THE EFFECT OF NITROGEN FERTILIZATION ON THE PHENOLOGY OF ROOTS IN A BARRIER ISLAND SAND DUNE COMMUNITY

by

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Abstract

Little work has been done on the phenology of root growth and senescence largely due to methodological difficulties. The application of minirhizotron technology has enaWed the tracking of individual roots through an entire growing season. As a result, direct measures of turnover, root growth, and an analysis of cohorts were made. Small plots on a 36 year old dune on Hog Island, a barrier island in the Virginia Coast Reserve Long Term Ecological Research Site, were fertilized with nitrogen. Minirhizotron tubes were installed in each fertilized and control plot. Each tube was sampled monthly for nine months, March through October of 1992. Root length density increased throughout the growing season with the highest root length density in the top 0-20 cm of the soil profile. The fertilized plots had higher root length densities (14.05 mm cm^{-2}) than the unfertlized plots (2.68 mm cm^{-2}). The turnover was higher in the unfertilized plots only in the top 0-20 cm of the soil profile (fertilized = 0.020 percent loss per day, unfertilized 0.024 percent loss per day). The cohort analysis found that the largest loss of roots for a cohort occurs within the first month. There was also a decline in root loss in the last sampling of the last cohort potentially indicating the roots were preparing ‘for the winter months. The overall low turnover rate, the decreased turnover rate with fertilization and the decreased turnover in the last cohort imply that roots tend to be conserved in this nutrient poor system.

Keywords: Root, Minirhizotrdn, Phenology, Turnover, Nitrogen Fertilization

Acknowledgements

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Introduction

Plants primarly acquire nutrients through root systems. Roots are also the largest heterotrophic portion of the plant. As a result, a balance must be reached between the needs of the plant for nutrients end the considerable energy drain thet roots place on the plant (Caldwell 1979, Bloom et al. 1985). Little work hes been done to explore the root distribution patterns that plants use belowground to survive in nutrient limited ecosystems. Barrier islands provide a particularly good environment to observe, nutrient limited root distribution responses. The sandy soil of the islands makes root observetion relatively easy end the low nitrogen status of the soil also makes nitrogen content of the soil easily manipulated.

Roots serve four major roles for most plants : holding the plant in the soil, resource storage, absorbing nutrients, end absorbing water. Caldwell (1979) described the high expense of root growth end maintenance end the significant resource drain placed on the plant. Because roots are an expensive portion of the plant to grow end maintain, plants heve developed strategic ways to distribute their roots. Harris end Wilson (1970) found that four grasses showed different strategies of root development and that the strategy's effectiveness wes directly related to the severity of stress placed upon the plant. The position of roots within the soil metrix should, therefore, reflect the strategies plants use to efficiently perform root functions.

Plants have evolved strategies for efficiently managing environmental stresses (Bloom et al. 1985). Caldwell (1979) suggested that short lived fine roots placed within the established root zone would be more effective at gathering ephemeral resources from microsites within the soil matrix than would long lived roots. He also suggested that nutrient adsorptive intensity may be inversely related to root longevity. Both Grime and Cambell (1991) and Sharpe and Rykiel (1991) described' productivity responses to resource availability within a site. They felt that highly productive, resource rich plants have flexible allocation patterns while resource poor, unproductive plants have a less flexible response. Sharpe and Rykiel (1991) also suggested that resource poor plants tend to allocate resources to storage when encountering a new supply, while Grime and Cambell (1991) detailed on a cellular level how this response would occur. Tilman and Wedin (1991) found an increase in root biomass correlated with increasing nitrogen status for five perennial grass species.

Previous research on roots has focused mostly on root/shoot ratios (Mooney and Winner, 1991; Bloom et al. 1986; Thornley, 1969; Orians and Solbrig, 1977; Hansson and Petterson, 1989; Bazzaz et al., 1987); however, little work has been done on the distribution of roots within the soil matrix after root/shoot partitioning has occurred. There has also been little work done on root length density variation with depth and time, and no studies have been performed in dune communities.

Turnover has also received little attention in dune communities. Aber et al. (1985) found turnover directly correlated with an increasing nutrient status in a forested ecosystem, which supports both Grime and Cambell's (1991) and Sharp8 et al.'s (1991) theory on resource allocation.

Traditional root research involves such time-consuming and destructive methods as soil coring or soil monolith removal. The destructive nature of traditional sampling makes repeated measurements of the same volume of soil impossible. Repeated coring also causes a significant impact on the research site. Current methods of turnover calculations through sequential corings are also known to be inaccurate (Singe et al. 1984). Minirhizotrons, clear tubes in which video tape recordings of roots are made, have been used at a variety of other sites including a hardwood forest (Hendrick and Pregitzer 1992a, Hendrick and Pregitzer 1993a, Hendrick and Pregitzer 19936) agricultural systems (Hansson and Andren 1987) and a simulated tropical forest (Körner and Arnone 1991). Minirhizotrons were used to observe root dynamics in the present study because their non-destructive nature allows direct measurement of turnover and root longevity. Because there is little site disturbance after the installation of the minirhizotrons, there is much less disturbance to the experimental plots. Repeated measurements can be made of the same section of soil and direct measurements of root longevity and turnover are obtained by measuring the same roots through time.

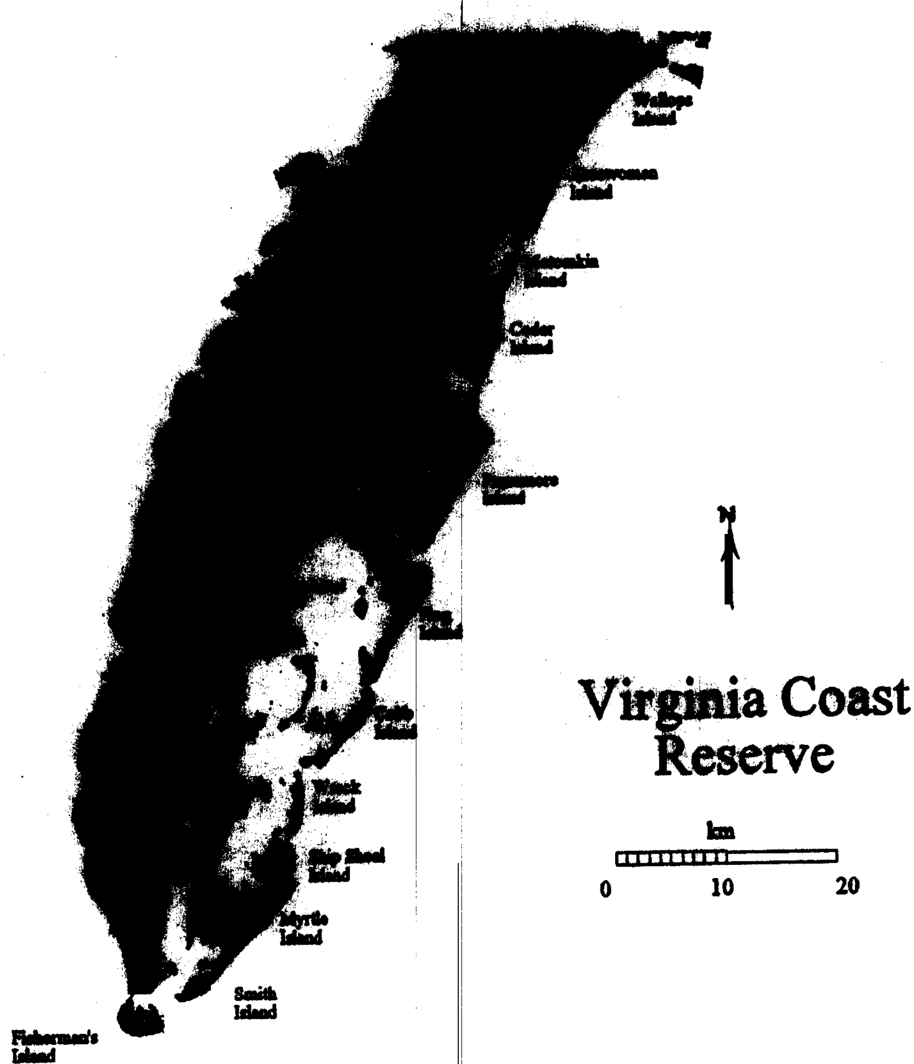
This study quantified the root dynamics of a barrier island dune community to determine what strategies are used by the plants to survive. The dune ecosystem on which the study site is located is a low productivity, low resource environment and the plants, primarily perennial grasses, should respond as previously described for low resource, low productivity plants. In addition, the location of nutrients within the soil profile should affect the location of the roots. There should be an increase in longevity of roots within resource rich patches, and a decrease in longevity in nutrient poor patches. The primary questions posed in this study included: Does turnover increase with nitrogen fertilization? Do ephemeral roots exist and does fertilization affect their longevity? Is there an increase in root length density which coincides with fertilization within the soil profile? Is there a seasonal change of turnover? Is there a seasonal change of root (length density? The primary objective of this study was to quantify root phenology, as expressed in root length denslty and turnover, in fertilized and control plots on a nutrient poor barrier island sand dune using minirhizotron observation tubes.

METHODS

Site Description

The study site is located on Hog Island, a barrier island off the eastern coast of the Delmarva Peninsula on the Virginia Coast Reserve (VCR) Long-Term Ecological Research (LTER) Site (Figure 1). The land is currently owned and managed by the Nature Conservancy of Virginia, located at Brownsville near Nassawadox. Although a small community existed on Hog Island in the

Figure 1. Map of Virginia Coast Reserve; research site is on Hog Island.


Wallops Island
Cedar Island
Wreck Island
Ship Shoal Island
Myrtle Island
Smith Island
Fisherman's Island
N
Virginia Coast Reserve
km
0
10
20

early part of this century, the island has been largely uninhabited since the late 1940's (Dueser et al, 1976). All e ful domestic livestock were removed from the island in the cattle drive of 1980 (Hayden et al. 1991).

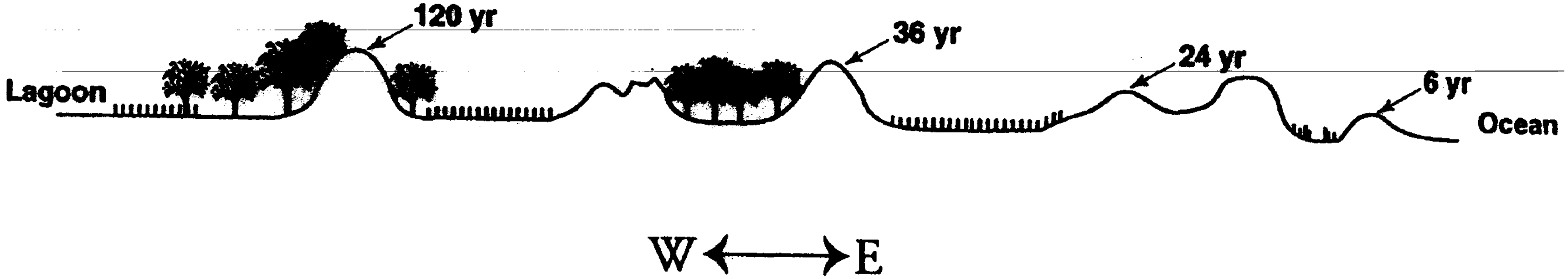
On north Hog Island accretion has produced distinct dune complexes as well as a foredune area. From the Atlantic Ocean to the bayside of the island, a chronosequence of dunes have been aged. from 6 to 124 years old (Hayden et al, 1991). The present study was located on the 36 year old dune ridge (Figure 2).

The plant community on this dune complex is dominated by *Ammophila breviligulata* Fernald, *Spartina patens* Muhl. and *Panicum amarum* Eli. (Table 1). The community is on a well-drained sand dune with surrounding wet areas. To the east Is a freshwater marsh of *Spartina patens*. To the west are wax myrtle (*Myrica cerifera* L.) thickets.

Monthly rainfall and temperature data for 1992 ranged from highs of 216 mm rainfall (August) and 25.1 °C mean temperature (July) to lows of 5.7 mm rainfall (December) and 0°C mean temperature (December) (Table2).

The soil of the study site is a Newhan-Corrolan complex (Dueser et al, 1976). This udipsamment is characterized by excessively drainedNewhan soil in the higher elevations and the well drained Corrolan soil in the lower elevations. The soil, therefore, provides few nutrients, low nutrient retention and limited water retention. All plots were placed within the Newhan series. A previous study (Day and Lakshmi, unpublished data) found a higher level of

Figure 2. **Diagram** of dune chronosequence on Hog Island; study plots are on the 36 year old dune.



Lagoon
120 yr
36 yr
24 yr
6 yr
Ocean
W
E

Table 1. Plant species in both fertilized and unfertilized plots ranked by mean cover class (data from two quarter meter square samples in each plot sampled 9/94). Ambr = *Ammophila breviligulata* Fernald, Paam = *Panicum amarum* Ell., Sppa = *Spartina patens* Muhl., Soca = *Solanum carolinense* L., Ansc = *Andropogon scoparius* Michaux, Livi = *Linum virginianum* L., Ruac = *Rumex acetosella* L., Sote = *Solidago tenuifolia* Pursh, Casp = *Carduus sp.*

Fertilized Plots			Unfertilized Plots		
Species	Stems m^{-2}	% Cover Class'	Species	Stem m^{-2}	% Cover Class'
Ambr	315	2.375	Sppa	82	1
Paam	39.5	1.125	Ambr	34	0.625
Sppa	10.5	0.5	Paam	9.5	0.375
Soca	2.5	0.375	Soca	0.5	0.25
Ansc	6	0.125	Ruac	13	0.25
Livi	0.5	0.125	Casp	0.5	0.125
Ruac	0.5	0.125	Ansc	0	0
sote	1	0.25	Livi	0	0
Casp	0	0	Sote	0	0

'Mean % Cover Classes (4 = 75-100%, 3 = 50-75%, 2 = 25-50%, 1 = 1-25%, 0 = 0%)

Table 2. Average monthly temperature and total monthly rainfall from the Hog Island weather station in 1992.[a]

Month	Temperature (°C) /Rainfall (mm)
Jananuary	4.9 / 40.8
Febuary	5.6 / 68.9
March	6.7 / 40.2
April	1 2.2/45.5[b]
May	15.1/131.9[b]
June	20.5 / 107.8
July	25.1 / 102.7
August	23.2 / 216.0
September	21.9 / 162.8
October	14.7 I 44.6
November	11.1 / 101.0
December	0.0/5.7[b]

[a]Data condensed from Krovetz and Porter (1992)
[b]Data from Brownseville Virginia to supliment missing weather data from Hog Island

soil water nitrogen at 15 cm than at 50 cm in areas with the same fertilization treatment used in this study and in areas without fertilization (Table 3).

Experimental Design

Eight 3x3 m plots were selected within the study site. The sites were chosen for both visually representing the dune ecosystem and being spaced far enough apart to allow for independent treatment of the plots. Four plots were randomly assigned to nitrogen fertilization treatment and four plots were left as untreated controls. Fifteen g N m^{-2} with a 70%-30% mix of coated temperature-release urea to uncoated urea was applied to the fertilized plots. There were three applications of fertilizer during the year (March 14, June 10, and October 3 of 1992). The site was monitored on an approximately monthly basis from March through October of 1992 (Table 4). Inclement weather and logistical problems prevented strict monthly sampling. Four minirhirotron tubes were placed in each plot, one meter from the adjacent sides, each tube perpendicular to one of the sides (Figure 3). The etched frames faced towards the center of the plot to reduce any possible edge effects,.

Ail eight 3x3 m plots were chosen within the study site to reflect the grassy drier regions of the dunes. The plots were, therefore, away from *Myrica* thickets and above any areas which appeared to have experienced standing water. in addition, the plots were distributed in such a way to assure that the fertilization treatment of any single plot would not affect an adjacent plot.

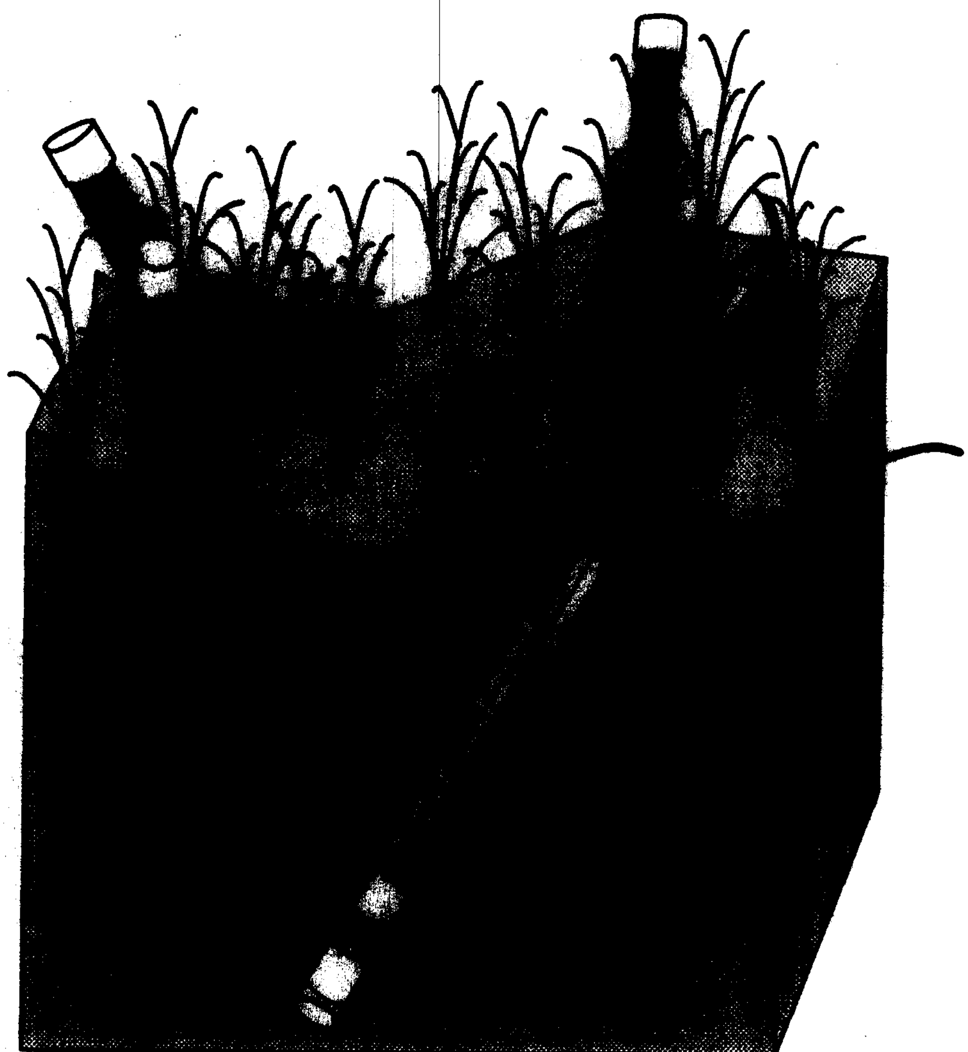
Table 3. Ppm ammonia and nitrate from porous cup lysimeters at two depths on the 36 year old dwne on Hog Island (Day and Laksmi, unpublished data)

	Control		Fertilized	
depth	Ammonia	Nitrate	Ammonia	Nitrate
15 cm	0.08	0.29	19.86	29.28
50 cm	0.05	0.07	0.36	18.16
Ratio	1.6	4.14	55.2	1.6

Table 4. Dates that observations were made from research site during 1992.

March 14,1992
April 25,1992
May 28,1992
June 24,1992
July 29,1992
August 25,1992
October 3,1992
October 24,1992

Figure 3. Installation of tubes within a plot. A minirhizotron camera is inserted in one of the tubes.



Minirhizotrons

Minirhizotrons are clear tubes placed in the ground from which observations are made of the roots in the surrounding soil. In the present study, observations were made with the video camera system described by Hendrick and Pregitzer (1992s).

Minirhizotron research requires three main steps: field installation and data collection, laboratory analysis/ and data processing. The first step involves preparing, installing, and monitoring the tubes. Laboratory analysis entails the creation of data sets from video tapes made in the field. Finally, the data processing provides ecologically significant information such as root length density, turnover rate, and phenology of cohort groups.

The minirhizotron tubes were 2 m long, clear, 5.08 cm inner diameter butyrate tubes with 0.65 cm thick watts. Butyrate tubes have been used by several other researchers (Hendrick and Pregitzer 1992*a*, Rygiewicz et al., 1991) and are more durable than glass tubes (Hendrick, pers comm.).

The bottom of each tube was capped with plexiglass to prevent soil and water from entering, and thu vent the accumulation of fungi, algae, or samplings. Two parallel lines, 18 mm apart, were etched down the surface of the tube, from the top to the bottom, with transverse lines etched every 13.5 mm. The result was a column of frames or "windows", 18 mm x 13.5 mm, stacked from the base to the top of the tube. A number was placed in the third frame from the bottom of each tube to provide unique identification.

Etch marks were filled with acrylic fluorescent green paint, as suggested by Pregitzer (1992). Thinned paint was brushed into the etchmarks and after partial drying, excess paint was wiped off with a damp cloth. This produced clear windows surrounded by fluorescent green lines.

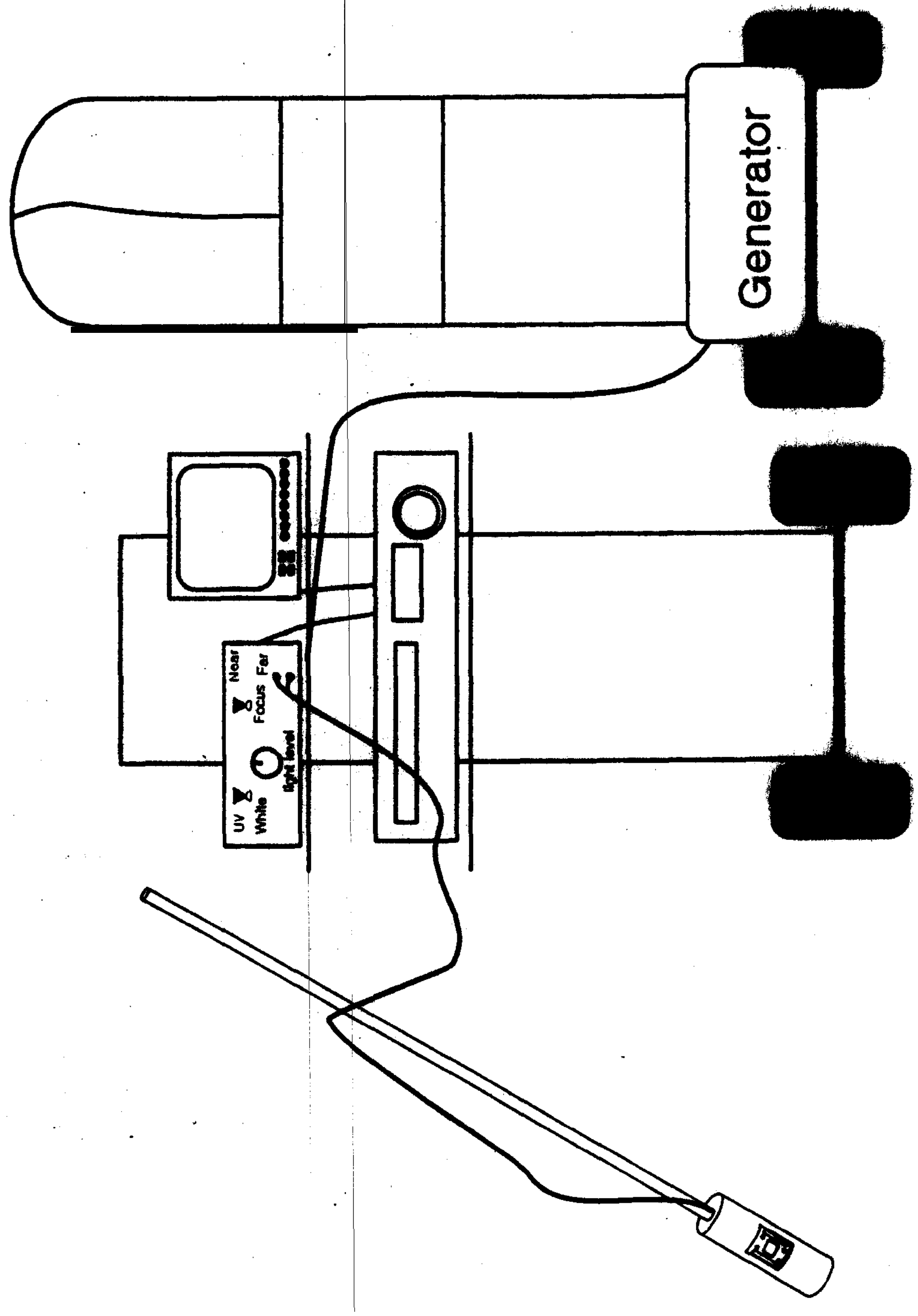
Light has been shown to Influence root growth (Lake and Slack 1961, Furuya and Torrey 1964) and several other studies have shown the effect of light on growth of roots along minirhizotron tubes (Levan et al. 1987, Vos and Groenwald 1987). To prevent light from penetrating the minirhirotron tubes, the top of each tube was painted to just below the soil surface with a flat black paint. In addition, the top of the tube was wrapped with electrical tape to provide a light-tight fitting with a PVC cap which was placed over the open end of the tube. The cap also prevented debris and rain from entering the tube.

Each tube was inserted into the soil at a 45° angle to the ground. The angle of insertion was assured with an auger stand built at the Old Dominion University science shop. Holes were augered to a depth which would allow observation through approximately 80 frames or 1 m of observable tube. After the depth was estimated to be correct for insertion of the tube, the tube was inserted in the augered hole. The top was then wrapped with tape and capped. Loose sand was replaced around the interface of the tube and the soil-surface. The tubes were installed Febuary 15-29, 1992.

Once the tubes had been in place for two to three weeks, the first readings were taken. A Bartz two-inch diameter minirhitotron video camera was inserted into the minirhizotron tubes. The camera was placed at the bottom of each tube and drawn along the etched marks pausing at each window. The image was viewed on a small color monitor and focused with an electronic control box while recording in S-VWS on a VCR (Figure 4). The tapes were dated and returned to the laboratory for analysls.

In the laboratory, the same VCR used in the field was hooked to a Targa plus video board in the computer. This board converts the signal from an analog television signal to a digital signal which can be interpreted by a computer monitor. The board also stores the images, frame by frame, in memory; thus, video frames were "frozen" for analysis. ROOTS software ver "1 (Michigan State University Remote Sensing Laboratory, 1989) was used to digitize the video images from the field to numeric data. A computer "mouse" was used to outline a skeleton (a line along the center and a single line along the width) of the root on the monitor and to store the coordinates, as well as length and width measurements, to a dbase file (see Appendix for detailed protocol). The root skeletons fr m the previous month were overlaid on the corresponding video frame from the current month. Whether the roots within

Figure 4. Illustration of minirhizotron field equipment **which includes: minirhizotron camera, minirhizotron** camera controller, super **VHS VCR, monitor, and generator.**


Generator
UV
White
light level
Focus
Near
Far

the current video frame matched that of a previous root skeleton was noted. After digitizing was complete, the files created by ROOTS were compiled into a single file whii was manipulated in Microsoft Foxpro (a database program).

Within Foxpro, programs were developed which produce cohort groups and turnover data. Because black roots were shown to produce new live roots, color was not useful for determining root longevity in this study. The program therefore assumed all visible roots were alive. The database programs placed each root into a cohort based on its first appearance (programs and required digitizing protocol available upon request from the author). The cohort group provided the basis for turnover calculations. The data were exported to an ASCII file and loaded into SAS for statistical analyses.

Root Length Density

Root length density (RLD) was calculated by summing all of the foot lengths (RL) for a given area (A) of the tube and dividing that quantity by the area of the tube observed. Root length density was calculated for each date and depth combination of each tube for analysis.

Turnover

To calculate turnover, the root length was compared between sample n and sample n+ 1. If a root was not observed at time n + 1 the root was assumed to have decomposed. The turnover was therefore 1.00 or 100%. If the root was longer at time n + 1 than at time n, in other words the root grew between time n and n + 1, there was no turnover and the turnover was 0.00.

If a root was smaller at n + 1 than at n, a simple calculation was made to determine the turnover (equation 1).

(1) (root length n - root length n + 1) / root length n = turnover

This measure was related to ndrick and Pregitter's (19928) mortality measure butbecause it was fig red on a per root basis, with root extension eliminated from the calculation, tr e turnover was actually measured. Turnover was calculated on a per root basis and averaged for each depth and date combination of each tube. These means were then used for statistical analyses.

Cohort Analysis

To determine root life expectancy, roots were placed into cohorts, or groups, based upon when the roots were initially observed. Percent change was calculated from time n to time n+ 1 for each cohort. The percent change was then divided by the number of days between sampling dates to control for different intervals between sampling dates. Root number density rather than root length density was used. This eliminated skewing. the data in favor of longer roots.

Not all of the data were used for the statistical analyses. The reasoning for the removal process folk)ws. It was believed that the largest percent change would occur between the irst and second month of any cohort. Three consecutive percent changes were felt to be the minimum to ensure that the changes observed were not transitory. Four months of data were, therefore, determined to be the minimum number of sampling dates within any cohort. The first, second, third, fourth, and fifth cohorts had enough sampled dates to meet the minimum criteria to be chosen for this analysis. The first two cohorts were eliminated from the analysis because they contained too many missing values (percent change could not be calculated if there were no roots observed within a cohort). Therefore, only the third, fourth, and fifth cohorts were used in the analysis.

Because interest was focused upon the longevity of the roots, the analysis used root age. Root age was given by the number of dates since the roots were first observed, as discussed above. There were three root age categories for the percent change: one, two, and three. Root age one represented the percentage change interval from time one to time two. Root *age* two represented the percentage change interval from time two to time three and so forth. If a root lives to be a certain age, it *may* have a greater likelihood of survival. A percent change would, therefore, decrease with root age. Another possibility is that as roots age their likelihood of survival would decrease. This would result in higher percent changes with root age.

Statistical Analyses

Originally, it was hoped that tubes could be nested within plots as suggested by Hendrick and Pregitzer (19926): however, there were not enough degrees of freedom with the number of factors and levels measured and the number of replicates to calculate an error term for the ANOVA. Therefore, the option of which factors and/or' levels to decrease had to be decided. Originally frames were classed into 12 depth classes; each depth class was roughly equivalent to 5 cm vertical depth. Because there were not enough-degrees of freedom available to look at all of the depths separately, the depths were pooled into three roughly 20 cm vertical depth classes (O-22 cm, 22-46 cm, 46-68 cm). This provided enough degrees of freedom for the error term to perform the analyses. Because /depth class one was necessarily related to depth class two and depth class two was related to depth class three, depth was analyzed as a repeated measure. Dates were also analyzed as a repeated measure for this study. The final ANOVA model, therefore, had plots nested within treatments and both time and depth class as repeated measures.

A nested, crossed, repeated measures model wes used to analyse the cohort data. Plots were nested within treatments as was done with the other measures. However, cohorts were crossed with treatment and root age was a repeated measure.

Initial testing of the root length density data showed that a depth*date*plot(treat) interaction was significant. When the data were plotted depth*date for each plot, it was apparent that plot 2 was significantly different from all of the other plots. This plot, although an unfertilized plot, had the highest root length density of any depth and date combination for either the fertilized or the unfertilized plots. This plot also had a higher root length density in the 20-40 cm depth class than the O-20 cm depth class. All other plots had the highest root length density in the first O-20 cm depth class, nearest the surface. Both of these trends showed clear differences not only from the other unfertilized plots but from the fertilized plots as well. Since the plot was initiated in the spring of 1992, *Myrica cerifera* began to overhang the plot and possibly had roots extending into the dune edge. Thus, because plot 2 differed not only in the quantity of roots but also in the pattern of root distribution from all the other plots, it was considered an outlier. Because it clearly was not representitive of the dune community, plot 2 was removed from all analyses.

A log transformation was used in an attempt to normalize the root length density data. Biological data, such as root length density, are often distributed along a log-linear scale. Because the analysis was an unbalanced design (plot 2 was removed), a sum of squares IV was used. A test for sphericity of orthogonal components was found to be significant ($p < .0001$); therefore, the Greenhouse-Geiser adjusted F was used rather than using the split plot F probabilities. The Greenhouse-Geiser adjusted F deflates the degrees of freedom to compensate for the interrelatedness often found in repeated measures. It also adjusts to aptly measure the repeated factor. It takes into account the fact that time one is more highly correlated with time two than with time n and similarly for the depths.

Because turnover is a rate, an arcsine transformation was used in an attempt to normalize the data. Missing values reduced the degrees of freedom so that it was not possible to calaculate the error term for the ANOVA. The first three turnover intervals were removed from the analysis to remove enough missing values to allow the calculation of the error term; missing values were highest in the first few months of sampling. A test for sphericity of orthogonal components was not found to be significant ($p > 0.5943$). As a result, the split-plot F probability was used rather than the adjusted F used in the root length density analysis.

Results

Root Length Density

The results showed three main effects and two interactions to be significant (Table 5). Figure 5 shows root length density for all depths and dates for both the fertilized and unfertilized treatments. The same depth pattern of root length density can be seen in both treatments with the highest root length density occuring at ≈15 cm. The higher variation seen in the unfertilized plots can be attributed to the fine scale shown for root length density in the unfertilized graph than in the fertilized graph. Lower root numbers mean that fewer roots can provide information regarding root length density Both depth*date ($F = 4.40, p < 0.01$) and date*treatment ($F = 10.51, P < 0.01$) interactions were significant. The fertilized treatment increased in root length density over time; whereas, the unfertilired treatment only increased slightly over time (Figure 6). As a result there was also a higher root length density in

Table 5. Nested doubly repeated measures analysis of variance examining the effect of fertilizer on root length density over time and across three depth classes. DF = Degrees of freedom, SS = sums of squares, F Value = calculated F value, Adj Pr > F = Greenhouse-Geisser adjusted F value.

Source of variation	DF	Type IV SS	Mean square	F value	Pr>F
Treat'	1	343.763	343.763	43.16	0.0001
Plot (Treat)[a]	5	125.385	25.077	3.15	0.0282
Error	21	167.246	7.964		
Depth"	2	310.933	155.467	30.71	0.0001
Depth *Treat	2	1.433	0.716	0.14	0.8680
Depth*Plot(Treat)	10	56.187	5.619	1.11	0.3776
Error(depth)[b]	42	212.626	5.063		
Date[a]	7	1043.13	149.019	69.1'8	0.0001
Date *Treat[a]	7	158.404	22.629	10.51	0.0001
Date*Plot(Treat)	35,	118.880	3.397	1.58	0.0529
Error(Date)[c]	147	316.646	2.154		
Depth *Date[a]	14	70.265	5.019	4.40	0.0001
Depth*Date*Treat	14	11.357	0.811	0.71	0.6688
Depth*Date*Plot(Treat)	70	112.903	1.613	1.42	0.0757
Error(Depth *Date)[d]	294	334.983	1.139		

[a] significant at the $p<0.05$ level, [b] Greenhouse-Geisser Epsilon = 0.9977,
[c] Greenhouse Geisser Epsilon= 0.7607, [d] Greenhouse Geisser Epsilon = 1.0664

Figure 5. Contour plot of root density by both depth and date for fertilized and unfertilized plots.

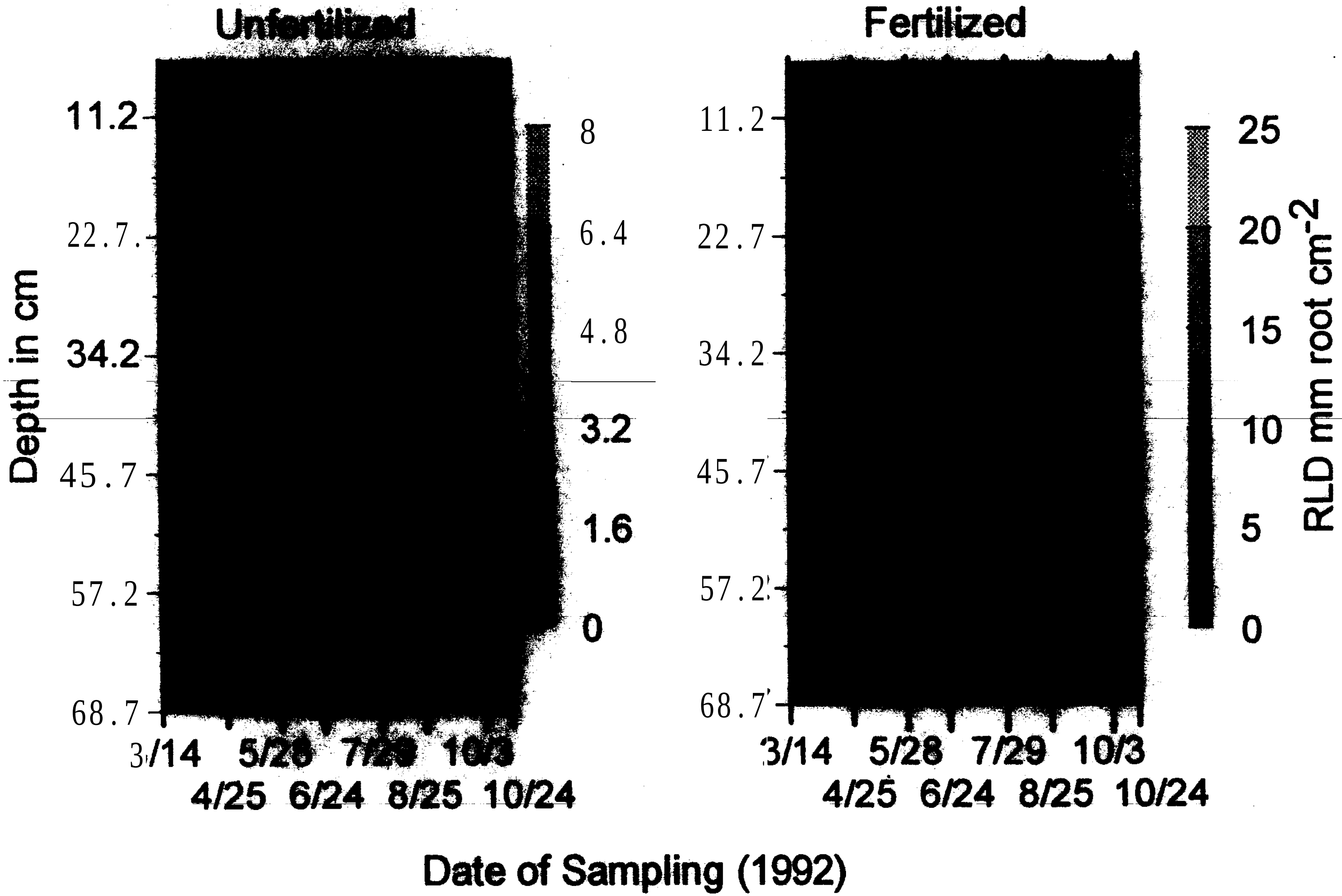
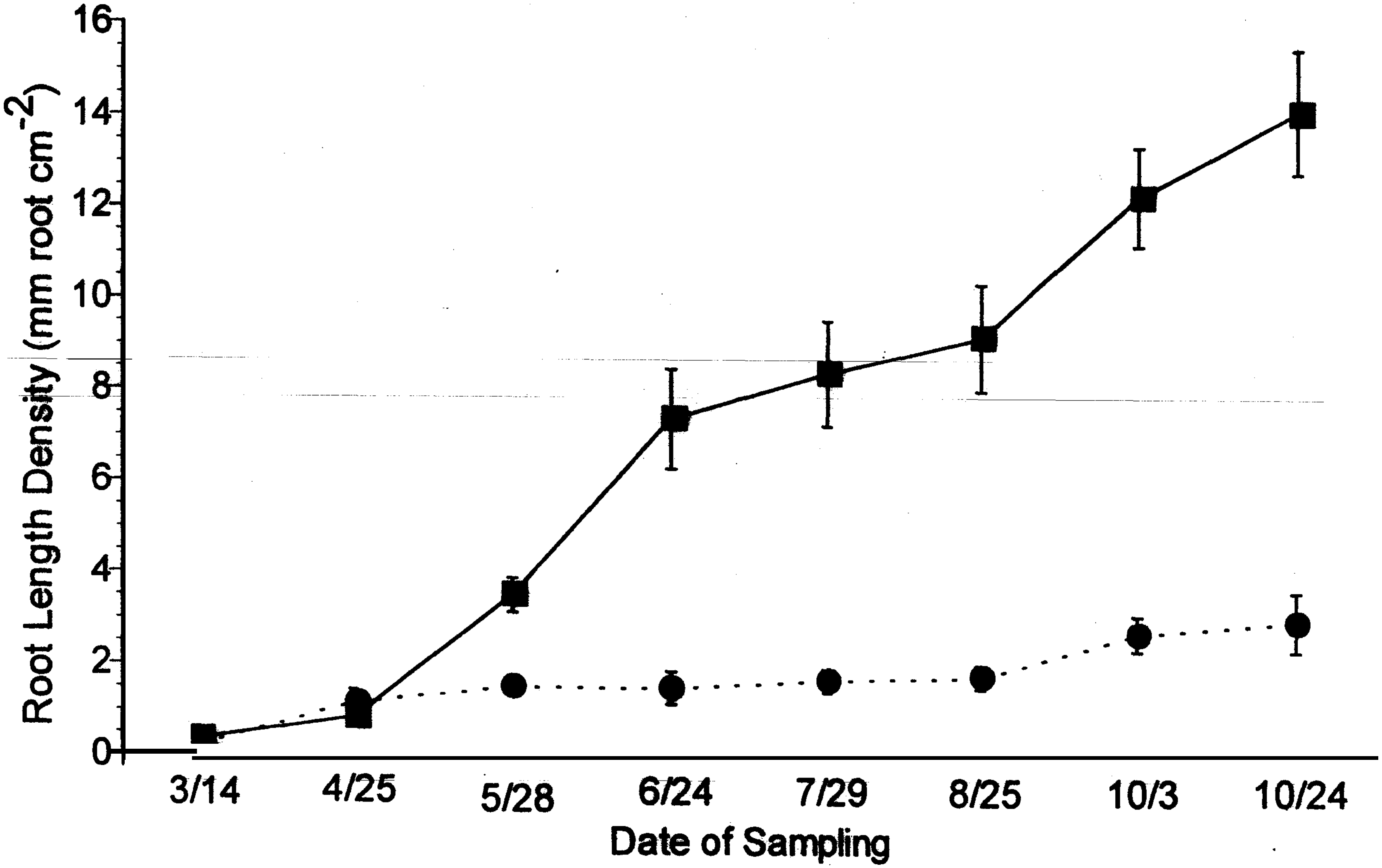

Unfertilized
Fertilized
Depth in cm
11.2
22.7
34.2
45.7
57.2
68.7
8
6.4
4.8
3.2
1.6
0
25
20
15
10
5
0
RLD mm root cm^-2
3/14
5/28
7/29
10/3
4/25
6/24
8/25
10/24
Date of Sampling (1992)

Figure 6. Root length density for fertilized plots (■) and unfertilized plots (●) from March 14, 1992 to October 24, 1992. Means and standard errors calculated from mean of plots, with root length density from entire tubes used to calculate plot means.


Root Length Density (mm root cm-2)
0
2
4
6
8
10
12
14
16
3/14
4/25
5/28
6/24
7/29
8/25
10/3
10/24
Date of Sampling

the fertilized treatment (root length density = 14.05 mm cm^{-2}, on 10/24/92) than in the unfertilized treatment (root length density = 2.88 mm cm^{-2}, on 10/24/92). The depth*date interaction was somewhat less clear (Figure 7). However, the 0-20 cm depth class showed a higher rate of increase with time then the two deeper depth classes. The 20-40 cm and 40-60 cm depth class do not appear to be different from each other. The 0-20 cm depth class had the highest root length density.

Turnover

The turnover analysis provided fewer significant interactions than did the root length density analysis. The analysis of variance found one main effect and one interaction to be significant (Table 6). The treatment*depth interaction was significant ($F = 2.59, P < 0.05$). Turnover tended to decrease with depth in the unfertilized plot while the turnover in the fertilized plot showed no clear change from the beginning to the end of the growing season (Figure 8). The fertilized plots appeared to have a lower turnover than did the unfertilized plots in the 0-20 cm depth class (fertilized = 0.020 SE 0.0011, unfertilized = 0.024 SE .0013). There did not appear to be a difference between treatments for the other depth classes. Date was a significant main effect ($F = 3.31, P < 0.05$). Turnover in March was higher than turnover in either April or May (Figure 9).

Cohort Analysis

Figures 10 and 11 show the cohort root number density by date. The patterns and relative values were similar to root length density for the plots

Figure 7. Root length density (RLD) for 0-20 cm depth (■), 20-40 cm depth (●), and 40-60 cm depth (▲) for both fertilized and unfertilized plots. Means and standarb errors calculated from mean of plots. Note that the scales on the y-axes; are different.

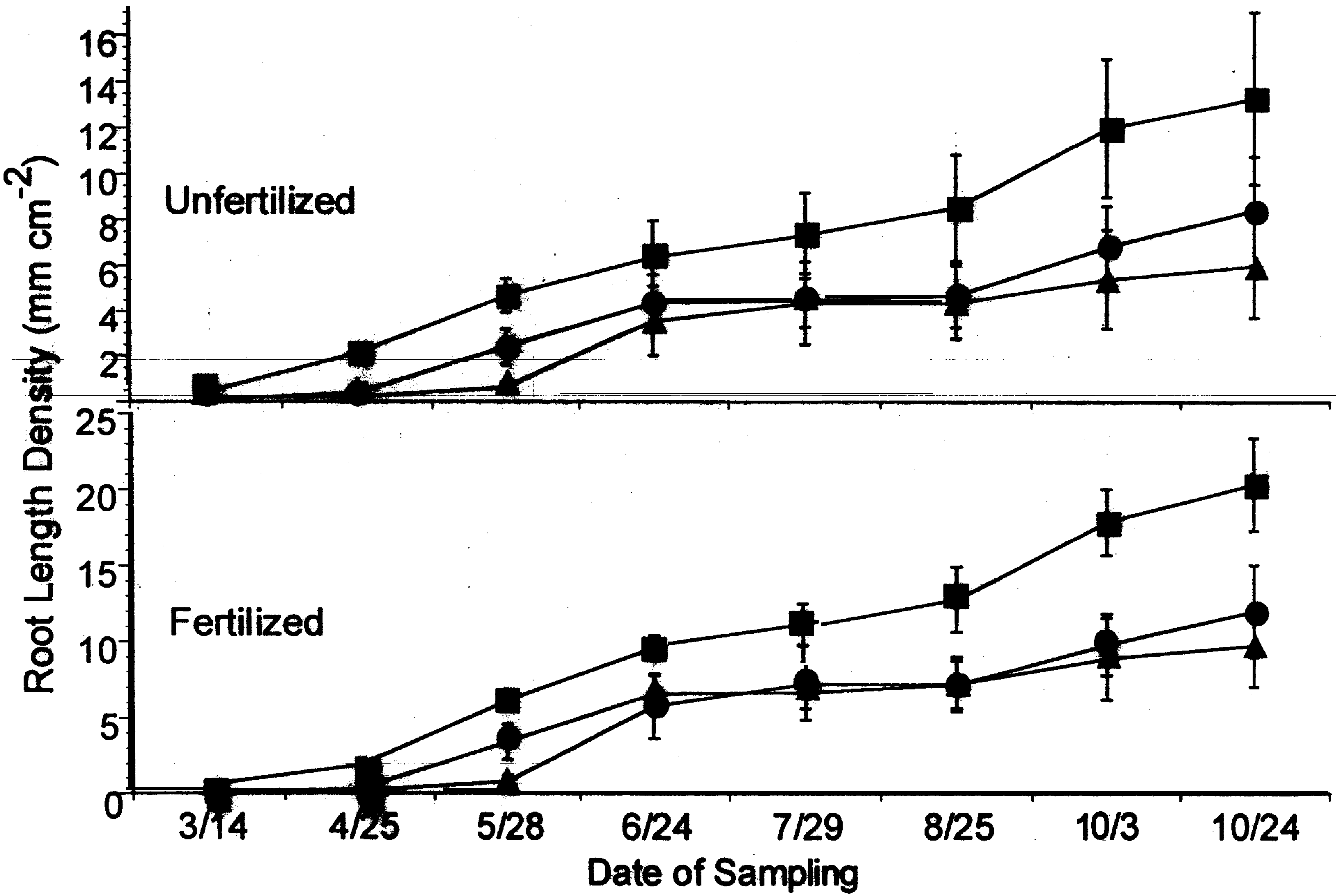
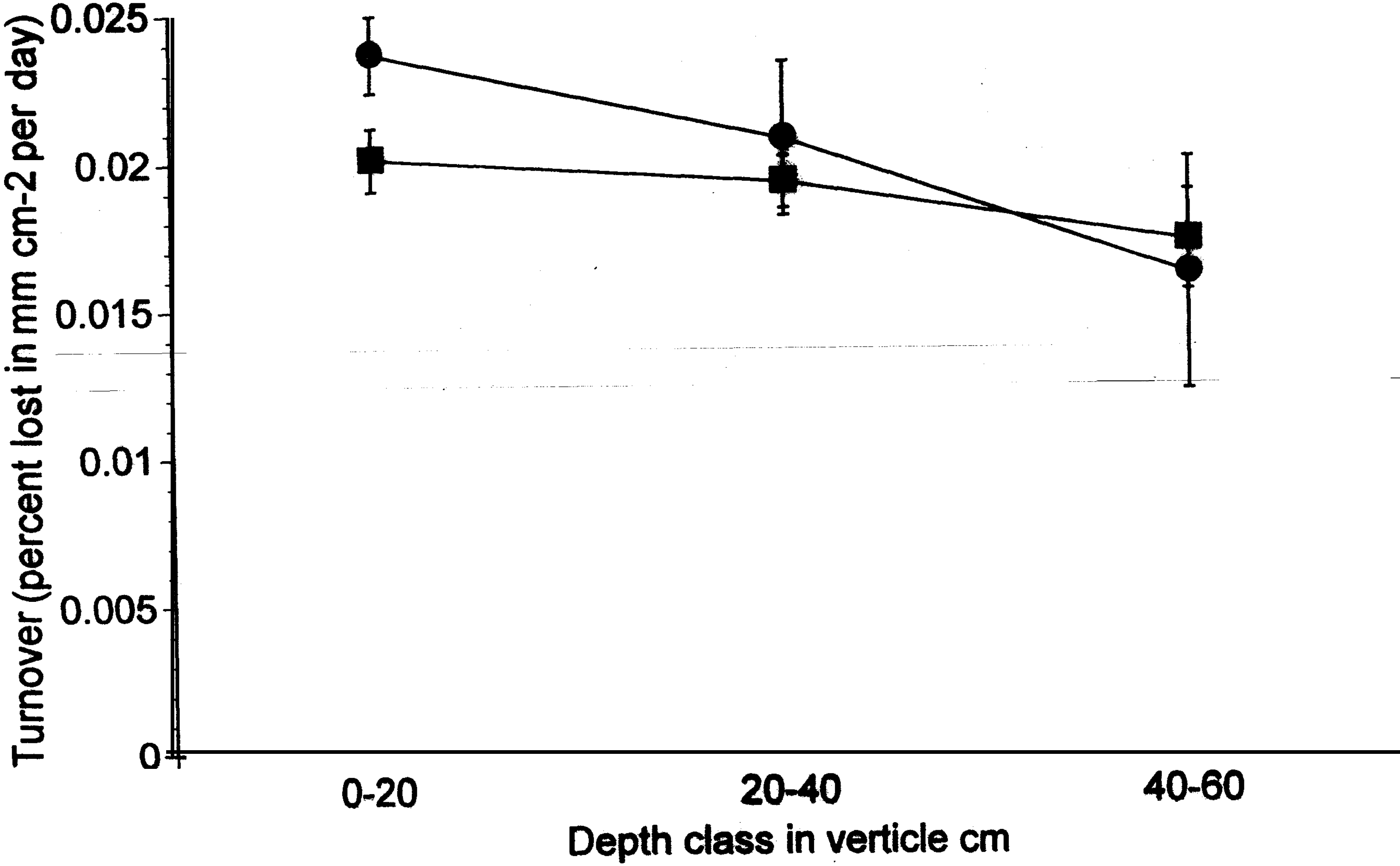

Unfertilized
Fertilized
Root Length Density (mm cm-2)
16
14
12
10
8
6
4
2
25
20
15
10
5
0
3/14
4/25
5/28
6/24
7/29
8/25
10/3
10/24
Date of Sampling

Table 6. Nested doubly repeated measures analysis of variance examining the effect of fertilizer on turnover over time and across three depth classes. DF = Degrees of freedom, SS = sums of squares, F Value = calculated F value. SS and Mean square in ten thousandths.

Source of variation	DF	Type IV SS	Mean square	F value	Pr>F
Treat	1	0.061	0.061	0.05	0.8377
Plot(Treat)	3	0.131	0.044	0.03	0.9910
Error	5	6.618	1.323		
Depth	2	2.304	1.152	2.37	0.1438
Depth *Treat"	2	4.613	2.306	4.74	0.0356
Depth*Plot(Treat)	6	1.686	0.281	0.56	0.7410
Error(depth)	10	4.864	0.487		
Date'	3	1.271	0.424	10.76	0.0005
Date *Treat	3	0.120	0.040	1.02	0.4130
Date *Plot(Treat)	9	0.010	0.011	0.27	0.9743
Error(Date)	15	0.069	0.039		
Depth*Date	6	0.170	0.028	1.04	0.4205
Depth*Date*Treat	6	0.295	0.049	1.81	0.1313
Depth*Date*Plot(Treat)	18	0.585	0.033	1.19	0.3261
Error(Depth*Date)	30	0.817	0.027		

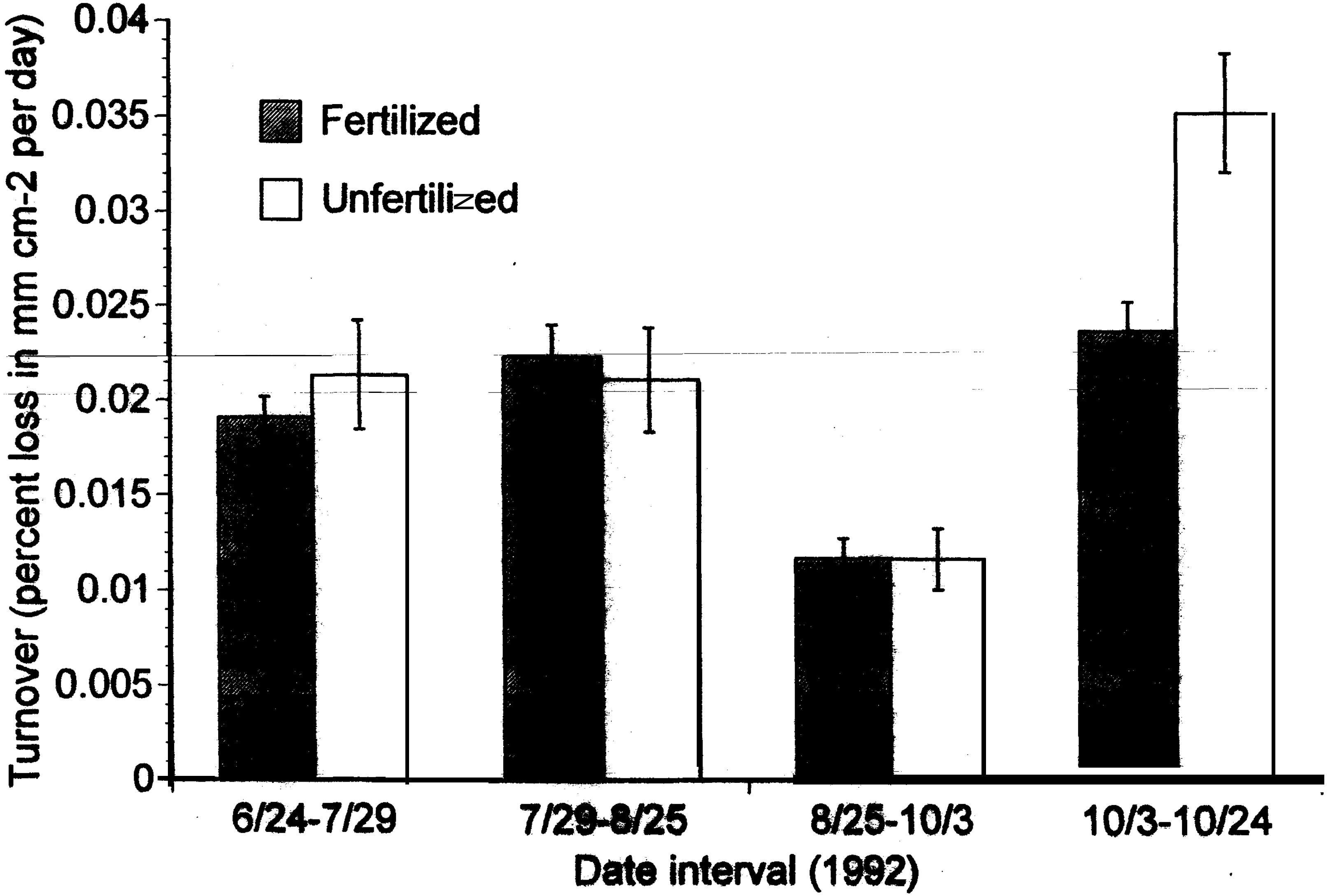
. significant at the $p<0.05$ level.

Figure 8. Turnover for fertilized plots (■) and unfertilized plots (●) for all three depth classes. Means anti standard errors calculated from mean of plots from 6/24/92- 1 0/24/92.



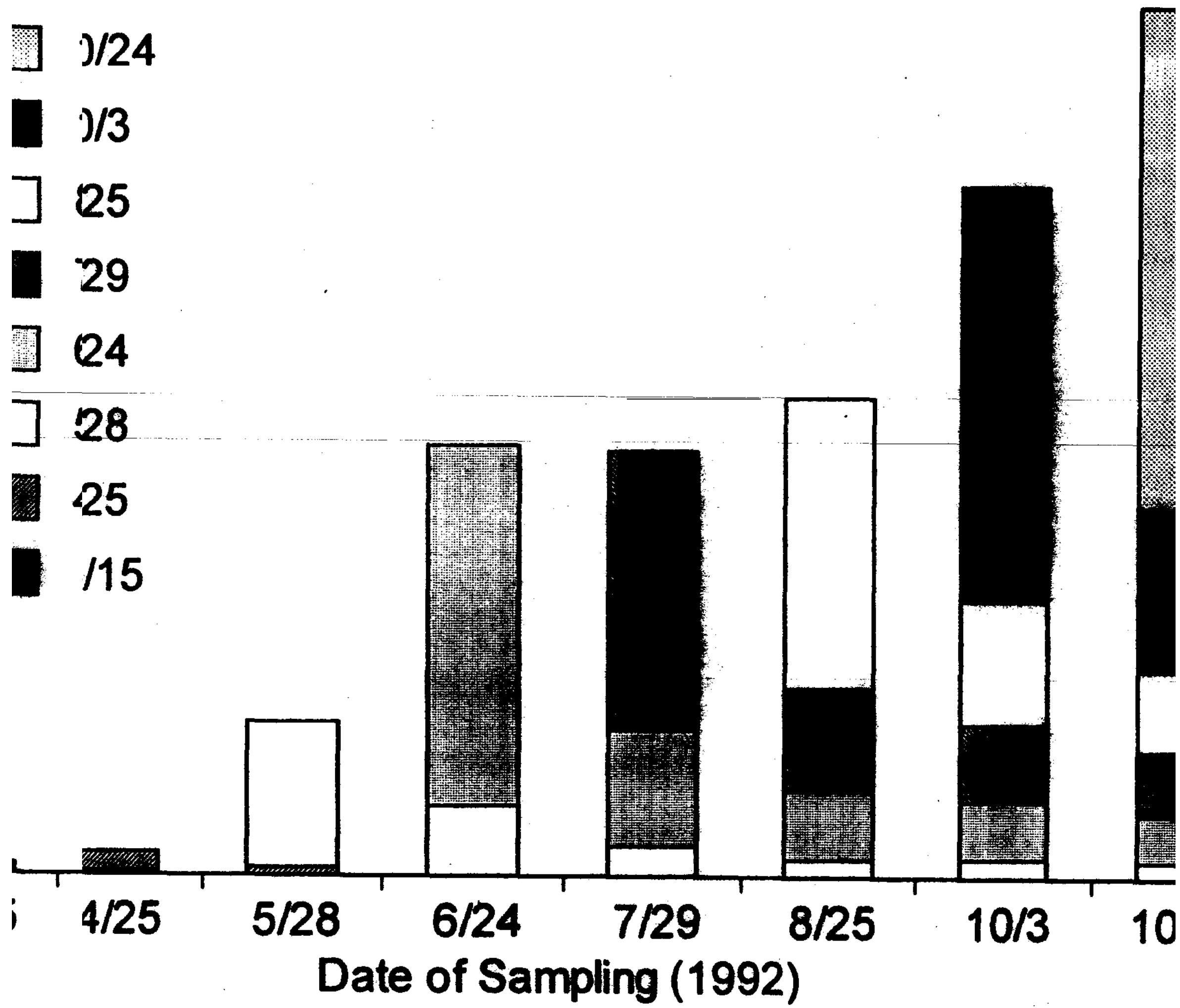
Turnover (percent lost in mm cm-2 per day)
0.025
0.02
0.015
0.01
0.005
0
0-20
20-40
40-60
Depth class in verticle cm

Figure 9. Turnover for date Intervals of fertilized and unfertilized plots. Means and standard errors calculated from mean of plots with root length density from entire tubes used to calculate plot means.



Turnover (percent loss in mm cm-2 per day)
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Fertilized
Unfertilized
6/24-7/29
7/29-8/25
8/25-10/3
10/3-10/24
Date interval (1992)

Figure 10. Cohort data by date for fertilized plots. Each color represents a cohort. Each column represents a sample date.


/24
/3
25
29
24
28
25
/15
4/25
5/28
6/24
7/29
8/25
10/3
10
Date of Sampling (1992)