The effects of thinning and soil disturbance on enzyme activities under pitch pine soil in New Jersey Pinelands

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A R T I C L E   I N F O

Article history:
Received 30 January 2012
Received in revised form 28 June 2012
Accepted 1 July 2012

Keywords:
Tree thinning
Soil disturbance
Soil enzyme activities
Active C
Microbial biomass

A B S T R A C T

Disturbance is an important factor in changing ecological processes. Forested ecosystems undergo natural disturbances of fire and windthrow and disturbances involved in management (thinning, harvesting, control burning). Using the New Jersey pine barrens as model system for eastern US mixed forests, we have observed that under conditions of repeated physical soil disruption or control burning, the pine barrens forest understory can change from ericaceous to graminoid species dominance through suppression of the ericaceous plants. In order to predict changes in soil properties and assess the potential to maintain this alternate herbaceous layer community, replicate manipulation plots were established the Franklin Parker Preserve in the New Jersey pine barrens. The manipulations consisted of: intact forest controls (CONT), canopy tree thinning (THIN) and canopy tree thinning plus ericaceous stem removal and soil tilling (THIN & PLOW). The manipulations were designed to increase sunlight penetration to the forest floor and to encourage graminoid plant species establishment. Soil active carbon, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), six hydrolytic enzyme activities and two oxidative enzyme activities under three different treatments were measured. In the 0–10 cm soil depth, we observed that protease and arylsulfatase activity showed a significant decrease in the THIN and THIN & PLOW treatments compared to CONT, the activities of cellulase and phenol oxidase were significantly lower in THIN than CONT and THIN & PLOW, whereas the trend of peroxidase activity was opposite. There were no significant differences between treatments for glucosaminase, glucosidase and acid phosphatase activities. For the 10–20 cm soil depth only acid phosphatase and phenol oxidase activity significantly differed between treatments. We found that all enzyme activities except for peroxidase were significantly lower in the 10–20 cm soil horizon than in the 0–10 cm; peroxidase activity was significantly higher in the lower soil horizon. We demonstrated the activities of cellulase, phenoloxidase, arylsulfatase and protease were significantly and positively related to active C, but peroxidase activity was negatively correlated. Less expected is that only phenol oxidase was related to soil MBC, whereas the correlations between peroxidase, phenol oxidase, arylsulfatase, protease activities and soil MBN were obvious. By understanding the relationships between microbial activity (enzyme production) and disturbance, we can determine the beneficial effects of many management practices on the potential nutrient availability for subsequent tree growth, since these enzymes are key to nutrient mineralization and carbon dynamics in soil.

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1. Introduction

Although the New Jersey pine barrens is protected as a unique natural forest in the center of America’s most populous region (Forman and Boerner, 1981; Good and Good, 1984; Motzkin and Foster, 2002; Russell, 1994), the forest is representative of a series of similar pine dominated mixed forests on nutrient poor sandy soils that exist along the eastern seaboard of the USA. The forest has similar characteristics to coniferous forests on sandy soils in other parts of the world. Ecosystem restoration and sustainable forest management are important research issues under the Pinelands Comprehensive Management plan (NJ Pinelands Commission, 1980), an agency created to protect this region. A renewed emphasis on commercial timber extraction from these forests is currently a controversial issue among conservationist, so forest manipulations to increase forest productivity or alter structural properties of forest stands and promote ecosystem functions such as vegetative diversity are an important part of current debates.
Forest management in the NJ pine barrens includes thinning and harvesting along with low intensity prescribed burning to reduce fuel load (Skowronski et al., 2007). In both cases repeated disturbance has been shown to reduce the ericaceous shrub community dominance and conversion to a graminoid understory. Conventional thinning can accelerate understory regeneration and increase shrub layer density (Bailey and Tappeiner, 1998) by enhancing soil nutrient resource availability (sensu Latham et al., 1996; Johnson and Wedin, 1997), which may restrict tree growth. Under conditions of physical soil disturbance or repeated disturbance by fire, such as annual control burning, or by, the pine barrens ecosystem changes to a graminoid dominated ground vegetation with suppression of eri- caceous plants. Renewal of tree thinning and forest harvesting practices in this forest as a management technique is becoming more important, questions are being asked about the long term benefits or disbenefits of these practices.

Tree thinning changes forest microclimate conditions (Ma et al., 2010), which both directly and indirectly influences soil properties. Soil nutrient availability is the dominant driver in long-term site productivity and maintenance of forest ecosystem structure and function (Jokela et al., 2004). Thinning intensity and residue removal often result in changes in nutrient cycling by reducing nutrient pools resulting from removal of large quantities of organic matter (Blanco et al., 2008; Finkral and Evans, 2008; Gautam et al., 2003; Jacobsona et al., 2000; Valinger et al., 2000; Walmsley et al., 2009). Smith et al. (2000) reported that whole-tree harvest plus forest floor removal treatment reduced nutrient availability to trees, whereas slash addition may improve their nutrient status. Thinning reduced nutrient returns via litterfall, however, significant differences in nutrient availability were seldom created with thinning intensities of less than 30% of standing basal area (Smith et al., 2000). The reason may be that the remaining vegetation benefits from reduced competition for nutrients in the mineral soil and remaining forest litter layer (Blanco et al., 2008). However there is large site to site variability (Vesterald et al., 1995), but the negative effects of residue removal have been shown to be greater on nutrient poor sites than on fertile sites in Finnish forests (Smolander et al., 2010).

We elected to investigate the impact of thinning and soil disturbance treatments on soil enzymes, as soil processes determine forest ecosystem functions (van Bruggen and Semenov, 2000; Schoenholtz et al., 2000; Knoepf et al., 2000). Soil enzymes participate in almost every transformation process of litter decomposition and play a central role in maintaining forest soil fertility by releasing mineral (plant available) nutrients from complex organic resources (Baldrian and Stursôvá, 2010). Additionally the mycorrhizal symbionts of a number of tree and ericoid plant species produce protease and acid phosphatase enzymes, enabling them to access complex forms of N and P (Read, 1991), which are likely to be disrupted by mechanical soil disturbance. Changes in nutrient availability affect tree performance, especially in oligotrophic ecosystems. A wide range of enzymes have been found in soil, which are liable to change due to soil management techniques (Mosca et al., 2007; Maassen et al., 2006), Boerner et al. (2006) and Gial and Boerner (2007) reported some soil enzyme activities in post-thinning treatment were greater than in pre-thinning, however, opposite trends were shown by Boerner et al. (2008) and Hassett and Zak (2005). This inconsistency of results probably resulted from many different factors such as soil type, sampling time after thinning and research methods. Limited and controversial evidence exists as neither short- or long-term impacts of harvest practices on soil enzymes have been systematically studied.

Few enzyme studies have been conducted in the NJ pine barrens (Koutrev et al., 2002; Lucas et al., 2007; Sedia and Ehrenfeld, 2006). We chose eight soil enzyme assays based on their function in organic matter decomposition and nutrient cycling and their sensitivity to soil management (Bandick and Dick, 1999). Cellulase and β-glucosidase were chosen because they hydrolyze cellulose, the most prominent organic compound in forest litter, particularly as the final product of β-glucosidase is glucose which is an essential carbon source for microbial growth. β-Glucosaminase which is involved in chitin degradation and carbon and nitrogen cycling by hydrolyses of N-acetyl-β-D-glucosamine residues. Protease is related to organic nitrogen mineralization, and is also produced by ecto- and ericaceous mycorrhizae (Smith and Read, 1997). Acid phosphatases dominate in acid soil and hydrolyze organic phosphate esters and are also produced by ectomycorrhizae. Aryl-sulfatase is involved with the mineralization of aromatic sulfated esters to phenols and sulfate, releasing plant available SO4–-. These six soil enzymes function in carbon, nitrogen, phosphorus and sulfate cycling, especially as incorporation of organic matter into soil and mechanical disturbance of roots has been shown to influence organic matter decomposition rates (Sollins et al., 1996; Conn and Dighton, 2000; Salinas-Garcia et al., 2002). In order to assess the potential of establishing an alternate state of graminoid understory vegetation, we established a forest manipulation experiment of tree thinning, shrub removal and soil disturbance to encourage grasses.

2. Materials and methods

2.1. Site description

The study site was located at the Franklin Parker Preserve which encompasses almost 14 square miles in Woodland Township, Burlington County, in the New Jersey pine barrens. Mean monthly temperatures are 0.3 ºC and 23.8 ºC in January and June, respectively (1930–2004; State Climatologist of NJ, USA). Mean annual precipitation varies between 1123 and 1820 mm. The mixed conifer–hardwood forests consist of pitch pine (Pinus rigida), short-leaf pine (Pinus echinata) and black oak (Quercus velutina) with an understory of mostly ericaceous shrubs of huckleberry (Gaylussacia spp.), blueberry (Vaccinium spp.), and scrub oak (Quercus ilicifolia) (Robichaud-Collins and Anderson, 1994; Jordan et al., 2003; Landis et al., 2005; Matlack et al., 1993). The topography lies on a coastal plain with gentle undulations with a mean attitude of 40 m. The soils have developed from the unconsolidated sandy geologic deposits and are classified as Entisols (Markley, 1971) with low levels of soil nutrients, low water holding capacity and a low pH.

2.2. Field designing and sampling

In 2009, we selected a site with overstory dominated by pitch pine and understory dominated by ericaceous shrubs in the Franklin Parker Preserve, which is representative of large areas of upland pitch pine dominated ecosystems within the NJ pine
barrens and many other similar ecosystems along the eastern seaboard of the USA. Nine 20 m × 20 m plots, three per row, separated from each other by at least 2 m were laid out in an orthogonal design containing 3 treatments, randomly assigned per row. This resulted in 3 replicates per treatment. Treatments consisted of (1) CONT: control intact forest; (2) THIN: random trees removal, resulting in reduced of canopy cover from ca. 60% to ca. 35%. Control plots had 64.2% tree canopy cover, which was reduced in thinning treatments to between 29.7% and 30.6%. The harvested tree material (bole and brash) were removed from the plot; (3) THIN & PLOW: after thinning, all understory shrubs were clear cut by use of brush cutter and the plots were roto-tilled to a depth of approximately 15 cm. All plots were established within a forest area of similar canopy and ground cover characteristics to minimize within site heterogeneity, as the treatment effects were of primary concern, not a comparison between forests. The disturbances were designed to investigate the impact of selective harvesting operations with or without mechanical damage to the soil. Table 1 gives an outline of the stand characteristics following manipulation. Stem counts per unit area were converted to tree stems per hectare and ground cover by these stems calculated from allometric equations from stem density and DBH. Canopy cover was determined by regression of 120 points from 6 mid days transect lines against an open canopy reference, by a Photosynthetically Active Radiation (PAR) light wand, on cloudless days the summer after stand thinning.

In August 2010 we collected nine randomly placed replicate soil cores (5 cm diameter) to 20 cm depth from each plot. The cores were divided into upper (litter, humus and mixed humic/mineral horizon 0–10 cm) and lower (mineral sand 10–20 cm) depths and each sample placed in a plastic bag and transported to the laboratory. The cores were composited to yield three replicate samples per plot.

Gravimetric water content by drying soil at 70 °C for 48 h, and soil bulk density were determined. Subsamples of soil were passed through a 2 mm mesh sieve, after removing leaf litter and roots and then stored at 4 °C and analyzed for soil enzyme within four weeks. Each enzyme was determined within one or two days during the four week period such that each enzyme could be compared between treatments. Protease was analyzed first, followed by glucosidase, glucosaminase, arylsulfatase and phosphatase, then phenol oxidase and peroxidase and lastly cellulose. Another set of subsamples were prepared for extractable inorganic nitrogen analysis and microbial biomass.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stem density (stems ha⁻¹)</th>
<th>Basal area (m² ha⁻¹)</th>
<th>Tree height (m)</th>
<th>Canopy cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONT</td>
<td>1025 ± 281.7</td>
<td>20.3 ± 3.34</td>
<td>10.6 ± 0.079</td>
<td>57.9 ± 2.32</td>
</tr>
<tr>
<td>THIN</td>
<td>741.7 ± 312.6</td>
<td>16.7 ± 4.90</td>
<td>11.1 ± 0.217</td>
<td>35.5 ± 3.00</td>
</tr>
<tr>
<td>THIN &amp; PLOW</td>
<td>783.3 ± 351.2</td>
<td>17.6 ± 5.99</td>
<td>11.1 ± 0.742</td>
<td>38.7 ± 5.82</td>
</tr>
</tbody>
</table>

(MBC) and microbial biomass nitrogen (MBN) content using a Shimadzu TOC-Vcsh, non-dispersive infrared gas analyzer and TNM1 chemiluminescence nitrogen monoxide analyzer (Kyoto, Japan), following high temperature combustion.

Six hydrolytic enzyme activities (cellulose, glucosidase, glucosaminase, protease, arylsulfatase and phosphatase) and two oxidative enzyme activities (peroxidase and phenol oxidase) were measured. The cellulase activity (EC 3.2.1.4) was estimated using the substrate of carboxymethylcellulose and expressed activity in mg glucose produced g⁻¹ soil 24 h⁻¹ (Deng and Tabatabai, 1994). Somogyi reagents consisting of 4 parts KNa tartarate:Na₂CO₃:Na₂SO₄:NaHCO₃ (1:2:12:1.3) and 1 part CuSO₄·5H₂O:Na₂SO₄ (1:9) and the Nelon reagent (25 gm ammonium molybdate 450 ml H₂O + 21 ml H₂SO₄ + 3 g Na₃HAsO₃ 7H₂O dissolved in 25 ml H₂O) were prepared according to the method of reducing-sugar assay (Alef and Nannipieri, 1995). The activities of 1.4-β-glucosidase (EC 3.2.1.21) β-glucosaminase (NAG) (EC 3.2.1.52), acid phosphatase (EC 3.1.3.2) and arylsulfate (EC 3.1.6.1) were determined by the concentration of p-nitrophenol with pNP-linked substrates and expressed as mmol pNP released g⁻¹ dry weight of soil h⁻¹ (Sinsabaugh et al., 1999; Verchot and Borelli, 2005). In brief, 1 or 2 g fresh soil was incubated with 2.5 ml of acetate buffer and 2.5 ml of their corresponding substrates at 25 °C for 2 or 4 h. At the end of incubation, 2 ml of the soil and substrate slurry filtrate were mixed with 0.2 ml NaOH to terminate color development. The absorbance of the supernatant was measured spectrophotometrically (Spectrojet 70, Bausch & Lomb) following diluting or directly at 410 nm. Activities of phenol oxidase (EC 1.10.3.2) and peroxidase (EC 1.11.1.7) were determined by the method of Sinsabaugh et al. (1999). In brief, 2 g fresh soil was mixed 3 ml l-dehydroxyphenylalanine (DOPA) substrate for phenol oxidase and 3 ml DOPA and 0.3 ml of hydrogen peroxide for peroxidase, and then add 3 ml of acetate buffer, incubated 1 h in 25 °C. The absorbance of supernatant was measured directly at 460 nm. Micromolar extinction coefficient for l-dehydroxyphenylalanine (DOPA) was calibrated using mushroom tyrosinase (SigmaT3824) (Allison and Vitousek, 2004). These enzyme activities were expressed as mmol DOPA converted g⁻¹ dry weight soil h⁻¹. Protease activity was determined by the method of Folin-Ciocalteu reagent (Alef and Nannipieri, 1995) and expressed as μg tyrosine equivalents per gram dry matter h⁻¹.

#### 2.4. Statistical analysis

For each parameter, the average value of the three replicates per plot was used in the ANOVA to avoid pseudoreplication. One-way ANOVA analyses were used to test the effects of treatment on soil physical–chemical properties and soil enzyme activities. According to the assumptions of ANOVA transformations were performed, where necessary, to resolve heteroscedasticity or non-normal data distributions. Tukey’s HSD test was used for post hoc means separation. Paired t-tests were used to compare the differences of soil enzyme activities at both the 0–10 cm and 10–20 cm soil depth. Using Pearson’s correlation test we further investigated relationships between soil physical–chemical property and each enzyme activity. All statistical tests were performed using SAS 9.1 (SAS, 2003–2004).
3. Results

Analysis of variance showed that there were no significant differences in bulk density ($F=1.77$, $P=0.248$; $F=1.93$, $P=0.225$), soil moisture ($F=0.88$, $P=0.462$; $F=2.25$, $P=0.787$), pH ($F=0.16$, $P=0.857$; $F=2.34$, $P=0.178$), extractable ammonium ($F=2.29$, $P=0.182$; $F=1.29$, $P=0.343$) or extractable nitrate+nitrite ($F=0.03$, $P=0.967$; $F=0.14$, $P=0.872$) between treatments at either the 0–10 or 10–20 cm soil depths, respectively (Table 2). Microbial biomass N was significantly lower in both THIN and THIN & PLOW in the 0–10 cm soil horizon (Fig. 1). A similar significant response was seen for microbial biomass C in the 10–20 cm soil horizon along with a significantly lower extractable N in the THIN & PLOW treatment (Fig. 1).

Differences in enzyme activity between treatments are shown in Table 3. With exception of peroxidase, all enzyme activities were significantly higher in the 0–10 cm soil horizon than in the 10–20 cm. Peroxidase activity was significantly higher in the lower soil horizon (196.6 ± 0.23 upper and 4.26 ± 0.19 lower).

Table 3 also shows that of the cellulose decomposing enzymes (cellulase, glucosidase and NAG) in the 0–10 cm soil depth, there was little response in enzyme activity to treatment. A slight and significant decline in cellulase activity was seen in the THIN treatment, compared to the rest. There were differences in the activities of phenol degrading enzymes between treatments with peroxidase activity being significantly higher in the THIN and, conversely, phenol oxidase activity lower in the THIN treatment than the others. Protease and arylsulfatase showed a significant decrease in activity with both the thinning and THIN & PLOW, although there were no significant effects of treatment on acid phosphatase activity.

There were fewer differences in enzyme activity between treatments in the 10–20 cm soil depth than the surface soil. Phenol oxidase activity was reduced in the THIN treatment as was the case for the 0–10 cm soil horizon. There was also a trend of reduced acid phosphatase activity in the THIN & PLOW treatment.

Pearson correlations between soil characteristics and enzymes for the 0–10 cm soil horizon are shown in Table 4. Bulk density is negatively correlated to active carbon and microbial biomass N and C. Phenoloxidase, arylsulfatase, acid phosphatase and protease activities were all significantly negatively related to soil bulk density. Cellulase, phenoloxidase, arylsulfatase and protease enzyme activities were significantly and positively related to active C, but peroxidase enzyme activity was negatively related. Microbial biomass N was positively related to phenoloxidase, arylsulfatase and protease activities.

4. Discussion

Within a year of applying the forest manipulation, we saw little changes in soil bulk density, soil moisture and soil pH. In these sandy soils, rainfall events tend to readily compact soil and it appears that the mild soil disturbance imposed by the tilling process was insufficient to cause a change in bulk density. The capability of holding water for sandy soil is very low, so we were not surprised to see little difference in soil moisture taken at this one time, despite changes in evapotranspiration by reducing the trees density and increasing incident solar radiation (Hassett and Zak, 2005).

Significant short-term losses of soil organic C after thinning or harvesting have been reported (Carter et al., 2002; Gail and Boerner, 2007; Nave et al., 2010). Although little is known of the composition of soil active C as measured by KMnO$_4$ extraction, the method has been shown to be more sensitive to soil management practices than a measure of total soil C and more closely related to soil biological properties than the other measures of soil organic C (Mirskey et al., 2003). In this study there was a trend toward reduced soil active C in both the THIN and THIN & PLOW treatments levels relative to CONT. One year post treatment may not have been enough time for significant changes in soil C to have materialized. Plant litters in this ecosystem degrade slowly due to the presence of recalcitrant compounds such as lignin, cellulose, hemicelluloses, condensed tannins, lipids and phenolics (Jonsson et al., 2006). The plowing treatment incorporated plant residues and roots into the mineral soil, presumably the increase soil–residue contact and improved microclimate conditions, such as increased oxygen availability, accelerated decomposition rates of soil labile organic carbon (Salinas-Garcia et al., 2002; Sollins et al., 1996; Zibilske et al., 2002). This, together with possible reduced root exudates and reduced turnover of extraradical hyphae of mycorrhizae, could account for the reduced active C in the THIN and THIN & PLOW treatments compared to the CONT. Also the physical breakdown of detritus into smaller pieces by the mechanical action of plowing promotes the decomposition process. Previous work in a similar pine barrens upland site showed that leaf litter decomposition was significantly greater in leaf litter placed below-ground than on the soil surface (Conn and Dighton, 2000).

Enzymes produced by soil microbiota are freely available proteins and nitrogen sources for immobilization by microbes or plant roots. However, the determination of instantaneous quantification of enzymes between treatments within one experiment is a routine measure of microbial activity. The methods by which these assays are conducted may be improved by quality control and standardization of method (German et al., 2011), but it appears that for many of the enzymes assayed in our study (glucosidase, phosphatase, peroxidase, phenol oxidase) there is no differential loss in activity with storage at 4 °C compared to −20 °C for at least 21 days (DeForest, 2009). Thus we have compared an instantaneous measure of enzyme activity to compare between treatments within the same experiment.

The effects of thinning and plowing in our study did not reduce or increase enzyme activity consistently between the enzymes studied. This heterogeneity of enzyme response has been seen in other studies. However, each group of enzymes have different functions and not all resources they utilize are likely to change in the same way due to a single management treatment. For example, Boerner et al. (2006) observed that thinning significantly stimulated acid phosphatase, chitinase, and phenol oxidase activity during the ninth year post-treatment; the stimulation having gradually increased to a maximum by the fourth year post-treatment. In a study of fire, soil mechanical disturbance and a combination of both, Boerner et al. (2008) showed that there were different responses of soil enzymes to treatment and that these responses varied between forests and soil types. The authors found that among eight sites where fire and mechanical thinning treatments were performed, phenol oxidase activity was significantly affected by treatment only in three sites. Phenol oxidase activity was significantly reduced by fire in one, fire plus mechanical treatment in a second and by mechanical treatment alone in the third. In contrast, when the eight sites were pooled, chitinase activity was not significantly affected by treatment. Caldwell et al. (1999) found that annual-harvest treatments significantly reduced activities of β-glucosidase and phosphomonoesterase, with β-glucosidase activity declining more than phosphomonoesterase. Furthermore, reduction in enzyme activity was greater in the more acidic, highly weathered upper-terrace soil than in the more fertile lower-terrace soil. Hassett and Zak (2005) reported that aspen harvest intensity decreased microbial biomass and extracellular enzyme activity involved with litter decomposition. In our work, thinning decreased the activities of cellulase, phenol oxidase, protease and arylsulfatase, but increased peroxidase activity. There was no significant
Table 2
Soil physical and chemical properties of the experimental plots. Values are means ± SE, except pH which is percent difference of H+ concentration. n = 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil depth (cm)</th>
<th>Soil bulk density (g/cm³)</th>
<th>Soil moisture (%)</th>
<th>pH</th>
<th>Active carbon (mg kg⁻¹)</th>
<th>NH₄-N (mg kg⁻¹)</th>
<th>NO₂-N + NO₃-N (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>0–10</td>
<td>0.61 ± 0.03</td>
<td>13.7 ± 1.7</td>
<td>4.16 ± 5%</td>
<td>659.8 ± 28.23</td>
<td>0.812 ± 0.142</td>
<td>0.354 ± 0.019</td>
</tr>
<tr>
<td>THIN</td>
<td>0.74 ± 0.03</td>
<td>11.0 ± 1.56</td>
<td>4.29 ± 3%</td>
<td>552.9 ± 36.26</td>
<td>0.078 ± 0.036</td>
<td>0.342 ± 0.015</td>
<td></td>
</tr>
<tr>
<td>THIN &amp; PLOW</td>
<td>0.73 ± 0.07</td>
<td>11.7 ± 1.20</td>
<td>4.29 ± 3%</td>
<td>210.8 ± 6.64</td>
<td>0.052 ± 0.042</td>
<td>0.297 ± 0.011</td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.36 ± 0.02</td>
<td>4.1 ± 0.14</td>
<td>4.79 ± 2%</td>
<td>154.0 ± 9.32</td>
<td>0.568 ± 0.040</td>
<td>0.297 ± 0.019</td>
<td></td>
</tr>
<tr>
<td>THIN</td>
<td>1.29 ± 0.02</td>
<td>4.0 ± 0.60</td>
<td>4.87 ± 1%</td>
<td>196.6 ± 27.43</td>
<td>0.775 ± 0.162</td>
<td>0.285 ± 0.025</td>
<td></td>
</tr>
<tr>
<td>THIN &amp; PLOW</td>
<td>1.27 ± 0.05</td>
<td>4.4 ± 0.46</td>
<td>4.67 ± 7%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Mean (±SE) soil enzyme activity of the experimental treatments (n = 9). Values sharing the same superscript letter between treatments within the same soil depth for each enzyme are not statistically significantly different (Tukey’s Honestly Significant Difference test).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil depth</th>
<th>CON</th>
<th>THIN</th>
<th>THIN &amp; PLOW</th>
<th>CON</th>
<th>THIN</th>
<th>THIN &amp; PLOW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–10 cm</td>
<td>2.68 ± 0.16</td>
<td>1.91 ± 0.19</td>
<td>2.32 ± 0.12</td>
<td>0.09 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Cellulase</td>
<td>2.68 ± 0.16</td>
<td>1.91 ± 0.19</td>
<td>2.32 ± 0.12</td>
<td>0.09 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Glucosidase</td>
<td>187.33 ± 34.02</td>
<td>163.99 ± 12.03</td>
<td>170.18 ± 2.69</td>
<td>26.45 ± 1.22</td>
<td>28.89 ± 4.63</td>
<td>26.97 ± 2.60</td>
<td></td>
</tr>
<tr>
<td>NAG</td>
<td>279.45 ± 46.16</td>
<td>174.00 ± 34.72</td>
<td>223.90 ± 10.09</td>
<td>33.95 ± 8.07</td>
<td>22.39 ± 5.67</td>
<td>30.11 ± 5.71</td>
<td></td>
</tr>
<tr>
<td>Peroxidase</td>
<td>1.11 ± 0.12</td>
<td>2.97 ± 0.56</td>
<td>1.80 ± 0.35</td>
<td>4.29 ± 0.46</td>
<td>4.68 ± 0.39</td>
<td>3.82 ± 0.37</td>
<td></td>
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<tr>
<td>Phenol oxidase</td>
<td>4.48 ± 1.84</td>
<td>2.84 ± 0.10</td>
<td>3.82 ± 0.34</td>
<td>2.71 ± 0.24</td>
<td>1.42 ± 0.18</td>
<td>1.95 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>52.29 ± 7.28</td>
<td>29.03 ± 4.01</td>
<td>30.29 ± 1.57</td>
<td>8.24 ± 0.90</td>
<td>8.20 ± 0.67</td>
<td>7.71 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>Arylsulfatase</td>
<td>21.80 ± 1.44</td>
<td>11.70 ± 2.25</td>
<td>17.80 ± 1.98</td>
<td>5.95 ± 1.93</td>
<td>4.00 ± 0.34</td>
<td>7.01 ± 0.69</td>
<td></td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>1.27 ± 0.10</td>
<td>1.22 ± 0.09</td>
<td>1.05 ± 0.11</td>
<td>0.43 ± 0.02</td>
<td>0.50 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Mean microbial biomass N (MBN) in the 0–10 cm soil horizon (left) and C (MBC) in the 10–20 cm soil horizon (right) from the forest treatments CONT (C), THIN (T) and THIN & PLOW (TP). Bars are SE of means.

Table 4
Pearson Correlation Matrix for soil enzymes and nutrients at 0–10 cm depth. Bulk density g cm⁻³ (BD), active carbon (car); cellulose (cel), peroxidase (per), phenol oxidase (phen), NAG, arylsulfatase (ary), phosphatase (pho), protease (pro), glucosidase (glu), MBC mg cm⁻³ (MBC), MBN μg cm⁻³ (MBN). Note: No correlations were found for H+, NH₄-N mg kg⁻¹, or NO₂-N mg kg⁻¹.

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*  Sig. at α < 0.1.
** Sig. at α < 0.05.
*** Sig. at α < 0.01.
**** Sig. at α < 0.0001.

Impact of thinning on glucosidase, NAG and phosphatase activity. We also found reductions in MBN at 0–10 cm and MBC at 10–20 cm soil depths due to thinning. Thus, the response of enzymes to disturbance varies between sites and type and level of disturbance. In general, the lack of change of cellulose degrading enzymes between treatments likely reflects the fact that these oligotrophic systems are more nutrient than carbon limited. Slight changes in cellulose availability have little effect on enzymes that degrade it.
Activities of peroxidases, chitinase, cellulase and phosphatases have been found to be significantly higher in litter than humus and mineral soil horizon (Alarcón-Gutiérrez et al., 2009; Andersson et al., 2004), and a significant correlation between cellulose concentration and cellulase level has been reported (Pavel et al., 2004). Therefore soil organic carbon content is related to soil enzyme activities. Our work also demonstrated soil cellulase, phenol oxidase, arylsulfatase and protease enzyme activities to be positively related to soil organic carbon concentration (Table 4). Peroxidase activity was negatively related to soil active carbon and is likely to have been more closely related to the increased structural carbon entering the THIN and THIN & PLOW plots as root turnover from harvested trees; carbon that has not yet entered the active carbon pool. This result may be related to the method used for peroxidase activity as Johnsen and Jacobsen (2008) argued the phenol oxidase was denatured by reactive oxygen species in the presence of hydrogen peroxide when using L-DOPA oxidation as substrate. Peroxidase enzyme activity was significantly higher in THIN and THIN & PLOW plots at 0-10 cm soil depth. Since this enzyme is linked with wood decomposition it is probable that root turnover and small woody residues from the tree harvest stimulated enzyme production.

There were positive correlations between peroxidase, phenol oxidase, arylsulfatase, protease enzyme activities and soil MBN, however only phenol oxidase was related to soil MBC.

In the THIN treatment, reduction of protease and phosphatase enzymes might be related to the reduction in tree and ericoid root activity, where root death reduced the influence of ericoid- and ecto-mycorrhizal production of these enzymes (Read, 1991). Elsewhere, decreased belowground fine root biomass following thinning has been reported (Campbell et al., 2009; Ryu et al., 2009; Silver and Vogt, 1993; Tian et al., 2010). Several studies have shown that pitch pine roots associate with mycorrhizal fungi which, together with ericoid mycorrhizal roots, are able to excrete extracellular enzymes, such as proteases and acid phosphatase, involved in the direct mobilization of nutrients from organic substrates (Conn and Dighton, 2000; Courtey et al., 2005; Cumming, 1996; Trasar-Cepeda and Gil-Sotres, 1987). Protease enzyme activity was lower in both the THIN and THIN & PLOW treatments, possibly resulting from the reduced ericoid root activity in these treatments (Chalot and Brun, 1998). In our experiment, however, we did not see a change in acid phosphatase expression in the 0–10 cm soil horizon, but a significant decrease in THIN & PLOW in the 10–20 cm depth, possibly influenced by root mass loss due to physical disturbance. Phenol oxidase enzyme was the only other enzyme showing significant difference between treatments at both soil depths, with the same pattern of greater reduction in THIN than THIN & PLOW, compared to CONT.

Our results that show cellulase and phenol oxidase enzyme activities in THIN & PLOW treatment were greater than THIN treatment and equivalent to the CONT. It is possible that fractionation of organic matter and incorporation to greater depth could account for more organic matter microbial surface contacts, thus increased enzyme activity compared to the THIN treatment.

5. Conclusions

In the first year after thinning manipulation there were no significant differences at either the 0–10 or 10–20 cm soil depths for bulk density, soil moisture, pH, active carbon, NO3 and NH4; but the change of soil biological parameters were distinct. Microbial biomass N, cellulase, phenol oxidase, protease and arylsulfatase activities were significantly reduced in the upper soil horizon. Peroxidase activity, however, increased in thinned and disturbed treatments. Our result show the THIN & PLOW treatment increased the activities of cellulase and phenol oxidase, and decreased peroxidase activity; however, no differences were found for microbial biomass N and C, glucosidase, NAG, protease and acid phosphatase activities in the upper soil horizon with respect to the THIN treatment.

In this study changes in soil enzyme activities in soil resulting from tree thinning and soil disturbance were found one year post site establishment. We predict that greater changes in both soil chemistry and enzyme expression will occur over time. If graminoid plant species successfully establish in the THIN & PLOW plots, we predict that there will be significantly more changes in soil enzymes due to the changes in the nature of root chemistry entering the decomposition cycle and the development of a different microbial community in graminoid species rhizospheres. Although our study is limited to the NJ the pine barrens, our results may be applicable to other systems where plant community change has been achieved by management or disturbance. The results will help us to understand what changes are likely to occur wherever similar change takes place as a result of succession, disturbance, alien species invasions or climate change and that will have an effect on nutrient availability for plant growth. The activity of soil enzymes gives an indication of substrate utilization potential. Although the enzymes are substrate specific, we can group them into families of similar function. However, it is not really possible to expect them to all show similar trends in abundance with any one soil manipulation. For example, many enzymes are produced by rhizospheric microorganisms, so their activity must be related to changes in root abundance and health. Other microbial activity is related to soil processing and grazing by soil fauna. Hence the full interpretation of the impacts of soil management on enzyme activity has to be part of a holistic study of soil changes. We present here a snap-shot of initial changes of enzyme abundance upon which we can build with measures of other edaphic properties, over time. Our current aim is to present information about soil disturbance effects that are somewhat consistent with other publications.

Acknowledgements

We thank the New Jersey Conservation Foundation for letting us use their land for this experiment and USDA McIntire Stennis program for funding.

References


