



Uptake and distribution of ¹⁴C-TNT in conifers

TV5-associated Project A1:

Title: „Dendrotolerance to explosive specific substances in soils and long-term fate of [¹⁴C]-trinitrotoluene and [¹⁴C]-RDX in coniferous trees.“



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Faculty for Horticulture and Agriculture,
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Section Phytomedicine

www.schoenmuth.de/kora/schoenmuth-BBA2007.pdf

2) Federal Research Centre for
Agriculture and Forestry (BBA),
Institute for Ecotoxicology and
Ecochemistry in Plant Protection



Background

Background is the fact, that many formerly as well as currently used military sites are contaminated with TNT.

Often these areas are covered with woodlands.

In Germany, and other European countries these woodlands are dominated by conifers, especially by Norway spruce (*Picea abies*) and by Scots pine (*Pinus sylvestris*).

Moreover, we recently found that coniferous trees are more tolerant to TNT and other explosive specific substances than broadleaf trees or herbaceous plants.

It is necessary to investigate the potential of (conifer) trees for their ability for natural reduction of TNT soil contamination.

While for broad-leaved trees some information about TNT uptake and TNT metabolism is available, up to now, for conifers almost no results may be found.





Material

4-5-years-old conifers used for [14C]-TNT uptake experiments:



Botanical: ***Pinus sylvestris***
English: Scots pine
German: Gemeine Kiefer



Botanical: ***Picea glauca „Conica“***
English: Canadian white spruce
German: Zuckerhutfichte





Application system for [¹⁴C]-TNT

gravimetrical control
of [¹⁴C]-input



dynamical
[¹⁴C]-spiking

Glas fibre wick application systems:

- # Allow both, permanent and pulse applications of explosive specific substances (XSS)
- # Gravimetical transpiration measurements in large series are possible
- # Dynamical mass input of explosives into soil/tree systems is quantifiable

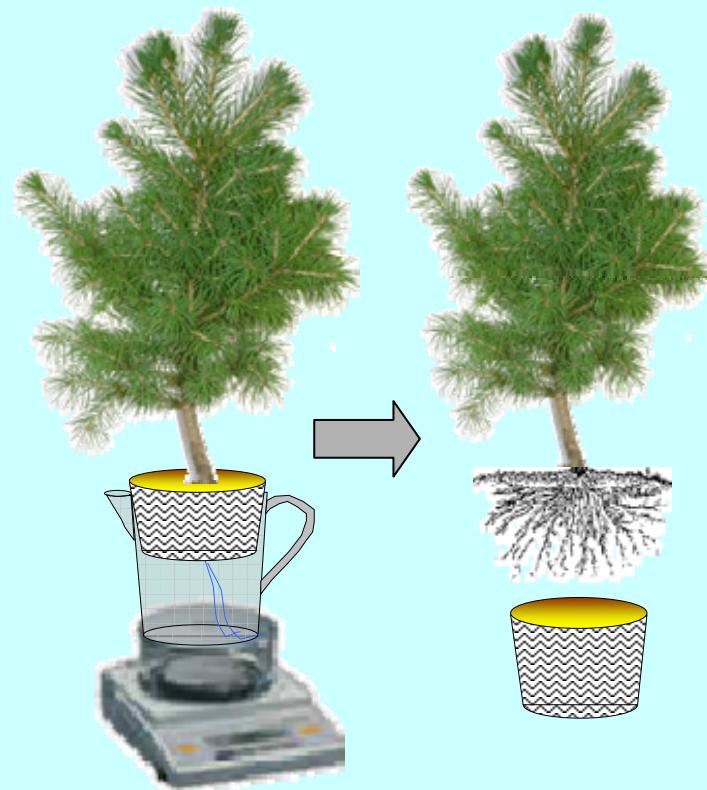
**Applied concentration:
45 mg/l TNT, spiked with [¹⁴C]-TNT**





Tree harvest, 5 weeks after single pulse of [¹⁴C]-TNT

gravimetrical control
of ¹⁴C-input



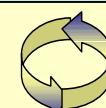
dynamical
[¹⁴C]- spiking

separation
of tree parts



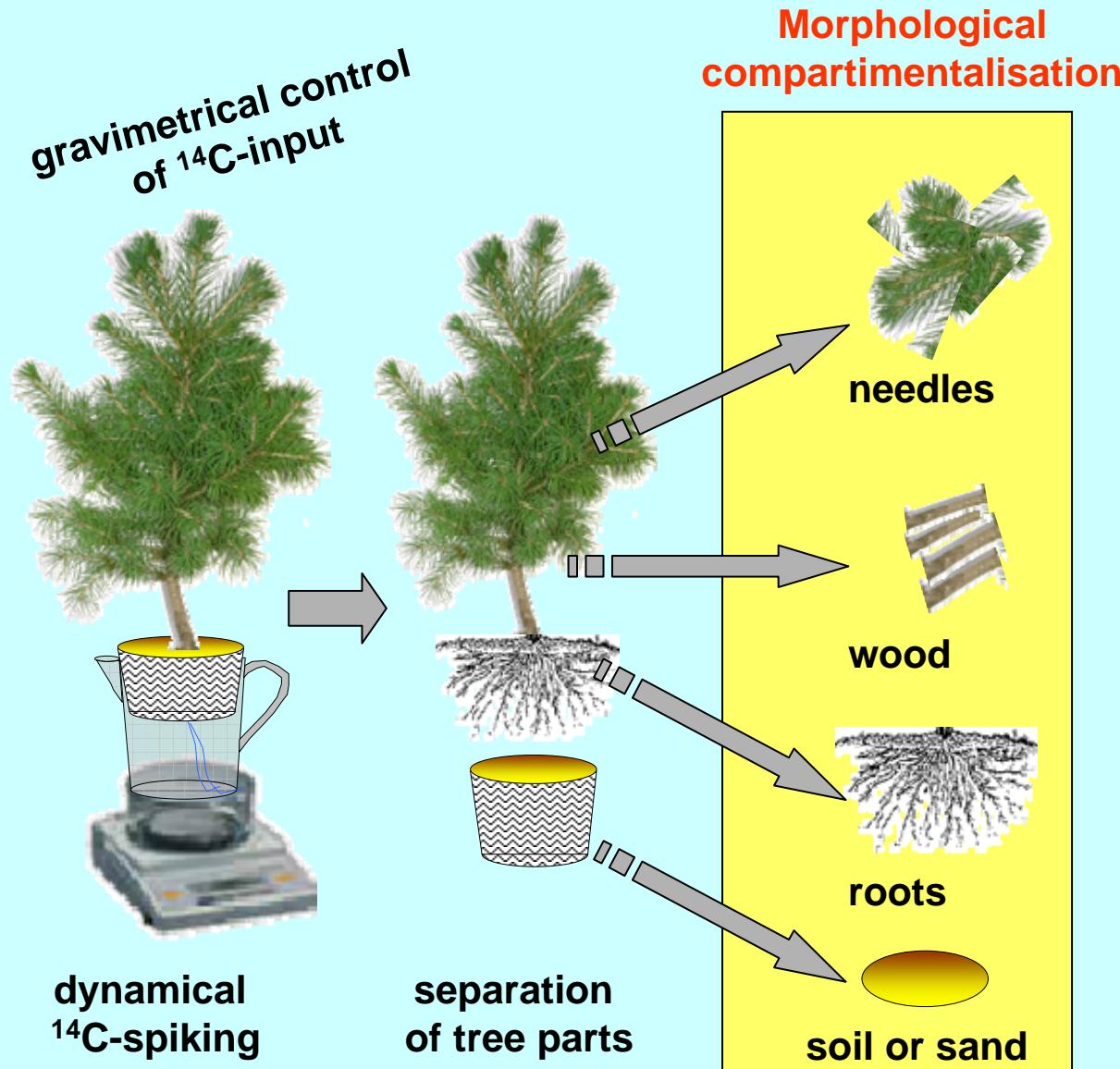
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Morphological mass fractionation for TNT uptake analysis



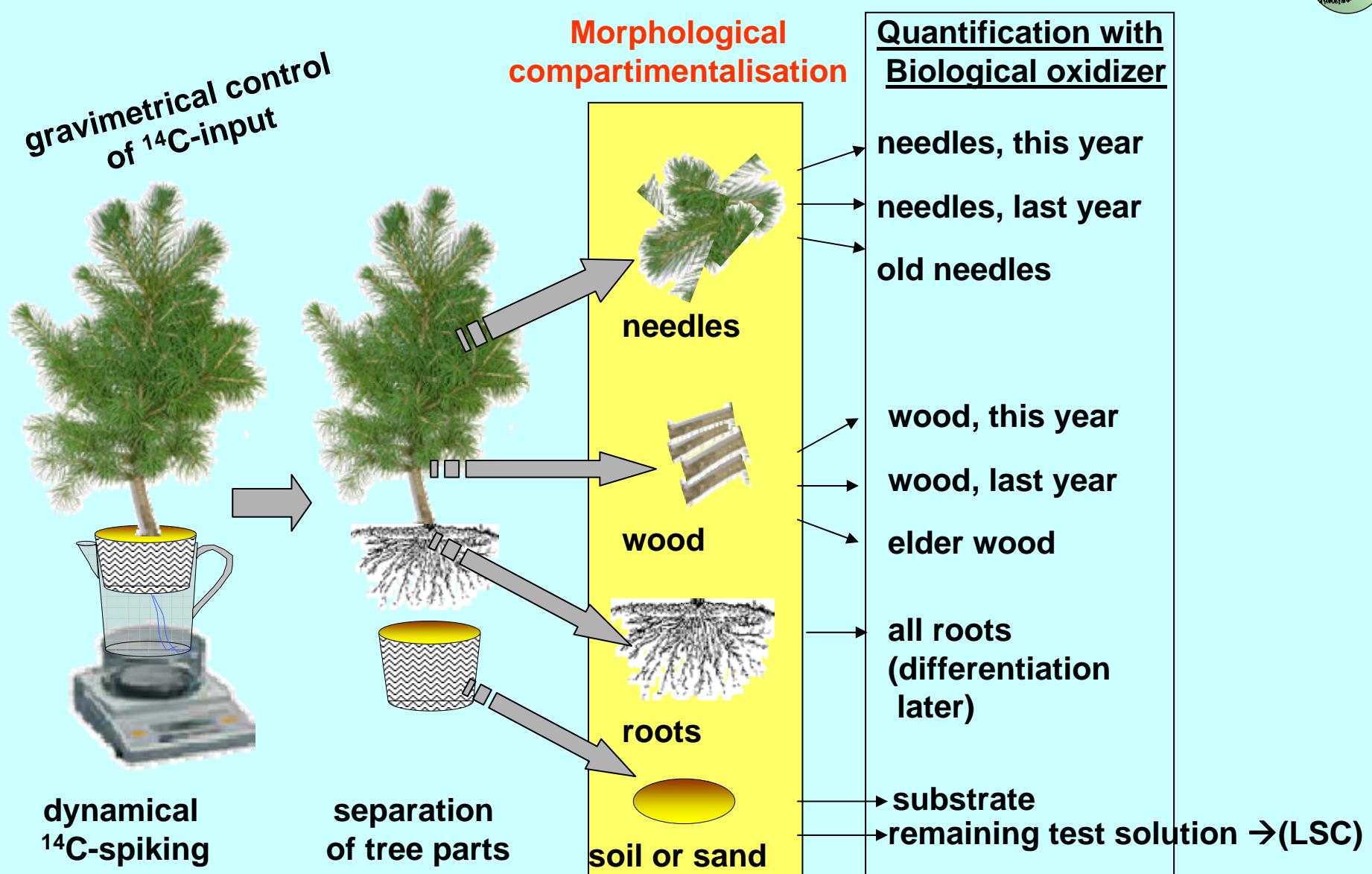
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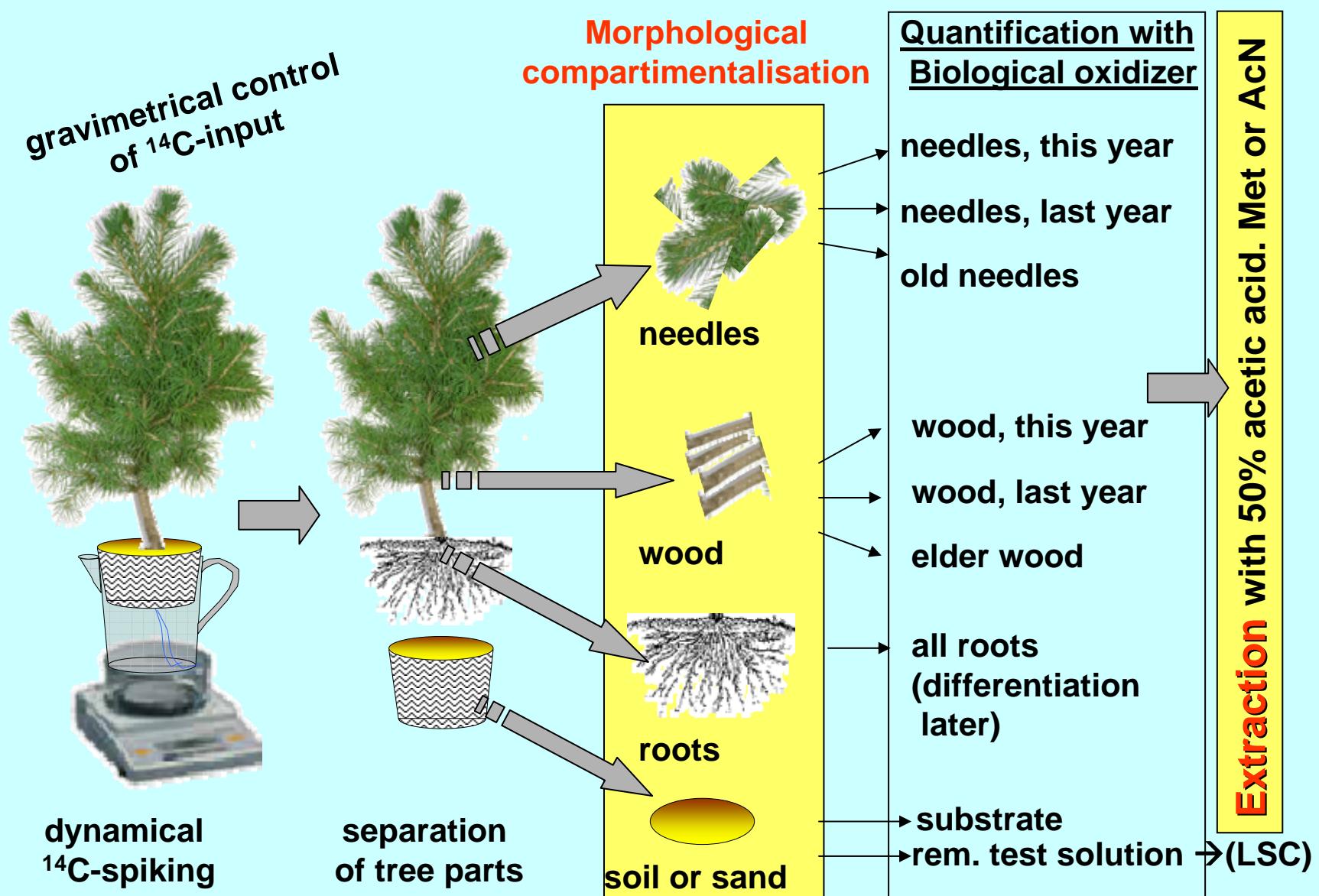
Morphological mass fractionation of TNT uptake



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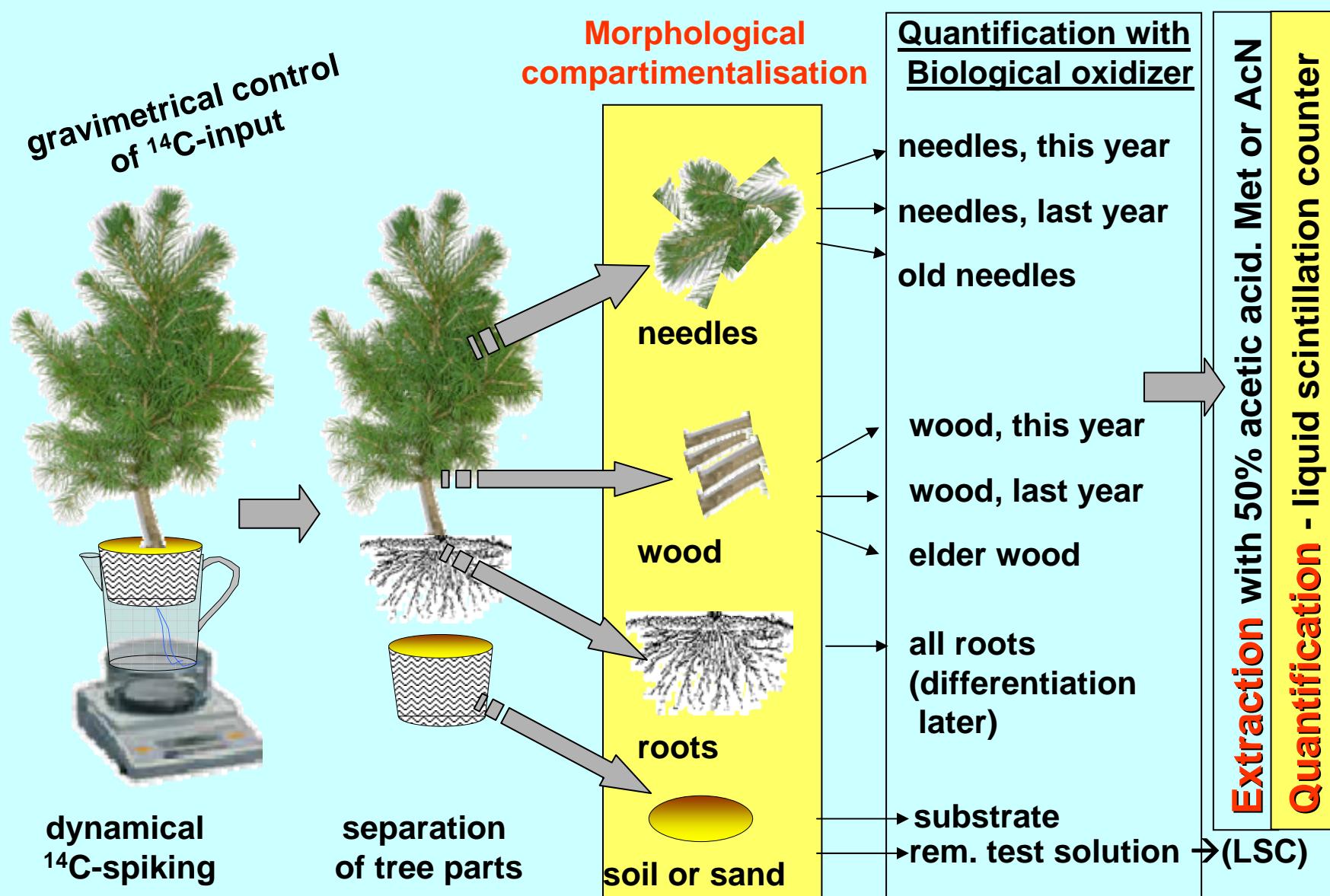


Morphological mass fractionation of TNT uptake analysis





Morphological mass fractionation of TNT-uptake



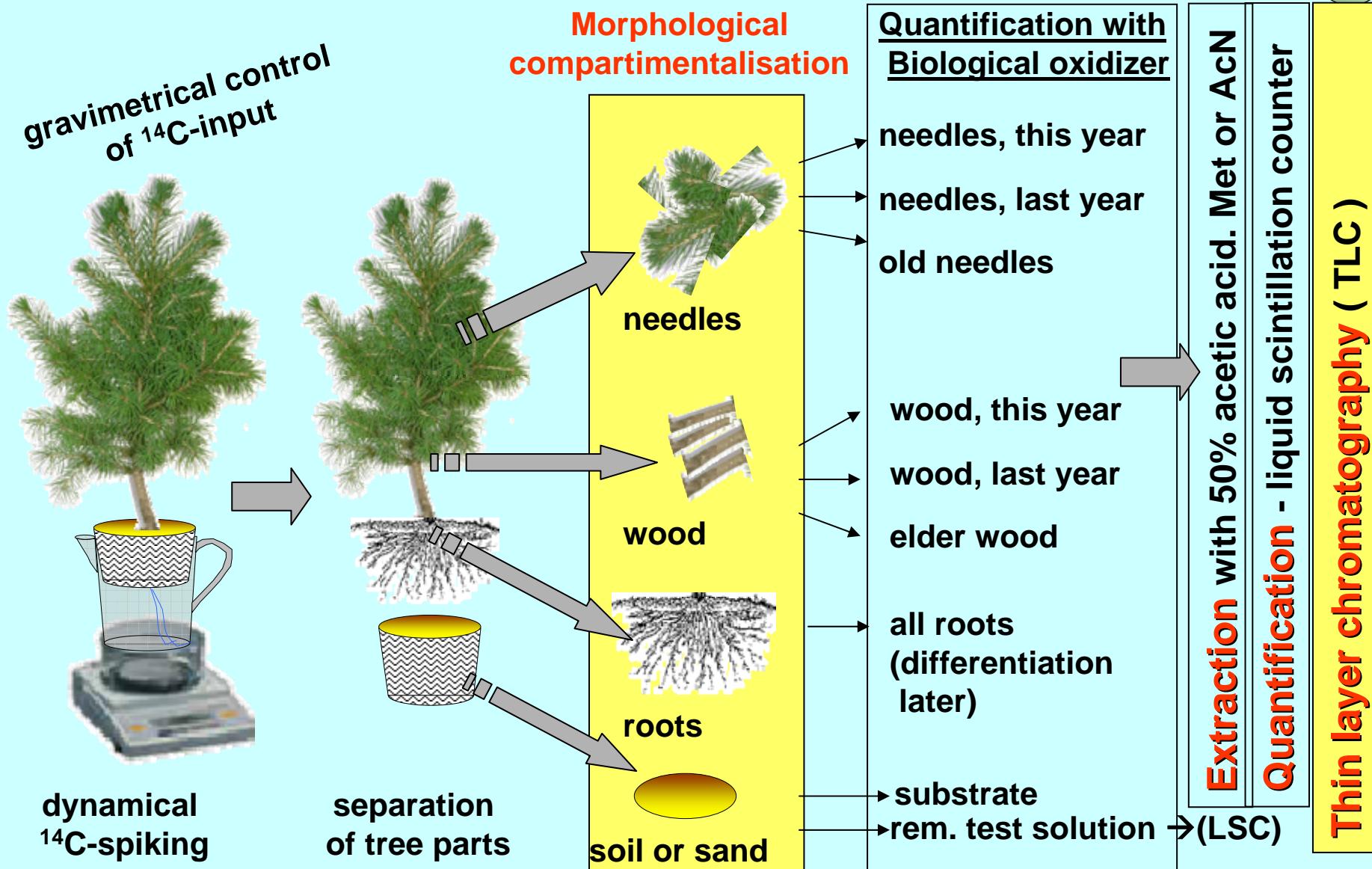
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Morphological mass fractionation of TNT-uptake



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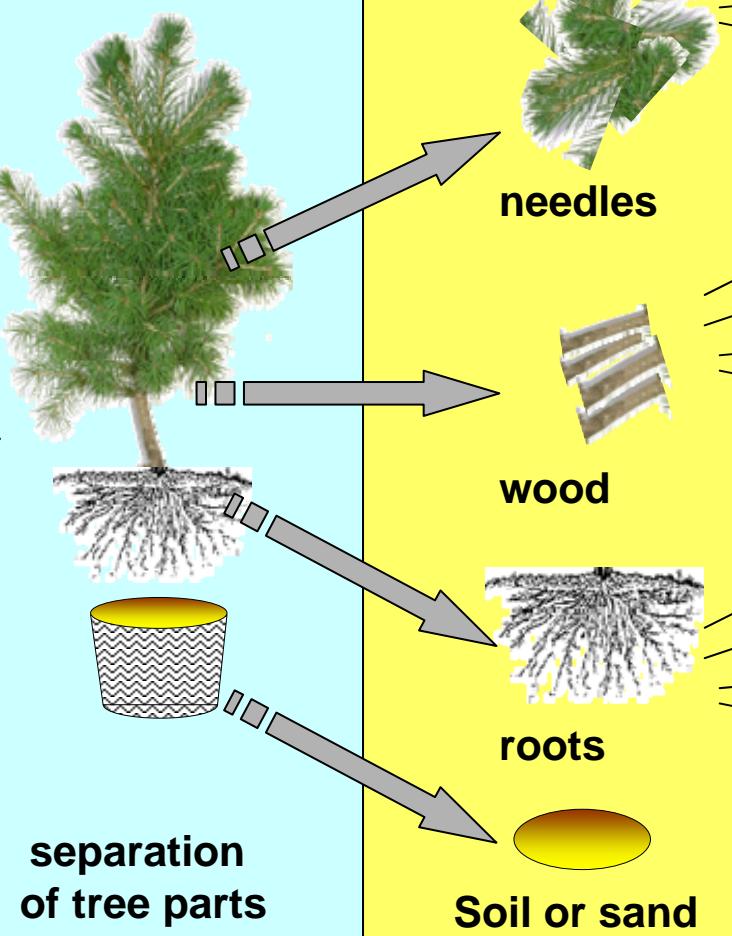


Chemical fractionation of [¹⁴C]-TNT uptake

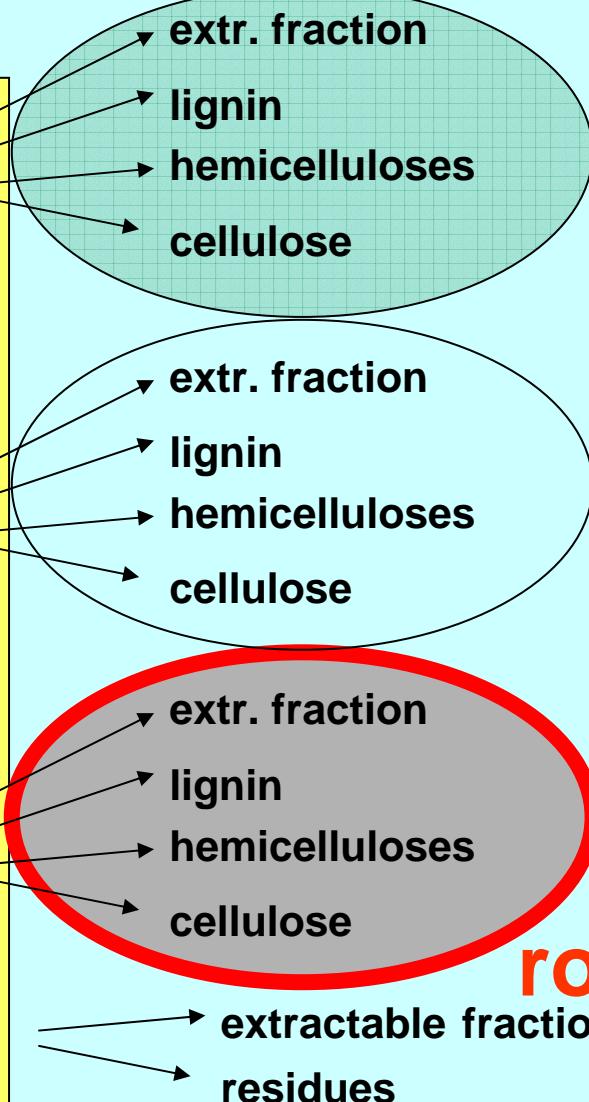
gravimetrical control
of ¹⁴C-input



1. Morphological compartmentalisation

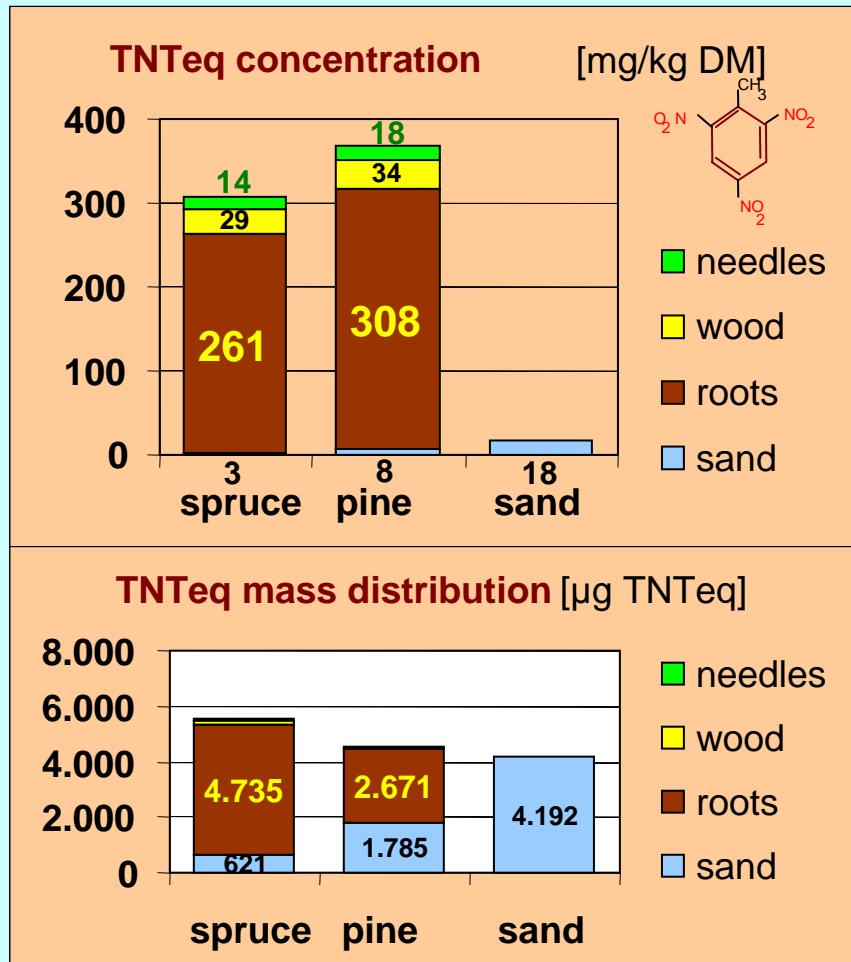


2. Biochemical compartmentalisation





Results: Morphological [¹⁴C]-TNT distribution in conifers



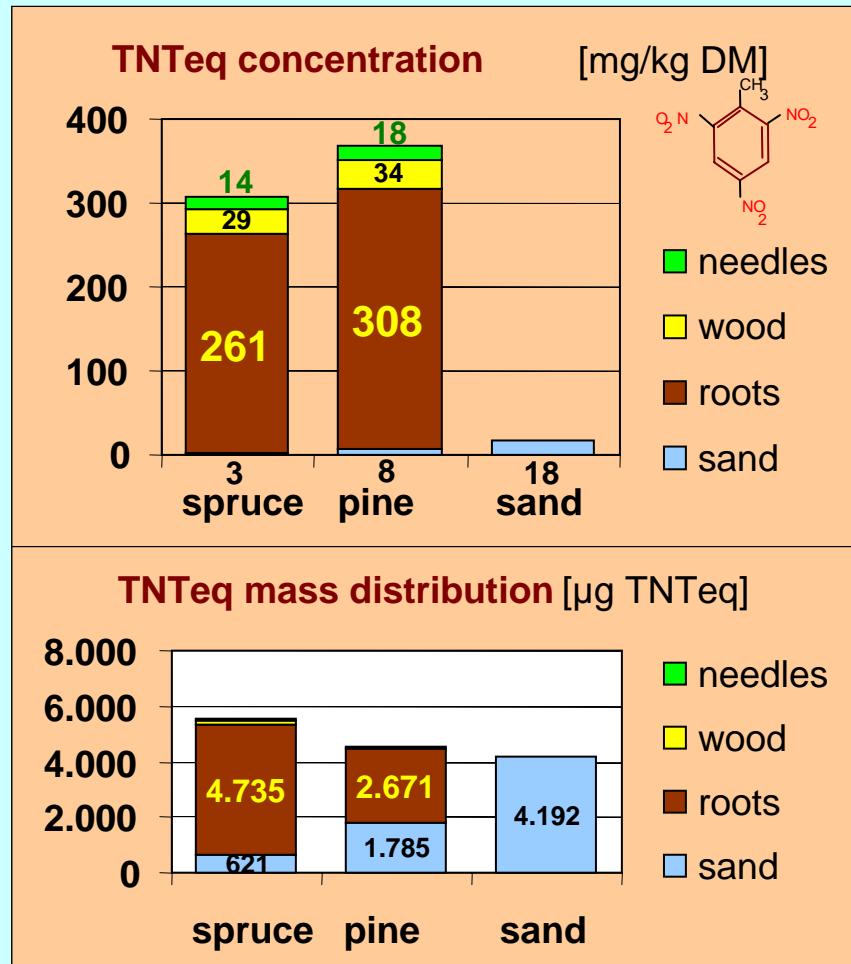
TNT is predominantly accumulated in roots

Up to 300 mg TNTeq per kg DM in pine roots

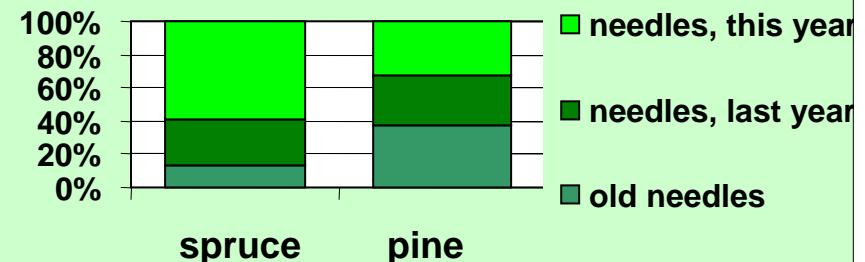
Conifers can lower TNT content remarkably in TNT containing substrates.



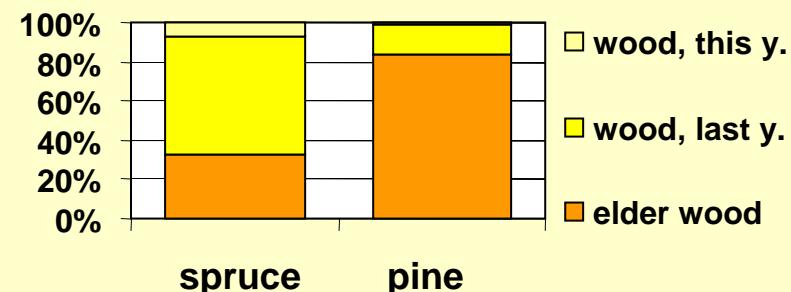
¹⁴C-TNT localisation in conifers



rel. TNTeq mass in needles [%]



rel. TNTeq mass in wood [%]

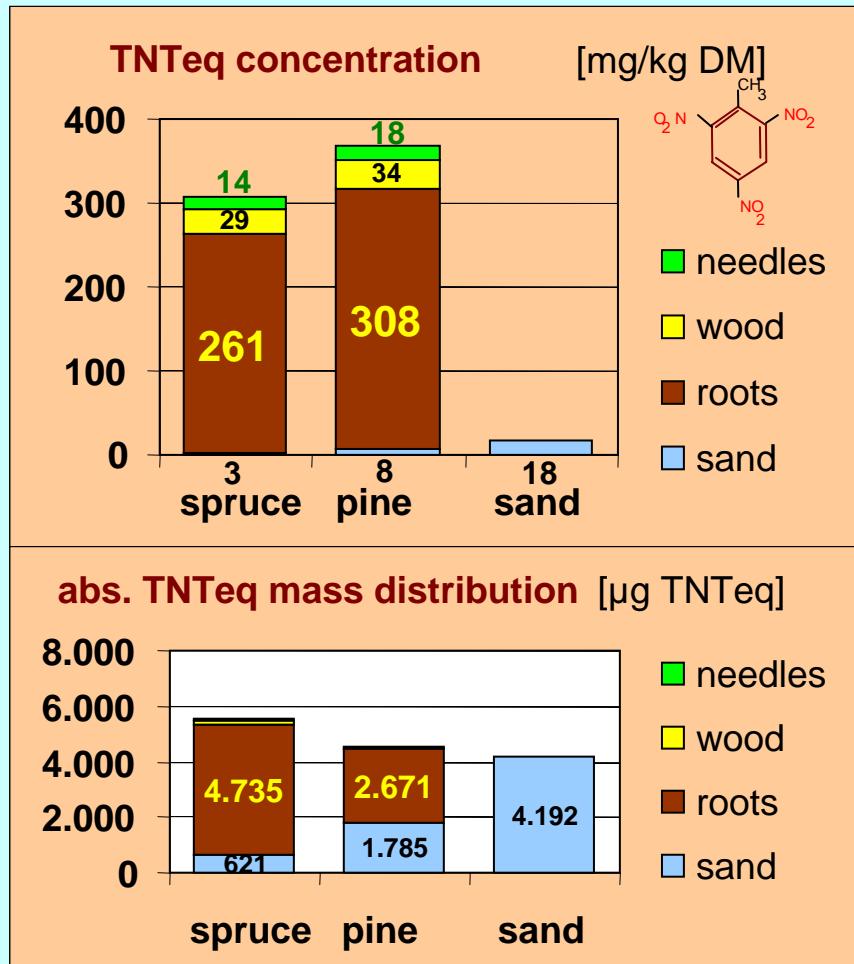


TNT is predominantly accumulated in conifer roots

Up to 300 mg TNTeq per kg DM in pine roots

Conifers can lower TNT content remarkably in TNT containing substrates.

¹⁴C-TNT localisation in conifers

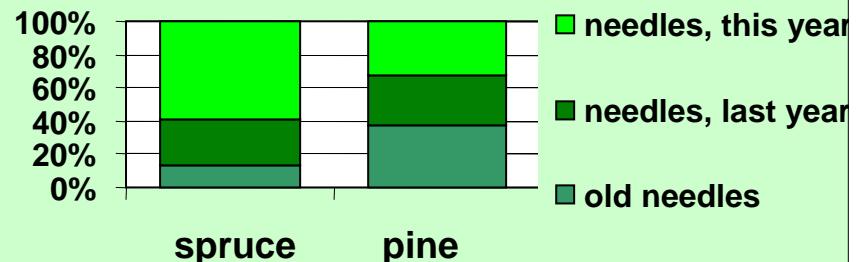


TNT is predominantly accumulated in conifer roots

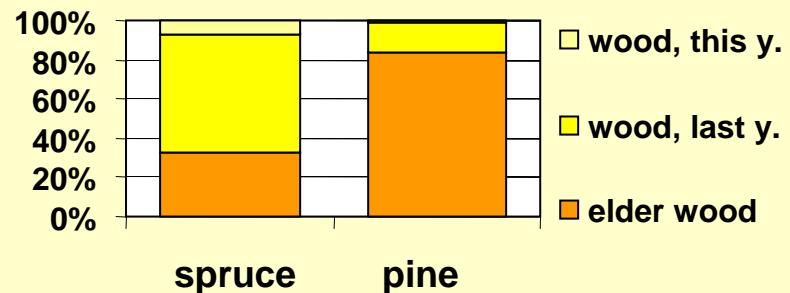
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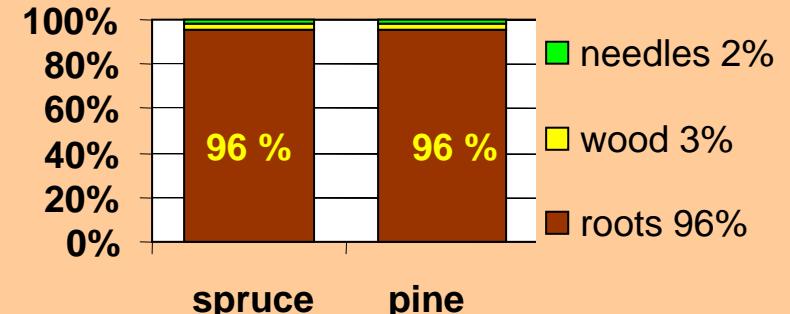
rel. TNTeq mass in needles [%]



rel. TNTeq mass in wood [%]



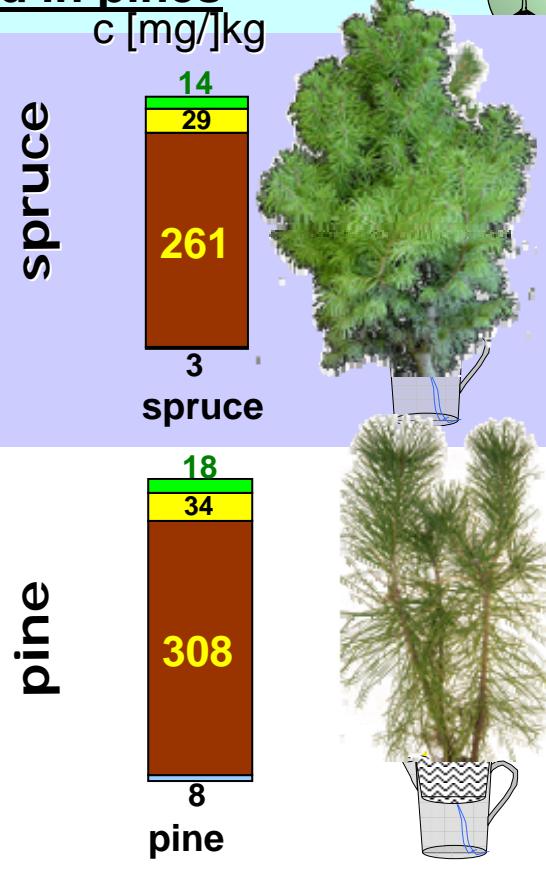
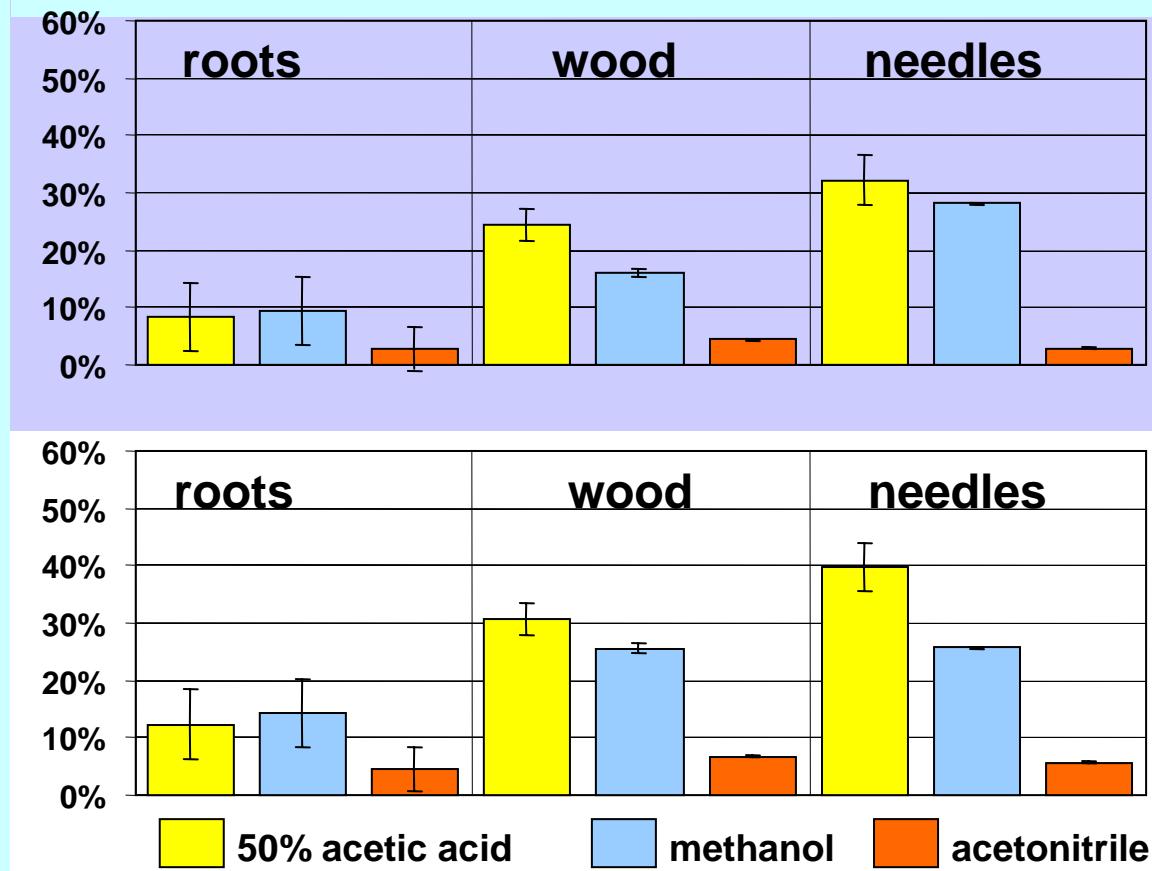
rel. TNTeq mass distribut. [%]



High degree of „fixation“ of ^{14}C -TNT in spruces and in pines



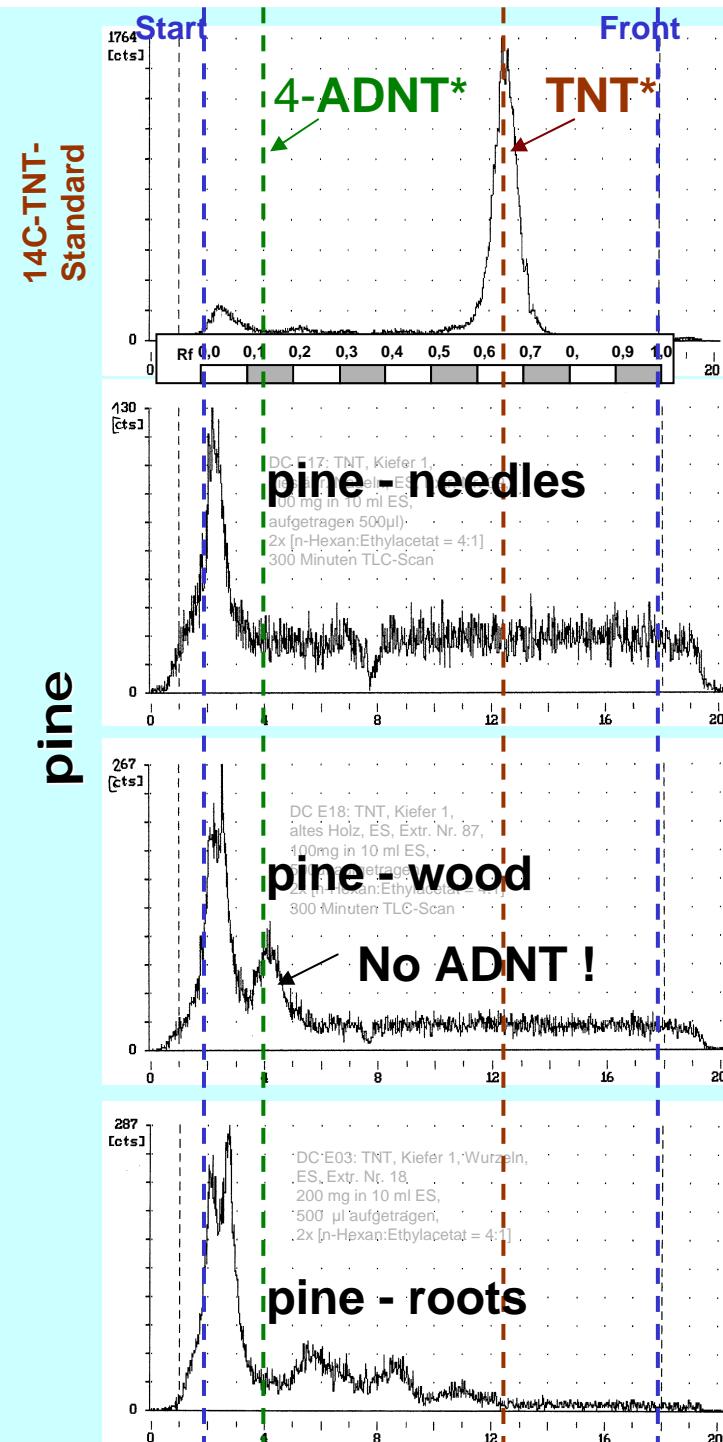
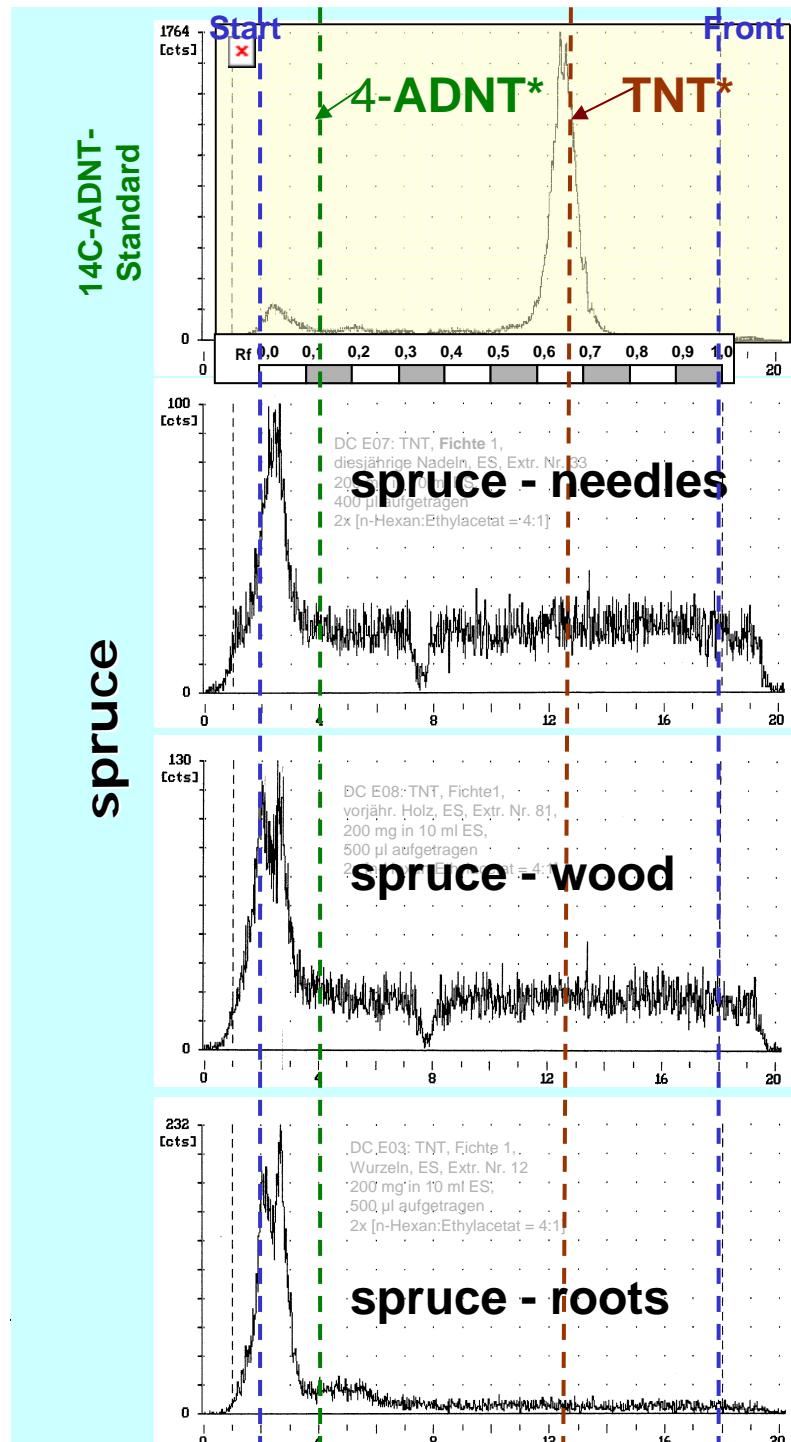
extraction efficiency [%]



TNT → high metabolism (and/or sequestration?) in roots (approx. 90 %)

- Strong „binding“ of TNTeq in root tissue prevents upwards transport
- „Binding sites“ in root tissue must be localised (fine roots, coarse roots, root surface, rhizodermis, central cylinder within the Caspary strip)
- Long term fate of TNTeq in roots has to be elucidated (mineralisation ?)





AA-Extracts
mobile TLC phase:
n-hexan:ethyl acetate
= 4:1
(2 runs)
scan time: 300 min



needles



wood

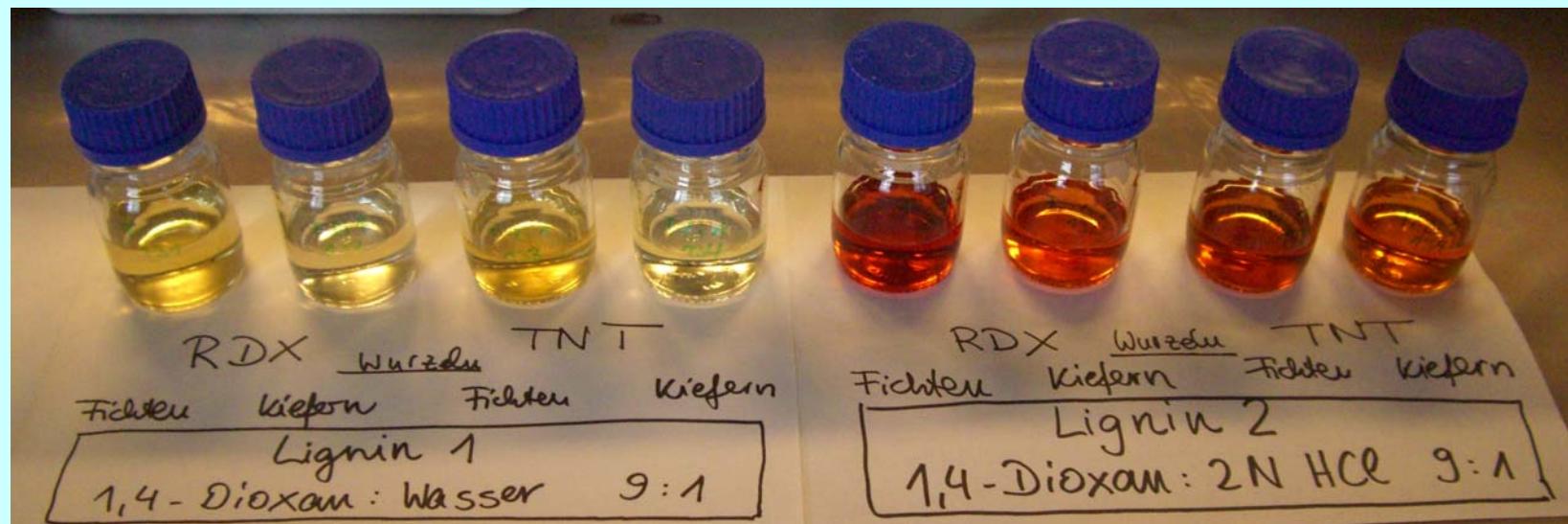
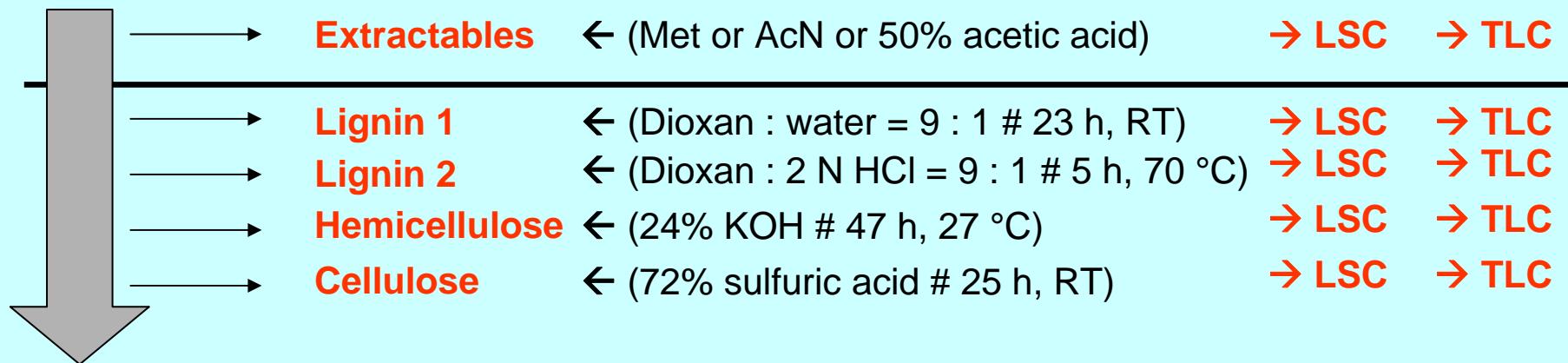


roots



Extraction of „ONER“ „Organic Non-Extractable Residues“

Tree root tissue extraction scheme



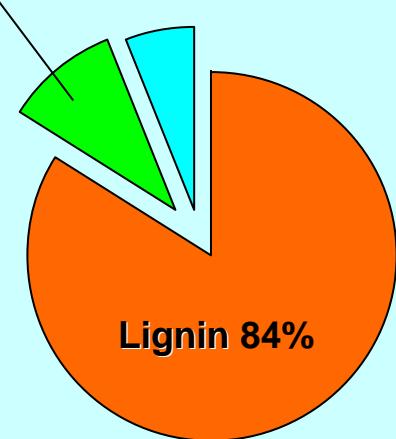


Chemical fractionation of non-extractable residues

TNT fate in root cell wall constituents

Hemicellulose 10%

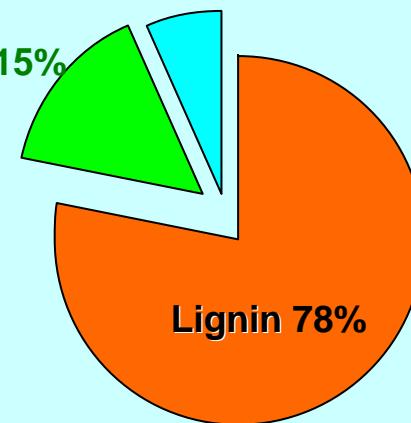
Cellulose 6%



Picea roots + TNT

Hemicellulose 15%

Cellulose 7%



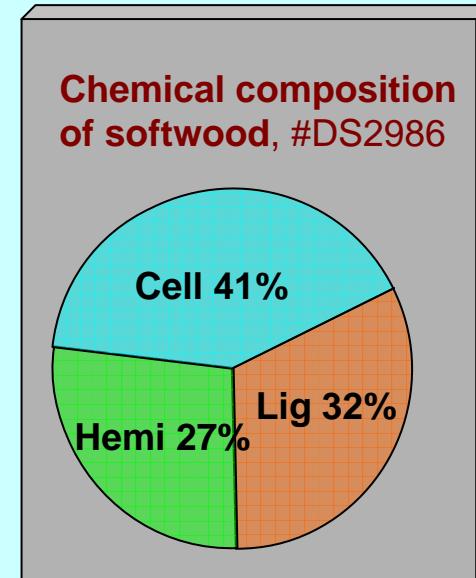
Pinus roots + TNT

Chemical composition
of softwood, #DS2986

Cell 41%

Hemi 27%

Lig 32%



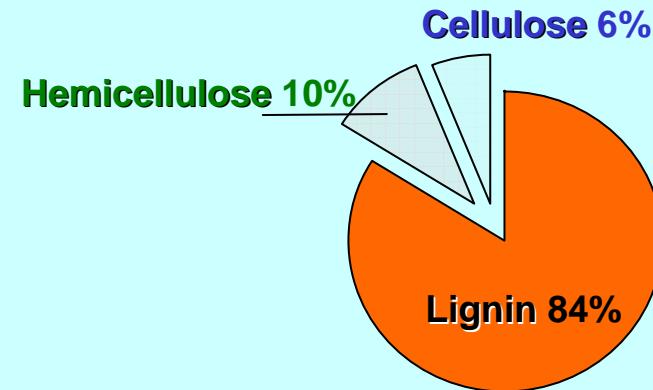
The bound TNT-derived radioactivity is mainly located (appox. 80%) in the lignin fraction.

Hemicellulose and cellulose fractions play only a minor role.

This distribution does not reflect the chemical composition of conifer wood.

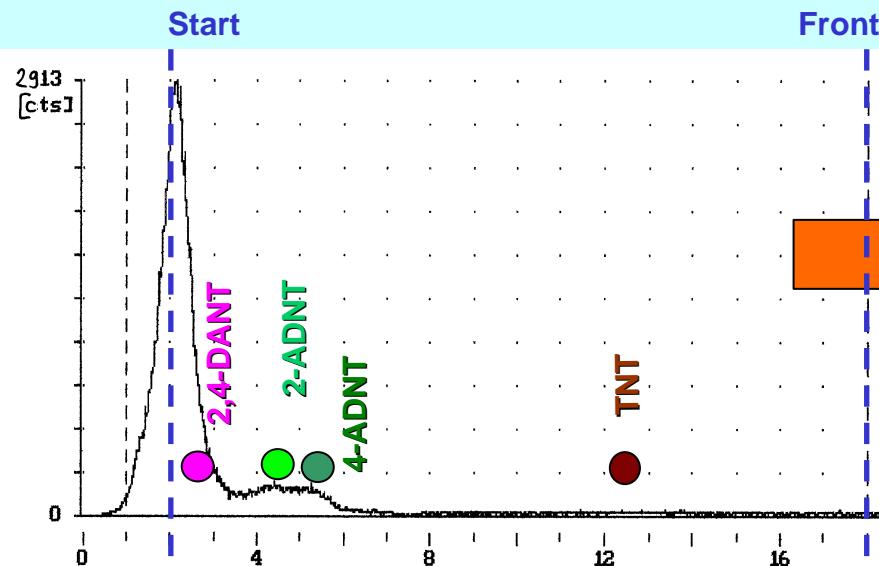


Lignin 2-fraction, *Picea* roots

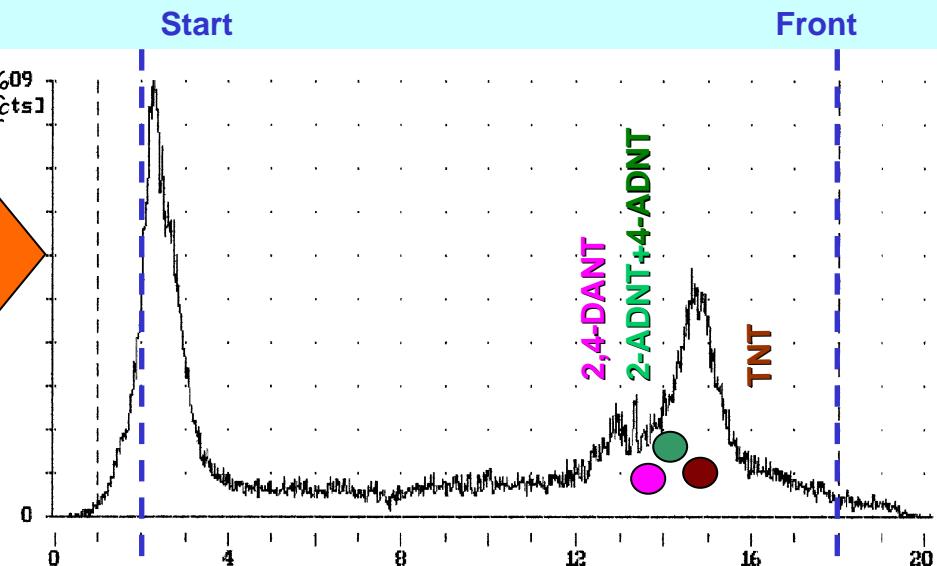


TLC-Radiochromatograms

n-hexan:ethyl acetate = 4:1, 2 runs



acetone:methanol:water = 100:15:10, 1 run



Obviously, sequestration of ¹⁴C-TNT eq into lignin occurred.



Hemicellulose fraction, *Picea* roots

Hemicellulose 10%
Cellulose 6%

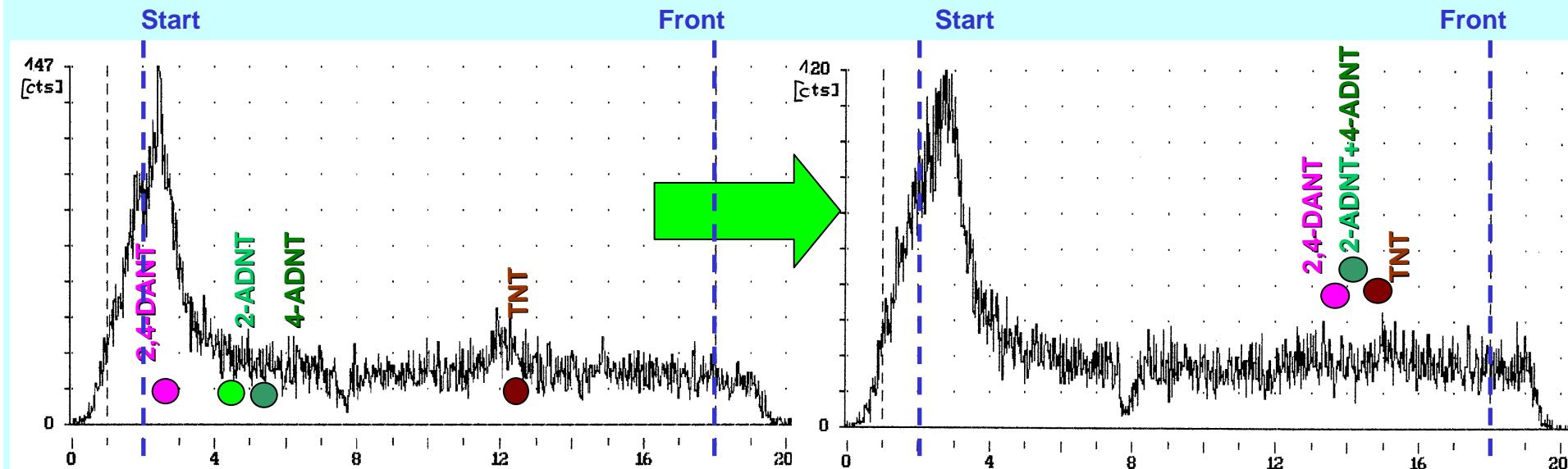


Lignin 84%

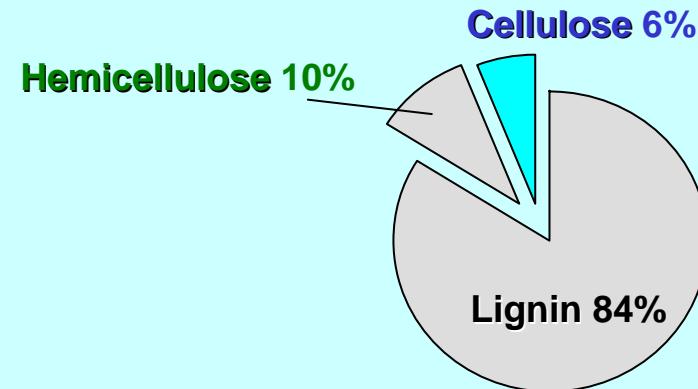
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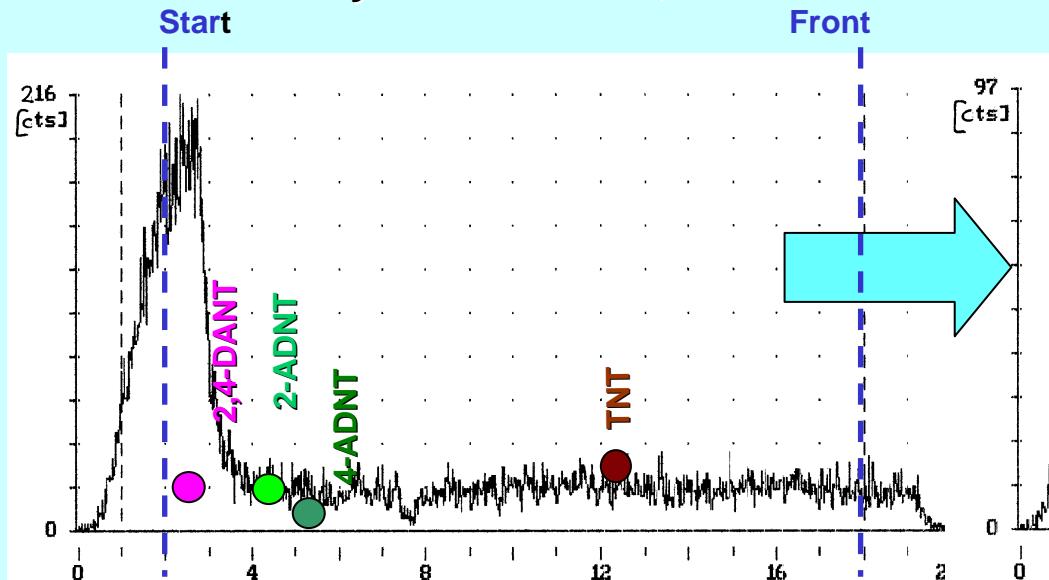


Cellulose fraction, *Picea* roots

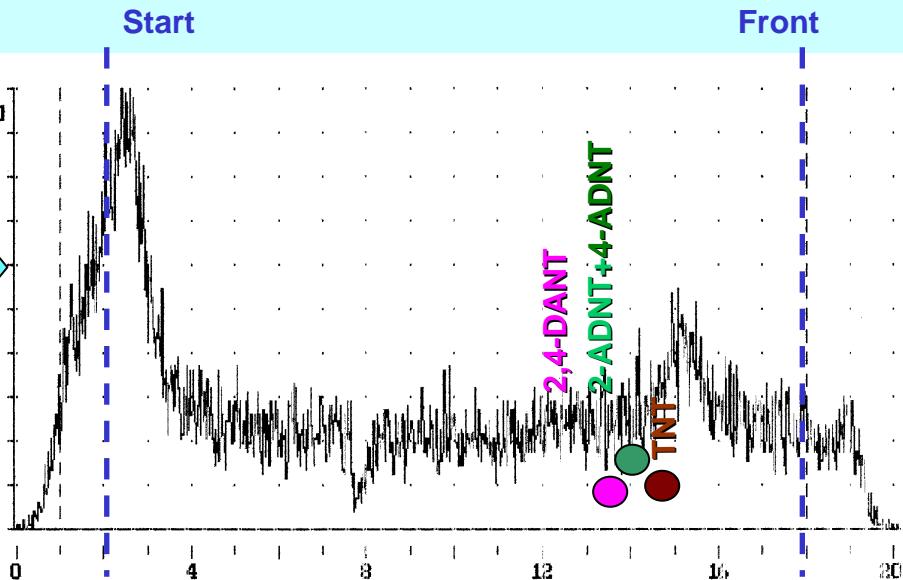


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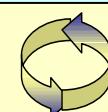
acetone:methanol:water = 100:15:10, 1 run



Summary and conclusions



1. In comparison to broadleaf trees and to herbaceous plants, **conifers** (spruce, pine) **are more tolerant** to TNT and other explosive specific compounds.
2. Both, **spruces and pines** are able to **take up considerable amounts of TNT** from soil solutions and TNT derivatives are accumulated in conifer plants. Metabolized TNT derivatives are mainly accumulated in roots where **90-95%** of them are **non-extractable „bound“**.
3. The non-extractable bound TNT derivatives are mainly localized in the lignin fraction of conifer roots. This opens the possibility for slowly degradation of bound TNT derivatives by soil born lignolytic fungi (e.g. *Phanaerochaete chrysosporium*), which are known to mineralize both, TNT and lignin.
4. Toxikological risks for wood utilization **are (preliminary) considered as low**.





Thank you
for your attention!

**Special thanks to Tanja Scharnhorst
for her excellent experimental work!**



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