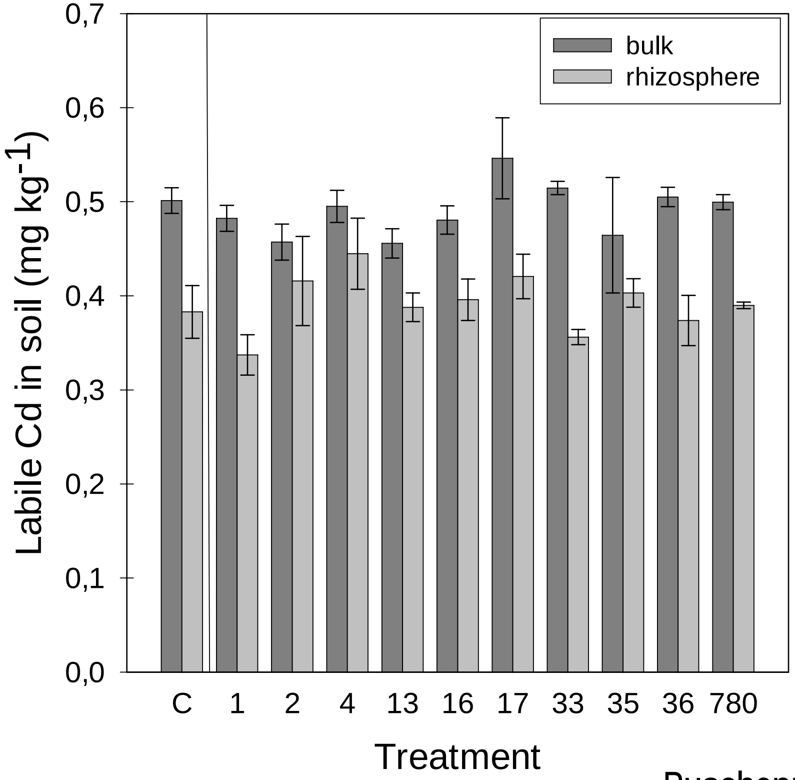


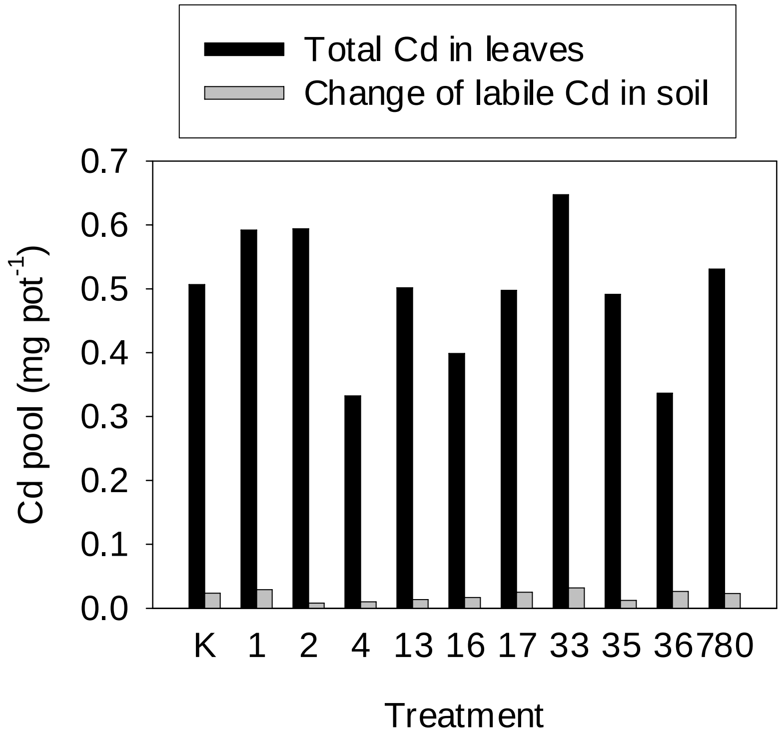
Phytoextraction of heavy metals:

Studies on metal-accumulating willows

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Influence of rhizosphere microbes on Zn/Cd accumulation by *Salix caprea*





Puschenreiter et al., unpublished

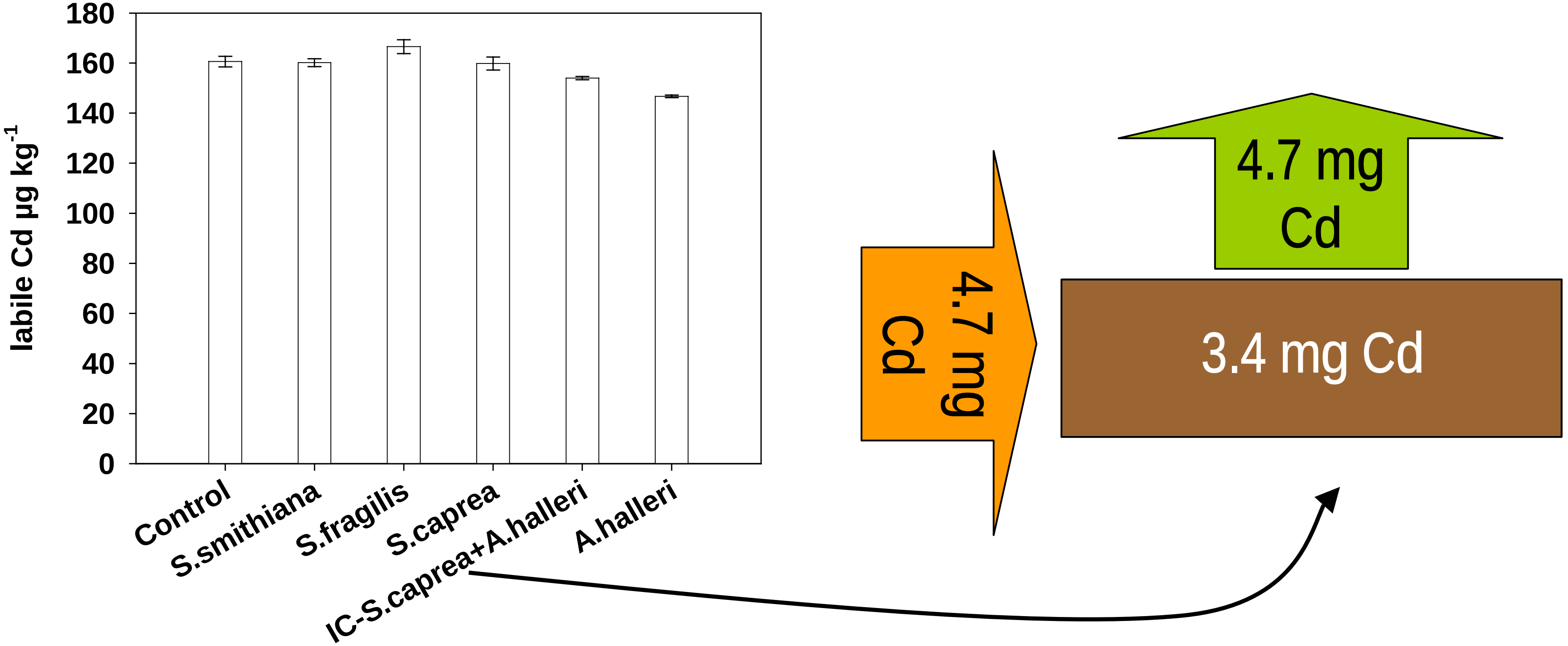
Phytoextraction of heavy metals:

Studies on metal-accumulating willows

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- labile Cd and calculated metal fluxes after first phytoextraction period (pot experiment)

Wieshammer et al., unpublished

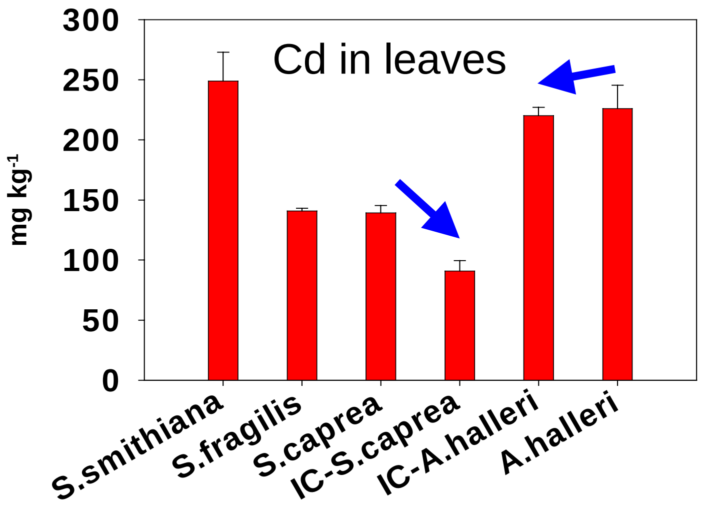


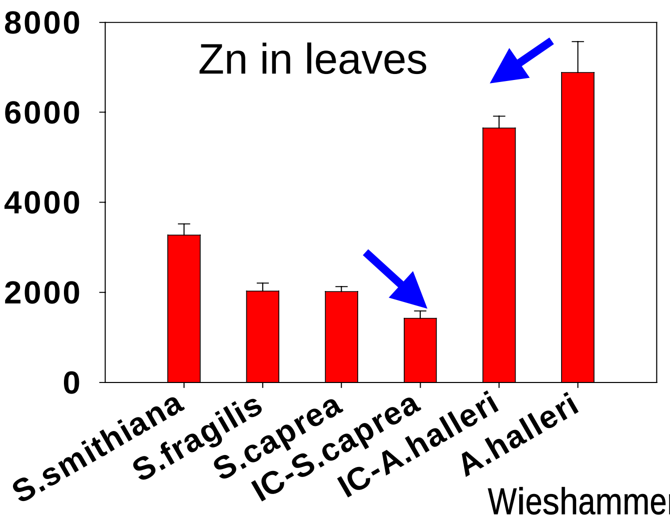
Phytoextraction of heavy metals:

Studies on metal-accumulating willows

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- **Intercropping:** Two (or more) plant species / cultivars share the rhizosphere → mutual influences
- competition for metals in the lysimeters with limited root zone
- likely less critical in field conditions as roots explore different zones
- enhanced total metal removal
- reduced leaching / enhanced overall transpiration rates
- understorey plants can minimise surface run off





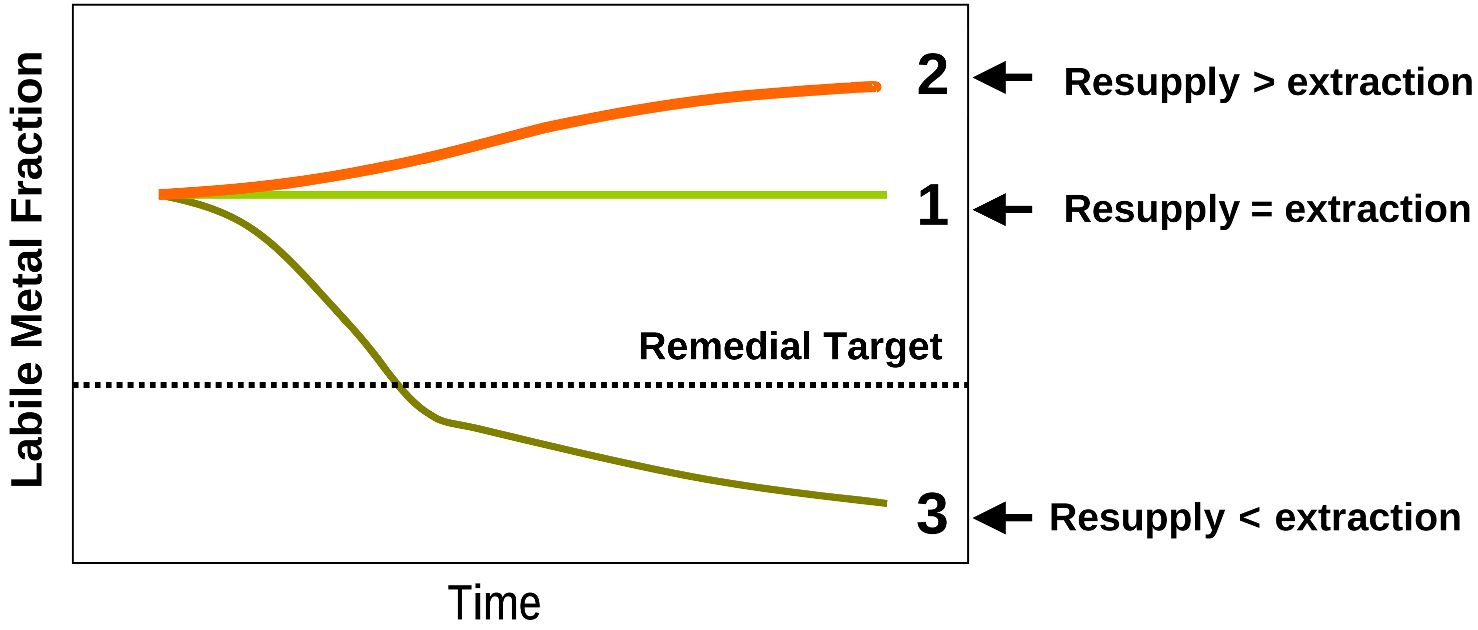
Wieshammer et al., unpublished

Phytoextraction of heavy metals:

The concept of bioavailable contaminant stripping (BCS)

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Reducing the bioavailable fraction of contaminants in soil (according to Hamon and McLaughlin, 1999)



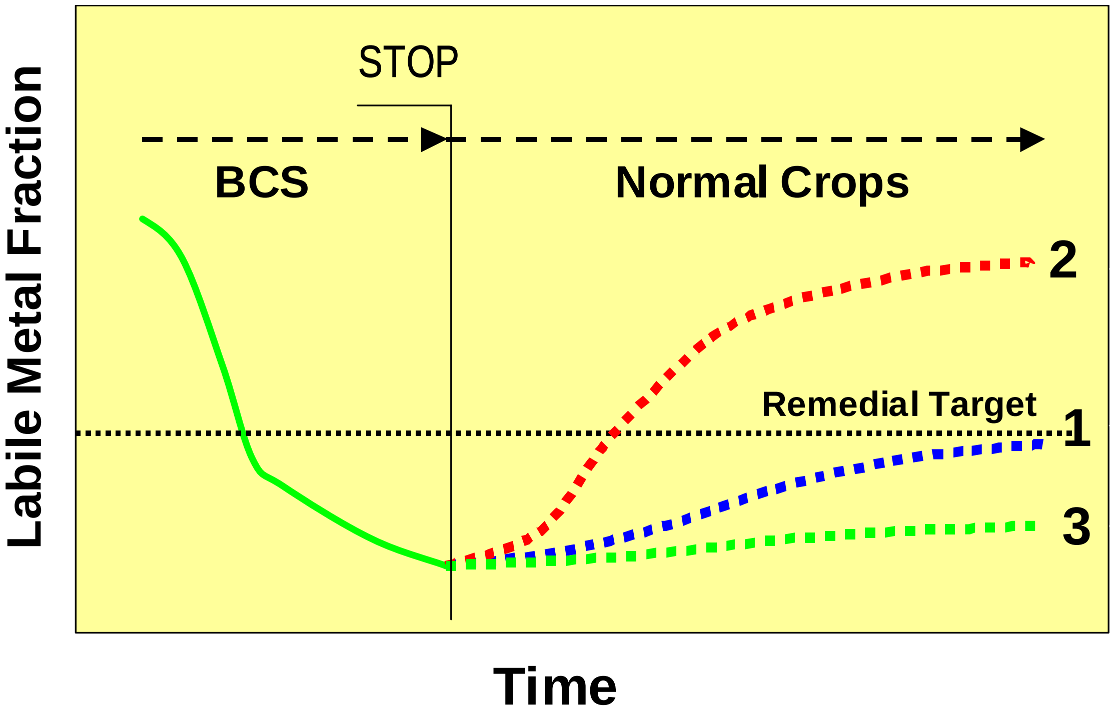
Phytoextraction of heavy metals:

The concept of bioavailable

contaminant stripping (BCS)

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Sustainability?



No success

At risk

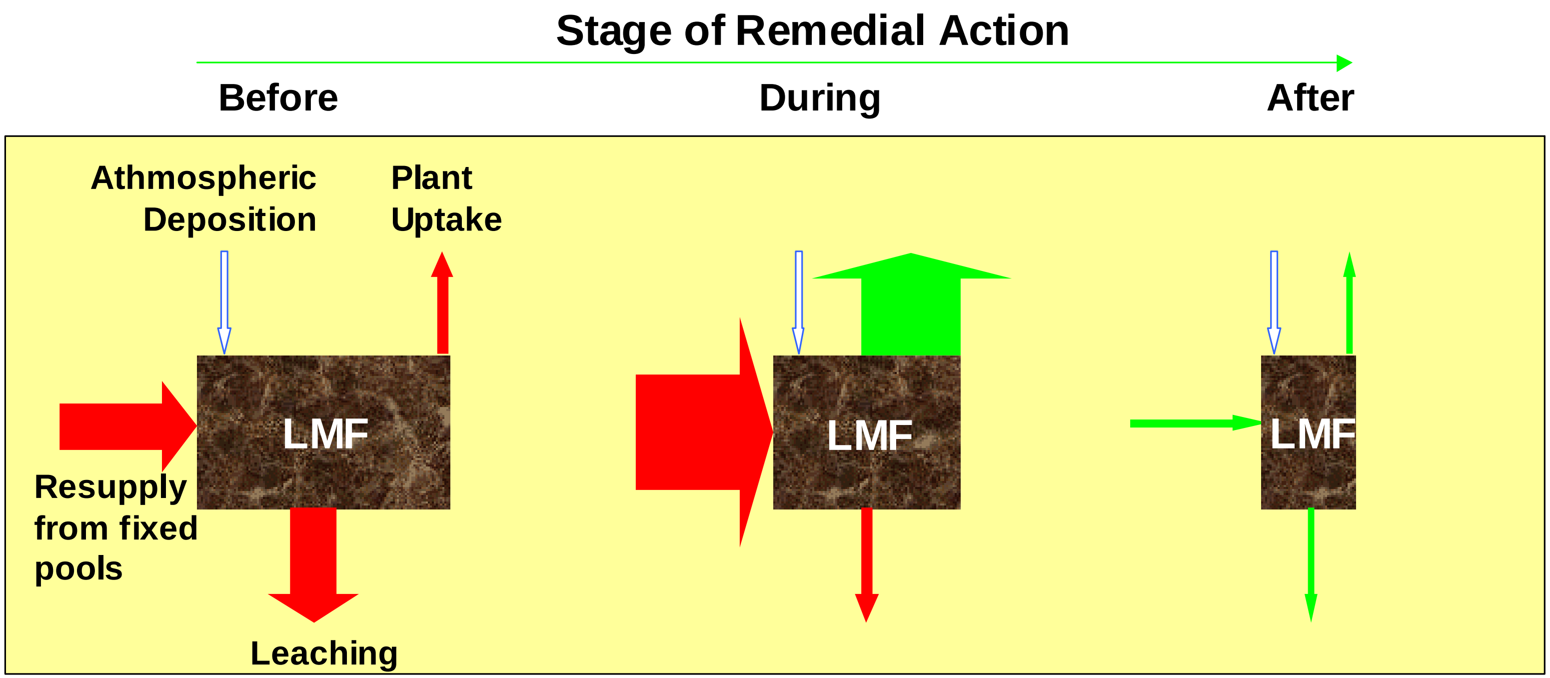
Sustainable remediation

Phytoextraction of heavy metals:

The concept of bioavailable contaminant stripping (BCS)

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Target of BCS-based phytoextraction

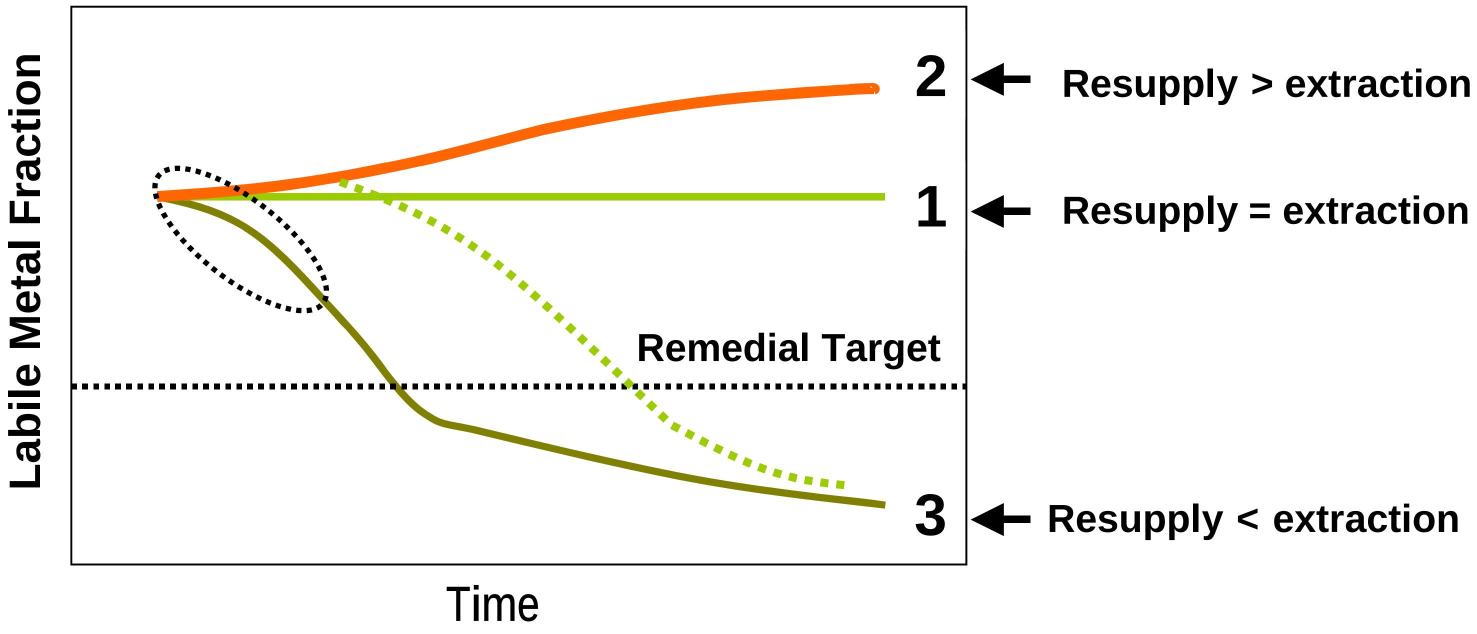


Phytoextraction of heavy metals:

The concept of bioavailable contaminant stripping (BCS)

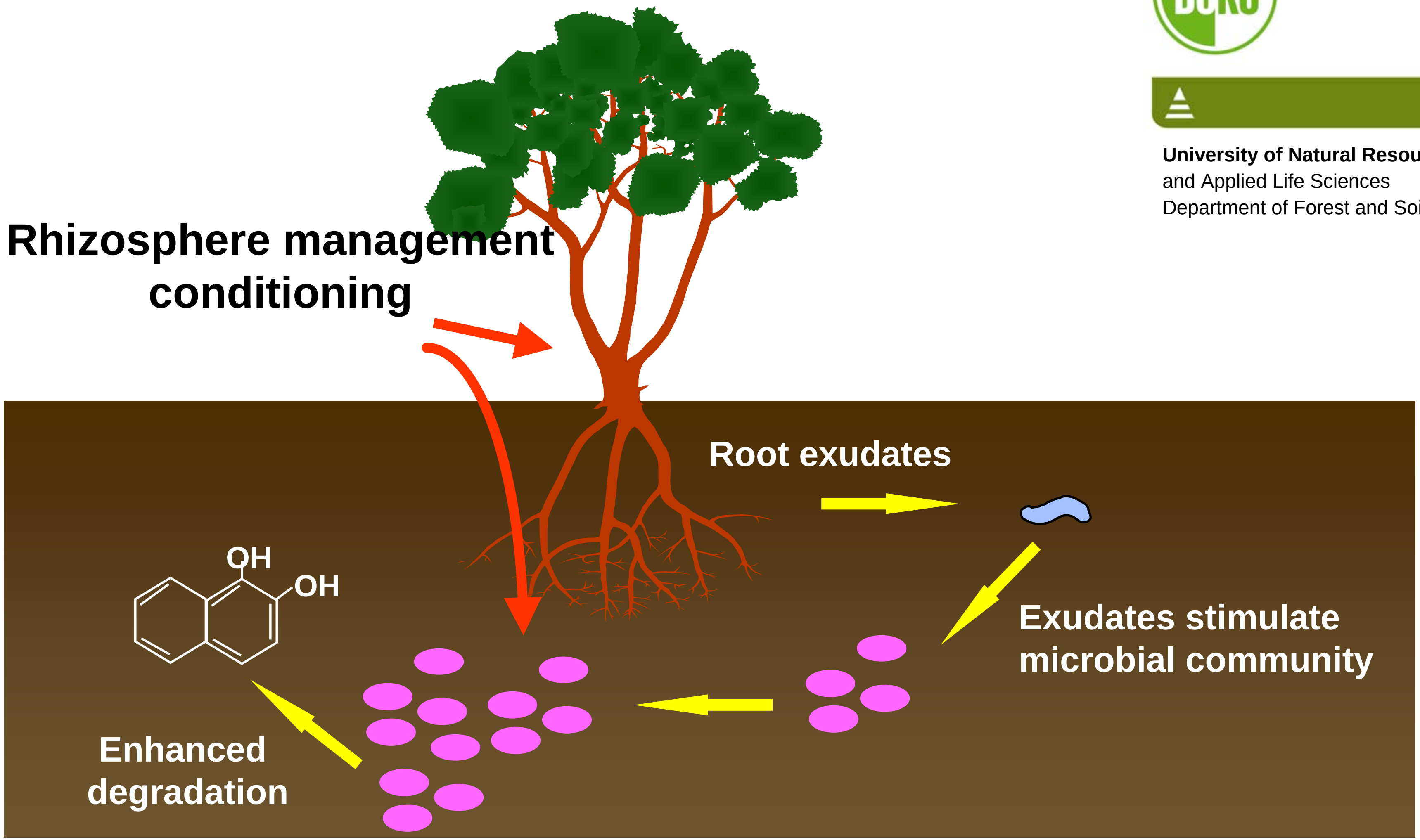
University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- High buffer capacity of the soil → delayed decrease of the labile (bioavailable fraction)



Phytoremediation

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

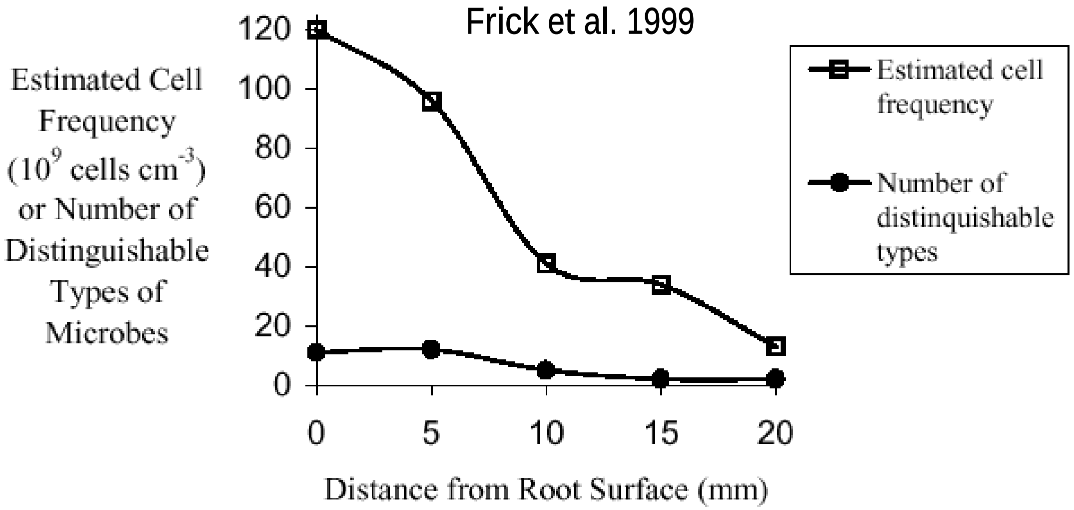


Phytodegradation of organic pollutants

The rhizosphere effect

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Stimulation of specific microbial activity in the rhizosphere; specific populations
- Root exudates as microbial carbon source and signal carriers
- microbial populations and activities 5 – 100 times greater in rhizosphere compared to bulk soil

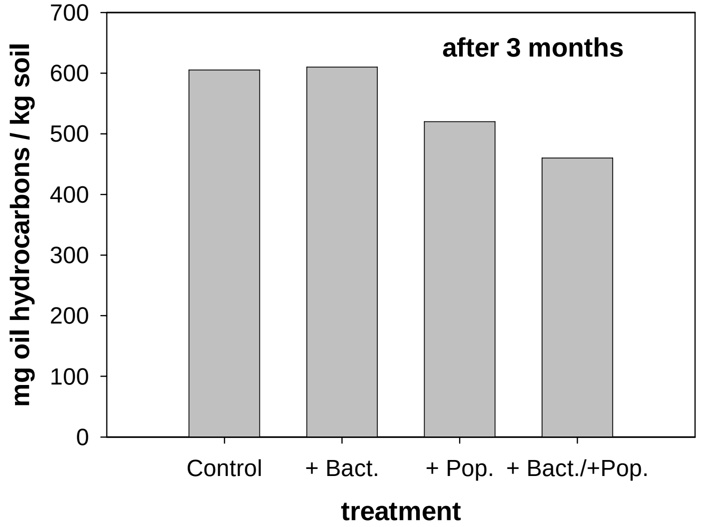


Phytodegradation of organic pollutants

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- small pot experiment
- inoculation of hydrocarbon-degrading bacteria
- combination of plant+bacteria most successful

Tesar et al. 2003



Phytodegradation of organic pollutants

Role of plant enzymes released by roots

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

Table 3
Plant enzymes that have a role in transforming organic compounds

Enzyme	Plants known to produce enzymatic activity	Application
Dehalogenase	Hybrid poplar (*Populus* spp.), algae (various spp.), parrot feather (*Myriophyllum aquaticum*)	Dehalogenates chlorinated solvents
Laccase	Stonewort (*Nitella* spp.), parrot-feather (*Myriophyllum aquaticum*)	Cleaves aromatic ring after TNT is reduced to triaminotoluene
Nitrilase	Willow (*Salix* spp.)	Cleaves cyanide groups from aromatic rings
Nitroreductase	Hybrid poplar (*Populus* spp.), Stonewort (*Nitella* spp.), parrot feather (*Myriophyllum aquaticum*)	Reduces nitro groups on explosives and other nitroaromatic compounds, and removes nitrogen from rings structures
Peroxidase	Horseradish (*Armoracia rusticana* P. Gaertner, Meyer & Scherb)	Degradation of phenols (mainly used in wastewater treatment)
Phosphatase	Giant duckweed (Spirodela polyrhiza)	Cleaves phosphate groups from large organophosphate pesticides

Susarla et al. 2002

Phytodegradation of organic pollutants

Supporting the microbial degradation – Rhizosphere management

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Choosing proper plant species
- Supporting the plant microbe system by fertilization, adjusting pH (mostly liming), irrigation, drainage
- Inoculation with hydrocarbon-degrading bacteria (adapted or genetically engineered). Successful establishment may require repeated introduction.
- Additional inoculation with biosurfactant producing bacteria

Rhizosphere processes involved in phytoremediation of contaminated soils - Summary

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

- Plant roots deplete the bioavailable heavy metal fraction
- Root exudates may change heavy metal bioavailability
- Root exudates may stimulate microbial activity
- Microbial activity may change heavy metal bioavailability
- Microbial activity may enhance the process of hydrocarbon degradation
- Microbial activity may enhance plant growth (rooting density)
- Combining plants and microbes (Inoculation / Intercropping) to enhance the phytoremediation efficiency

Acknowledgements

University of Natural Resources
and Applied Life Sciences
Department of Forest and Soil Sciences

Colleagues: Alex Dellantonio, Maria Noel Dos Santos, Walter Fitz, Ingrid Langer, Peter Schweiger, Reinhard Unterbrunner, Gerlinde Wieshammer, Michael Zivkovic, etc.

Project Partners: Othmar Horak, Angela Sessitsch, Joseph Strauss, etc.

Those who paid for research work: Austrian Science Fund (FWF), Vienna Science and Technology Fund (WWTF), Austrian Federal Ministry for Agriculture, Forestry, Environment and Water Management, City of Vienna

My family

Thanks for your attention!