# How to statistically treat disappeared fine roots for longevity estimates from minirhizotrons

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## ABSTRACT

Minirhizotrons, transparent acrylic tubes inserted in the soil, are well suited for long term, non destructive, *in situ* observations of fine roots. In minirhizotrons, the fine roots are regularly photographed and the root images are visually evaluated according to their status as living, dead or disappeared. This evaluation gives the background for further statistical treatment to estimate the fine root longevity. It is inherent in the minirhizotron technique that a large group of roots will be described as “disappeared” due to grazing, overgrowing by other roots, unclear images or other reasons. Because the fraction of disappeared roots is substantial in some cases, this has consequences for the interpretation of the longevity results.

We processed three years of minirhizotron images from Norway spruce stands in southeast Norway (30 yr old) and northern Finland (70 yr old). Of all processed fine roots 32 and 23% was evaluated as disappeared in Norway and Finland, respectively. When roots labeled as disappeared were pooled together with dead ones, the fine root longevity estimates, using the Kaplan-Meier method, decreased almost by a factor of two (401 and 433 days), as opposed to labeling them as *censored observations* (770 and 777) days for Norway and Finland, respectively).
Here we demonstrate how the early decision making on the fine root status bears consequences on the resulting longevity estimates.
KEYWORDS: fine root longevity, Kaplan-Meier survival analysis, minirhizotrons, Norway spruce, *Picea abies*

## 1. INTRODUCTION

Fine roots have been estimated to represent 33% of the global annual net primary productivity under the assumption that roots have a mean lifetime of about 1 year (Jackson et al. 1997), which means that they are born and die within a year. Hence, the accuracy of the values for the fine root longevity has a direct impact on the validity of the productivity estimate. However, despite the urgent need for more accurate estimates of root lifespan, it is still poorly documented, mostly due to methodological problems in observing fine roots.

Minirhizotrons, transparent acrylic tubes embedded in the soil, designed to monitor fine roots *in situ*, without disturbance, are well adapted for longevity studies. The fine roots are regularly observed, photographed and the images taken are evaluated according to their life condition as newly appeared, living, dead or disappeared. The Kaplan-Meier survival analysis, the statistical method widely used for calculating the fine root longevity (Majdi and Damm, 2001; Majdi and Öhrvik, 2004; Majdi and Andersson, 2004; Pritchard et al. 2008; Strand et al. 2008; Tierney and Fahey 2001), pools all the observed roots in two groups: censored and not-censored. As censored are considered all the roots that are still living at the last observation time point. As non-censored are all the roots that died during the observation period. However, during the root evaluation a large group of roots is described as “disappeared”. These are the roots that disappeared from the view or are not any longer discernable in the image (Fig. 1).

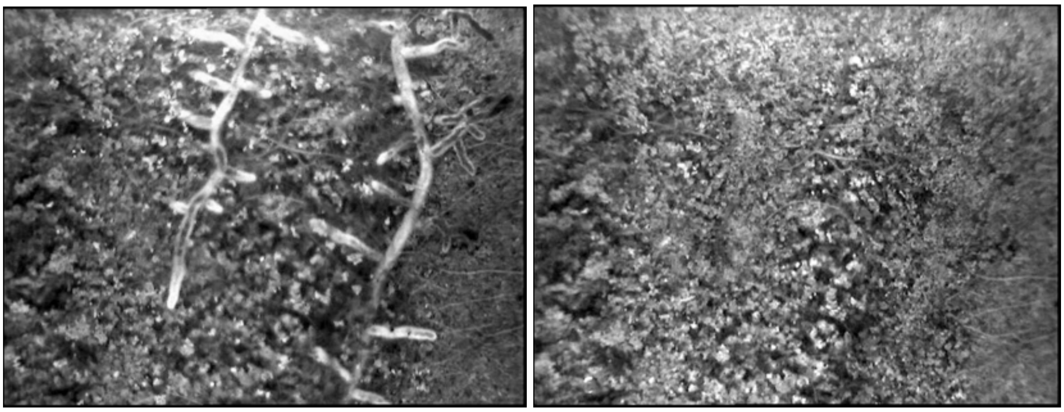

Figure 1. Fine roots photographed in July (left panel), disappeared from the view when photographed one month later (right panel).

The fate of the disappeared roots is uncertain as these roots may well have been dead and decomposed, in which case it would be correct to pool them together with the dead roots. However, the disappeared roots may also remained living and only vanished from the field view because of the changed growing angle or because of superposition by other roots. In this case the excluding of disappeared roots from the analysis, and treating them as the missing data, would have been more correct. By labelling the roots as disappeared we really do not know their status, and the choice of including or excluding them in the analysis may have rather large consequences for the results and their interpretation. In our study we show how KM estimates of fine root longevity change when including or excluding the disappeared roots from the analysis.

## 2. METHODS

### 2.1. Site description

In southeast Norway (Nordmoen) the study was established in 30 year old Norway spruce (*Picea abies* (L.) Karst.) stand, during three years (2001-2003). The study area is located on a homogenous sandy plain. Three replicates (21m x 21m) were established within the stand. Within each replicate 3 vertical minirhizotrons were installed.

In northern Finland (Kivalo), the study was established in 70 year old Norway spruce stand, on mesic mineral soil, during three years (2004-2006). The stand had 3 replicate plots, and within each plot 3 vertical and 2 horisontal minirhizotrons were installed.

### 2.2. Minirhizotron installation

On both sites acrylic minirhizotron tubes (1m long, 5 cm internal diameter and 6 cm external diameter) were installed vertically (90° to the soil surface), about 1-1.5m from the nearest tree. In total, nine vertical minirhizotrons were installed in each stand. To allow the stabilization of tubes and roots, the image taking started one year after the installation. Images from the tubes were collected monthly during the growing season and during 15 and11 sessions, in Norway and Finland, respectively. Approximately 3000 images were taken from each stand.

The images were taken with a Bartz minirhizotron camera system (Bartz Technology Corporation, Santa Barbara, CA, USA) using the associated image capture software BTC I-CAP (Bartz Technology Corporation) and stored on the portable computer.

The images were later processed using the Windows-based software RooTracker (Duke University, Durham, NC, USA). All individual roots visible in the image were assigned a specific number, they were manually traced with a mouse for root length and diameter. Roots that were white in colour the first time they were observed were considered “new”, while at subsequent viewings were classified as “living”. Roots were defined as “dead” if they appeared black, shrivelled and produced no new roots upon subsequent viewings or if they disappeared after previously being considered dead. Roots that disappeared from the view field without being previously considered as dead were classified as “disappeared”, which opened the possibility to treat them either as missing data (excluded from further analysis) or as additional dead roots. In our study we present results of both these approaches and show how excluding/including of disappeared roots effects the calculations of longevity.

### 2.3. Statistical analysis

To estimate longevity, the fine root observations were classified according to (1) living already at the first recording, (2) newly appearing during subsequent uptakes, (3) dead before the last uptake, (4) still alive at the last uptake, (5) disappearing during the measurements. All roots classified according to (1) or (4) were considered as right censored, those fulfilling both (2) and (3) were not censored; for disappeared roots, one set of analysis considered them as censored and another one as non-censored (thus giving them the same status as the dead ones). The lifetime duration of each fine root together with the censoring variable provided the input to a Kaplan-Meier survival analysis.

## 3. RESULTS

Of all the observed roots in minirhizotron images, 32 and 23 % were labeled as disappeared in Norway and Finland, respectively (Table 1).

When treating the disappeared roots as dead ones, the longevity estimates were 401 and 433 days in Norway and Finland, respectively (Table 2). However, when disappeared roots were considered to be live (censored) the longevity became almost the double; 770 and 777 days, in Norway and Finland, respectively (Table 2).

Thus, the initial decision on how the disappeared root will be treated statistically has a profound effect on the lifetime estimates.

Table 1. Number of non-censored roots observed in the minirhizotrons after excluding or including the roots labelled as disappeared.

| | | Excluding disappeared roots | | Including disappeared roots | |
|---|---|---|---|---|---|
| Stand age (years) | Total no. of roots | non-censored (n) | % non-censored | non-censored (n) | % non-censored |
| 30 | 817 | 215 | 26.3 | 476 | 58.3 |
| 70 | 1943 | 576 | 29.6 | 1022 | 52.6 |

Table 2. Fine root lifetime (days) in Norway spruce stands using the Kaplan-Meier estimates, including or excluding roots labelled as disappeared.

| Stand age (years) | Excluding disappeared roots | Including disappeared roots |
|---|---|---|
| 30 | 770 ± 40 | 401 ± 27 |
| 70 | 777 ± 33 | 433 ± 13 |

## 4. DISCUSSION

In minirhizotrons it is impossibile to determine whether a root which has disappeared from the view is dead (decomposed), or still living, but invisible due to causes such as superimposition by other roots, condense or biofilm on the tube surface. Thus, the disappearance of the roots from view does not always mean that the roots are dead. The decision to consider these roots as dead (and decomposed), has a considerable consequences on their life span estimates. We show that this decision may cause underestimation of the life span by almost 50 %, 401 days as opposed to 770 in Norwegian stand, and 433 days versus 777 days in the Finnish stand.

Based on our data, it seems that a statistical convention of pooling disappeared and dead roots (instead of excluding disappeared roots from further analysis) gives statistically sounder results. On the other hand, excluding the disappeared roots from further analysis may give more realistic lifetime estimates, providing that the study lasted for sufficiently long time to have enough of uncensored observations.

## REFERENCES

Jackson R.B., Mooney H.A. and Schulze E.D. 1997. A global budget for fine root biomass, surface area, and nutrient contents. Proceedings Of The National Academy Of Sciences Of The United States Of America 94: 7362-7366.

Majdi H. and Andersson P. 2004. Fine root production and turnover in a Norway spruce stand in northern Sweden: Effects of nitrogen and water manipulation. Ecosystems 7: 1-7.

Majdi H., Damm E. and Nylund J.E. 2001. Longevity of mycorrhizal roots depends on branching order and nutrient availability. New Phytologist 150: 195-202.

Majdi H. and Ohrvik J. 2004. Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. Global Change Biology 10: 182-188.

Pritchard, S.G., Strand, A.E., McCormack, M.L., Davis, M.A. and Oren, R. (2008). Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-$CO_2$-enrichment. Global Change Biology 14: 1-13.

Strand A.E., Pritchard S.G., McCormack M.L., Davis M.A. and Oren R. 2008. Irreconcilable Differences: Fine-Root Life Spans and Soil Carbon Persistence. Science 319: 456-458.

Tierney G.L. and Fahey T.J. 2001. Evaluating minirhizotron estimates of fine root longevity and production in the forest floor of a temperate broadleaf forest. Plant And Soil 229: 167-176.