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RESEARCH ARTICLE

Elevated root retention of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in coniferous trees

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Abstract For decades, the explosive RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) has been used for military and industrial applications. Residues of RDX pollute soils in large areas globally and the persistence and high soil mobility of these residues can lead to leaching into groundwater. Dendroremediation, i.e. the long-term use of trees to clean up polluted soils, is gaining acceptance as a green and sustainable strategy. Although the coniferous tree species Norway spruce and Scots pine cover large areas of military land in Central Europe, the potential of any coniferous tree for dendroremediation of RDX is still unknown. In this study, uptake experiments with a ^{14}C-labelled RDX solution (30 mg L^{-1}) revealed that RDX was predominantly retained in the roots of 6-year-old coniferous trees. Only 23 % (pine) to 34 % (spruce) of RDX equivalents (RDXeq) taken up by the roots were translocated to aboveground tree compartments. This finding contrasts with the high aerial accumulation of RDXeq (up to 95 %) in the mass balances of all other plant species. Belowground retention of RDXeq is relatively stable in fine root fractions, since water leaching from tissue homogenates was less than 5 %. However, remobilisation from milled coarse roots and tree stubs reached up to 53 %. Leaching from homogenised aerial tree material was found to reach 64 % for needles, 58 % for stems and twigs and 40 % for spring sprouts. Leaching of RDX by precipitation increases the risk for undesired re-entry into the soil. However, it also opens the opportunity for microbial mineralisation in the litter layer or in the rhizosphere of coniferous forests and offers a chance for repeated uptake of RDX by the tree roots.

Keywords Phytoremediation · Dendroremediation · Explosives · Hexahydro-1,3,5-trinitro-1,3,5-triazine · ^{14}C-RDX uptake · Leaching · *Pinus sylvestris* · *Picea abies* · Tree compartments

Abbreviations

DM	Dry matter; dry mass
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LSC	Liquid scintillation counting
PET	Polyethylene
RDX	Research Department Explosive; Royal Demolition Explosive

Background, aim and scope

Large-scale soil contamination with energetic compounds is of environmental concern worldwide. Contamination of the environment may result from improper handling of these compounds during manufacture and packaging, but also from regular explosions, partial detonations, corroding of unexploded ordnance (UXO) and open burning of outdated

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munitions. Widely dispersed residues of explosive compounds such as 2,4,6-trinitrotoluene (TNT) and Research Department Explosive/Royal Demolition Explosive (RDX; cyclonite; hexahydro-1,3,5-trinitro-1,3,5-triazine; Fig. 1) can be found on present and former military firing ranges, in war zones, and in areas where energetics are used for industrial purposes (Panz and Miksch 2012).

Currently, RDX has replaced TNT as the most widespread, conventional explosive used in military applications, due to its higher detonation power and better storage stability (Joos et al. 2008). Both explosives are classified as persistent organic pollutants (POPs) and as priority contaminants (USEPA 2010). Similar to other triazinic compounds, RDX is considered a potential human carcinogen (cancer group C; USEPA 2004). Acute toxicity of RDX is only observed at elevated concentrations (Talmage et al. 1999; Robidoux et al. 2002, 2004; Zhang and Pan 2009; ATSDR—Agency for Toxic Substance and Disease Registry 2010, 2012). Whereas TNT can be tightly bound to clay minerals and the humic matrix of soils, RDX is highly mobile. Drinking water supplies in the vicinities of RDX pollution are threatened due to RDX leaching into groundwater (Rylott et al. 2011). Several authors (Steuckart et al. 1994; Godejohann et al. 1998) have detected RDX downstream of aquifers near former ammunition plants and live firing ranges. Maximum recommended limit for RDX in drinking water in the USA is 2 μg L^{-1} (USEPA 2004).

In Germany alone, former ammunition plants and military training areas comprise almost 10,000 km^2 and represent 2.8 % of the entire country (Schröder et al. 2003). The heterogeneous distribution of RDX pollution over such large areas has generally limited the remediation of sites. High costs and the technical drawbacks of currently available technologies, such as soil excavation followed by incineration or land filling (Rylott and Bruce 2009; Marmiroli et al. 2011), have also impacted negatively on soil remediation. Phytoremediation is considered a low-cost strategy that makes use of plants with the ability to take up, accumulate, degrade and detoxify environmental pollutants. Mass balance has been established for the uptake and accumulation of (radiolabelled) RDX by numerous annual species, predominantly agronomic plants (Harvey et al. 1991; Cataldo et al. 1995; Vila et al. 2007a; Vila et al. 2007b; Chen et al. 2011), as well as for perennial grasses (Thompson and Polebitski 2010; Brentner et al. 2010) and young poplar cuttings (Thompson et al. 1999; Yoon et al. 2006; Brentner et al. 2010; Thompson and Polebitski 2010). A high accumulation of RDX-derived radioactivity in aboveground plant parts of up to 95 % was observed. However, accumulation was predominately localised in leaves, which is regarded as a main potential threat to food chain biomagnification of the contaminant.

Phytoremediation systems should "to a certain degree be self-maintaining" (Rock 2003) over decades to confront the longevity of the RDX contamination problem. It is self-evident that, for a sustainable reduction of environmental pollutants, the usability of long living trees (dendroremediation) should be investigated. Trapp et al. (2001) were the first to propose that the enormous biomass of forests (up to 300 t ha^{-1}) could serve "as a safe sink for organic chemicals".

Coniferous trees appear to be particularly beneficial for dendroremediation, owing to their intrinsic growth tolerance to explosives and concomitant soil pollutants. Moreover, they have minimal soil cultivation, soil quality, nutrient and water requirements (Schoenmuth and Pestemer 2004a,b). Conifers are also of interest because they lower contaminant percolation in an indirect manner by all-season transpiration and canopy interception of rain and snowfall (Schulze 1982). In addition, many conifers (particularly pine species) seem to be

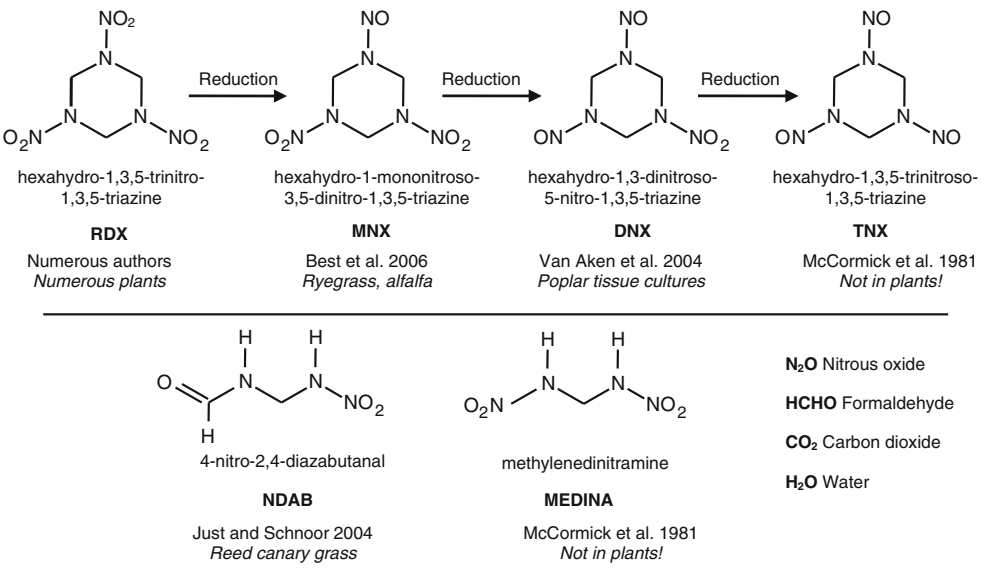

Fig. 1 Structure of RDX and some RDX degradation products with examples of experimental detection in plants

adapted by evolution to climate change and initiate the rehabilitation of abandoned military land as pioneer plants. Conifer stands offer extensive potential as renewable resources for raw material and energy supply on devastated soils without competing with food production.

Uptake of RDX by coniferous trees or shrubs has not yet been proven, with the exception of a short table notice by Schneider et al. (1995) concerning red cedar (*Juniperus virginiana*) grown on RDX-contaminated soil (16.8 mg RDX kg^{-1}) at the Iowa US Army Ammunition Plant. The objective of this paper was to balance uptake and mass distribution of ^{14}C-labelled RDX in the coniferous species Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*), since these species dominate the vegetation of large military areas in Central Europe, particularly in Germany. Soil-potted spruces and pines were pre-grown for a period of 6 years under outdoor conditions, because it was assumed that RDX balance studies are transferable to adult tree stands. Carbon14-RDX uptake experiments were conducted in aqueous solution to allow a quantitative separation of the biomass of each compartment, particularly of the fully differentiated root system. Subsequent leaching studies with tissue homogenates should reveal the stability of RDX retention in different compartments of the trees.

Materials and methods

Chemicals

Unlabelled RDX (CAS-No. 121-82-4) was purchased from Promochem (Wesel, Germany). Uniformly ring labelled ^{14}C-RDX (specific activity 56 mCi $mmol^{-1}$; purity>95 %) was delivered by Joerg Kix Sales Agency (Volxheim, Germany). The neat ^{14}C-RDX was dissolved in acetonitrile. A 60-μL aliquot of this acetonitrile solution was added to 100 mL deionised water to prepare an aqueous stock solution of ^{14}C-RDX. Oxysolve C-400 scintillation cocktail (Zinsser Analytic, Berkshire, UK) was used for $^{14}CO_2$ trapping, while Lumasafe Plus (Lumac LSC, Groningen, Netherlands) served as scintillation cocktail for aqueous ^{14}C-solutions. All chemicals were analytical grade reagents.

Radioactivity measurements

A "Biological Oxidizer" OX 500 (Zinsser Analytik, Frankfurt/M., Germany) was used for the determination of ^{14}C of solid organic material. Emerging $^{14}CO_2$ was trapped in Oxysolve C-400 scintillation cocktail. Aqueous samples were mixed with Lumasafe Plus scintillation cocktail. ^{14}C-radioactivity was quantified using a liquid scintillation counter (LSC; LS 6500, Beckman, Fullerton, USA). For details of ^{14}C measurements, see Gong et al. (2012).

Coniferous trees

Two-year-old seedlings of Norway spruce (*P. abies* (L.) H. Karst.) and Scots pine (*P. sylvestris* L.) were planted in a loamy sand in 3-L plastic pots. Spruces were potted as single seedlings, while pines were planted in clumps of three plants per pot. For safe stand stability under outdoor conditions, pots were embedded in field soil. During the vegetation period, pots were rotated every 6 weeks to prevent growth of roots into the surrounding soil. By the age of 6 years, the stubs of the three pines had grown tightly together and the pines could be considered as one tree. For experimental use, adherent soil was completely removed from the 6-year-old trees. Three spruces and four pines with similar stem height (Norway spruce, 60 cm; Scots pine, 105 cm) and comparable transpiration rates (spruce, ~100 g day^{-1}; pine, ~80 g day^{-1}) were selected for RDX uptake experiments.

Incubation conditions

Uptake experiments for RDX were conducted in a temperature-controlled greenhouse at 20±2 °C and an average relative humidity of 45–60 % under natural long-day light conditions (15 h) in late spring in the absence of artificial light. Incubation solutions were prepared with the same tap water used for outdoor irrigation. Elemental analysis of the calcium-rich tap water (pH 7.6) revealed the following concentrations [mg L^{-1}]: As 0.01; B 0.098; Ca 110; Co <0.001; Cr 0.001; Cu 0.791; Fe 0.308; K 5.04; Mg 12.5; Mn 0.007; Mo 0.001; Na 43; Ni 0.022; P 0.01; Pb 0.004; S 36; V 0.001; Zn 0.879. The concentrations of Al, Be, Cd, Sb, Se and Sn were below the detection limits. No nutrient solutions were applied. The addition of sulphate and nitrate was avoided, since both ions could serve as electron donors for enhanced microbial degradation of RDX (Joos et al. 2008). Freezer bags (4-L) of polyethylene (PET) were used for tree root incubation. Tree-bearing PET bags were positioned in black plastic jars. Bag openings were loosely closed around the base of the tree stems by plastic ties. The tops of the jars were covered with aluminium foil to prevent photo-oxidative degradation of RDX. The use of PET bags, rather than compact vessels, limits root desiccation at low water levels. It also simplifies the quantification of surface-adherent RDX after oxidative combustion of the thin PET material. Tree transpiration (=evapotranspiration minus evaporation of tree-free controls) was recorded gravimetrically at 2- to 3-day intervals.

Application of ^{14}C-RDX

Tree-containing PET bags were filled with 900 mL unlabelled RDX solution (30 mg L^{-1}, i.e. 27 mg RDX per tree). Thereafter, 8.6 mL (~400 kBq per tree) of aqueous ^{14}C-RDX stock solution was transferred to each tree bag. The homogeneity of the applied

^{14}C-activity was determined by LSC and the ^{14}C was quantified and corrected for each replicate. Calculated ratios for applied RDX mass and initial ^{14}C-activity were 67.7±1.9 ng RDXeq Bq^{-1} for Norway spruce and 67.0±1.0 ng RDXeq Bq^{-1} for Scots pine. Since RDX, RDX metabolites and bound forms of RDX could not be distinguished, the ascertained RDX data (mass and concentration) were preferentially expressed as equivalents of the parent compound (RDXeq) (Cataldo et al. 1995; Vila et al. 2007b). Spruce and pine took up the ^{14}C-RDX-containing application solution, almost completely, after 9 and 14 days, respectively. Thereafter, only RDX-free water was applied until the termination of incubation on Day 29.

Sampling and tree tissue analysis

Control of RDX uptake in apical needles

On Day 0, 1, 2, 5, 9, 22, and 28 after initiating the RDX application, samples of 200–300 mg of the previous year's needles were taken from shoot tips of each replicate to monitor the appearance of the RDX-derived radioactivity. Samples were dried at 50 °C for 2 days. The activity of ^{14}C was determined by LSC following oxidative combustion.

Tree compartmentalisation and analysis of RDX distribution

Trees were sacrificed 29 days after RDX application. The roots were rinsed with deionised water. The rinsing water and residual incubation solution was filtered (paper filter No. 1450; Whatman Schleicher Schuell, Dassel, Germany), whereafter the radioactivity of each solution was determined by LSC. Paper filters containing tree root residues and aliquot pieces of PET bags (4 cm×4 cm) were combusted in the oxidizer. Trees were separated into seven compartments with pruning shears. The compartments included freshly emerged spring sprouts ("pine candles"), older needles, stems plus twigs, root stubs, coarse roots (Ø≥1 mm), and living as well as dead fine roots (Ø<1 mm). Dead fine roots could be distinguished from the live ones by their dark brown to black colour and smooth structure. Roots were pre-dried at 22 °C for 1 day. The fresh biomass of all compartments was measured and all tree parts were chopped into pieces of 1 cm length and dried for 5 days at 50 °C. After weighing for dry mass distribution, plant material was ground with an analysis mill (A 11, IKA, Schwäbisch Gmünd, Germany) and sieved (250 μm=60 mesh). Five aliquots of 50–100 mg dry homogenised material from each tree were combusted in the oxidizer and the corresponding radioactivity was quantified by LSC.

Remobilisation experiments

For leaching experiments, 500 mg dry matter of homogenised plant tissue from each compartment were mixed, in triplicate, with 20 mL deionised water in a 50-mL centrifuge tube (Sarstedt, Nümbrecht, Germany). Samples were incubated on a rotation shaker (Certomat, Braun, Melsungen, Germany) at 180 rpm for 24 h at room temperature (20±2 °C). After incubation, the aqueous suspension of tree tissue was passed through paper filters (No. 595, Ø 90 mm, Whatman Schleicher Schuell). The radioactivity of the filtrate was measured by LSC. All filters and solid plant residues were oxidatively combusted prior to LSC analysis to determine the remaining radioactivity.

Statistical analysis

Statistical differences were calculated using Statgraphics software (Centurion XVI.I, StatPoint Technologies, Inc., Warrenton, VA, USA). A 95 % confidence level was applied for all statistical analyses ($p \leq 0.05$). Differences between Norway spruce and Scots pine were evaluated by two-sample comparison with the Student's T test. Analysis of variance (ANOVA) was used to determine whether differences between results from the seven tree compartments of each tree species were significant or not. The Student–Newman–Keuls multiple comparison procedure was also used to identify significantly different parameters.

Results and discussion

Tree transpiration and RDX uptake control

Norway spruce and Scots pine were able to take up and translocate ^{14}C-RDX-derived radioactivity rapidly to aerial parts. Carbon-14-radioactivity could be measured in apical needles within 2 days after application of the radioactive solution. Initial detection and increase of ^{14}C-activity at the endpoint of the transpiration stream took place considerably faster in spruce than in pine (Fig. 2b). A maximum needle concentration of 44.5 mg RDXeq kg^{-1} was measured in spruce. The values were four- to five-fold higher than those measured in pine (maximum 10.4 mg RDXeq kg^{-1}). Since the dry biomass of needles in both species was similar (spruce, 58 g; pine, 63 g per tree; Fig. 3a), the higher RDX concentrations in spruce needles may be due to the one and a half times higher transpiration intensity of *Picea* (Fig. 2a). The lower tree height of spruce (60 cm versus 105 cm of pine) may also contribute to distinctly higher concentrations of RDXeq in apical spruce needles.

In numerous angiosperm species, RDX exposition has resulted in RDX-specific damage to the leaves. Typical symptoms in monocotyledonous plants such as maize, sorghum and rice, included tip yellowing and browning of leaf edges. In dicotyledonous soybean, bush bean, maple and willow, RDX induced chlorophyll loss and inter-veinal necrotic spots

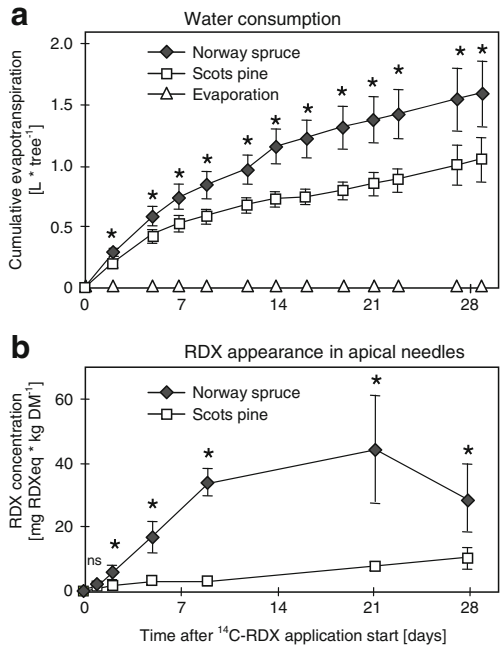

Fig. 2 Evapotranspiration (**a**) and appearance of ^{14}C-label in apical needles (**b**) of Norway spruce and Scots pine during the uptake experiment. *Symbols* for Norway spruce (*solid diamonds*) and Scots pine (*open squares*) represent the mean±standard deviation of triplicates (*spruce*) and quadruplicates (*pine*). *Asterisks* indicate significant differences between both tree species (*T* test, $p \leq 0.05$; *ns* not significant)

(Winfield et al. 2004; Vila et al. 2007a; b; Chen et al. 2011; own observations). Throughout the uptake experiments conducted during the current study, no evidence of RDX-specific symptoms of phytotoxicity could be found. This was confirmed by a range of other RDX exposition studies with gymnosperm trees, including *P. sylvestris*, *P. abies* and *Picea glauca* (unpublished results).

Recovery and disappearance of RDX

The mean total recoveries after 29 days for pine (37 %) and spruce (41 %) were remarkably low (Table 1a). Literature data show that RDX recoveries appear to decline over time. This is supported by findings of Thompson et al. (1999) in ^{14}C-RDX uptake experiments with young poplar cuttings in hydroponics, where recovery of ^{14}C declined from 88 % on Day 2 to 74 % on Day 7. Yoon et al. (2006) reported a similar decline in ^{14}C-recovery from 89.6 % (Day 14) to 57.4 % (Day 30), also with exposed poplar cuttings in hydroponics. Low RDX recoveries can also be deduced from data of Chen et al. (2011), who explored the fate of RDX in crop plants, grown for 4 weeks in RDX-spiked soil. Based on the author's data, weak recoveries could be calculated for maize and sorghum (44 %), wheat (41 %), and soybean (28 %).

The high proportion of undetected RDXeq may be explained by the degradation of the explosive to carbon dioxide or other volatile compounds by microbial communities in the root zone. It is known that RDX may be mineralised in soil to CO_2, inorganic nitrogen and water (McCormick et al. 1981; Hawari et al. 2000; Kwon and Finneran 2008). In addition, the reduction of nitro-groups to *N*-nitroso-groups accompanied by the formation of the metabolites hexahydro-1-mononitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine(DNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), is possible (Fig. 1). Mineralisation of RDX demands a cleavage of the triazinic ring and may be performed by fungi, as well as bacteria. For example, fungal mineralisation was reported for the white rot fungus *Phanerochaete chrysosporium* (Sheremata and Hawari 2000). Bacterial mineralisation is possible by *Desulfovibrio* (Clint and Adrian 2009, *Gordonia* and *Williamsia* (Thompson et al. 2005). Decomposing dead fine roots of spruce and pine (Table 1; Fig. 3a), which are common elements in soil grown tree root systems (Leigh et al. 2002), may serve as a habitat and as a source of substrate for RDX-degrading microorganisms.

A plant-mediated metabolisation of RDX via phytophotolytic degradation to N_2O, HCHO and 4-nitroso-2,4-diazabutanal (NDAB) was evidenced in leaves of reed canary grass (*Phalaris arundinacea*) by Just and Schnoor (2004). Thompson and Polebitski (2010) observed the "transformation of RDX to a volatile (unknown) organic chemical" in switchgrass (*Panicum virgatum*) and in hybrid poplar trees (*Populus deltoides* × *nigra* DN-34). Direct evidence for mineralisation by the plant itself could only be found for RDX metabolites in axenic cell cultures of poplar (*P. deltoides* × *nigra* DN-34) (Van Aken et al. 2004a; Schnoor et al. 2006). However, RDX may be mineralised indirectly to CO_2 in plants by an endophytic bacterium (*Methylobacterium populi*), which has been isolated from poplar tissue (Van Aken et al. 2004b).

The assumption, in this study, that the disappearance of ^{14}C radioactivity during RDX uptake in spruce and pine could be due to enhanced mineralisation, is supported by an independent experiment with 7-year-old Scots pines. Preliminary results with pines planted in quartz sand and in loamy sand soil indicate that 31 % of applied RDX label could be trapped as $^{14}CO_2$ from the pine rhizosphere during the first 4 months. Release of $^{14}CO_2$ from the phyllosphere, however, was less than 0.1 % of applied ^{14}C (data not shown). Besides phytoextraction, the "disappearance effect" of RDX is beneficial for the aim of dendroremediation regardless of whether RDX is mineralised independently, or its metabolites are volatilised by plants itself or by plant-associated consortia of microorganisms.

Uptake and distribution of RDX in plants

Pines exhibited a higher mass of roots and aerial parts than spruces (Fig. 3a), which led to about 25 % higher overall

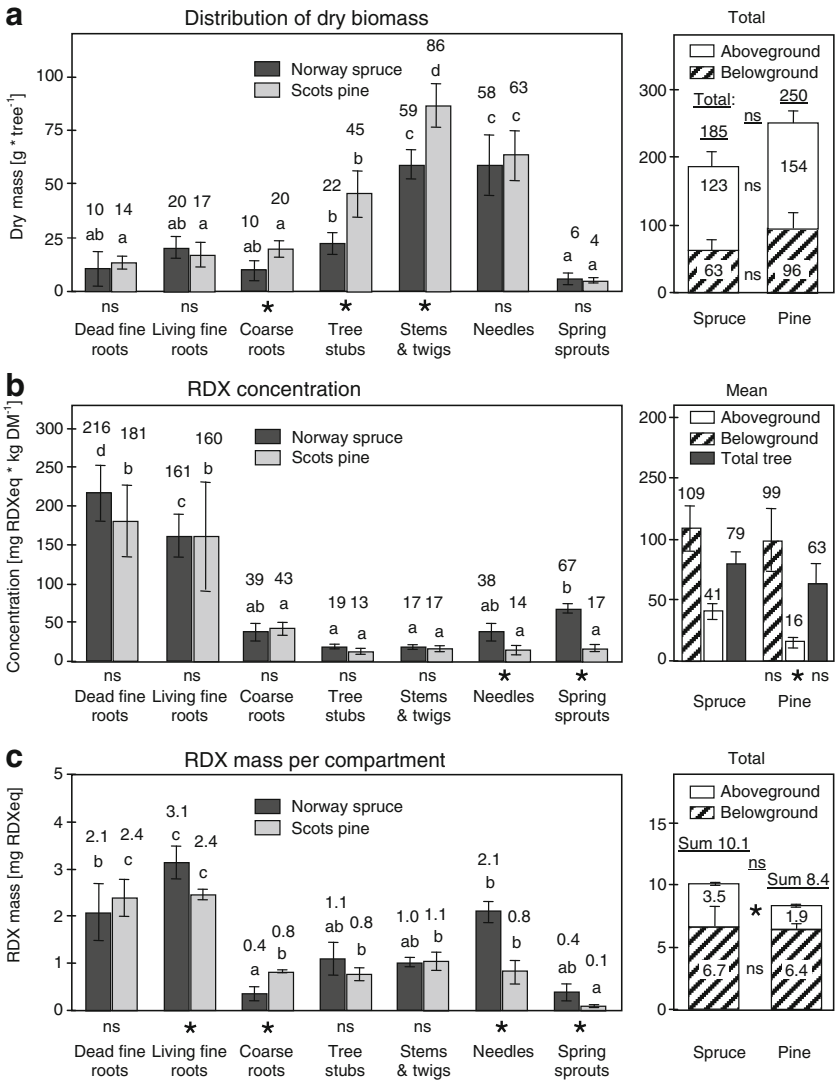

Fig. 3 Dry mass distribution (**a**), RDX concentration (**b**), and accumulated RDX mass (**c**) in different tree compartments of Norway spruce and Scots pine. Values represent the mean± standard deviation of three replicates for spruce (*black shaded columns*) and four replicates for pine (*grey shaded columns*). *Hatched columns* show belowground compartments. Aboveground tree parts are presented as *white columns. Asterisks* indicate significant differences between both tree species (*T* test; $p \leq 0.05$; *ns* not significant). Means of columns with different lowercase letters are statistically significant ($p \leq 0.05$; ANOVA; Student–Newman–Keuls multiple comparison procedure) for each tree compartment

biomass. The significantly higher biomass of pines was primarily due to the accumulation of mass in the wood of stems plus twigs, and stubs as well as in coarse roots. The aerial woody compartments (stems and twigs) and needles were dominant in the overall biomass of both species.

The comparison of equivalent RDX concentrations revealed that the uptake of RDXeq by spring sprouts and older needles was significantly higher in spruces than that in pines (Fig. 3b). In combination with the dry biomass, higher concentrations were also responsible for the higher mass accumulation of RDXeq in spruce (Fig. 3c). Differences in RDXeq concentrations between spruce and pine were negligible for dead or alive fine roots, coarse roots, tree stubs, stems and twigs (Fig. 3b).

Average root concentration factors (RCF=RDXeq concentration in roots/initial RDX concentration in external solution) were 3.6 (spruce) and 3.3 (pine). Mean translocation factors for RDXeq (TF=shoot concentration/root concentration) were 0.4 (spruce) and 0.2 (pine).

The highest concentrations of RDXeq were determined in living, as well as in dead fine roots of both tree species and ranged from 160 to 216 mg RDXeq kg^{-1} DM (Fig. 3b). Although the biomass proportions of fine roots were relatively low (Fig. 3a), elevated concentrations of RDXeq caused the highest mass accumulation in both fine root fractions (Fig. 3c). More than 50 % of RDXeq mass of the total plant uptake were recovered (Table 1) from the two fine root compartments alone. Living fine roots are mainly responsible for water uptake in coniferous trees (Lindenmair 2004). Thus, rhizofiltration of substantial water fluxes in the fine root fraction could retain considerable amounts of dissolved RDX. The surprisingly high concentrations of RDXeq in dead fine roots could possibly be explained by RDX-induced cell death in formerly living roots. However, fine root turnover measurements in mulberry trees demonstrated that 58 % of the fine roots had died after a 6-month growing season (Leigh et al. 2002) without any influence of phytotoxic contaminants. Thus, a second reason for the high accumulation of RDXeq is

Table 1 Mass balance of recoveries of ^{14}C from Norway spruce and Scots pine exposed to ^{14}C-RDX for 29 days

	a) General recovery [% of initial ^{14}C]		b) RDX in trees [% of total ^{14}C uptake]	
Tree species	Norway spruce	Scots pine	Norway spruce	Scots pine
Compartment				
Spring sprouts	1.39 [a]±0.53	0.28±0.11	3.73±1.73	0.92±0.36
Needles	7.66±0.87	2.99±0.93	20.59±2.20	9.73±3.03
Stems and twigs	3.71±0.33	3.85±0.70	9.99±1.00	12.53±2.24
Total (aboveground)	12.76±0.35	7.12±0.84	34.30±0.63	23.17±2.62
Tree stubs	4.00±1.31	2.84±0.51	10.73±3.51	9.23±1.59
Coarse roots	1.32±0.57	3.02±0.11	3.55±1.53	9.83±0.36
Living fine roots	11.51±1.41	8.97±0.46	30.92±3.45	29.22±1.44
Dead fine roots	7.68±5.48	8.77±1.44	20.50±14.25	28.55±4.80
Total (belowground)	24.52±6.47	26.60±1.37	65.70±16.32	76.83±4.58
Total (whole tree)	37.28±6.72	30.72±1.89	100.00±16.32	100.00±6.05
PET bags	0.25±0.20	0.64±0.42	–	–
Filters and solutions	3.47±2.32	5.21±1.91	–	–
Total recovery	40.99±5.68	36.57±0.19	–	–

[a] Data represent means of three replications (Norway spruce) and four replicates (Scots pine)±standard deviation

possibly due to an unspecific absorption of RDX on the spongy surface and in the parenchyma of the rhizodermis of dead fine root tissue.

The low aboveground accumulation of 23–34 % of equivalent RDX mass in coniferous trees (Table 1; Fig. 3) contrasts with the high aerial mass accumulation reported for all other species previously investigated. For example, in annual crop plants, the aerial mass accumulation of ^{14}C-RDXeq reached 86 % (Harvey et al. 1991), 84–95 % (Cataldo et al. 1995), and 78–95 % (Vila et al. 2007a,b). In perennial switchgrass (*P. virgatum*; Brentner et al. 2010; Thompson and Polebitski 2010) 58–66 % were found, while in young cuttings of hybrid poplar (*P. deltoides* × *nigra*, DN-34) 70–95 % of ^{14}C-RDXeq were balanced in aerial parts (Thompson et al. 1999; Yoon et al. 2006; Brentner et al. 2010). However, the large age differences between the root systems of tested 6-year-old conifers and the mentioned young angiosperm plants do not permit an assessment of the differences in their RDX uptake. Instead, a comparison between angiosperm and gymnosperm species should consider that they differ considerably in terms of genetics, morphology, anatomy and physiology. Differences may include tissue structuring cell wall composition, variation of lignin-monomers and water economy.

Potential remobilisation of ^{14}C-RDX by water leaching

The sustainability of the retention of RDX, following uptake, depends on the stability of the RDX binding in the respective tree tissue. Therefore, leaching of the tree tissue with deionised water should imitate the potential for remobilisation of incorporated RDXeq by precipitation. The leaching time was restricted to 24 h to minimise possible microbial degradation of RDXeq. Since the entire tree biomass (185–250 g DM of each tree) could not be combusted for analysis of unleachable ^{14}C-RDX residues, homogenised tree material had to be used. This may not be the best scenario for leaching experiments. However, it should be considered that fine materials, in the form of sawdust, are produced during tree felling, in the production process of biofuel pellets, and in the pulp and paper industry.

Only slight differences in the degree of compartment-related leaching between the two coniferous species could be ascertained (Fig. 4a). In addition, the percentage of total leached RDXeq mass per tree varied from 21.4 % (pine) to 28.8 % (spruce) (Fig. 5). However, the degree of leaching of RDXeq differed considerably between the conifer compartments of each species (Fig. 4). Despite the highest compartment concentrations of RDXeq in both live and dead fine roots (160–216 mg kg^{-1}; Fig. 3b), only 3–5 % was leached from these compartments (Fig. 4a). The degree of leaching from coarse roots was moderate (16–18 %), but increased to 47 to 64 % in stubs, stems and twigs, needles and spring sprouts (Fig. 4a).

A stable root accumulation of RDX in poplar cuttings was described by Yoon et al. (2006), who reported that only 2 % of RDX taken up by roots were leachable by water. However, the degree of leaching from poplar leaves was 24 % after an uptake period of 30 days. Considering the absolute mass of leached RDXeq per tree (Fig. 4b), only tree stubs, stems and twigs, and needles are of interest. The leachability of RDXeq from homogenised samples of woody twigs, trunks and stubs might exceed realistic values, unless the trees are felled and

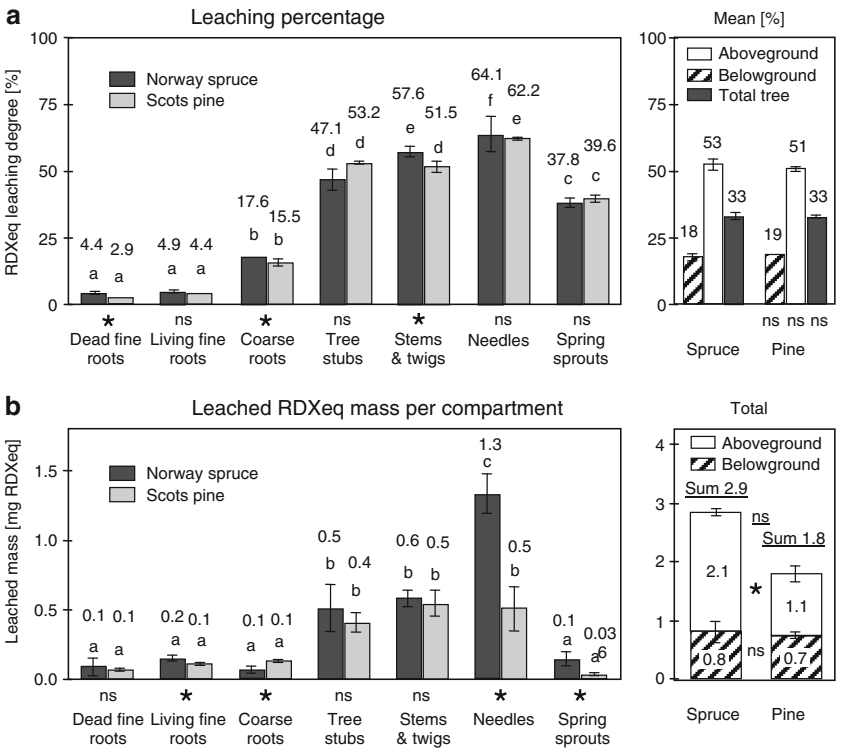

Fig. 4 Leaching percentage (**a**) and leached RDXeq mass per tree compartment (**b**) from Norway spruce (*black shaded columns*) and Scots pine (*grey shaded columns*). *Hatched columns* show data of belowground compartments. Values of aboveground tree parts are shown as *white filled columns*. Data represent the mean±standard deviation of three replications. *Asterisks* indicate significant differences between both tree species (*T* test; $p \leq 0.05$; *ns* not significant). *Different letters* show statistically significant differences among the compartments of the respective tree species ($p \leq 0.05$; ANOVA; Student–Newman–Keuls multiple comparison procedure)

processed. In complete trees, leaching is limited due to the compact nature of these compartments. In this context, the proposal of Trapp et al. (2001) for "safe" dendromass accumulation of xenobiotics is only viable for RDX in unground woody tree parts.

There is a potential risk for leaching of RDX from live trees, because the specific surface area of needles is large. The needles have a 3-year lifespan and falling needles may allow leaching into the litter layer of forest soils. The mass balance of leached RDXeq (Fig. 5) indicates that a third (pine) to a half

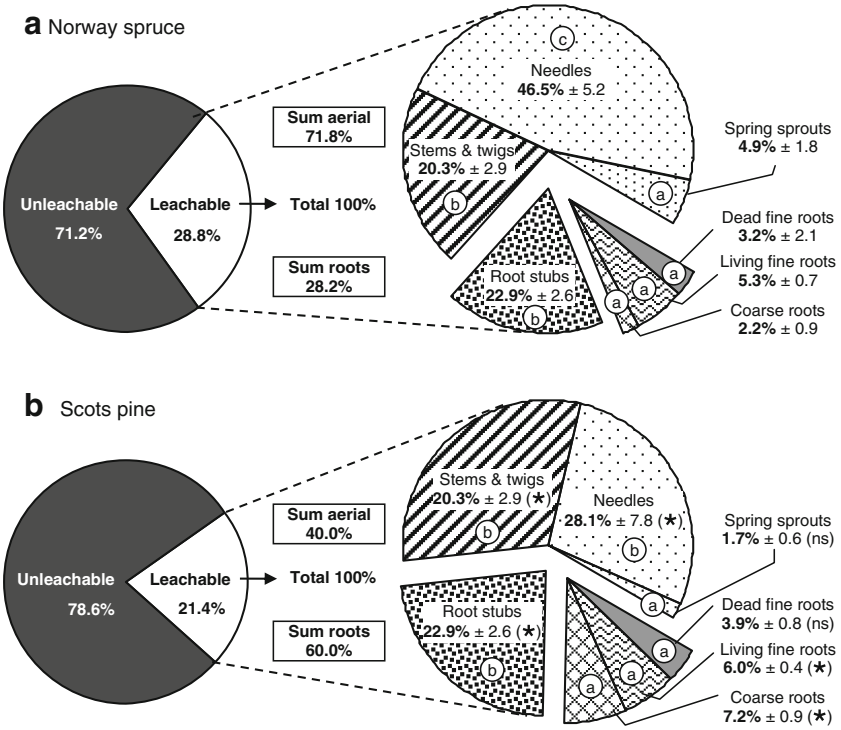

Fig. 5 Mass distribution of leachable RDX equivalents from Norway spruce (**a**) and Scots pine (**b**). Values represent means± standard deviation of three replications. Segments in pie charts: Total unleachable RDX residues (*black*); total leachable RDX residues (*white*); dead fine roots (*grey*); living fine roots (*wave-like*); coarse roots (*squared*); tree stubs (*confetti*); stems and twigs (*hatched*); needles (*wide dots*); spring sprouts (*dense dots*). *Asterisks* in parentheses indicate that pine data differ significantly from those of spruce (*T* test; $p \leq 0.05$; *ns* not significant). *Different letters* in circles show statistically significant differences among the compartments of the respective tree species ($p \leq 0.05$; ANOVA; Student–Newman–Keuls multiple comparison procedure)

Table 2 Influence of degree of homogenisation on relative recovery of ^{14}C from leached needles

	Relative recovery	
Homogenisation level	Norway spruce	Scots pine
Fine milled needles	1.00	1.00
Coarse milled needles	0.84[a]±0.02	0.58±0.01
Intact needles	0.38±0.03	0.38±0.02

[a] Data represent means of three replications±standard deviation

(spruce) of leachable RDXeq may be allocated to needle leaching. Leaching from intact needles was 40 % for both conifers, a value substantially lower than that obtained for the respective needle homogenate (Table 2).

Yoon et al. (2006) detected RDX and MNX in leachates from RDX-treated poplars, but none of the known microbial metabolites DNX, TNX, MEDINA and NDAB (Fig. 1). The occurrence of "an adduct of MNX" after a 5-day leaching procedure was thought to be responsible for extensive RDX transformation. In addition to this present study, methanolic extracts of ^{14}C-RDX-laden needles, wood and roots of 3-year-old *P. sylvestris* and *P. glauca* (Dwarf Alberta spruce) were analysed by radio thin layer chromatography. Evaluation of the R_f-values indicated that the likelihood of the presence of parent RDX was very high, followed by lower levels of the reductive RDX metabolites, MNX, and possibly DNX. The metabolites TNX and MEDINA could not be detected (data not shown). In other preliminary experiments aqueous leachates from needles of sand-grown, 7-year-old Scots pines, treated with unlabelled RDX, were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS). After 24-h needle leaching, high portions of parent RDX (peak area ratio: RDX/MNX/DNX=99:1:0.1) were identified, but DNX only occurred occasionally. The TNX and MEDINA metabolites were absent (data not shown). The RDX transformation in poplar leachates observed by Yoon et al. (2006) could possibly be explained by microbial effects during the long leaching period of 5 days.

Conclusion

This study describes, for the first time, the establishment of a mass balance for RDX uptake in fully differentiated 6-year-old trees. The uptake and distribution of RDX in gymnosperm coniferous trees has also not been described before. Previous balance studies with ^{14}C-RDX were restricted immature poplar cuttings and herbaceous plants. In 6-year-old trees of Norway spruce and Scots pine, the retention of uptaken ^{14}C-RDX was 66–77 % in the root system, mainly in live and dead fine roots. Average RCF ranged from 3.3 to 3.6. High root accumulation of equivalent RDX and low translocation to aerial plant parts (translocation factor TF=0.2–0.4) is in contrast to all previous mass balance studies in plants where up to 95 % of RDX-derived-radioactivity was transported to aerial plant parts.

Root accumulation of RDX residues was reliable in both conifers, since leaching from living as well as dead fine roots was less than 5 %. As a consequence of RDX retention in dead fine roots, it was concluded that even after tree felling, roots of coniferous trees could continue their contribution to dendroremediation of RDX-contaminated areas. Furthermore, long-term rot of RDX-laden fine roots might lead to an indirect mineralisation of incorporated RDX residues by soil microbial consortia.

The higher degree of leaching (47–58 %) found in homogenised samples of woody twigs, trunks, root stubs, and coarse roots may exceed the realistic values of uncrushed material due to the compact nature of the material. The ecological impact of this higher potential leaching is only expected if these tree compartments would be harvested and processed. The utilisation of RDX-laden softwood for bioenergy is recommended if sawdust exposition could be avoided. Because of the risk of potential release of RDX residues, the exploitation of RDX-laden wood is not recommended for timber and paper manufacture.

Leaching from the large relative surface of older needles (62–64 %) and spring sprouts (38–40 %) involves the risk of undesired remobilisation of RDX by precipitation. However, increased contaminant transport, via leaf litter, opens the opportunity for microbial mineralisation in the litter layer. The percolation of RDX-containing leachates through the root zone of conifer forests offers a chance for rhizosphere degradation of RDX residues and for repeated uptake of RDX and its metabolites by tree roots. Regardless of whether they are newly planted or have grown for decades, stands of coniferous trees may serve as efficient tools for dendroremediation and groundwater protection on RDX-contaminated sites.

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