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The impact of material used for minirhizotron tubes for root research

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Summary

- A wide variety of transparent materials are currently used for minirhizotron tubes. We tested the null hypothesis that minirhizotron composition does not influence root morphology and dynamics.
- Minirhizotron data were compared for glass, acrylic and butyrate tubes in apple (*Malus domestica*) and acrylic and butyrate tubes in a study with six forest tree species.
- Root phenology and morphology were generally similar among tubes. Apple root production was greatest against glass; these roots became pigmented later and lived longer than roots near acrylic or butyrate. Roots generally became pigmented faster next to butyrate than next to acrylic. Root survivorship was shorter near butyrate tubes in three of the four hardwood species; however, survivorship was shorter near acrylic tubes for the three conifer species. Comparison of minirhizotron standing crop data with root standing crop from cores showed that the acrylic data matched more closely than the butyrate data.
- This study reveals that the transparent material used often has little effect on root production but can substantially influence root survivorship in some plants.

Key words: conifers, fine roots, hardwoods, minirhizotrons, root life span, root morphology, root pigmentation, root survivorship.

Introduction

Many of the least destructive approaches to observing roots and soil organisms involve transparent materials placed in the soil. Once installed, transparent-wall techniques permit repeated, nondestructive observation of individual roots for growth, phenology and demography (Fahey *et al.*, 1999). Traditionally, large underground chambers, often referred to as rhizotrons, were used for root studies. With the development of miniature video cameras, boroscopes and fiberscopes, minirhizotrons are becoming the method of choice to study individual root demography both in pot studies and in the field (McMichael & Taylor, 1987; Hendrick & Pregitzer, 1996; Joslin & Wolfe, 1999; Johnson *et al.*, 2001).

Rigid minirhizotron tubes have been made of materials such as glass (Richards, 1984; Eissenstat & Caldwell, 1988; Fitter *et al.*, 1999), acrylic (polymethylmethacrylate known as Perspex, Plexiglas or Acrylite; Vos & Groenwold, 1983; Itoh, 1985), polycarbonate (Lexan; Box & Johnson, 1987) and cellulose acetate butyrate (butyrate or CAB; Box *et al.*, 1989; Hendrick & Pregitzer, 1992; Wells & Eissenstat, 2001). Materials used to make minirhizotrons with flexible walls include: cellulose acetate (Merrill *et al.*, 1987), polyvinyl film (Merrill, 1992), FEB Teflon film (Kosola, 1999) and rubber inner-tubes for motorcycle tires (Gijsman *et al.*, 1991; López *et al.*, 1996). While borosilicate glass (SiO_2) is probably the most similar in chemical composition to elements present in most mineral soils, plastic chemistry is variable and not similar to soil constituents. Plastics are chains of repeating carbon monomers with various backbones and side-chains; in some plastics, the side-chains are easily hydrolysed and released into solution. Plastics have been preferred because plastic minirhizotrons are less prone to breaking or because field conditions or experimental objectives required minirhizotrons with flexible walls to promote good soil contact or allow access to the soil environment (Kosola, 1999). In addition, it is often desirable to scribe or drill holes on the minirhizotron tubes, which is much easier to accomplish with plastics than with glass. The most important assumption regarding the estimation of fine root growth dynamics with minirhizotrons is that roots seen next to the tubes are behaving in a manner similar to those in the bulk soil, but little research has been devoted to potential effects of the minirhizotron material on the data collected. There is also no known standard for material used for rhizotron or minirhizotron tubes. The acceptance that tubes are benign may be why many authors do not indicate the type of material used in their minirhizotron studies.

Tierney & Fahey (2001) compared root production and longevity estimates using butyrate tubes and soil screens in a temperate broadleaf forest. They found similar estimates for root longevity using the two methods and concluded that minirhizotrons do not affect the longevity of fine roots. Johnson *et al.* (2001), in their review of minirhizotron studies, cited unpublished data that showed no difference between fine root biomass density in the bulk soil and either root density against polycarbonate (Mojave Desert) or butyrate (Douglas-fir stands) minirhizotron tubes. They also concluded from this data that minirhizotrons did not affect root production.

However, there is limited evidence that the type of transparent material may differentially influence root growth and death. The only paper of which we are aware that specifically compares transparent materials was by Taylor & Böhm (1976). They compared acrylic rhizotrons with large windows built in Ames, IA, USA to those made of glass in studies in Auburn, AL, USA. They concluded, based on a variety of field crops in a variety of soils, that soil adhesion tended to be higher against glass than acrylic windows. Root length density against glass was comparable to that in the bulk soil; however, root length density against acrylic was greater than that in the bulk soil. They speculated that the larger air gaps observed to form between soil and acrylic windows tended to cause preferential root growth, leading to artificially increased root length densities by acrylic windows.

We are not aware of any study that has compared different plastics as materials for minirhizotron tubes, despite the wide variety of materials currently in use. We compared three of the most common materials used for minirhizotron field studies: acrylic, butyrate and glass. Our first experiment used all three of these materials for tubes in a project on apple root growth and survivorship. We assumed that glass represented the most natural environment because it is made from silica sand and because of the results of Taylor & Böhm (1976). In a larger experiment comparing root dynamics in six tree species, we compared acrylic and butyrate tubes, which are the most widely used materials for minirhizotron studies, to determine (1) if the tube material affects the number of roots or root mass observed against a minirhizotron tube, (2) if the tube material affects the time to pigmentation, death and disappearance in these roots, and (3) if differences in tube material are found, whether these effects are uniform across tube type for a variety of species and soil types.

Materials and Methods

Experiment 1: Apple

Experiment site The first experiment was conducted in an apple orchard at the Russell E. Larson Agricultural Research Center in Rock Springs, PA, USA (40.80° N 77.86° W, altitude 356 m), using 14, 20-yr-old *Malus domestica* 'Gold Spur delicious'/M26 trees. Trees were *c.* 2.5 m tall and planted at a 2-m spacing in a 'Penn State four-wire low-hedgerow' trellis system with 3.7-m spacing between rows. The soil at this site is a Hagerstown silt loam (Typic Hapludalf) and is characterized by a 20-cm surface layer of dark brown silt and a 93-cm layer of reddish brown silty clay subsoil. The soil is moderately permeable and has a high available water capacity. State College has an average yearly precipitation of 967 mm with the largest amounts falling in May, June and July. The mean annual temperature is 9.3°C with average summertime highs of 25–28°C.

Fifty-seven minirhizotron tubes (19 of each tube type) were placed in the ground 40 cm from the trunks of the trees, 20 cm apart and inserted at a 30° angle from vertical in May 1997. The design was a completely randomized block with each tree (block) having three tubes placed in the adjacent soil, one of each material: glass, acrylic and butyrate. Plastic tubes were purchased from Pena-Plas (Jessup, PA, USA) 3 months before installation. Each tube was 38 cm long with an internal diameter of 1.9 cm, an external diameter of 2.2 cm and had two columns of 35, 8 × 8 mm windows scribed on the surface. For plastic tubes, lines were scribed with a soldering iron and then painted black. For glass tubes, black-line decals (0.79 mm) were baked on each tube. Each tube bottom was sealed with a tight-fitting rubber stopper to prevent water from entering the tube, and the tops were sealed with a stopper and black tape to prevent the entrance of light. When not in use, tubes were covered with white plastic to minimize solar heating. To encourage root growth, 200 ml of standard-strength Miracle-Gro nutrient solution were added once to the soil around the tube following tube installation.

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Data collection An 8-mm rigid, swing-prism boroscope (Olympus America Inc., Lake Success, NY, USA) with a video camera attached to the eyepiece (Bartz Technology, Santa Barbara, CA, USA) and a fiber-optic light source was used to take the videos (a Sony Hi-8 video deck) beginning in June 1998. The 2-yr lag time between tube installation and video measurement allowed roots to adjust to the initial soil disturbance (Joslin & Wolfe, 1999). The videos were taken two times per week for the first two weeks and then once a week through August 1998. Roots were recorded a total of nine times. Videos were viewed using a Sony Hi-8 video deck and a Macintosh 7500 computer. Roots were tracked from birth to death and then analysed for survivorship. The number of neighbor roots (number of roots in a frame minus 1) was determined on the last sampling date before a root died or on the last sampling date for roots that were still alive. The date a root became pigmented was recorded in the same fashion. Root diameter was determined on the first date of appearance using RooTracker software (Dave Tremmel, Duke University Phytotron, Durham, NC, USA).

Experiment 2: six forest trees

Experiment site This experiment was conducted in a common garden planting at The Morawina Experimental Station in The Siemienice Experimental Forest near Kçpno, in central Poland (51°14.87′ N, 18°06.35′ E, altitude 150 m). Before establishment of the current planting, the vegetation was an 81-yr-old Scots pine (*Pinus sylvestris* L.) stand. Average precipitation for the area is about 580 mm/year with most of it falling in June, July and August Mean annual temperature is 7.5°C with average summer high temperatures ranging from 18 to 22°C.

The planting consisted of two adjacent sites with three blocks each. There were a total of 14 tree species in the planting (Szymanski, 1982). Within each block there were nine, monospecific 20 × 20 m plots, with a total of nine species per site. Trees were planted in 1970 and in 1971 as 1- and 2-yr-old-seedlings, respectively, at 1 × 1 m spacing; there had been some self-thinning since planting. Each area had a fairly uniform topography and soil. However, there were differences in soil properties between the two sites. The first site had a 'grey-brown podzolic soil' with a much higher proportion of small fractions (< 0.02 mm) and much higher content of macro- and micro-elements compared with the 'brown podzolic soil' of the second site. Soils in both sites are nutrient poor with a plowed A horizon (unpubl. data). Average mineral soil pH (in water) ranged from 3.8 to 4.1 in the conifer plots and from 4.1 to 4.4 in the hardwoods. For this experiment, we chose three deciduous broad-leaved species in the first site (*Acer pseudoplatanus* L., *Fagus sylvatica* L. and *Quercus robur* L.) and three evergreen conifers in the second, adjacent site (*Picea abies* (L.) Karst., *Pinus nigra* Arnold and *Pinus sylvestris* L.).

The minirhizotron tubes were made at Penn State and shipped to Poland. The large number of tubes involved, the potential problems with shipping and the earlier difficulty with glass tubes breaking, restricted this experiment to an examination of the two plastics. The acrylic and butyrate tubes were purchased in 1.8-m lengths (Thermoplastic Processes, Stirling, NJ, USA, distributed by Total Plastics/Garron Plastic, Harrisburg, PA, USA) and cut into thirds. The minirhizotron tubes had an inside diameter of 5.2 cm and a wall thickness of 6.4 mm. Using a soldering iron and a guide, they were scribed with a strip of 1 × 1.25 cm windows, and the windows were numbered. Black forester paint was used to fill the indentations and then the excess wiped off to leave clear windows. Tubes were numbered at the top and coded for plastic type to prevent confusion later. Solid PVC rod, cut and lathed to make a bottom plug, was sealed in place with caulk. Tops of the tubes were wrapped in black electrical tape and sealed with a rubber stopper to keep light and rain from entering the tubes; no other covers were used because the tubes were shaded > 80% of the time.

In November 1998 (when the acrylic was 3 months old and the butyrate was 6 months old), the 60 cm tubes were installed randomly in the plots at an angle of 30° from vertical. Three tubes of each type were installed per plot, three plots per species. The tubes were at least 3 m from the plot borders, and the butyrate and acrylic were interspersed within each plot.

Data collection Minirhizotron images were collected using a minirhizotron camera and associated image-capture software (Bartz Technology Corp., Santa Barbara, CA, USA) starting in May 1999, 6 months after tube installation to allow for the system to recover from the installation disturbance (Joslin & Wolfe, 1999). Images were collected 10 times from May through December 1999 at 2- to 4-wk intervals. They were collected three times each in 2000 and 2001 at 4-month intervals because the 1999 data indicated very long-lived roots.

Captured images of the windows in the tubes were later viewed as a time sequence. The date a root was first observed and the date of disappearance were recorded. Root birth and root death were estimated as the day halfway between successive imaging dates. If a root disappeared at some point because another root grew in front of it or if a root was still alive at the end of the data collection period, then it was marked as censored. Individual root life span was calculated as the number of days from root birth to root death. In this system, root death was often associated with disappearance; no outward signs of loss of cortical tissue were evident before such disappearances. Only fine roots born in 1999 and 2000 were used in the analyses for life span, but followed through 2001. Soil depth was calculated from the position of the window down the tube and the installation angle.

Root production was recorded as the total number of new roots observed per unit area of observation surface per year. Root diameters were determined by direct measurement on a computer screen using the image from the date a root was first observed. This study only examined the production and demography of the finest two orders of roots. Root diameter was used to estimate root order. Using roots collected from the same plots and separated by order, we determined the upper limits of root diameter of the finest two root orders for each species individually using WinRhizo software (Regent Instruments Inc., Quebec, Canada) (unpubl. data). The root diameters of the scanned first- and second-order roots were graphed, and the diameter value for the 50th percentile was chosen as the upper limit.

Converting root counts to root length and then dividing by specific root length converted minirhizotron root counts at the end of the study to standing crop in terms of root mass. Root length was calculated using WinRhizoTron (Regent Instruments Inc.) for each tube for five dates over the course of the experiment. The regression equation of the relationships within each species (all $R^2 > 0.92$) was used to convert root number to root length. Specific root length was calculated separately by scanning roots of known order for each species with WinRhizo and dividing sample length by sample dry weight.

Subsamples of 30 roots per plot per species in minirhizotron images from spring and summer 1999 (180 roots total per species) were studied for root pigmentation, which often indicates decrease in root metabolic activity and absorptive capacity (Comas *et al.*, 2000; Bouma *et al.*, 2001). Pigmentation may also indicate an increase in defensive compounds. The subsampling of roots was deemed sufficient to cover the range of variability in the pigmentation rates. A random number generator was used to choose tube and window numbers in each season. All of the roots seen in that window on that date were then noted. This procedure was repeated until enough roots were recorded. Only roots that were white at first appearance were used for this analysis. The date when at least 50% of a root's length was pigmented was recorded as well as the date of death. Roots generally only changed color once before dying. Many roots were pigmented at the time of first appearance and were not included in this analysis.

Root standing crop After 3 yr, estimated root mass visible in minirhizotrons should reflect the balance between fine root production and turnover, in essence an index of standing crop. To better understand the differences in data between the two tube types, we compared minirhizotron root standing crop (SC_{mrt}) with soil fine root standing crop (SC_{cores}). Core samples for root biomass were collected between rows of trees and otherwise randomly located within the plot using a 15-cm long, 4.7-cm diameter soil core sampler (Arts Mfg. & Supply, American Falls, ID, USA). Soil cores were taken in July 1999 from three plots per species (three cores per plot) from the same hole at two consecutive depths (0–15 cm and 15–30 cm). In addition, eight cores per plot were taken in July 2002 from a depth of 0–15 cm. In this paper we averaged data from both sampling periods.

Following collection, soil core samples with roots were stored at −3°C for later processing in the laboratory. Soil samples with roots were washed over 1 mm sieves, and roots were manually separated from soil and divided into two categories, < 2 mm and > 2 mm in diameter, and oven-dried at 65°C for 1 wk. In this paper we present only data on fine roots < 2 mm. Average SC_{cores} was calculated in g m^{-2} of projected surface area of the soil corer used; SC_{mrt} was calculated in g m^{-2} of imaging surface area of the tubes.

Statistical methods

Data are reported as significant if $P < 0.05$. We examined the possible effect of blocking the tubes by tree in our statistical models for the apple experiment. In no case was this factor found significant ($P > 0.4$). Glass tubes were considered the control. Because more than one-third of our glass tubes broke during the study, we decided not to include the block effect in the models. A general linear model (one-way ANOVA) was used to analyse root diameter and root number in the apple experiment.

In the forest tree experiment, differences in root production between tube type and year were analysed using a general linear model with a split-plot design. Wilcoxon tests were performed to compare root life spans between tube types within each species in the forest tree experiment.

Survivorship curves and root life span estimates for each species were calculated using the BASELINE statement of PROC PHREG in SAS (SAS Institute, Cary, NC, USA). Cox proportional hazards models were used to test for differences within each species for the influence of tube type, diameter, soil depth and time of birth on root life span (Allison, 1995; Wells & Eissenstat, 2001). Separate hazard models were also performed where acrylic and butyrate tubes were analysed separately to evaluate any possible interactions of a covariate (e.g. diameter) with tube type. The differential risk of white roots browning for the two types of plastics was tested with a Cox proportional hazards model. In the apple experiment, the Cox proportional hazard model was run with comparisons of acrylic and butyrate to glass and then a comparison of the parameter estimates of acrylic and butyrate with a linear hypothesis.

Cox's partial likelihood method (Cox, 1972) estimates regression models of time until 'failure' of an individual without specifying an underlying distribution, and the estimate depends only on the ranks of these event times, not their numerical values. It allows for censored data and time-dependent covariates, making it very useful for root demography data that is randomly censored. Two kinds of data are presented: hazard functions and survivorship curves. The hazard function estimate quantifies the instantaneous risk that an event (e.g. root death) will occur at time ($t + \Delta t$) given that the individual (a root) has survived to time (t). For a sample or population, the hazard function reveals overall trends of the

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individuals over the sampling period. The survivorship curves are calculated from the baseline of the hazard function for an individual whose covariate values (e.g. plastic type and root diameter) are all zero (Allison, 1995).

Results

Experiment 1: apple

The type of transparent material had a significant effect on cumulative apple root production ($P = 0.035$, Fig. 1). From June through August 1998, cumulative root production was greatest for glass and least for butyrate, with acrylic intermediate. Seasonal patterns of production were similar for the three types of materials (data not shown).

We also examined whether the type of transparent material might affect root morphology. Mean root diameter ranged from 0.34 to 0.35 mm (Pooled SE = 0.20 mm) among the three materials, with no evidence that the plastics altered root diameter (data not shown; $P > 0.60$). We did not observe any other features of the roots (e.g. branching) that qualitatively differed among the three transparent materials. Time to root pigmentation was significantly influenced by tube material (Table 1, Fig. 2a). Roots became pigmented significantly faster next to butyrate tubes, followed by acrylic and then glass. There was a strong effect of type of transparent material on root survivorship (Table 1, Fig. 2b). Roots visible on glass showed the highest survival rates and those visible on butyrate had the lowest rates, with acrylic intermediate.

Experiment 2: forest trees

Root production Seasonal root production patterns between acrylic and butyrate tubes did not differ significantly. The largest amount of roots was produced in the summer for all species and tube types. Year had a greater effect on root production than plastic type (Fig. 3). The number and mass of roots produced significantly increased from 1999 to 2001 for *Q. robur*, *P. nigra* and *P. sylvestris* ($P < 0.04$). For one species, there was a significant year–plastic interaction (*A. pseudoplatanus*, $P = 0.02$), with the production in 1999 and 2000 being

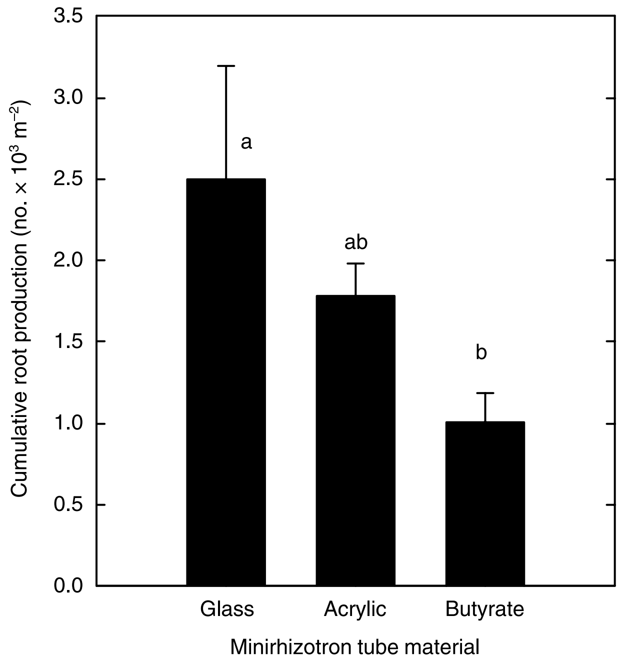

Fig. 1 Cumulative number of apple (*Malus domestica*) roots produced (+ SE) per unit area of observation surface for three minirhizotron materials from June to August 1998. Number of tubes of each material: acrylic, $n = 19$; butyrate, $n = 19$; and glass, $n = 12$. Total surface area on a minirhizotron used for observation was 45 cm^2. Different letters above the bar indicate differences significant at $P < 0.05$ using Duncan's multiple range test.

Table 1 The effects of tube type on risk of pigmentation and life span in apple (*Malus domestica*) roots adjacent to glass, acrylic and butyrate minirhizotrons

	α	SE	χ^2	*P*	Hazards ratio	Risk
Risk of pigmentation						
Acrylic vs glass	0.68	0.20	11.18	0.0008	1.97	Acrylic > glass
Butyrate vs glass	1.46	0.21	48.80	0.0001	4.32	Butyrate > glass
Acrylic vs butyrate[1]	–	–	20.12	0.0001	–	–
Risk of mortality						
Acrylic vs glass	0.75	0.29	6.51	0.01	2.11	Acrylic > glass
Butyrate vs glass	1.34	0.30	19.78	0.0001	3.81	Butyrate > glass
Acrylic vs butyrate[1]	–	–	6.17	0.013	–	–

[1]This contrast was tested as a linear hypothesis in the proportional hazards model. Results of Cox proportional hazards regression are indicated, including hazards ratios (HR, HR = e^{β}), parameter estimates (β), SE, χ^2 and P-values. A positive β indicates an increased risk of mortality with an increase in the parameter. 'Risk' is the relative magnitude of the risk in a particular contrast df = 1 for all. Number of neighboring roots and root diameter were also significant covariates in the model (data not shown). Note: The risk for bivariate, discrete variables is interpreted as the ratio of the risk of one state to another and calculated as [HR × 100] (e.g. a HR of 2.11 indicates an increased risk of mortality of 111% in the sampling interval for roots near acrylic vs those near glass).

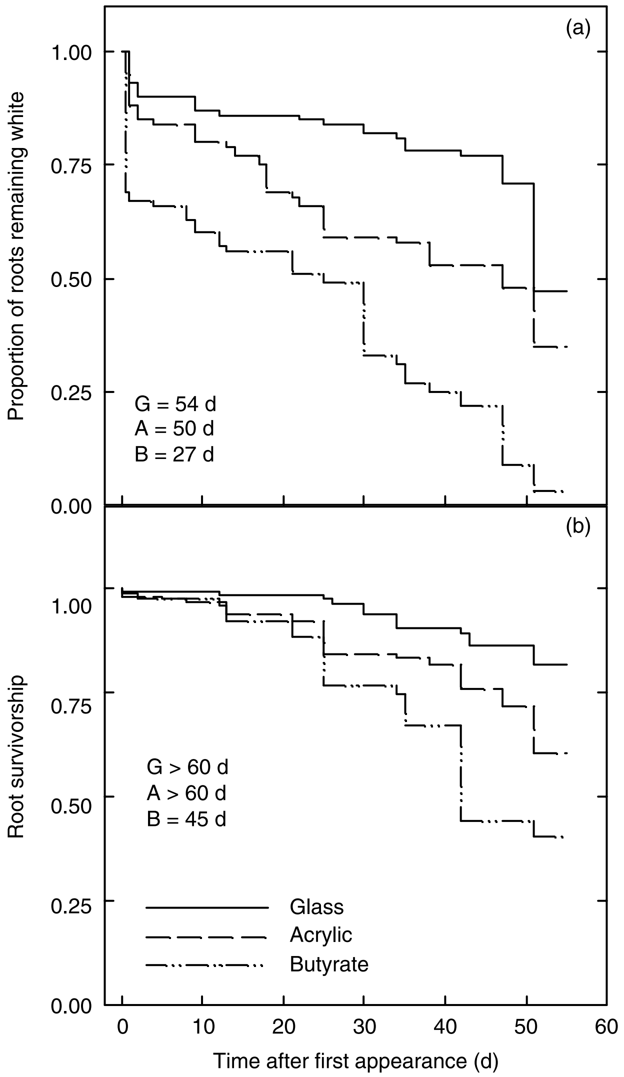

Fig. 2 (a) Proportion of apple (*Malus domestica*) roots not pigmented (still white) growing adjacent to minirhizotron tubes made of three different transparent materials. Curves were generated using the BASELINE statement in PROC PHREG in SAS software, which produces the baseline survivor functions for the chosen covariate (plastic) evaluated at the means of the other covariates, in this case birth date. Number of days at which 50% of the roots became pigmented is shown for each material (G, glass; A, acrylic; B, butyrate). (b) Apple root survivorship against minirhizotrons of different transparent materials. Significant differences were found between tube type and the number of roots, tube type and root diameter and tube type and the number of neighbors. Median life span estimates in days shown.

similar but the production next to butyrate in 2001 being less than half that next to acrylic. Cumulative root production tended to be greater by acrylic tubes for *P. abies* ($P = 0.055$). There was no difference between cumulative production near butyrate and acrylic for the other five species.

Morphology

Mean root diameter was significantly influenced in two of the six species. *Fagus sylvatica* roots were significantly thicker near butyrate tubes (0.37 mm vs 0.27 mm). However, *P. sylvestris* roots were significantly thicker next to acrylic tubes (0.42 mm vs 0.32 mm). Mean root diameters for the other species were within 0.02 mm for both tube types. Root branching, as indicated by the percentage of first-order roots, was not influenced by tube type.

Pigmentation The risk of pigmentation was significantly influenced by tube type (Table 2). The time from birth (white) to pigmentation was significantly decreased against butyrate tubes for four of six species (Fig. 4). Roots remained white about 10–42 d longer by acrylic than butyrate tubes depending on the species. Fine roots that remained white and never became pigmented had a significantly increased risk of dying near butyrate tubes for four of six species (Table 2).

Root life span Tube material affected root survivorship in a species-specific way in the forest trees (Table 2, Fig. 5). For butyrate plastic, roots of *A. pseudoplatanus* and *Q. robur* exhibited increased risks of death between sampling dates compared to acrylic (Table 2). Median life span of *Q. robur* roots was twice as long against acrylic tubes as against butyrate tubes (580 d vs 290 d, $P < 0.01$). Median life span of *A. pseudoplatanus* roots was at least three times longer against acrylic than butyrate (> 900 d vs 300 d, $P < 0.01$). By contrast, the three conifer species had decreased risks of root death near butyrate compared with acrylic (Table 2, Fig. 5). *Picea abies* and *Pinus* spp. roots lived at least two to three times longer near butyrate tubes than near acrylic tubes (> 900 d vs 340–500 d, $P < 0.01$).

For individual species, the differential influence of material on patterns of survivorship among individual plots (three per species) was relatively consistent. Using 900 d as the end-point of the experiment, median root life span differences between acrylic and butyrate tubes (life span near acrylic minus life span near butyrate) among the three plots ranged from 600 d to > 700 d for *A. pseudoplatanus*, from 140 d to > 600 d for *Q. robur* and from −200 d to 100 d for *F. sylvatica*. For conifers, differences in median root life span between butyrate and acrylic tubes among the three plots ranged from 500 d to > 550 d for *P. abies*, from 400 d to > 500 d for *P. nigra* and from 200 d to > 650 d for *P. sylvestris*. Thus, no single plot overly influenced the direction of response, although the magnitude of response was influenced by one plot for *Q. robur* and *P. sylvestris*.

There was no correlation of tube type and root production with root life span. Increased root production was coupled with a longer root life span (*A. pseudoplatanus*, acrylic), with shorter root life span (*P. abies*, butyrate) and with no change in root life span (*F. sylvatica*) for different species.

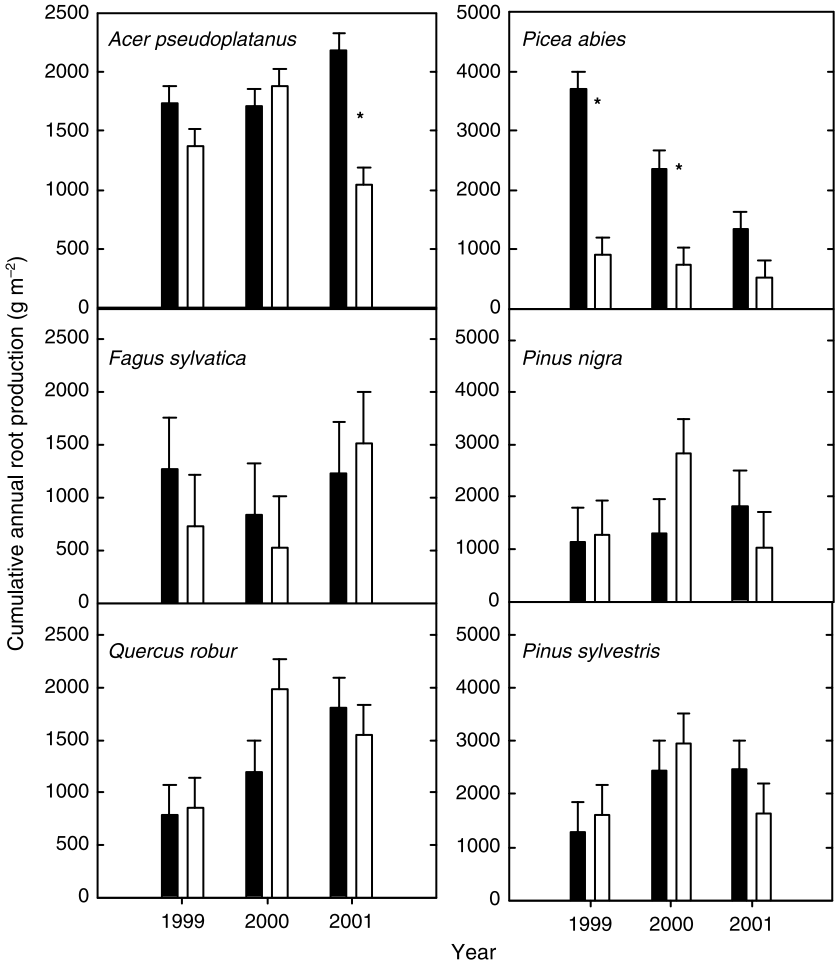

Fig. 3 Cumulative annual root production (+ SE) for the forest tree experiment in 1999, 2000 and 2001. There were three tubes of acrylic (closed bars) and three tubes of butyrate (open bars) for each species replicated in three separate plots (nine tubes total of each tube material and for each species). Asterisks indicate a significant difference between acrylic and butyrate production within the marked year ($P < 0.05$). Total cumulative production over the experiment was only marginally significant for *Picea abies*. Note the different scales of the y-axes.

Potential interactions of tube type with root diameter, depth and time of birth were examined for each tube type separately using Cox proportional hazard models. When data were separated by tube type, the relationships with diameter, depth and time of birth were similar to the overall model and significant for four or five out of the six species (data not shown). Most of the significant relationships were for acrylic tubes. Near butyrate tubes, root diameter and soil depth were not risk factors for root mortality, and time of birth was significant for only two of six species (*Q. robur* and *P. sylvestris*).

Standing crop We compared the relationship between root standing crop determined by soil coring to a root standing crop index against the tubes at the end of the experiment in order to assess the relative accuracy of the data collected near the different tube types. The SC_{cores} varied sevenfold while SC_{mrt} varied tenfold near acrylic and sixfold near butyrate tubes (Fig. 6). SC_{cores} explained 67% of the variation in SC_{mrt} near acrylic tubes ($P < 0.0001$, $F_{1,17} = 34.9$), but only 10% of variation near butyrate tubes ($P = 0.11$, $F_{1,17} = 2.81$). The lower adjusted R^2 value for the butyrate tubes was in part a result of the standing crop indexes of *P. nigra* and *F. sylvatica* being similar in magnitude to those near *A. pseudoplatanus* even though the SC_{cores} of *P. nigra* and *F. sylvatica* were three- to six-fold lower.

Discussion

Minirhizotrons are currently one of the most commonly used methods in field root research. Our results provide an unpleasant reminder of the potentially adverse effects that an observer may have on organism behavior. In particular, root pigmentation and root survivorship were often strongly affected by the type of tube material. Roots adjacent to butyrate tubes usually had a greater risk of becoming pigmented in both experiments (Table 2; Figs 2a and 4). Also, those roots born next to butyrate tubes that did not become pigmented usually had a greater risk of dying (Table 2). Root pigmentation has been observed in minirhizotron and developmental studies to be caused by an accumulation of phenolic compounds (McKenzie & Peterson, 1995; Comas *et al.*, 2000) as well as associated with a marked decrease in root respiration rates and metabolic activity (Comas *et al.*, 2000). If pigmentation represents a condition of reduced root absorptive capacity (Comas *et al.*, 2000; Volder *et al.* unpubl. data), then roots,

Table 2 The effects of tube type on risk of pigmentation of white roots, the risk of mortality of white roots that never became pigmented, and the overall risk of root mortality for six species using acrylic and butyrate minirhizotrons calculated using Cox proportional hazard regression

	β	SE	χ^2	*P*	Hazards ratio	Risk
Pigmentation						
Acer pseudoplatanus	0.072	0.15	0.22	0.64	1.07	–
Fagus sylvatica	0.96	0.29	10.86	0.001	2.6	Butyrate > acrylic
Quercus robur	2.30	0.39	33.97	< 0.001	9.96	Butyrate > acrylic
Picea abies	1.30	0.21	37.23	0.001	3.67	Butyrate > acrylic
Pinus nigra	0.62	0.32	3.63	0.056	1.85	Butyrate > acrylic
Pinus sylvestris	0.47	0.25	3.66	0.05	1.6	Butyrate > acrylic
White root mortality						
Acer pseudoplatanus	1.77	0.22	64.78	< 0.001	5.84	Butyrate > acrylic
Fagus sylvatica	0.80	0.30	7.28	0.007	2.23	Butyrate > acrylic
Quercus robur	0.82	0.27	9.14	0.003	2.27	Butyrate > acrylic
Picea abies	0.26	0.21	1.54	0.21	1.29	–
Pinus nigra	0.77	0.33	5.43	0.02	2.15	Butyrate > acrylic
Pinus sylvestris	0.16	0.25	0.42	0.52	1.18	–
Root mortality						
Acer pseudoplatanus	1.01	0.19	28.22	< 0.001	2.74	Butyrate > acrylic
Fagus sylvatica	–0.06	0.20	0.08	0.78	0.94	–
Quercus robur	0.53	0.17	9.60	0.002	1.70	Butyrate > acrylic
Picea abies	–2.21	0.34	42.46	< 0.001	0.11	Acrylic > butyrate
Pinus nigra	–3.16	0.58	29.57	< 0.001	0.04	Acrylic > butyrate
Pinus sylvestris	–2.04	0.31	43.43	< 0.001	0.13	Acrylic > butyrate

Hazard ratios (HR), parameter estimates (β), SE, χ^2 and *P*-values for the effect of tube type are reported. Risk is the direction of the risk relationship; df = 1.

whether they became pigmented or remained white, had a greater risk of lost absorptive function and/or death near butyrate tubes.

Root life span was also strongly influenced by tube type, but the specific response differed among species. In Experiment 1, apple root survival was longest by glass and shortest by butyrate, and intermediate by acrylic (Table 1, Fig. 2b). In the forest tree experiment, mortality of white roots that never became pigmented was higher by butyrate than acrylic in three hardwood species and one of the conifer species (Table 2). The remaining two conifer species exhibited no difference between the two tube types. In terms of total root survivorship, two of the three hardwoods exhibited longer survival by acrylic than butyrate (*F. sylvatica* exhibited no difference), whereas the three conifers exhibited much longer survival by butyrate than acrylic. These species-specific responses make generalizations about tube influence difficult.

Although for the forest tree experiment we did not have a specific control, we could compare a minirhizotron standing crop index to soil core standing crop as a direct assessment of differences in results among tube types. The SC_{cores} exhibited a much stronger positive correlation with biomass of roots observed at the end of the experiment by acrylic than by butyrate tubes (Fig. 6); however, SC_{mrt} for a number of species was not influenced by tube type. The unusually high SC_{mrt} near butyrate tubes for some species appears to be an artifact of the tubes.

As a second assessment of differences, we assumed that basic tree physiology requires that annual fine root production and foliage production should be in some type of rough balance. For example, for 20 grassland, mixed and forested plots in North America, fine root production represented from 40 to 70% of total fine tissue (fine roots plus foliage) production (Reich *et al.*, 2001). For the six forest species in Poland, we estimated the fraction of total fine tissue production (fine root plus leaf production) contributed by fine roots, using the fine root turnover estimates calculated for both tube types (see median root life spans in Fig. 5) and the SC_{cores} (Fig. 6) and litter-fall production data (J. Oleksyn, unpubl. data). Using the acrylic tube data, per cent fine root production of total fine tissue production varied 3.5-fold across the six species, from 12% for *A. pseudoplatanus* to 44% for *P. abies*. The per cent estimate for *A. pseudoplatanus* was low compared with the rest of the species whose per cent production estimates ranged closer to twofold, from 20% to 44%. Using the butyrate data, per cent fine root production of total fine tissue production ranged 94-fold, from 0.5% for *P. nigra* to 47% for *A. pseudoplatanus* and *Q. robur*. While some of the butyrate per cent production estimates fall within the range of other reported estimates, some species were very low. The data from butyrate tubes indicate that for *P. nigra*, *P. abies* and *P. sylvestris*, only 0.5, 5 and 6%, respectively, of total fine tissue production was root production. It is difficult to envision how these estimates could be accurate. Summarizing, by using total tissue

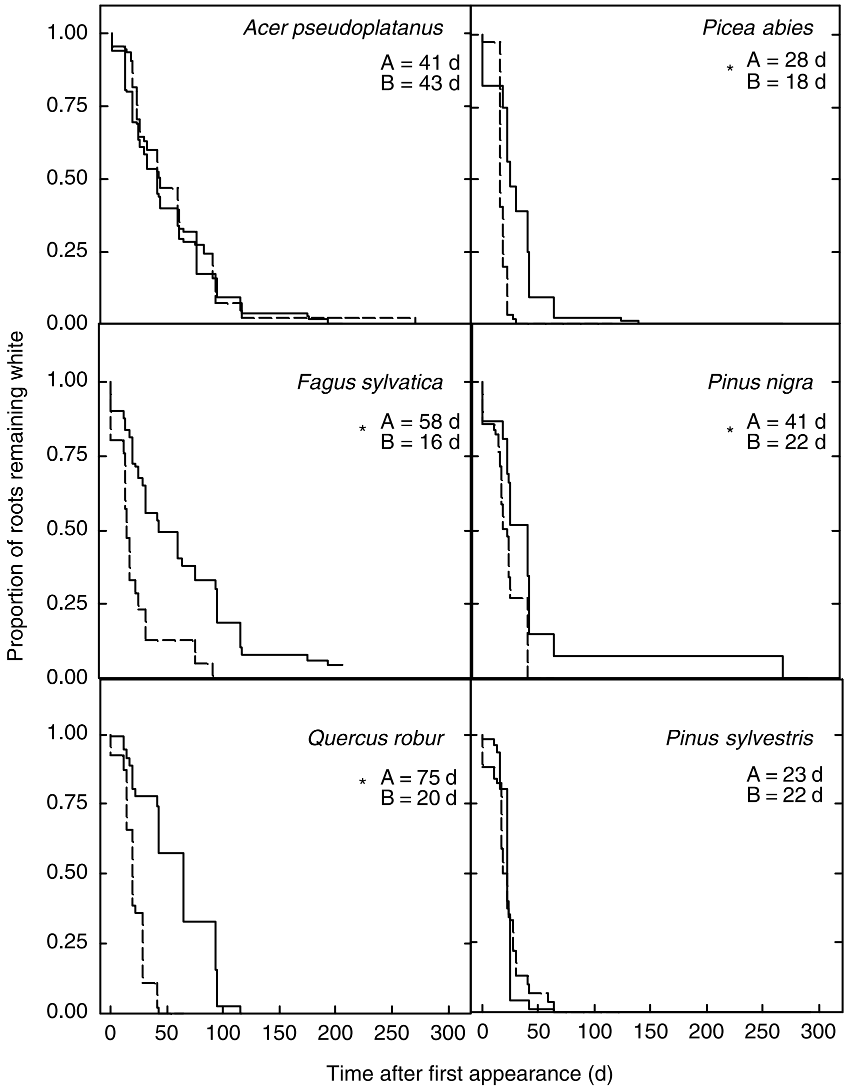

Fig. 4 Proportion of roots not pigmented (still white) growing adjacent to minirhizotron tubes made of two different plastics, acrylic (solid line) or butyrate (broken line). Curves were generated using the BASELINE statement in PROC PHREG in SAS software, which produces the baseline survivor functions for the chosen covariate (plastic) evaluated at the means of the other covariates, in this case plot and season of birth. A random subsample of 120 roots of each species was used for this estimate. Roots were born in spring or summer 1999 or 2000. Number of days after which 50% of the roots became pigmented is indicated for each material (A, acrylic; B, butyrate). Asterisks indicate significant differences ($P < 0.05$).

production to establish boundary conditions on the overall productivity of the trees, more species exhibited dubious estimated root production estimates near butyrate than acrylic.

For some species and some factors, there were no significant differences between minirhizotron tube types. Intrinsic properties such as diameter and morphology were not affected by tube type. Mean root diameter was generally very similar among tube types for a given species. In only two forest trees were differences observed, and the results were mixed. Root branching was also unaffected in both experiments. Extrinsic properties such as time of birth and soil depth were similarly unaffected.

New root production was also only modestly affected by tube material. Seasonal root production patterns were unaffected by tube type. Total new root production was only affected by tube type consistently in two of seven species. Apple roots were most numerous near glass followed by acrylic and butyrate tubes, but there were no seasonal differences (Fig. 1). In Experiment 2, only *P. abies* exhibited fairly consistent higher root production by acrylic than by butyrate over the three years of the study (Fig. 4).

Although they did not make a comparison of the two materials at the same time, and in the same place, Taylor & Böhm (1976) predicted higher root production against acrylic windows than glass windows because of greater numbers of soil gaps with acrylic tubes. We did not observe different sized soil gaps among glass, acrylic and butyrate minirhizotrons. With the small-diameter minirhizotron tubes used in our studies, we had good soil contact with the minirhizotron surface regardless of the transparent material used. In addition, the sandy soil at the forest site enhanced tube-soil contact.

We considered other possible reasons for the observed tube effects and tube differences. The composition of the plastics was a possible explanation, although we can only speculate on the influences. Butyrate and acrylic plastics used for the tubes are similar in physical properties, such as hardness, specific

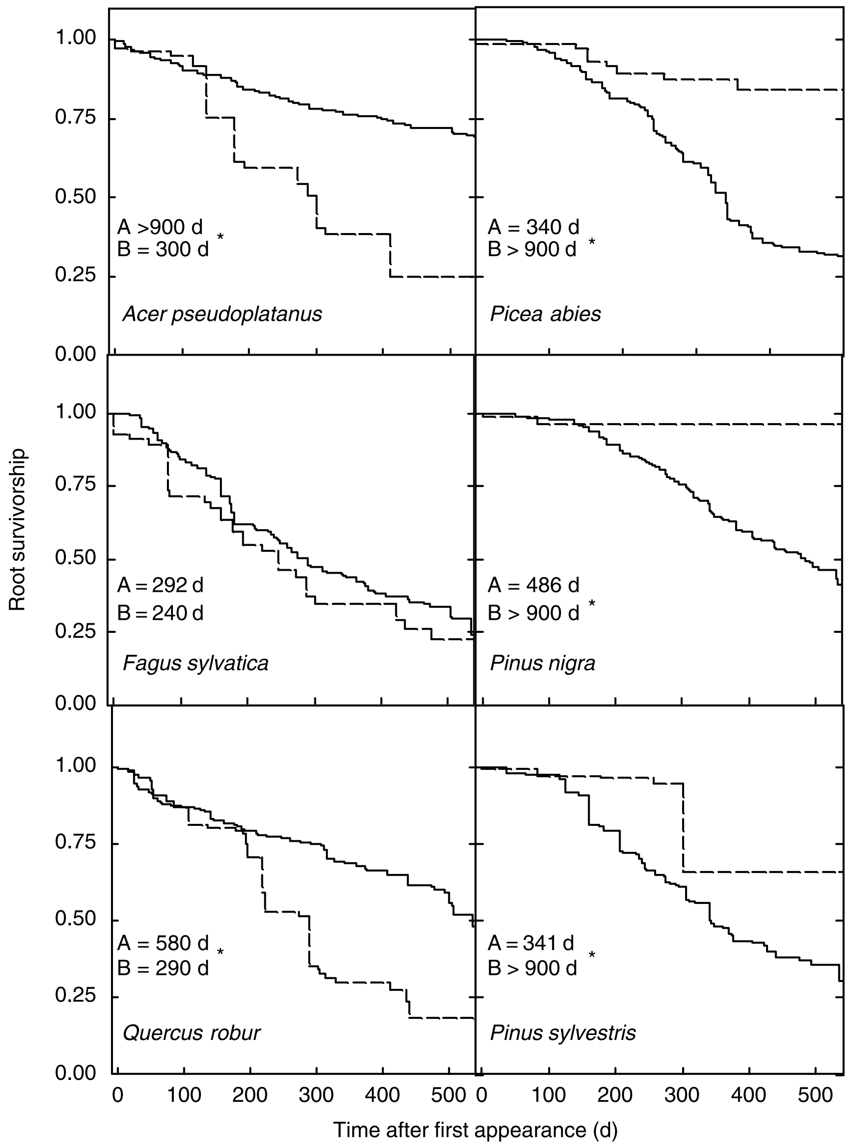

Fig. 5 Survivorship probabilities for fine roots growing adjacent to minirhizotron tubes made of two different plastics, acrylic (solid line) or butyrate (broken line). Curves were generated using the BASELINE statement in PROC PHREG in SAS software, which produces the baseline survivor functions for the chosen covariate (plastic) evaluated at the means of the other covariates, in this case soil depth, root diameter and time of birth. Only fine roots born in 1999 and 2000 were used for these estimates. Experiment was run 930 d, but curves are only shown to 500 d so as not to bias for roots born early. Median life span estimates in days are indicated (A, acrylic; B, butyrate). Asterisks indicate significant differences ($P < 0.05$).

gravity and tensile strength (data sheets from Thermoplastic Processes, Inc., Stirling, NJ, USA, and CYRO Industries, Rockaway, NJ, USA). However, from personal experience, we know that acrylic is more likely to shatter and crack than butyrate and must be handled more carefully. Acrylic (polymethylmethacrylate) has a polyethylene backbone with methyl ester groups present as side-chains, and these groups can hydrolyse to produce methanol. Cellulose acetate butyrate is a polyester with a cellulose backbone. During production, the hydroxyl groups on the glucose molecules are replaced with esters of acetic acid and butyric acid. These esters readily hydrolyse and degrade to their alcohol and acid constituents and may be responsible for the characteristic smell associated with butyrate. Some microorganisms, such as saprophytic fungi, release cellulases which would breakdown CAB and can release organic acids, which can breakdown acrylic. Therefore, for both plastics, chemical reactions on the surfaces and the release of small molecular weight chemicals are possible, but probably more common for butyrate (Robert Minard, pers. comm.). Consequently, chemicals being released at the surface of the butyrate and/or acrylic tubes may interact with the soil solutes and microfauna to influence root pigmentation and survival.

Conclusions

Because minirhizotron studies are so labor intensive, there has been a lack of investigation of such basic questions as 'What is the best type of transparent material to use in minirhizotron research?'. We feel that there is need for more study in this area to explain the reasons behind the differential responses that our study found and to determine if there is a best material for

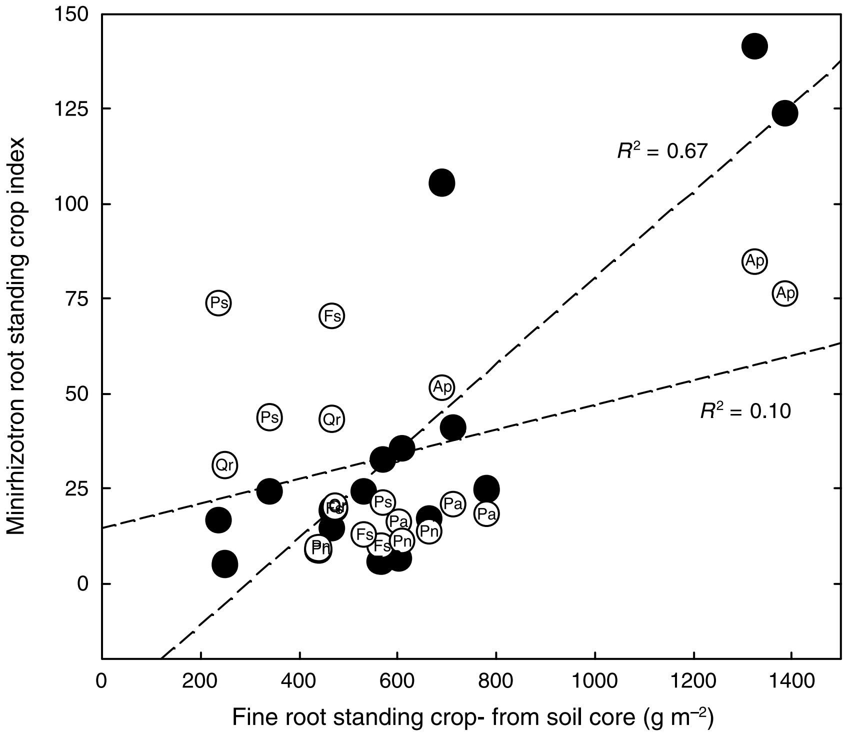

Fig. 6 Relationship between standing fine root (< 2 mm) biomass from soil cores taken to 45 cm depth in 1999 and an index of standing fine root (< 1 mm) crop against the acrylic (closed circles) and butyrate tubes (open circles), also 0–45 cm, in the same plots at the end of the experiment in 2001. Each data point represents either the acrylic or butyrate standing crop index (average of three tubes, calculated in g m^{-2} of imaging surface area of the tubes) with the soil core standing crop estimate of the plot (average of 11 cores, calculated in g m^{-2} of projected surface area of the soil corer used). Species labels are shown for butyrate tubes (Ap, *Acer pseudoplanatus*; Fs, *Fagus sylvatica*; Pa, *Pinus abies*; Pn, *Pinus nigra*; Ps, *Pinus sylvestris*; Qr, *Quercus robur*). Plant species for corresponding acrylic point will be the same as that for butyrate for a given soil standing crop.

rigid minirhizotron tubes. Of the material we tested, we assumed glass to be the most inert, but it is difficult to use. In our study in Pennsylvania, about one-third of our tubes broke after only one winter.

How can we evaluate the reliability of the two plastic tube materials? In the forest tree experiment, acrylic was less detrimental than butyrate in terms of survival rates of white roots and time to root pigmentation, which is associated with reduced root function. Although the contrasts of SC_{mrt} with SC_{cores} provides only an indirect means of gauging the reliability of the minirhizotron data, they clearly suggest that data obtained using butyrate tubes is more problematic than that near acrylic for some species. The SC_{mrt} values from butyrate tubes did not correlate as well as expected with SC_{cores}. Also, a comparison of root productivity with total fine tissue productivity suggests that data for butyrate tubes yielded dubious estimates of root production for some species. Finally, differences in plastic chemistry also suggest that butyrate tubes were more likely than acrylic tubes to chemically influence the rhizosphere. Although each of these lines of evidence is circumstantial, they collectively suggest that there is a differential response of roots of different tree species near minirhizotron tubes of different materials. Hence, we suggest the use of glass tubes whenever conditions allow, and we emphasize the importance of researchers reporting the type of minirhizotron tube material used. Tube type probably does not affect relative differences in root survival within a species, such as the consistent evidence that finer roots are shorter lived than coarse roots (Wells & Eissenstat, 2001). Minirhizotron researchers, however, should be aware of the potential problems when comparisons are made across species. In our study, the root standing crop index across all species appeared less problematic for acrylic than butyrate tubes.

Researchers are continually frustrated by the lack of good methods for understanding root dynamics. Although there have been great technological improvements in minirhizotron cameras and analysing software, there are still some artifacts as identified in this study. We feel minirhizotrons are still the preferred method for observing roots *in situ*. However, our results suggest more research should be conducted to refine this important tool for studying below-ground dynamics.

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