

**Study Area,
Design,
and Methods**

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Study Area

The K, Rb, ^{133}Cs concentrations and ^{137}Cs activity concentrations in soil fractions and fungal compartments were studied in an area located in a forest ecosystem on the east coast of central Sweden (N 60°22'; E 18°13'). The soil was a sandy or clayey till and the humus mainly occurred in the form of mull. A more detailed description of the study area is presented in Vinichuk et al. (2010b).

Sporocarps of ectomycorrhizal fungi *Suillus variegatus* were studied in an area located about 40 km north-west of Uppsala in central Sweden (N 60°08'; E 17°10'). The forest, located on moraine, is dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), with inserts of deciduous trees, primarily birch (*Betula pendula* and *Betula pubescens*). The field layer consisted mainly of dwarf shrubs – bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.), and heather (*Calluna vulgaris* L.); details about the area and sampling are presented in Dahlberg et al. (1997).

Study Design

For determining K, Rb, and ^{133}Cs concentrations in soil fractions and fungal compartments, samples of soil and fungal sporocarps were collected from 10 sampling plots during September to November 2003. Four replicate soil samples were taken, by a cylindrical steel tube with a diameter of 5.7 cm, from around and directly underneath the fungal sporocarps (an area of about 0.5 m²), and within each 10 m² area to a depth of 10 cm. Soil cores were divided horizontally into two 5-cm thick layers. Sporocarps of 12 different fungal species were collected and identified to species level, and the ^{137}Cs activity

concentrations in fresh material determined with calibrated HPGe detectors in laboratories at Department of Soil and Environment of Swedish University of Agricultural Sciences. The sporocarps were dried at 35 °C to constant weight and concentrations of K, Rb, and ^{133}Cs were determined.

In addition, a selection of dried sporocarps of *Suillus variegatus* (n=51), retained from a study by Dahlberg et al. (1997), on the relationship between ^{137}Cs activity concentrations and genotype identification, was used. These sporocarps were collected once a week during sporocarp season (end of August through September) in 1994, and were taken from five sampling sites (100 to 1600 m² in size) within an area of about 1 km². Among mentioned above sporocarps eight genotypes with 2 to 8 sporocarps each were tested (in total 32 sporocarps): these are referred to as individual genotypes. Sporocarps within genotypes were spatially separated by up to 10-12m. All genotypes were used in the estimation of correlation coefficients, but only genotypes with at least four sporocarps were included in the alkali metal analyses. In addition, 19 Dahlberg's individual sporocarps with unknown genotype (i.e. not tested for genotype identity) were included: these sporocarps consisted of both the same and different genotypes. The combined set of sporocarps refers to all sporocarps: for further details about the sampling and identification of genotypes see Dahlberg et al. (1997). The ^{137}Cs activity concentration values were corrected to sampling date and expressed as kBq kg⁻¹ dry weight (DW) for each sporocarp, as reported by Dahlberg et al. (1997).

Methods

For ^{137}Cs activity concentrations and concentrations of selected metals in soil fractions and fungal compartments, fungal mycelia were separated with forceps

from the soil samples (30-50 g, 0-5 cm layer depth) under a dissection microscope (magnification X64), small amounts of distilled water were added to disperse the soil. The prepared fraction of mycelium (30-60 mg DW g⁻¹ soil) was not identified to determine whether the mycelia extracted from the soil samples and the sporocarps belonged to the same species, as it was assumed a majority of the prepared mycelia belonged to the same species as the nearby sporocarps. The method for mycelium preparation is described in Vinichuk & Johanson (2003). Mycelium samples were dried at 35 °C to constant weight for determining ¹³⁷Cs activity concentration and metal concentrations.

The soil samples (0-5 cm layer) were partitioned according to the method described by Gorban & Clegg (1996). First, the soil was gently sieved through a 2 mm mesh to give a bulk soil fraction. The remaining soil aggregates containing roots were further crumbled and gently squeezed between the fingers: this was called the rhizosphere fraction. The residue (which is the finest roots with adhering soil particles) was called the soil-root interface fraction. Nine samples of bulk soil fraction and mycelium, 12 samples of fungal sporocarps, and 6 samples of rhizosphere and soil-root interface fraction were analyzed for ¹³⁷Cs activity concentration and metal concentrations.

The ¹³⁷Cs activity concentrations in bulk soil samples and sporocarps were determined with calibrated HP-Ge detectors, corrected to sampling date, and expressed as Bq kg⁻¹ DW. The measuring time used provided a statistical error ranging between 5 and 10%. For element analyses, a 2.5 g portion of each sample was analyzed by inductively coupled plasma at the laboratories of ALS Scandinavia (Luleå, Sweden). Plant certified reference material, peach leaves NIST 1547 (NIST, Gaithersburg, USA), which has a matrix sufficiently close to fine roots and fungal material, was used for accuracy assessment: the recoveries were 102.4% for Co, 101.4% for Ni, 103.5% for Cu; 99.4% for Zn, 104.6% for

Cd, and 101.9% for Pb. For soil, CRM SO-2 (heavy metals in soil) was used, but this has no certified values for the metals studied. The detailed measurement procedure is presented in Rodushkin et al. (2008). Bioconcentration ratios (BCR) were defined as the concentration of the element (mg kg^{-1} DW) in the specific fraction divided by the concentration of the element (mg kg^{-1} DW) in bulk soil for the 0-10 cm soil layer. Element concentrations in the fractions analyzed are reported as mg kg^{-1} DW.

For element analyses (K, Rb and ^{133}Cs) of *S. variegatus* sporocarps, aliquots of about 0.3 g of each sample were analyzed by inductively coupled plasma technique. Element concentrations are reported as mg kg^{-1} DW. The isotopic ratio of $^{137}\text{Cs}/^{133}\text{Cs}$ was calculated with Equations 1 and 2 (Chao et al. 2008):

$$\frac{^{137}\text{Cs}}{^{133}\text{Cs}} = \frac{A}{C} \times \frac{\alpha}{\lambda \times N} \times 10^3 \quad (\text{Equation 1})$$

where: A is the ^{137}Cs radioactivity (Bq kg^{-1}); λ is the disintegration rate of ^{137}Cs $7.25 \times 10^{-10} \text{ s}^{-1}$; α is the atomic weight of cesium (132.9); N is the Avogadro number, which is 6.02×10^{23} ; and, ^{133}C and C are the ^{133}Cs concentration (mg g^{-1}). Equation (1) can be simplified to Equation 2:

$$\frac{^{137}\text{Cs}}{^{133}\text{Cs}} = 3.05 \times 10^{-10} \times \frac{A}{C} \quad (\text{Equation 2})$$

where: A is the ^{137}Cs activity concentration in Bq kg^{-1} and C is the ^{133}Cs concentration in mg kg^{-1} . Thus, the units of the isotope ratio are dimensionless.

The relationships between ^{137}Cs activity concentrations and metals concentrations in different soil fractions, mycelia, and sporocarps of *S. variegatus* were identified by Pearson correlation coefficients. Correlation coefficients for sporocarps of *S. variegatus* were analyzed in five separate sets of samples: in four sets, all samples had known genotypes and, the last set was a combined set of samples containing both genotypes tested by somatic

incompatibility sporocarps and genotypes that had not been tested. Correlation analyses for genotypes with three or less sporocarps were omitted. All statistical analyses were run with Minitab® 15.1.1.0. software (© 2007 Minitab Inc.); the level of significance was 5% (0.05), 1% (0.01) and 0.1% (0.001).

