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Restoration and Canopy Type Influence Soil Microflora in a Ponderosa Pine Forest

Sarah I. Boyle, Stephen C. Hart,* Jason P. Kaye, and Mark P. Waldrop

ABSTRACT

In ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) forests of the western USA, fire exclusion by Euro-American settlers facilitated pine invasion of grass openings, increased forest floor detritus, and shifted the disturbance regime toward stand-replacing fires. We evaluated the impacts of two replicated ecological restoration treatments involving tree thinning alone (thinning restoration) and a combination of tree thinning, forest floor reduction, and prescribed burning (composite restoration) on soil microbial activity, biomass, and function approximately 8 yr after initial treatments. Microbial-N levels in the two restoration treatments were not significantly different from the control during either the dry or wet periods of the growing season. Soil respiration measured in situ was significantly higher in the restoration treatments than in the control only during the dry period, while soil enzyme activities were generally higher in the composite restoration treatment than in the thinning restoration or control treatments during the wet period. Community-level physiological profiles suggested differences in the physiological capacities of bacteria and fungi in the composite restoration treatment compared with the other treatments. We also compared microbial characteristics under different canopy types to evaluate the impacts of pine invasion and establishment in grass openings on soil microorganisms. Soil respiration rates (dry period only) and enzyme activities (wet period only) were higher in grass openings than under presettlement trees, with intermediate values found under postsettlement pines that have invaded grass areas. Taken together, our results suggest that restoration treatments have long-term impacts on the soil microflora in these forests.

Before Euro-American settlement of the southwestern USA in the late 1800s, ponderosa pine forests were characterized by widely spaced trees, large grass openings, and low-intensity surface fires that burned every 2 to 5 yr (Covington et al., 1997). Euro-American settlement disrupted fuel continuity through the establishment of roads and trails, as well as the reduction of herbaceous fuels by domestic livestock grazing (Covington et al., 1997; Neary et al., 1999). Subsequent favorable weather conditions and active fire suppression activity allowed for exceptionally high pine seedling establishment. The resulting high tree densities have heightened competition for moisture, nutrients, and light among trees, decreasing individual tree growth. Other consequences include the loss of herbaceous and shrub species in the understory and reductions in habitat for some wildlife species. The risk of stand-replacing fire has also greatly increased due to heavy fuel loading and high tree density, threatening local plant and animal life (Covington et al., 1997; Kaye and Hart, 1998a; Neary et al., 1999).

Ecological restoration, which involves the removal of trees that established in grass areas after Euro-American settlement and the reintroduction of low intensity fires, is being undertaken throughout the western USA to restore the structure and function of these forests to within their natural range of variability (Kaye et al., 2005). Although much research has been conducted on the macroscale impacts of ecological restoration in this region (e.g., Stone et al., 1999; Bailey and Covington, 2002), little emphasis has been given to possible impacts on the soil microflora. Soil microorganisms play fundamental roles in many ecosystem processes, including decomposition and nutrient cycling, and affect many important soil hydrological and chemical properties (Gallardo and Schlesinger, 1994; Hart et al., 2005). Hence, changes in the soil microbial community may lead to changes in the structure and function of the overall ecosystem, and ultimately determine ecosystem sustainability (Bossio and Scow, 1995).

Soils disturbed by tree removal often have reduced microbial diversity compared with undisturbed areas (Atlas et al., 1991; Arunachalam et al., 1999; Byrd et al., 2000). Furthermore, prescribed fire has been shown to alter the structure (Pietikiänen and Fritze, 1995), activity (Fritze et al., 1993), and function (Staddon et al., 1998) of the soil microflora. However, the conclusions of these studies may not be applicable to ponderosa pine forests of the southwestern USA for a variety of reasons. For instance, the effect of tree removal on soil microorganisms covaries with the extent of the disturbance (Morris and Boerner, 1998), with clearcutting causing substantially greater changes in microbial activities than low intensity thinning (Dahlberg et al., 2001). Ecological restoration removes numerous trees, but these trees are typically small in diameter, and the total site disturbance is generally much lower than that found in commercial tree harvests (Covington et al., 1997). Prescribed fires used in restoration are typically lower in intensity than most other prescribed burns and wildfires because of fuel reductions or manipulations before burning (Covington et al., 1997). Further, repeated burning used to maintain restoration treatments may have different impacts than a single fire event (Neary et al., 1999), and the interactive effects of both tree removal and fire on the soil microflora have rarely been considered in any forest (Acea and Carballas, 1996; Boerner et al., 2000). Finally, all of these previous studies have been conducted in more mesic forests; microbial responses to thinning and burning treatments may differ in semiarid ponderosa pine forests where water availability is a strong ecological factor (Kaye et al., 2005).

S.I. Boyle and S.C. Hart, School of Forestry & Merriam-Powell Center for Environmental Research, Northern Arizona Univ., Flagstaff, AZ 86011-5018; J.P Kaye, Crop & Soil Sciences Dep., The Pennsylvania State Univ., University Park, PA 16802; M.P. Waldrop, School of Natural Resources & Environment, Univ. of Michigan, Ann Arbor, MI 48109. Received 21 Jan. 2005. *Corresponding author (steve.hart@nau.edu).

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In this study, we evaluated the impacts of 8-yr-old ecological restoration treatments on soil microbial activity, biomass, and function within a mature ponderosa pine stand in northern Arizona (Covington et al., 1997). Replicated restoration prescriptions included tree thinning alone or a combination of tree thinning, forest floor reduction, and prescribed burning. Within restoration treatments, we stratified our sampling under major canopy cover types to assess potential changes in the soil microflora due to pine invasion into grass openings (Kaye and Hart, 1998a, 1998b). We focused our sampling before and after the summer monsoon rains because biological activity in this semiarid region is strongly influenced by precipitation patterns. This experimental design allowed us to test effects of restoration treatments, dominant vegetation, and seasonal rainfall patterns on soil microbial communities.

MATERIALS AND METHODS

Study Site

Our study site (35°16′11″ N long., 111°44′30″ W lat.) was a 3-ha portion of the Gus Pearson Natural Area (GPNA), approximately 10 km northwest of Flagstaff, AZ, in the Coconino National Forest (Bailey and Covington, 2002). The area is characterized by pole-sized (10- to 36-cm diam. at breast [~1.4-m] height; DBH) ponderosa pine trees in the overstory and bunchgrasses in the understory (see Kaye and Hart, 1998b for a list of major species). About half of the 567 mm of mean annual precipitation falls in the form of winter snow and the other half as summer monsoon rains, with an average of 94 frost-free days and a mean annual air temperature of 7.5°C (Schubert, 1974; Savage et al., 1996). The summer growing season is characterized by a dry period of little precipitation from May to early July, followed by heavy rainfall in the latter half of the summer (Bailey and Covington, 2002). The site ranges in elevation from 2195 to 2255 m, with slopes of <5% (Schubert, 1974). The soil is classified as a Brolliar stony clay loam, and is a member of the fine, smectitic, Typic Argiboroll USDA Soil Taxonomic Family. The site has never been harvested, but was grazed between 1876 and 1910. The last reported natural fire at the site was in 1876, before which fires occurred every 2 yr on average (Dieterich, 1980).

Treatments

Fifteen 0.25-ha plots were established in 1992, and five of the plots were assigned to each of three treatments (control, thinning restoration, and composite restoration). A fuel break was needed to protect buildings of the historical Fort Valley Experiment Station, so the 10 restoration treatment plots were assigned randomly (five as thinning and five as composite) to plots closest to the buildings. The remaining five plots were assigned to the control treatment. All areas had statistically similar aboveground wood growth (stem, bark, and branches) and tree biomass (Kaye et al., 2005), mineral soil organic matter and N concentrations (Stone et al., 1999), and anaerobically mineralizable N in the mineral soil (S.C. Hart, unpublished data, 1993) before treatment.

Thinning restoration plots were thinned to test whether re-establishment of aboveground stand structure alone could restore ecosystem function. The thinning restoration treatment was designed not only to reduce overall tree density by removing most of the trees that became established post-Euro-American settlement (ca. 1876), but was also implemented in such a way as to restore the clumpy spatial pattern of the trees. The thinning restoration treatment resulted in the removal of the entire aboveground biomass of 2226 trees ha^{-1} from the site, more than half of which were between 2.5 and 12.5 cm DBH (Covington et al., 1997). Thinning reduced the stand basal area from ~35 to <13 m^2 ha^{-1} (Covington et al., 1997; Bailey and Covington, 2002). The composite restoration plots were thinned as described for the thinning restoration plots. Additionally, the forest floor (O horizon) was manipulated to create a fuel load similar to presettlement conditions before the reintroduction of fire via prescribed burning. Previous research has suggested that that old growth tree mortality is high when prescribed burns are not preceded by the reduction of forest floor fuels that accumulated over 120 yr of fire suppression (Covington et al., 1997). The Oa and Oe layers were removed from the site, and the remaining litter layer (Oi) amended with ~672 kg ha^{-1} of native grass and forb clippings (~1 yr of herbaceous production) from a nearby prairie. Thinning and composite restoration plots underwent thinning in 1993, and composite restoration plots were burned in 1994 and 1998 (~4-yr return frequency) to mimic the historic fire return interval at the site. The control plots were left untreated. More information on thinning treatments and prescribed burning can be found in Covington et al. (1997) and Kaye and Hart (1998b).

Within each plot, we further stratified sampling beneath three or four canopy types based on previous research in ponderosa pine forests showing that ecosystem response to thinning and fire treatments is dependent on canopy type (Covington and Sackett, 1986). Additionally, comparison of treatment responses among canopy types provides insight into how pine invasion into grass areas since Euro-American settlement has altered these ecosystems (Kaye and Hart, 1998a, 1998b). Control plots included presettlement pine, postsettlement pine retained, and grass opening canopy types. The thinning and composite restoration plots included these three canopy types along with the additional canopy type of postsettlement pine removed. Canopy type sampling areas (subplots) were selected randomly from the population of potential areas for a given canopy type within each treatment plot. This sampling design resulted in a total of 15 subplots in the control treatment (3 canopy types × 5 plots = 15 subplots), while thinning and composite restoration treatments had 20 subplots each (4 canopy types × 5 plots = 20), for a total of 55 subplots across the entire study area. Canopy type classifications did not change during the study period.

Soil Sampling

Within each subplot, four, 2-cm diam. soil cores (Oakfield Apparatus Company, Oakfield, WI) were taken randomly (0-to 5-cm mineral soil depth) within a 0.30-m^2 area adjacent to soil respiration measurements (see below). These soil samples were then composited, resulting in one soil sample for each subplot. The sampling depth was chosen based on studies showing that microbial biomass and activity are greatest in the uppermost portion of forest soils (Nohrstedt et al., 1989; Mergel et al., 2000), and we expected treatment differences to be greatest in this mineral soil layer. Sampling occurred on 30 June and 13 Aug. 2001, before and after the onset of the summer rains (hereafter referred to as dry and wet periods, respectively) to assess the impact of seasonal fluctuations in soil water content on soil microbial communities and their responsiveness to restoration treatments (Fig. 1a). Hence, we sampled soils approximately 8 yr after tree thinning in the thinning and composite restoration plots. Further, our sampling in the composite restoration plots occurred roughly 7 yr

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after the first prescribed burn, and about 3 yr since the second prescribed burn; therefore, the direct impacts of the burn via soil heating on the soil microflora would have been minimal at this point in the fire cycle (i.e., 4-yr return frequency; Hart et al., 2005).

Soil Characteristics

Soil samples were stored as intact cores at 4°C for <12 d before processing. All soils were sieved (≤2 mm) field-moist immediately before use in analyses. Soil water content was determined gravimetrically at 105°C for each sample, and all concentration data are expressed on an oven-dry mass basis except where noted. Soil pH was determined in a 1:2 (w/v) suspension of air-dried soil to 0.01 *M* $CaCl_2$ on an Orion 720A pH meter (Allometrics, Inc., Baton Rouge, LA). Soil total (organic) C and soil total N were determined on finely ground, air-dried samples on a Flash EA 1112 N/protein analyzer (CE Elantech, Lakewood, NJ).

Soil temperature and volumetric soil water content were measured continuously using thermisters and time domain reflectometry probes (CS615) linked to dataloggers (CR10; Campbell Scientific Inc., Logan, UT). Soil temperature (7.5-cm mineral soil depth) and volumetric soil water content (0- to 15-cm mineral soil depth) were measured in all canopy types within two plots of each treatments (n = 8 in each restoration treatment and n = 6 in the control). Daily mean soil temperatures were generated from measurements taken every minute, while daily mean volumetric soil water contents were determined from hourly measurements.

Microbial Biomass-Nitrogen and Inorganic Nitrogen

Microbial biomass-N was measured using a modification of the chloroform fumigation-extraction method described by Haubensak et al. (2002). A 10-g field-moist soil subsample from each subplot was immediately extracted with 50 mL of 0.5 *M* K_2SO_4. A corresponding 10-g subsample was fumigated with hydrocarbon-stabilized $CHCl_3$ in a vacuum desiccator for 5 d. The fumigated subsample was then extracted with 50 mL of 0.5 *M* K_2SO_4. Soil suspensions were shaken for 1 h on a mechanical shaker and then filtered through Whatman no. 1 filter paper that had been rinsed with deionized water. Filtered extracts were frozen immediately until processed further. Unfumigated, filtered extracts were analyzed colorimetrically for ammonium (NH_4^+) and nitrate (NO_3^-; Lachat Instruments, Inc., 2001 and 2000, respectively) using a Lachat AE Flow Injection Auto-analyzer (Lachat Instruments, Milwaukee, WI) to determine inorganic-N pool sizes at the time of sampling. Total-N (organic-N + NH_4^+) in fumigated and unfumigated extracts was determined (salicylate method; Lachat Instruments, Inc., 1994) on a Lachat AE Flow Injection Auto-analyzer after digestion of 20-mL aliquots using a modified micro-Kjeldahl procedure (Haubensak et al., 2002). The N-flush caused by fumigation was calculated by subtracting the total N in the unfumigated samples from the corresponding total N in fumigated extracts. A k_{EN} of 0.20 was used to convert chloroform labile-N data to biomass-N data (Davidson et al., 1989; Hart et al., 1994).

Soil Respiration

Soil respiration was measured using the soda-lime closed chamber technique (Edwards, 1982; Grogan, 1998; Kaye and Hart, 1998b). Kaye and Hart (1998b) also used the soda-lime technique at this site to measure soil respiration in 1995 and 1996, and found that soda-lime-derived soil respiration rates were well correlated with those determined with an Infrared

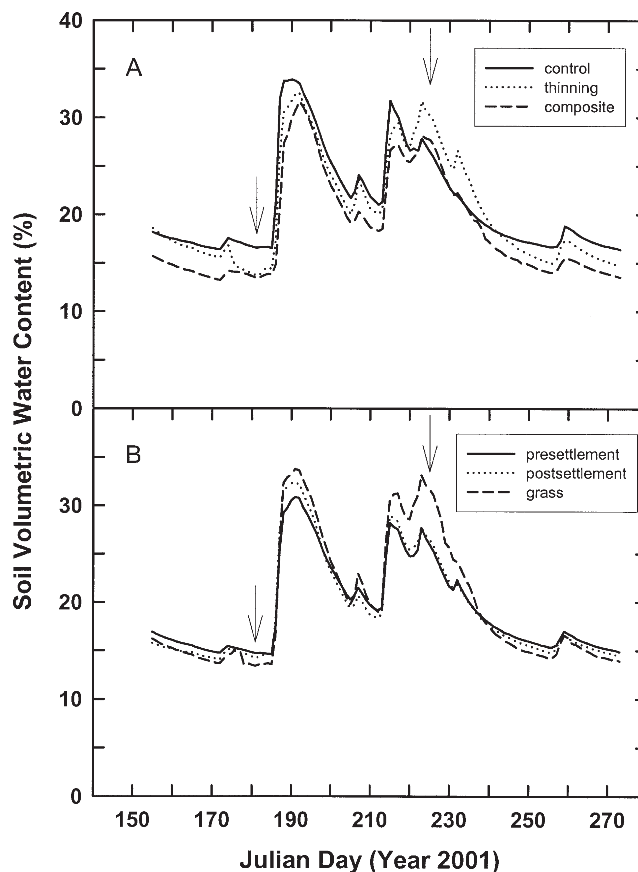

Fig. 1. Mean volumetric soil water content for the 2001-growing season (0- to 15-cm mineral soil depth) for (A) treatments and (B) canopy types. Vertical arrows indicate sampling dates. Repeated measures ANOVA on ranks showed that treatment had a significant effect (P < 0.001) on soil water content across the growing season, with median values from control > thinning restoration > composite restoration plots (Dunn's method, n = 2). Repeated measures ANOVA on ranks also indicated that canopy type had a significant effect (P < 0.001) on soil water content across the growing season, with median values from grass > postsettlement pine retained = presettlement pine areas (Dunn's method, n = 6).

Gas Analyzer, although soda-lime values, on average, underestimated CO_2 fluxes.

Soil respiration was measured 3 d before each soil sampling date at every subplot. We established our soil respiration chambers in the same locations used by Kaye and Hart (1998b). A place-holding ring extending 1 cm into the ground marked the subplot location for future measurement dates and minimized lateral gas flow near the ground. The soda-lime method uses a mixture of NaOH and CaO or $Ca(OH)_2$ (granular 1.68–3.36 mm, 6–12 mesh soda-lime; Fisher Chemical, Pittsburgh, PA), which absorbs the CO_2 that is evolved from the soil and trapped in the chamber. We dried the soda lime (60 g oven-dry weight) at 105°C for 24 h in polypropylene containers (8-cm diam.). After cooling in a desiccator, the containers were weighed to within one ten-thousandth of a gram, transported to the field, opened, and placed under an opaque chamber (27.5 cm diam., 20 cm tall, 0.59 m^2). After 24 h, the containers were removed and sealed, and returned to the laboratory for drying, cooling, and weighing. Six blanks were included to account for CO_2 gain during handling. Blanks were handled the same way as the samples, except that they were placed under chambers for only 30 s. The CO_2 flux integrated over the time period was calculated by subtracting the mean net blank weight gain from

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the net sample weight gain and multiplying by a 1.69 correction factor that accounted for the theoretical mass of water released as soda-lime reacts with CO_2 (Grogan, 1998). Further details describing the efficacy of the soda lime method, as applied here, for measuring soil respiration can be found in Kaye and Hart (1998b).

Enzyme Assays

The activities of eight ecologically important enzymes were assayed as indices of microbial activity and function. Utilization of β-1, 4-glucosidase (EC 3.2.1.21), α-1, 4-glucosidase (EC 3.2.1.20), β-galactosidase (EC 3.2.1.22), β-xylosidase (EC 3.2.1.37), cellobiohydrolase (EC 3.2.1.91), N-acetyl-glucosaminidase (EC 3.2.1.30), alkaline phosphatase (EC 3.1.3.1), and sulfatase (EC 3.1.6.1) were measured using the MUB-linked substrates 4-methylumbelliferyl β-D-glucosidase, 4-methylumbelliferyl-α-D-glucoside, 4-methylumbelliferyl β-D-galatoside, 4-methylumbelliferyl 7-β-D-xyloside, 4-methylumbelliferyl β-D-cellobioside, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide, 4-methylumbelliferyl phosphate disodium salt, and 4-methylumbelliferyl sulfate potassium salt, respectively (Sigma Chemical, St. Louis, MO). The first six enzymes are involved in the degradation of carbohydrate polymers (Eivazi and Tabatabai, 1988; Sinsabaugh, 1994; Eivazi and Bayan, 1996; Boerner et al., 2000). Alkaline phosphatase hydrolyzes phosphate esters and anhydrides of phosphoric acid to release phosphate groups (Eivazi and Tabatabai, 1977; Bergmeyer et al., 1983), while sulfatase hydrolyzes organic sulfate esters to release inorganic forms of S (Tabatabai and Bremmer, 1970; Ganeshamurthy and Nielsen, 1990; Eivazi and Bayan, 1996). All enzyme analyses were conducted on microtiter plates using a modification of a microtiter method proposed by Sinsabaugh et al. (2000) for the testing of urease activity (Waldrop et al., 2004).

A soil suspension of 1 g of soil per 100 mL of 5 m*M* bicarbonate solution (pH 8.2) was prepared for each sample. Each test well was inoculated with 100 μL of soil suspension and 100 μL of one of the enzyme substrate solutions. The enzymes were incubated for 1 h and assayed at 20°C, which is within the range of diel temperature fluctuations in the surface mineral soil at our site during the growing season (Dick, 1997). The level of enzyme activity was indicated by the intensity of fluorescence (Miller et al., 1998), measured by a Fluoromax fluorimeter (Jobin Yvon-Spex, Edison, NJ) with an attached MicroMax Microwell plate reader (Jobin Yvon-Spex, Edison, NJ) at an excitation of 360 nm and an emission of 450 nm. Standards for quenching were included on each plate, and each enzyme was replicated six times per plate to reduce the variability of readings for each sample (Sinsabaugh et al., 2000). Enzyme activities are reported as μmol product kg^{-1} oven-dry soil h^{-1}.

Community-Level Physiological Profiles

Commercially available microtiter plates from Biolog, Inc. (Hayward, CA) were used to assess the physiological capacities of the cultural soil microflora through sole C source (substrate) utilization patterns (Garland and Mills, 1991; Balser et al., 2002). The ECO plate type, which contains 31 environmentally relevant C substrates replicated three times per plate, was used to provide community-level physiological profiles (CLPPs) of bacteria (Classen et al., 2003). Biolog SF-N2 plates, which have 95 unique C substrates per plate, were used to assess CLPPs of fungi (Classen et al., 2003). The ECO plates quantify the utilization of the C substrates based on a colorimetric reaction, while C utilization on fungal plates is assessed using turbidity measurements (Garland and Mills, 1991; Buyer et al., 2001; Classen et al., 2003).

Plates were prepared as described in Classen et al. (2003). Briefly, 4 g of sieved (≤2 mm) soil were extracted in 36 mL of 50 m*M* K_2HPO_4 buffer, and allowed to settle for 0.5 h before a 0.4-mL aliquot of this extraction was removed. Aliquots were then diluted in 39.6 mL of inoculating solution to give a final dilution of 1:1000 (A.C. Kennedy, USDA-ARS, personal communication, 1999). To inhibit bacterial growth, fungal inoculations also contained 1 μg of streptomycin sulfate and 0.5 μg of chlortetracycline per microtiter plate well (Dobranic and Zak, 1999). Both plate types were inoculated with 100 μL of the 1:1000 dilution per well.

Plates were placed in polyethylene bags to reduce evaporation and incubated in the dark at 22°C. Incubation lengths were selected based on previous data collected using soils from this site showing that fungal growth begins on bacterial plates after 72 h, and that fungal plate utilization patterns require 168 h to develop fully (Classen et al., 2003). Thus, ECO plates were read at 590 nm after 72 h, and SF-N2 plates were read at 750 nm after 168 h on a MicroMax Plate Reader (Molecular Devices, Sunnydale, CA). One ECO plate and three SF-N2 plates were used for each soil sample.

Biolog well absorbance values that were <0.06, the detection limit of the spectrophotometer (Biolog, Inc., personal communication, 2000), were set to zero. For ECO plates, data for within-plate replicates for each sample were averaged, and data from the three replicate SF-N2 plates for each sample were also averaged. We normalized the data by dividing the color or turbidity development of each well by the total color or turbidity development of the entire plate, such that the sum of all the individual well values from a plate equaled one (Classen et al., 2003). This normalization procedure reduces the influence of differences in initial inoculum densities on CLPPs (Garland and Mills, 1991; Garland, 1996; Classen et al., 2003). We also analyzed normalized Biolog data by substrate guilds rather than individual substrates, using separate analyses of variance for each guild (Dobranic and Zak, 1999; Selmants et al., 2005). These groupings allowed us to assess which types of substrates contributed the most to community separation determined by non-metric multi-dimensional scaling ordinations and multi-response permutation procedures (see below).

Statistical Analysis

All canopy-type (subplot) data were scaled to the plot level using a geographic information system (GIS) to analyze plot-level treatment effects (Kaye and Hart, 1998a, 1998b). The GIS file contained the area of each canopy type within each plot, which allowed us to calculate the proportion of the plot represented by each canopy type. These proportions were used to scale the data to the plot level. The data were then analyzed using a standard one-way analysis of variance (ANOVA). In cases where data did not meet the assumptions of parametric ANOVA (e.g., the enzyme data), Kruskal–Wallis ANOVAs on ranks were used. If the main effects were significant, means were compared using the Holm-Sidak method (parametric ANOVA) or medians were compared using Dunn's test (ANOVA on ranks). Non-metric multi-dimensional scaling (NMDS) ordinations with the multi-response permutation procedures (with Sorensen distance measure) were also used to evaluate plot-level treatment effects on the activities of all enzymes combined, as well as on CLPPs. Non-metric multi-dimensional scaling condenses multivariate data into single points represented within a two-dimensional space (Mather, 1976). Multi-response permutation procedures (MRPP) compare the distances in ordination space between points in two

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Table 1. Mean (and one standard error) for selected soil (0- to 5-cm mineral soil depth) characteristics at the Gus Pearson Natural Area.

	Treatment†			Canopy type†		
Soil characteristic	Control	Thinning restoration	Composite restoration	Presettlement	Postsettlement retained	Grass
pH‡	5.5 (0.1)	5.5 (0.1)	5.4 (0.1)	5.3 (0.1)	5.5 (0.1)	5.5 (0.1)
Total C, g kg^{-1}	31.0 (2.9)	40.8 (1.8)	32.6 (3.0)	50.7 (5.9)	28.6 (1.4)	35.2 (2.3)
Total N, g kg^{-1}	1.3 (0.1)a	1.7 (0.1)b	1.5 (0.1)ab	2.0 (0.2)	1.3 (0.1)	1.7 (0.1)
C/N mass ratio	23.0 (0.7)	23.3 (1.0)	21.3 (0.8)	24.8 (0.8)b	21.7 (0.5)a	20.9 (0.7)a

† Values from the same row with different lowercase letters are statistically different ($P \leq 0.033$, Bonferroni corrected); when no lowercase letters are given, values are statistically similar. Due to a significant ($P < 0.10$) treatment by canopy type interaction, no statistical results are shown for total C or total N by canopy type. $n = 5$ for treatment comparisons and $n = 15$ for canopy-type comparisons.
‡ Measured in a 1:2 (w/v) suspension of air-dried soil to 0.01 M $CaCl_2$.

different groups with those distances within each of the groups to determine whether or not the two groups are statistically different (Mielke and Berry, 2001). Data were also tested for differences between dry and wet periods at the plot level using paired t tests.

The canopy type of postsettlement pine removed was not present in the control areas; therefore, canopy type differences were assessed by analyzing only data from the presettlement pine, postsettlement pine retained, and grass canopy types (Kaye and Hart, 1998a, 1998b). Differences in soil variables were determined using a two-way ANOVA, with canopy type, treatment, and their interaction as factors. Non-metric multidimensional scaling ordination with MRPP was used to evaluate canopy-type effects on the activities of all enzymes combined and on CLPPs, as was done for plot-level data. For both plot and canopy-type analyses, enzyme data were analyzed per unit dry mass of soil as well as per unit mass of microbial-N. The latter approach was used to evaluate whether any differences in enzyme activities among treatments or canopy types were due to compositional differences in the soil microbial community or differences in microbial community size (Boerner and Brinkman, 2003). Within sampling dates, Pearson correlation analyses were used to test for covariance among selected soil measurements at the plot level ($n = 15$, except for soil temperature where $n = 6$) and at the canopy level ($n = 55$, except for soil temperature where $n = 22$).

One-way repeated measures ANOVAs on ranks were used to assess differences in soil temperature and volumetric soil water content (0–15 cm) among treatments and canopy types over the summer of 2001 (June 4 to September 30), because the assumptions of parametric ANOVAs were not met. Medians were then compared using Dunn's method.

The experiment-wide α was set at 0.10 before the start of the experiment to balance between the risks of committing Type I and II errors. Bonferroni corrections were used for the post-hoc Holm-Sidak method, Dunn's method, and MRPP comparisons (three pairwise comparisons for both plot and canopy-type comparisons, $P \leq 0.033$). All statistical analyses were performed using SigmaStat (version 2.03, Systat Software, Inc., Pt. Richmond, CA) except for the multivariate analyses, which were performed using PC-ORD software (Version 4, MjM Software Design, Gleneden Beach, OR).

RESULTS

Effects of Restoration Treatments on Soil Microflora

Restoration treatments did not significantly alter soil pH, total C, or C/N mass ratio (Table 1). Total N concentration was higher in the thinning restoration treatment than in the control, but the composite restoration treatment was statistically similar to the other two treatments. Soil temperature was significantly higher in the composite restoration treatment than in the control during both the dry and wet sampling periods, while soil temperature in the thinning restoration plots was intermediate (Table 2). This pattern was also found over the 2001 growing season (June 4 to September 30; RM ANOVA on ranks, $P < 0.001$; Fig. 2a). Gravimetric soil water content (0–5 cm) was similar among treatments during both sampling periods (Table 2); however, soil

Table 2. Mean (and one standard error) for selected soil characteristics (0- to 5-cm mineral soil depth) during dry and wet periods at the Gus Pearson Natural Area.

	Treatment†			Canopy type†		
Sampling period/ soil characteristic	Control	Thinning restoration	Composite restoration	Presettlement	Postsettlement retained	Grass
Dry period‡						
Soil temperature, °C§	14.1 (0.9)a	16.8 (0.9)ab	19.4 (1.0)b	16.6 (1.2)	15.8 (1.4)	18.7 (1.0)
Soil water content, %¶	9.7 (1.0)	10.0 (1.0)	9.8 (0.2)	11.8 (0.8)b	10.5 (1.4)ab	9.1 (1.0)a
Nitrate-N, mg m^{-2}	21 (6)	12 (5)	38 (11)	12 (4)	11 (3)	17 (4)
Ammonium N, mg m^{-2}	294 (97)	206 (75)	348 (70)	114 (23)	198 (35)	145 (12)
Microbial-N, g m^{-2}	3.36 (0.36)	2.96 (0.32)	3.25 (0.55)	4.04 (0.54)	3.37 (0.52)	4.30 (0.70)
Wet period‡						
Soil temperature,°C§	15.9 (0.1)a	17.1 (0.4)b	17.6 (0.1)b	16.7 (0.4)	16.7 (0.5)	17.3 (0.3)
Soil water content, %¶	29.5 (0.9)	31.0 (0.7)	30.8 (1.3)	27.2 (2.1)a	31.0 (0.7)ab	32.1 (1.0)b
Nitrate-N, mg m^{-2}	12 (9)	14 (5)	24 (6)	12 (5)	13 (4)	24 (6)
Ammonium N, mg m^{-2}	198 (33)a	296 (20)b	217 (20)ab	218 (25)	296 (32)	220 (16)
Microbial-N, g m^{-2}	6.43 (0.89)	4.60 (0.84)	6.43 (0.65)	6.49 (0.76)ab	5.52 (0.67)a	8.22 (0.80)b

† Values from the same row with different lowercase letters are statistically different ($P \leq 0.033$, Bonferroni corrected); when no lowercase letters are given, values are statistically similar. Due to a significant ($P < 0.10$) treatment by canopy type interaction, no statistical results are shown for nitrate by canopy type. $n = 5$ for treatment comparisons and $n = 15$ for canopy-type comparisons except for soil temperature, where $n = 2$ for treatment comparisons and $n = 6$ for canopy-type comparisons.
‡ Dry period refers to June samples taken before the onset of summer rains; wet period refers to August samples taken after the onset of summer rains.
§ Soil temperature was measured using dataloggers and thermistors placed at a 7.5-cm mineral soil depth.
¶ Soil water content was determined gravimetrically.

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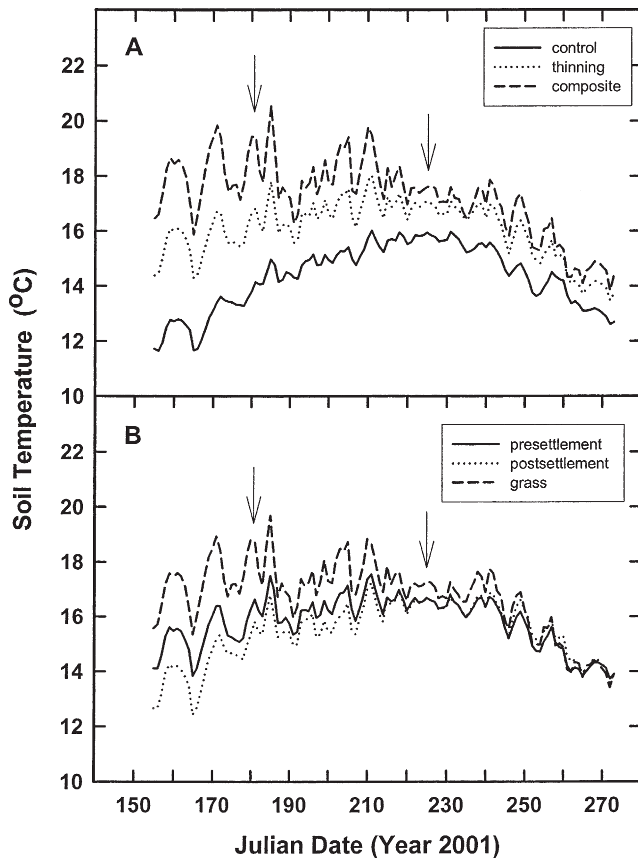

Fig. 2. Mean soil temperature for the 2001-growing season (7.5-cm mineral soil depth) for (A) treatments and (B) canopy types. Vertical arrows indicate sampling dates. Repeated measures ANOVA on ranks indicated that treatment had a significant effect ($P < 0.001$) on soil temperature across the growing season, with median values from composite restoration > thinning restoration > control (Dunn's method, $n = 2$). Repeated measures ANOVA on ranks also indicated that canopy type had a significant effect ($P < 0.001$) on soil temperature across the growing season, with median values from grass > postsettlement pine retained = presettlement pine areas (Dunn's method, $n = 6$).

volumetric water content (0–15 cm) over the 2001 growing season was significantly different among treatments (control > thinning restoration > composite restoration; RM ANOVA on ranks, $P < 0.001$; Fig. 1a). Ammonium N pool sizes were statistically similar among the treatments during the dry period, but NH_4^+–N pools were greater in the thinning restoration treatment than the control during the wet period; NH_4^+–N pool sizes in the composite restoration treatment were intermediate. Nitrate pool sizes were small across all treatments during both the dry and wet periods, and were statistically similar among treatments. Similarly, microbial-N was similar among treatments during both sampling periods.

The in situ soil respiration rate was higher in the two restoration treatments than in the control during the dry period, but no difference in soil respiration rates was observed during the wet period (Fig. 3a). Enzyme activities were generally low during the dry period (Fig. 4a). During this period, they did not differ significantly among any of the treatments when individual enzymes were compared (Fig. 4a), or when all enzymes

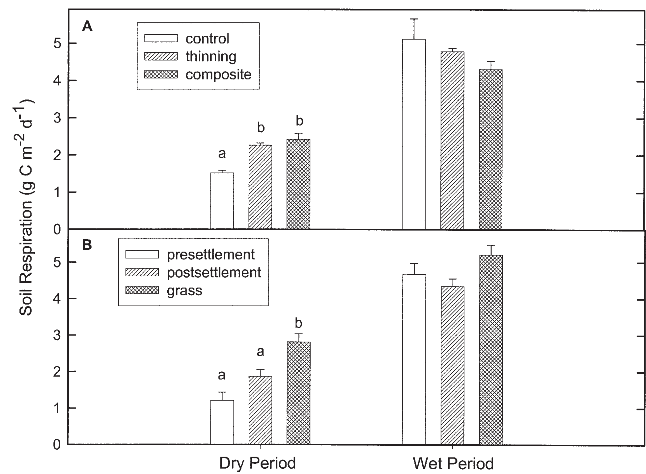

Fig. 3. Mean soil respiration rates for (A) treatments and (B) canopy types. Different lowercase letters indicate significant differences within each sampling period, as determined by ANOVA and the Holm-Sidak mean separation test ($P \leq 0.033$, Bonferroni corrected). Vertical lines represent one standard error of the mean. Respiration increased significantly from dry to wet periods for each treatment (A) and each canopy type (B), as determined by paired *t* tests ($P \leq 0.007$). There were no significant differences among treatments or among canopy types for the wet period.

were analyzed together by MRPP ($P = 0.714$). This pattern was also found when enzyme data were normalized to microbial-N values (data not shown). During the wet period, the activities of all of the enzymes assessed increased, and each of the enzymes tested except β-glucosidase and phosphatase showed significantly higher values in the composite restoration treatment than in the thinning restoration and control treatments (Fig. 4b). Similarly, when all of the enzyme activities during the wet period were analyzed together using MRPP, the composite restoration treatment was significantly different from both the control and thinning restoration treatments ($P = 0.007$ and 0.006, respectively), but the control and thinning restoration treatments were not different from each other ($P = 0.936$). Again, these patterns among treatments were similar when the enzyme data was normalized to microbial-N values (data not shown).

In the dry period, CLPPs were significantly different between control and composite restoration treatments for both bacteria and fungi ($P = 0.031$ for both; Fig. 5a, b). Bacterial and fungal CLPPs were also different between thinning and composite restoration treatments during the dry period ($P = 0.009$ and 0.007, respectively). Bacterial CLPPs were significantly different between the thinning and composite restoration treatments during the wet period ($P = 0.023$), with bacterial CLPPs of the control plots being intermediate between the two (Fig. 5c). However, fungal CLPPs were similar across all treatments during the wet period (Fig. 5d).

When ANOVAs were used to evaluate differences in substrate utilization by guilds, bacterial utilization of amino acids was significantly lower in the composite restoration treatment than the thinning restoration treatment during the dry period. Fungal utilization of amino acids was higher in the composite restoration treatment than in the thinning restoration or control treatments

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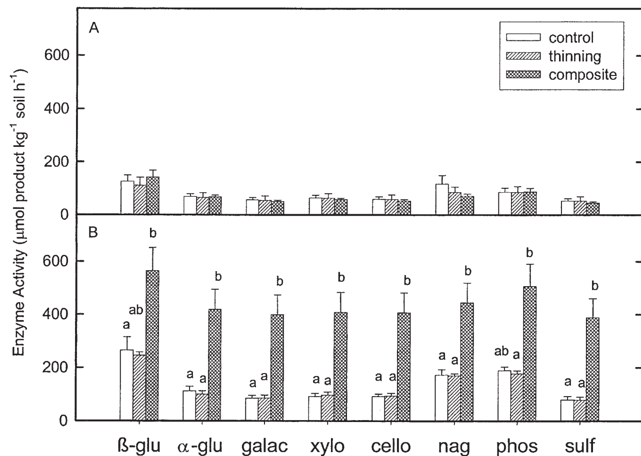

Fig. 4. Mean enzyme activities in treatment plots for (A) dry and (B) wet periods. Vertical lines represent one standard error of the mean. During the dry period, all enzyme activities were statistically similar among treatments (based on ANOVA on ranks, $P > 0.10$). During the wet period, treatment had a significant effect on all enzyme activities. For a given enzyme, values with different lowercase letters are statistically different ($P \leq 0.033$, Bonferroni corrected). All enzymes in each treatment increased significantly from dry to wet periods based on paired t tests at $P \leq 0.05$. Enzymes are abbreviated as follows: β-glucosidase (β-gluc), α-glucosidase (α-gluc), galactosidase (galac), xylosidase (xylo), N-acetyl-glucosaminidase (nag), phosphatase (phos), and sulfatase (sulf).

during the dry period. Amine and amide usage by fungi were higher in the thinning restoration treatment than the composite restoration treatment. During the wet period, we found no significant differences in substrate guild use for bacteria. However, for fungi, carbohydrate usage was greater in the thinning restoration treatment than the composite restoration treatment, and carboxylic acid use was lower in the thinning restoration treatment than both the composite restoration and control treatments (data not shown).

Effects of Canopy Type on Soil Microflora

Significant interactions between treatment and canopy type in two-way ANOVAs were only found for total C, total N, and NO_3^- pool size during the dry period. Hence, we pooled the results for a given canopy type across treatments to assess canopy-type effects on soil properties and microbial parameters. Soil pH values, temperature, and NH_4^+–N pools were statistically similar among the canopy types (Tables 1 and 2). However, the presettlement pine canopy type had a significantly higher C/N ratio than postsettlement pine and grass areas (Table 1). Furthermore, gravimetric water content was higher in presettlement pine canopy types than in grass openings during the dry period, but the reverse was true during the wet period (Table 2); postsettlement pine canopy types had intermediate gravimetric water contents during both periods. Over the 2001 growing season, volumetric soil water content (0- to 15-cm mineral soil depth; Fig. 1b) and soil temperature (7.5-cm mineral soil depth; Fig. 2b) were significantly higher in the grass canopy types than the two pine canopy types. Microbial-N was similar among canopy types during the dry period, but microbial biomass-N was higher in the grass openings than the postsettlement pine canopy type during the wet period; the presettlement canopy type had intermediate microbial-N pool sizes (Table 2).

In situ soil respiration was significantly higher in the grass areas during the dry period, with the two pine canopy types having similar rates (Fig. 3b). During the

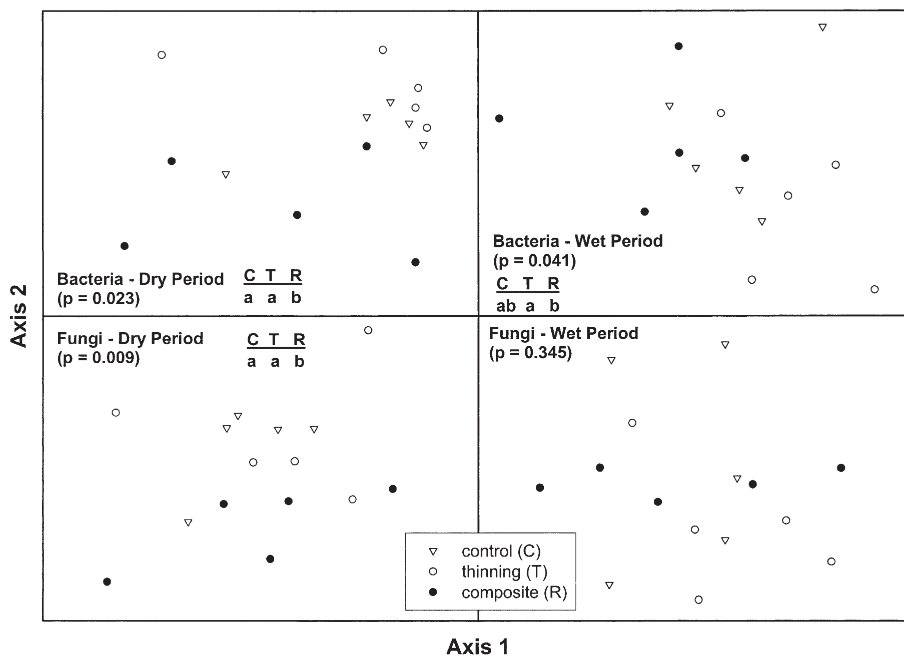

Fig. 5. Non-metric multi-dimensional scaling (NMDS) ordinations of bacterial and fungal community-level physiological profiles in restoration and control treatments during the dry and wet sampling periods. Each symbol represents a replicate plot ($n = 5$). Statistical differences among treatments were determined using multi-response permutation procedures (MRPP, $P < 0.10$), and are denoted by different lowercase letters ($P \leq 0.033$, Bonferroni corrected). Ordination stress level, a measure of the quality of representation of the data in the ordination, ranged from 8.6 to 13.3; these values suggest fair to good ordinations with no real risk of drawing false inferences (Clarke, 1993).

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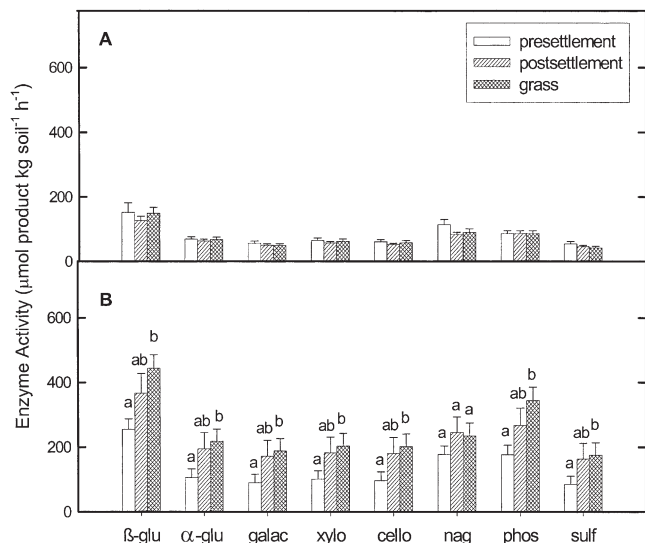

Fig. 6. Mean enzyme activities in canopy type during the (A) dry and (B) wet periods. Vertical lines represent one standard error of the mean. During the dry period, all enzyme activities were statistically similar among treatments (based on ANOVA on ranks, $P > 0.10$). During the wet period, canopy type had a significant effect on all enzyme activities except nag. For a given enzyme, values with different lowercase letters are statistically different ($P \leq 0.033$, Bonferroni corrected). All enzymes in each canopy type increased significantly from dry to wet periods, based on paired *t* tests at $P \leq 0.05$. Enzymes are abbreviated as follows: β-glucosidase (β-gluc), α-glucosidase (α-gluc), galactosidase (galac), xylosidase (xylo), N-acetyl-glucosaminidase (nag), phosphatase (phos), and sulfatase (sulf).

wet period, there were no significant differences in soil respiration among canopy types. When enzyme activities were compared among canopy types, no significant differences were found during the dry period for any individual enzyme (Fig. 6a), or for overall enzyme activity as assessed by MRPP multivariate analysis ($P = 0.891$). This pattern was also found when enzyme data were normalized to microbial-N values (data not shown). In the wet period, however, all of the enzymes except N-acetyl-glucosaminidase showed significantly higher enzyme activities in the grass canopy type than in the presettlement pine areas, with the postsettlement canopy types being intermediate (Fig. 6b). Similarly, when enzyme activities measured during the wet period were assessed simultaneously using MRPP, grass and presettlement pine canopy types were significantly different ($P = 0.003$), while postsettlement pine canopy types were similar to both grass and postsettlement areas ($P = 0.211$ and 0.252, respectively). Normalizing the enzyme data collected during the wet period to microbial-N generally resulted in similar patterns, except that pre- and post-settlement canopy types became significantly different from each other (data not shown). Pearson correlation coefficients between pairs of enzyme activities across all canopy types ($n = 55$) ranged from 0.33 to 0.97 in the dry period, and 0.88 to 0.99 in the wet period ($P < 0.02$). Non-metric multi-dimensional scaling and MRPP analyses of CLPPs found no significant differences among the canopy types for either sampling period for bacteria (dry period, $P = 0.485$; wet period, $P = 0.248$) or fungi (dry period, $P = 0.242$; wet period, $P = 0.219$). When substrate utilization patterns were analyzed by guilds, no significant differences were found for bacteria or fungi among canopy types during either sampling period (data not shown).

Seasonal and Environmental Influences on Microbial Responses

At the plot-level, gravimetric soil water content, soil respiration rate, all enzyme activities, and NH_4^+–N and microbial-N pool sizes increased significantly between the dry and wet periods (paired *t* tests, $P < 0.05$, $n = 15$). However, soil temperature and NO_3^-–N pool sizes were similar between dates ($P > 0.10$). At the canopy-type level during the dry period, gravimetric soil water content was correlated with microbial-N ($r = 0.30$, $P = 0.02$), soil temperature ($r = -0.44$, $P = 0.07$), β-glucosidase ($r = 0.24$, $P = 0.08$), N-acetyl-β-D-glucosaminide ($r = 0.27$, $P = 0.05$), and phosphatase ($r = 0.32$, $P = 0.02$). During the wet period, soil temperature was uncorrelated with soil respiration rate and any enzyme activity ($P > 0.10$). However, gravimetric soil water content correlated with the activities of each enzyme ($r = 0.44$ to 0.50, $P < 0.001$) during the wet period for canopy types. At the plot level, soil temperature and soil respiration were correlated ($r = 0.79$, $P = 0.06$) in the dry period only. In the wet period, gravimetric soil water content was found to be significantly correlated with several enzymes at the plot level: β-glucosidase ($r = 0.48$, $P = 0.07$), N-acetyl-β-D-glucosaminide ($r = 0.51$, $P = 0.05$), and phosphatase ($r = 0.46$, $P = 0.08$). No other significant correlations were found during the dry or wet periods at the plot level.

DISCUSSION

Restoration via tree thinning and prescribed burning may impact the soil microflora through a variety of mechanisms (Hart et al., 2005). Tree removal increases insolation to the ground and burning reduces the surface albedo and the thickness of the forest floor, all of which increase surface temperatures and heat transfer to the mineral soil (DeBano et al., 1998). Tree removal and burning can also change soil water regimes by altering rates of evaporation and transpiration, and eliminating interception of precipitation by the canopy and forest floor (Bissett and Parkinson, 1980; Arianoutsou-Faraggitaki and Margaris, 1982). These relatively immediate changes in soil microclimate may directly alter the composition and the activity of the soil microflora (Vázquez et al., 1993; Hart et al., 2005). Over the longer-term, thinning and burning can affect organic matter inputs and quality by changing plant productivity and vegetation composition (Kaye and Hart, 1998a, 1998b). Such changes in organic matter quantity or quality can, in turn, also alter microbial community structure and function (Lui et al., 2000; Hart et al., 2005).

Our results suggest that tree removal alone has only a modest impact on the soil microbial community 8 yr after treatment. Although soil respiration was higher in the thinning restoration treatment than in the control during the dry period, respiration rates were similar

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between these two treatments during the wet period. Kaye and Hart (1998b) also found that thinning increased soil respiration during the third growing season (1996) following treatment at this site. Because they found no significant differences in fine root biomass and only small differences in soil water content between thinning restoration and control treatments, they suggested that higher soil respiration in the thinning restoration treatment plots could be due to differences in microbial biomass, microbial community structure (e.g., bacteria to fungal ratio), or soil temperature. Eight years following the implementation of the treatments at this site, we found similar soil microbial-N concentrations, enzyme activities, and CLPPs of the microflora for thinning restoration and control treatments. We conclude that the higher soil respiration rates that we and Kaye and Hart (1998b) observed in the thinning restoration treatment are most likely the result of higher soil temperatures in this treatment compared with the control. Soil temperature was statistically similar between these two treatments on the day soil respiration was measured (Table 2) and soil temperature was uncorrelated with soil respiration at the canopy level. However, soil temperatures were significantly higher (difference in median values of 2.9°C) in the thinning restoration treatment than in the control treatment during the month preceding the dry period sampling (RM ANOVA on ranks, $P < 0.001$; Fig. 2a). Furthermore, Kaye and Hart (1998b) found moderately strong correlations ($r = 0.63$ to 0.65) between soil temperature and soil respiration within these treatments with a greater number of sampling dates (and thus more statistical power) than those used in our study.

Other studies have found that tree harvesting alone can both increase (Chang et al., 1995) and decrease (Entry et al., 1986; Morris and Boerner, 1998) soil microbial biomass. Additionally, a few previous studies have provided evidence consistent with a change in the composition of the soil microflora following tree harvesting. For instance, Staddon et al. (1998) found reductions in bacterial Shannon's Diversity Index values (based on Biolog plates) up to 5 yr after clear-cutting in a Canadian pine forest. Korb et al. (2003) found higher arbuscular mycorrhizae propagule densities but similar ectomycorrhizal inoculum potentials following restoration thinning in a ponderosa pine forest near our site, 1 and 2 yr following treatment. In our study, the longer intervening period between thinning and soil sampling, as well as the less severe thinning regime, may account for the lack of a substantial change in the soil microflora following tree removal compared with these previous studies. Overall, our results suggest that tree removal alone during restoration activities in southwestern ponderosa pine forests may alter soil microbial activity through increases in soil temperature, but likely does not have large effects on the function of the soil microbial community over the long term.

Although tree removal had only moderate effects on the soil microflora in this ecosystem, the restoration of the forest floor mass to presettlement levels and the reintroduction of fire in the composite restoration treatment had strong impacts on the soil microbial community. Soil respiration from the mineral soil is likely higher in the composite restoration treatment than in the thinning restoration treatment even though the measured soil respiration rates were similar. This is because both the forest floor and the mineral soil contribute to the net CO_2 efflux from the soil surface, but the composite restoration treatment plots had substantially lower forest floor areal densities than the thinning restoration plots (Kaye and Hart, 1998b). Furthermore, bacterial and fungal CLPPs and soil enzyme activities differed significantly between thinning and composite restoration treatments, while these microbial assays were generally similar between control and thinning restoration treatments where only thinning was conducted. Differences in enzyme activities between the two restoration treatments were maintained even when enzyme activities were expressed on a per unit microbial-N basis, and microbial-N was similar between these treatments. These results suggest that these differences in enzyme activities are driven by changes in the function of the soil microflora resulting from fire and are not simply due to changes in the size of the microbial community.

Previous studies evaluating the impact of fire on soil enzyme activities have often been contradictory. For example, β-glucosidase and acid phosphatase activities have been shown to increase (Ajwa et al., 1999), decrease (Eivazi and Bayan, 1996; Boerner et al., 2000; Boerner and Brinkman, 2003), or remain unchanged (Boerner et al., 2000) in response to fire. In our study, the additional disturbance of prescribed fire in the composite restoration treatment may have led to differences in enzyme activities by creating new substrates that alter the functional composition of the soil microflora relative to the thinning restoration treatment (Fritze et al., 1993), as suggested by our CLPP analyses. Based on analyses of substrate guilds, differences in substrate use among N-containing compounds (e.g., amino acids, amines, and amides) in these two restoration treatments appeared to account for most of the differences in CLPPs.

As was found by Kaye and Hart (1998b) 5 yr earlier, we observed higher soil respiration in grass areas than in the two pine canopy types. Based on these data, Kaye and Hart (1998b) suggested that soil respiration rates were likely to be much higher in presettlement forests than they are today because these forests had much greater grass cover than contemporary forests (Cooper, 1960; Covington et al., 1997). Our results are consistent with this conclusion, at least for periods of low soil water availability.

Kaye and Hart (1998b) also hypothesized that differences in the quality and quantity of litter input to the soil by vegetation, or differences in the size or composition of the soil microbial biomass in the contrasting canopy types, were likely responsible for observed soil respiration patterns because they failed to find any significant difference in soil microclimate or fine root biomass among them. We also did not find any soil microclimate differences among the various canopy types during the days that soil respiration were sampled. However, across the 2001 growing season, grass canopy types had signifi-

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cantly higher soil temperatures and volumetric water content (0–15 cm) than presettlement pine and postsettlement pine retained canopy types. Hence, at least for the grass areas, higher soil temperatures and water contents may explain in part the higher soil respiration rates observed in these areas during the dry period. Our results suggest that differences in the size or function of the soil microbial community are not responsible for any differences in soil respiration rates among canopy types. We did find significantly lower mineral soil C/N ratios in grass and postsettlement pine areas than in presettlement pine canopy types. This result may reflect differences in the quality of organic matter inputs to the soil among these contrasting canopy types, with grass and postsettlement pine areas receiving higher amounts of low C/N ratio herbaceous inputs to the soil than presettlement canopy types where almost all of the litter inputs are high C/N ratio pine litter (Kaye and Hart, 1998a, 1998b; Hart et al., 2005). Hence, the higher quality of organic matter inputs to grass and postsettlement pine canopy types might also account for the higher respiration rates observed in these areas.

Soil chemical and biological assays from postsettlement pine canopy types tended to either be similar to grass canopy types or intermediate in magnitude between presettlement and grass canopy types. Hart et al. (2005) also found that chemolithotrophic nitrifier population sizes were significantly higher in grass and postsettlement pine canopy types than in areas occupied by presettlement pine, three years after restoration activities were completed. In an area adjacent to our study site, Kerns et al. (2003) found that soil profile characteristics in grass and postsettlement pine patches were similar to each other, and that both differed from presettlement pine areas. Taken together, these results suggest that soils under postsettlement trees have retained the biological, chemical, and physical imprints of the grass vegetation that occupied these areas before their invasion by pines in the early 1920s (Covington et al., 1997). The persistence of this pattern suggests a strong linkage between plants and soils in southwestern ponderosa pine-bunchgrass ecosystems. The fact that pine invasion has not yet fundamentally altered the functional capabilities of the soil microbial community increases the chances that microbial communities that establish in restored grass openings (following postsettlement tree removal) will be similar to the communities present before Euro-American disturbances.

Our study indicates that water availability exerts a profound influence on soil microbial processes in semiarid ponderosa pine ecosystems. Microbial activity (as assessed by soil respiration measurements in situ), microbial-N, and the activities of a suite of soil enzymes all increased following the onset of summer rains. Interestingly, increases in soil water content that occurred following the beginning of the monsoon reduced or eliminated any observed differences in microbial activity or CLPPs among treatments and canopy types. However, significant differences in enzyme activity among treatments and canopy types were only observed during the wet period, and enzyme activities were only correlated with soil gravimetric water content during this sampling date. Our results are consistent with other studies in water-limited ecosystems where water availability has been shown to strongly control the function and size of the soil microflora (Kieft et al., 1987; Davidson et al., 1990; Liu et al., 2000). Hence, it is not surprising that the relative response of the soil microflora, as well as the vegetation, to these restoration treatments is tightly coupled to interannual variability in precipitation, which is extremely high in the southwestern USA (Kaye and Hart, 1998a, 1998b; Kaye et al., 2005). Changes in the microbial community following summer rains may have a large effect on plant processes at this time. For example, microbial biomass in the wet period contained 1 to 4 g m^{-2} more N than microbial biomass in the dry period. This large net flux of N into microbes is comparable with total annual net N mineralization and plant N uptake at this site (Kaye et al., 2005).

Our results suggest that ecological restoration treatments have significant, long-term effects on the soil microflora and support the need for long-term studies. The major impact of tree thinning alone on the soil microflora was an increase in their activity, likely driven by an increase in soil temperature. In contrast, forest floor manipulations and the reintroduction of fire to these forests affected the physiological capacities of the soil microflora, probably through changes in the availability of substrates. Differences in soil microbial activity and function among canopy types were also observed that were consistent regardless of treatment; this result suggests that, over the long term, the indirect effects of restoration treatments on vegetation composition may have a greater impact on the soil microflora than the direct effects of the treatments themselves (Hart et al, 2005).

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