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Effects of subsoiling on lateral roots, sucrose metabolizing enzymes, and soil ergosterol in two Jeffrey pine stands

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Summary We determined the effects of subsoiling on woody lateral roots and enzyme activities involved in stem carbon metabolism of 90- to loo-year-old Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) growing on the eastern side of the California Sierra Nevada Range. Twelve 1 .O-ha plots were established on each of two sites. Four site treatments-thinning and subsoiling entire plots, thinning and no subsoiling, thinning and subsoiling skid trails only, and no thinning or subsoiling (undisturbed control)-were replicated three times on each site. Root excavations and assays of stem cambium sucrose metabolizing enzymes were carried out during the summer and fall of 1994 and 1995. Subsoiled plots had more roots < 1 cm in diameter exhibiting dieback than undisturbed plots. No relationship was found for stem cambial sucrose synthase (SS) activity among treatments or sites; however, a strong seasonal pattern was observed. Activity of pyrophosphate-dependent phosphofrnctokinase (PPi-PFK) was seasonal, whereas ATP-dependent phosphofrnctokinase (ATP-PFK) activity was constant throughout the seasons. Neither of the PFK activities responded to treatment or site. Among plots, ergosterol, a surrogate for estimating live fungal biomass, was present in highest concentrations in soil and rhizosphere samples from subsoiled plots.

Keywords: *ergosterol, phosphofructokinase, Pinus jeffreyi, root dieback, sucrose synthase.*

Introduction

Biomass harvesting is often the only option available for thinning ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) stands (Kliejunas 1992) on the eastern side of the Californian Sierra Nevada Range. Harvesting equipment consists of mechanical, self-propelled shearers that fell and bunch the trees, which are then skidded to a whole-tree chipper. The majority of soils on these sites are of granitic or volcanic origin, and are susceptible to compaction by harvesting equipment (Froelich 1979, Davis 1992, Page-Dumroese 1993). Soil compaction is known to have a negative effect on ponderosa pine tree growth (Cochran and Brock 1985, Page-Dumroese 1993). To alleviate this problem, the practice of subsoiling or deep tillage is employed to decrease soil bulk density. However, there is evidence that the subsoiling implements cause root damage to the residual trees that has long-term effects on tree growth and stand health (Kliejunas 1992). In several pine species (e.g., *P. radiata* D. Don), fracturing compacted subsoil by creation of small channels has been found to improve growth (Nambiar and Sands 1992). No information is available on the effects of subsoiling treatments on small, woody roots and mycorrhizal fine roots in Jeffrey pine.

In loblolly pine (*Pinus taeda* L.) (Sung et al. 1993) and sweetgum (*Liquidambar styraciflua* L.) (Sung et al. 1989), cambial tissue sucrolytic enzymes, such as sucrose synthase, and glycolytic enzymes, such as pyrophosphate-dependent phosphofmctokinase, are sensitive to transplanting stress and seasons. However, it is not known whether these sucrolytic and glycolytic enzymes can also be used to determine the degree of stress caused by silvicultural treatments such as harvesting and subsoiling. If reliable and sensitive indicators of physiological status could be developed, then an important tool would be available for evaluation of certain forest management regimes.

The objectives of this study were to: (1) investigate the effects of thinning and subsoiling on woody roots of Jeffrey pine; (2) determine the activities of sucrolytic and glycolytic enzymes, such as sucrose synthase (SS), pyrophosphate dependent phosphofrnctokinase (PPi-PFK), and ATP-dependent phosphofrnctokinase (ATP-PFK), and their possible value in assessing tree responses to thinning and subsoiling; and (3) determine the effects of thinning and subsoiling on live soil fungal biomass.

Materials and methods

Site and treatment description

The two study sites, Poison and Dry Flat, are in the Milford Ranger District, Plumas National Forest, in northern California. The sites, located at latitutde 40°07′30″ N and longitude 120°30′00″ W, support stands of 88- to 114-year-old Jeffrey pine trees. The Poison site has volcanically derived soil belonging to the Sattley complex (ultic Argixeroll), and is characterized by a loam surface layer over a clay loam subsoil. The Dry Flat site has a granitic soil belonging to the Toem-Cagwin complex (dystric Xeropsamment), characterized by loamy

sand surface texture and a coarse sandy loam or coarse loamy sand subsoil. Both sites are at approximately 2,100 m elevation.

Within each site, 12 contiguous l.O-ha plots were established consisting of four randomly assigned treatments replicated three times. The four treatments were: (1) thinning and then subsoiling the entire plot to a depth of 50 cm, (2) thinning plots without subsoiling, (3) thinning and then subsoiling skid trails only, and (4) no thinning and no subsoiling (control plot). Thinning removed approximately 50% of the basal area of each plot, leaving residuals of 12.0 $m^2 ha^{-1}$ and 23.5 m^2 ha-' in the thinned plots at the Dry Flat and Poison sites, respectively. Thinning followed by subsoiling was carried out in August 1993. For the treatment in which the entire plot was subsoiled, at least 80% of the plot area was traversed by the subsoiling implement, based on visual estimates of disturbed soil surface. For the purposes of this study, only treatments 1, 2 and 4 were subjected to further observation and analysis.

During June 1995, trenches were dug perpendicular to the radial axes of two selected dominant or co-dominant trees from each plot at the Poison and Dry Flat sites. Orientation of the trench face was determined by random selection of quadrants defined by cardinal compass directions. The trenches were 2.2 $\pm$ 0.3 m long, 1.2 $\pm$ 0.2 m deep, and located 1 .O to 1.2 m from the root collar.

Lateral roots were evaluated by means of an acetate template that divided the face of the trench into five 20 cm long x 1 m deep rectangular sections. Root segments projecting from the trench face were divided into three size classes: < 1 cm, l-5 cm, and > 5 cm in diameter. Roots in each diameter class were counted in three randomly chosen sections of the template. The amount of dieback of lateral roots was evaluated by cutting longitudinal strips from each root and observing color and cambial condition. Roots were counted as undergoing dieback if discoloration and cambial death was observed at least 10 cm from the cut end of the root. Root numbers were expressed as the mean for the three sections. Dieback was expressed as a percentage of the total roots counted for a given size class. A total of 18 tree root systems were evaluated at each site. Additional data on depth of soil, tree age, and diameter at breast height (DBH) were recorded for the sampled trees.

Data were analyzed by the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC) in a two-way analysis of variance. We assumed a completely random design. Comparisons of least squares (LS) means, where appropriate, were interpreted in a Bonferroni manner at overall a = 0.05. Data presented in the text are LS means and their associated standard errors unless noted otherwise.

Sampling and analyses of sucrolytic and glycolytic enzymes

For enzyme analysis, cambium tissue samples were taken at breast height by cutting and peeling away one or two 4 x 10 cm bark strips from the stems of two dominant or codominant trees per plot. Xylem-side cambial tissues were then scraped off with a razor blade and immediately frozen in liquid nitrogen. Harvest dates were July 26, 1994; October 12, 1994; June 7, 1995; August 23, 1995; and September 12, 1995. No samples were taken from the control plots during 1994. Thus, 18 samples each were taken from the Poison and Dry Flat sites on the 1995 sampling dates and 12 samples from each of the two sites on the 1994 dates. Different trees were selected for cambium sampling on each date.

All tissues were processed within 72 h of harvest. Procedures for protein extraction and enzyme assays followed those used for loblolly pine seedlings (Sung et al. 1993). Protein concentration of each extract was determined with Bradford reagents using bovine serum albumin as a standard. Enzyme specific activities were expressed per mg protein.

Fine root samples and ergosterol analysis

After brushing away the litter layer under the drip line of the trees selected for enzyme samples, fine root samples were obtained by using a small shovel. Samples were taken to a depth of approximately 20 cm. Approximately 15 g (dry weight basis, range 4.3-23.5 g) of fine roots and adhering soil, defined as rhizosphere, was obtained from beneath one and two trees per plot in August and September 1995, respectively, and stored at -80 "C.

Ergosterol extraction and detection procedures were modified from those described by Sung et al. (1995). Only roots with diameters < 3 mm were included in the sample. Roots and soil were oven dried at 70 °C for 1 h before extraction. The roots were then cut into small pieces and placed in a 250-ml beaker containing the soil shaken off the roots. The root and soil material was homogenized in 95% ethanol containing 2 mM dithiothreitol at a ratio of one part root + soil to 10 parts ethanol mixture (w/v) for 1 min with a Polytron. The homogenate was stirred for 2 h at room temperature and then centrifuged at 20 °C for 20 min at 15,300 g. The volume of the supematant was measured and an 8-ml aliquot was saponified in 1.5 ml of 60% (w/v) KOH in a heating block for 30 min at 100 "C. After cooling to room temperature, 1 ml deionized water and 5 ml n-hexane were added to each tube. The tubes were vortexed for 1 min and the upper hexane layer containing the ergosterol was saved. The lower aqueous phase was re-extracted twice with 5 ml hexane. The combined hexane extract was dried at 40 °C in a heating block in a gentle stream of N_2 gas. After cooling, 5 ml methanol was added to the tubes which were then incubated at 40 °C for 10 min to dissolve the residue. The methanol extract was passed through a 0.45 micron nylon syringe filter before HPLC analysis.

A Dionex 4500 ion chromatographic HPLC with a Zorbax reverse phase C-18 column (4.6 mm x 12.5 cm) was used with a 25 µl sample loop and 100% methanol as the eluent. The UV detector was set at 282 nm and the retention time for ergosterol was 4.55 min. The ergosterol peak was verified by spiking the sample extract with standard ergosterol. Ergosterol solutions with concentrations in the range of 1 to 30 $\mu g\ ml^{-1}$ were used as external standards. Standardization was repeated every 12 samples. Ergosterol data are expressed as mean concentrations, $\mu g\ g_{dw}^{-1}$ of rhizosphere material (dry weight basis).

Statistical analysis of the ergosterol data followed a split plot design with site and treatment as main effects and month as subplots. Data were subjected to ANOVA using the GLM procedure of SAS. Where appropriate, multiple comparison tests of least square (LS) means were interpreted in a Bonferroni manner, with an overall $\alpha = 0.05$. Data values presented are LS means followed by their standard error.

Results

Trenches dug in August 1994 revealed that, in the subsoiled plots, dieback was extensive (25–30 cm) in roots < 1 cm in diameter. These roots had dried resin encrusted on the root ends which were assumed to have been cut by the subsoiling implement. Very few adventitious roots were observed growing near tips of severed roots that were still alive. In the 1995 excavations, significant treatment effects were found for percentage of roots exhibiting dieback (df = 2, SS = 905.1, MSE = 91.3, $P = 0.03$), but there were no differences in root dieback due to site (df = 1, SS = 37.4, $P = 0.53$). The site by treatment interaction was also non-significant (df = 2, SS = 232.4, $P = 0.32$). The percentage of roots < 1 cm that exhibited dieback was significantly greater ($\alpha = 0.05$) in the subsoiled plots (27.6 ± 4.4%) than in the undisturbed control plots (9.1 ± 3.9%). Dieback in the thinned only plots was not significantly different from that in subsoiled or control plots (17.7 ± 3.9%).

The total number of roots < 1 cm in diameter in the sampling quadrangles differed by site. The Poison site had a mean of 53.2 ± 4.0 roots versus 34.8 ± 4.0 for the Dry Flat site (df = 1, SS = 1521.7, MSE = 140.4, $P = 0.006$). No treatment differences or interaction between site and treatment were found for this variable (df = 2, SS = 286.4, $P = 0.39$, and df = 2, SS = 63 1 .0, $P = 0.14$, respectively), or for diameter at breast height (DBH), age at breast height, number of roots > 1 cm and depth of rooting zone in the excavated trees (analysis not presented).

Sucrose metabolizing enzymes

No relationship between treatments, sites, or tree DBH was found for stem cambial sucrose synthase (SS) activity, although trees at the Dry Flat site maintained slightly higher SS activity in August and September than trees at the Poison site (Table 1, Figure 1). Activities of PPi-PFK and ATP-PFK were not affected by treatment or site. The percentage of sampled trees with a total PFK/SS ratio less than or equal to 10/1 tended to be higher in August and September at the Dry Flat site than at the Poison site (Table 1). Throughout the sampling period, the percentage of PPi-PFK in the total PFK pool was never greater than 50%, i.e., ATP-PFK activity was always greater than PPi-PFK activity (Table 1).

Strong seasonal relationships were found for SS and PPi-PFK activities (Figures 1 and 2). The relationship between these two enzymes tended to be strongest in July and August, with r^2 equal to 0.70 and 0.61, respectively. There was little or no SS activity during June and October (Figure 1). Mean activity of PPi-PFK decreased 4-fold in these months compared to values in July (Figure 2). Activity of ATP-PFK exhibited no seasonal pattern, and remained between 140 and 210 nmol mg^{-1} protein min^{-1} during the sampling period.

Fine root samples and ergosterol analysis

Significant differences in ergosterol concentration (expressed as $\mu g\ g_{dw}^{-1}$ of rhizosphere material) were found between sites, treatments and months of sampling (df = 1, SS = 305.8, MSE = 113.0, $P = 0.013$; df = 2, SS = 416.2, MSE = 113.0, $P = 0.02$; df = 1, SS = 596.3, MSE = 87.8, $P = 0.02$, respectively). Interactions between combinations of these factors were non-significant ($P > 0.05$). In terms of the main effects, the Dry Flat site had an ergosterol concentration of 16.4 ± 1.4 versus 10.6 ± 1.4 $\mu g\ g_{dw}^{-1}$ for the Poison site. Also, the August samples contained higher concentrations of ergosterol than the September samples (17.6 ± 2.2 versus 9.4 ± 2.2 $\mu g\ g_{dw}^{-1}$). Overall, the subsoiled plots had significantly higher ($\alpha = 0.05$) ergosterol concentrations (18.3 ± 1.7 $\mu g\ g_{dw}^{-1}$) than the thinned only (11.1 ± 1.7 $\mu g\ g_{dw}^{-1}$) and control plots (11.1 ± 1.7 $\mu g\ g_{dw}^{-1}$).

The August and September mean total sample dry weights (± standard error) of rhizosphere material containing fine roots combined over all treatments and sites were similar (9.4 ± 5.2 g and 14.7 ± 4.7 g, respectively). This was also true for the August and September fine root dry weights combined over all treatments and sites (1.17 ± 0.67 g and 0.96 ± 0.28 g, respectively). Neither the August nor September samples exhibited significant differences ($\alpha = 0.05$) in total sample dry weights due to site, treatment, or interaction. Differences due to site were found in fine root dry weights in August (df = 1, SS = 2.1, MSE = 0.44, $P = 0.05$), and due to site and treatment for the September samples (df = 1, SS = 0.72, MSE = 0.08, $P = 0.001$; df = 2, SS = 0.67, MSE = 0.08, $P = 0.04$, respectively). For the August samples, the Dry Flat site had a mean of 1.6 ± 0.24 g_{dw}^{-1} of fine roots versus 0.82 ± 0.24 g_{dw}^{-1} for the Poison site. September mean dry weights of fine roots were 1.16 ± 0.09 g for the Dry Flat site and 0.77 ± 0.09 g for the Poison site. The subsoiled plots had the smallest dry weight of fine roots sampled, 0.71 ± 0.11 g versus 1.02 ± 0.11 g and 1.175 ± 0.11 g for the control and thinned only plots, respectively.

Discussion

Root dieback

Subsoiling resulted in a significant amount of root damage as expressed by percentage of root dieback. Root damage and disturbance are important factors in attracting root-feeding bark beetles that can serve as a vector for black-stain root disease in this region (Otrosina and Ferrell 1995). It is possible that some roots that died as a result of the site treatments decomposed and were sloughed off during the 1995 excavation. Thus, the sampling procedure and time may have contributed to an underestimate of root dieback. Although not statistically significant, the amount of root dieback in the thinned only plots was intermediate between that of the subsoiled and control plots.

Although the proportion of lateral root dieback observed in the trench faces did not differ between sites, the total number of lateral roots < 1 cm in diameter was greater at the Poison

Table 1. Monthly trends over the growing season for percentage of Jeffrey pine trees on the Poison and Dry Flat sites with a PFK/SS ratio ≤ 10/1 and percentage of PPi-PFK in the total PFK activity pool.

Month	Poison site		Dry Flat site	
	PFK/SS ≤ 10/1 (%)[1]	PPi-PFK/PFK (%)[2]	PFK/SS ≤ 10/1 (%)	PPi-PFK/PFK (%)
June	0	21.2 ± 7.2	0	25.2 ± 6.4
July	100.0	48.4 f4.5	100.0	46.5 ± 6.7
August	38.9	44.6 ± 10.2	83.3	43.4 ± 8.7
September	16.7	6.8 ± 9.1	27.8	42.8k7.8
October	0	18.0 ± 6.5	0	21.7 ± 5.8

[1] Percentage of trees sampled with a ratio of PFK (sum of PPi- and ATP-dependentphosphofructokinase specific activity) to SS (sucrose synthase) specific activity less than or equal to 10/1 in stem cambial tissues.

[2] Percentage of PPi-dependent phosphofructokinase in the total PFK activity pool of stem cambial tissues expressing their maximum potential for phosphorylating fructose 6-phosphate via PFK. Data are expressed as means ± SD.

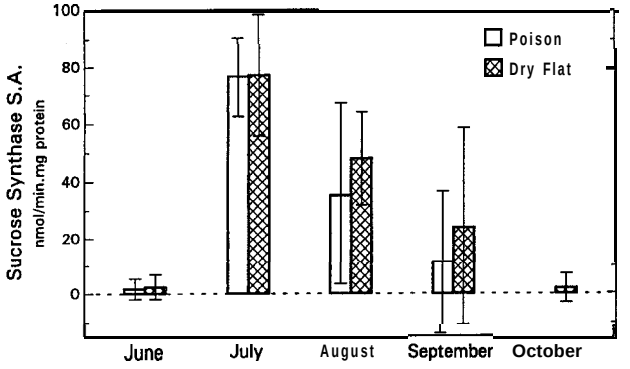

Figure 1. Seasonal patterns of stem cambial tissue sucrose synthase specific activity (S.A.) in Jeffrey pine trees from the Poison and Dry Flat sites. Means within each site were computed over the three site treatments. Bars represent the standard deviation.

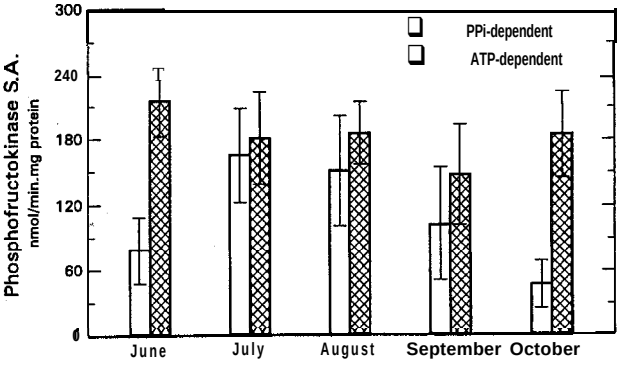

Figure 2. Means of seasonal PPi- and ATP-dependendent phosphofructokinase specific activities (S.A.) in stem cambial tissues of Jeffrey pine trees. Means were computed over the two sites and three treatments in this study. Tissues used for the sucrose synthase assay in Figure 1 were also used for the phosphofructokinase assay. Bars represent the standard deviation.

site than at the Dry Flat site. This may be associated with the higher pre-thinning stand density of about 46 m^2 basal area per ha on the Poison site. However, competition from increased stand density reportedly resulted in reduced extent and density of Douglas-fir root systems (McMinn 1963). The rooting zone depth was similar for both sites (O-90 cm) and is consistent with other root zone data reported for Jeffrey pine (Stark *1973).*

Sucrolytic and glycolytic enzymes

The activities of SS, PPi-PFK and ATP-PFK did not respond to site treatment, although the utility of measuring SS activity to assess stresses such as transplanting shock in loblolly pine seedlings has been demonstrated (Sung et al. 1993). We observed a strong seasonal relationship for SS activity in Jeffrey pine. In loblolly pine, SS activity is related to source-sink relationships in carbon metabolism and is also strongly correlated with growing season conditions (Sung et al. 1993). The patterns of SS activity in Jeffrey pine on these sites suggest a narrow window available for growth compared to that of loblolly pine (Sung et al. 1993).

Theoretically, the ratio between PFK (sum of PPi- and ATP-PFK) and sucrolytic activity (sum of SS and acid and neutral invertases) is 2/1. Because acid invertase and neutral invertase only play a small role in sucrolysis in loblolly pine (Sung et al. 1993), the ratio between PFK and sucrolytic activity can be replaced by the ratio between PFK and SS. In loblolly pine seedlings, there is an almost 2/1 PFK/SS ratio during the active growing months, and the ratio increases to 12/1 in quiescent months or during stress periods because SS decreases more than PPi-PFK, whereas ATP-PFK remains constant (Sung et al. 1993).

Although stem cambial SS, PPi-PFK and ATP-PFK activities in Jeffrey pine follow seasonal patterns similar to those of loblolly pine seedlings, the lowest ratio of PFK/SS for Jeffrey pine was 3.511 during July and the averages for July and August were 4.5/1 and 8/1, respectively. Nevertheless, the PFK/SS ratio in Jeffrey pine stems increased many fold during the slow-growing months, as it does in loblolly pine. A PFK/SS ratio of 10/1 was arbitrarily set (Table 1) in order to represent site effects on Jeffrey pine sucrose metabolism and

growth. At both the Poison and Dry Flat sites, the seasonal trends relating the proportion of sampled trees with PFK/SS ratios greater than 10/1 justified the use of a 10/1 PFK/SS ratio for determining tree growth activity. In August, there were twice as many trees on the Dry Flat site with active PFK/SS ratios (i.e., less than 10/1) than on the Poison site. Similarly, the Dry Flat site seemed to support greater and longer growth activity for Jeffrey pine than the Poison site (Table 1).

of fine root samples

Because ergosterol is present only in fungal cell membranes, its analysis provides a measure of living fungal biomass. The technique has proved useful for evaluating seasonal variations in soil, rhizosphere, and mycorrhizal fungal biomass in loblolly pine (Marx et al. 1995, Sung et al. 1995). The rhizosphere samples obtained from the subsoiled plots had higher ergosterol concentrations than those in the other treatments, suggesting that soil displacement and root severing caused by the subsoiling implement provided additional substrate for fungal decomposers. Alternatively, subsoiling may have increased ectomycorrhizal development by stimulating fine root production (Kozlowslci et al. 1991). Some studies indicate that fine root production and associated symbiotic fungal biomass may account for two-thirds of the annual biomass production in some forests (Marshall and Waring 1985). The ergosterol concentrations of loblolly pine rhizosphere samples reported in this study are in the range of those reported by Sung et al. (1995) and Marx et al. (1995).

Although we did not measure soil water content during the August or September sampling, soil was probably drier in September than in August (Stark 1973) and this may account for the higher ergosterol concentrations in August than in September.

We found no significant differences in total sample dry weights of rhizosphere soil containing fine roots obtained from the various treatments; however, recent studies have shown that ratios of ergosterol concentration found in mycorrhizal fine roots to that in soil approach 15/1 (Sung et al. 1995). Thus, mycorrhizal tine roots may contribute little toward total sample weight, but can dramatically increase total ergosterol. Significantly more fine root biomass was present in the samples from the Dry Flat site compared with those from the Poison site. Samples from subsoiled plots overall contained significantly fewer fine roots, yet had the highest ergosterol concentrations compared with samples from other plots. This may indicate increased mycorrhizal fungal activity, increased saprophytic fungal activity, or increased activities of both fungal groups. We conclude that the ergosterol analysis technique needs refining to include fixed volume sampling (Zaruoch et al. 1993), using root ingress cores and partitioning of soil and root components.

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