

Effects of ecological restoration on microbial activity, microbial functional diversity, and soil organic matter in mixed-oak forests of southern Ohio, USA

C. Giai^{*}, R.E.J. Boerner

Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH 43210, USA

Received 5 May 2006; received in revised form 28 July 2006; accepted 28 August 2006

Abstract

As a result of many decades of fire suppression and atmospheric deposition the deciduous forests of eastern North America have changed significantly in stem density, basal area, tree size-frequency distribution, and community structure. Consequently, soil organic matter quality and quantity, nutrient availability, and microbial activity have likely been altered. This study evaluated the effects of four alternative forest ecosystem restoration strategies on soil microbial activity, microbial functional diversity, soil organic C, and soil N status in two mixed-oak (*Quercus* spp.) forests in southern Ohio, USA. The soils of these forests were sampled during the fourth growing season after application of (1) prescribed fire, (2) thinning of the understory and midstory to pre-settlement characteristics, (3) the combination of fire and thinning, and (4) an untreated control. Prescribed fire, with or without thinning, resulted in increased bacterial but not fungal activity when assessed using Biolog[®]. In contrast, assays of acid phosphatase and phenol oxidase activity indicated greater microbial activity in the thinning treatment than in the other three treatments. Functional diversity of both bacteria and fungi was affected by restoration treatment, with the bacterial and fungal assemblages present in the thin + burn sites and the fungal assemblage present in the thinned sites differing significantly from those of the control and burned sites. Treatments did not result in significant differences in soil organic C content among experimental sites; however, the soil C:N ratio was significantly greater in thinned sites than in sites given the other three treatments. Similarly, there were no significant differences in dissolve inorganic N, dissolved organic N, or microbial biomass N among treatments. Bacterial and fungal functional diversity was altered significantly. Based on Biolog[®] utilization treatments the bacterial assemblage in the thin-only treatment appeared to be relatively N-limited and the fungal assemblage relatively C-limited, whereas in the thin + burn treatment this was reversed. Although effects of restoration treatments on soil organic matter and overall microbial activity may not persist through the fourth post-treatment year, effects on microbial functional diversity are persistent.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Restoration; Microbial activity; Microbial functional diversity; Soil organic matter; Soil enzymes

1. Introduction

Prior to settlement by Europeans, low intensity, dormant season fires were common features of mixed-

oak forests of eastern North America (Delcourt and Delcourt, 1997). Estimates of the pre-settlement fire return interval in the Appalachian Mountains region range from <5 years (Sutherland, 1997) to 10 years (Harmon, 1982). Since the establishment of effective fire suppression policies in the 1920s, the mixed-oak (*Quercus* spp.) forests that occupy much of the eastern United States have experienced changes in

^{*} Corresponding author. Tel.: +1 614.292.9373; fax: +1 614 292 2030.

E-mail address: [gaii.1@osu.edu](mailto:giai.1@osu.edu) (C. Giai).

stem density, basal area, tree size-frequency distribution, and community structure. Shade-tolerant but fire-intolerant tree species have increased in abundance while the fire-tolerant, shade-intolerant species that have dominated these forests for millennia have decreased significantly (Iverson et al., 1997). Both the direct suppression of fire and the indirect effects of changes in forest tree community structure have likely also altered key ecosystem properties such as soil organic matter quality and quantity, nutrient availability, and microbial activity (Yaussy, 2001; Sutherland and Hutchinson, 2003).

Efforts to restore oak forest ecosystems in the wake of decades of fire suppression have been initiated in a number of states over the last decade. In Ohio, these efforts are focused on the Ohio Hills site of the National Fire and Fire Surrogate Network (<http://www.fs.fed.us/ffs>), and include the experimental application of both functional restoration treatments (i.e. prescribed fire at historical intervals) and structural approaches (i.e. mechanical thinning to pre-settlement density and species composition) (Yaussy, 2001). Included in the restoration goals of this long term project was the goal of “no decrease, and, if possible, an increase, in floral, faunal, and soil microbial biodiversity [and] nutrient cycles adequate to sustain a mixed-oak ecosystem” (Yaussy, 2001). This criterion was established based on prior studies that concluded these oak-dominated forests were N-limited (Aber et al., 1989). More specifically, prior to the fire suppression and atmospheric deposition of the last century, these Appalachian mixed-oak ecosystems, like other forest dominated by trees dependent on ectomycorrhizal symbioses, were likely low in soil inorganic N with the majority of the N capital of the ecosystem in relatively recalcitrant organic matter (Vogt et al., 1991).

Previous studies in the region have analyzed the effects of prescribed fire on soil C, N content and soil enzyme activity in a landscape context (Decker et al., 1999; Boerner et al., 2000a,b; Boerner and Brinkman, 2003, 2004). However, to date there have been no specific evaluations of the effects of such restoration treatments on soil microbial functional diversity, or of the linkages among restoration treatments, N dynamics and the microbial community. Within this context, the specific questions we sought to answer were: (1) Do prescribed fire, thinning, and their combination affect soil microbial activity and microbial functional diversity? (2) Are changes in either microbial property linked to changes in organic matter C quantity and quality and soil N status?

2. Materials and methods

2.1. Study site

The Ohio Hills Site of the National Fire and Fire Surrogate (FFS) Network is located on the unglaciated Allegheny Plateau of southern Ohio. The climate of the region is cool, temperate with mean annual precipitation of 1024 mm and mean annual temperature of 11.3 °C (Sutherland et al., 2003). The forests of the region developed between 1850 and 1900, after the cessation of cutting for the charcoal and iron industries (Sutherland and Hutchinson, 2003). The current canopy composition differs little from that recorded in the original land surveys of the early 1800s (Yaussy et al., 2003). The most abundant species in the current canopy are white oak (*Quercus alba*), chestnut oak (*Q. prinus*), hickories (*Carya* spp.), and black oak (*Q. velutina*) (Yaussy et al., 2003).

Each of the study sites within the larger Ohio Hills FFS site was composed of four treatment units of 19–26 ha, each of which was surrounded by a buffer of approximately 10 ha. Both the treatment unit and its corresponding buffer received the experimental treatment. These treatment units were designed to include all combinations of elevation, aspect, and soil, and approximated the local watershed scale in area.

The two sites within the Ohio Hills FFS site chosen for this study were the Raccoon Ecological Management Area (hereafter REMA) (39°11'N, 82°22'W) and Zaleski State Forest (hereafter Zaleski) (39°21'N, 82°22'W). Both are located in Vinton County, OH, and are underlain by sandstones, siltstones, and shales of Pennsylvanian age (Boerner and Sutherland, 2003). The soils were formed in place from residuum and colluvium, and are dominated by Steinsburg and Gilpin series silt loams (typic hapludalfs) (Lemaster and Gilmore, 1993).

Analysis of fire scars in stems of trees that were cut as part of the establishment of this experiment indicated that fires were frequent from 1875 to 1930 (return intervals of 8–15 years). In contrast, few fires occurred after the onset of fire suppression activities in the early 1930s (T. Hutchinson, USDA Forest Service, personal communication).

Each treatment unit was stratified using a GIS-based integrated moisture index (IMI) developed by Iverson et al. (1997) for this region. The IMI stratification was achieved through integration of elevation, aspect, hill shade profile, solar radiation potential, downslope water accumulation, soil water holding capacity, and landscape curvature profile (Iverson et al., 1997).

Treatments were randomly allocated among treatment units within a site, and all treatment units were sampled through the pre-treatment year 2000. Treatments consisted of prescribed fire, thinning from below to a basal area comparable to that present prior to Euro-American settlement (14–16 m²/ha), the combination of prescribed fire and thinning, and an untreated control. Previous studies of fungal and bacterial abundance (Morris and Boerner, 1999) and enzyme activity (Decker et al., 1999) across a range of scales in these and neighboring sites have demonstrated no significant variations among treatment units prior to thinning or burning.

The prescribed fires were applied during March–April 2001 and April 2005. These dormant season fires were designed to be similar to the predominant mode of natural fires in the region. Flame lengths varied from <40 cm in parts of Zaleski to approximately 2 m at REMA (M. Bowden, Ohio Division of Forestry, personal communication). Maximum temperatures at 10 cm above the forest floor averaged 153–166 °C and the maximum temperatures recorded were ranged from 356–415 °C (Iverson et al., 2003, 2004). These fires consumed unconsolidated 66–74% of the Oi and Oe layers while leaving the majority of the coarse woody debris only charred. In areas where a distinct Oa layer was present, there was no significant consumption by the fires (T.F. Hutchinson, USDA Forest Service, personal communication). Although vegetation responses to these fires have not yet been published, similar fires in neighboring study sites produced little change in either herbaceous or woody species in the ground layer or understory (Hutchinson et al., 2005a,b).

Thinning was accomplished between September 2000 and April 2001, and focused on understory and midstory stems. The goal was a residual basal area of approximately 14 m²/ha, but this goal was not achieved at any of the study sites. Thinning removed an average of 27.9% of the basal area and left an average of 20.9 m²/ha in residual basal area (D. Yaussy, USDA Forest Service, personal communication). Units that were subjected to both thinning and burning were thinned at least 2 months prior to burning.

2.2. Field methods

Within each treatment unit ten sample plots of 0.10 ha were established such that the full suite of ten plots spanned at least 90% of the range of IMI in that treatment unit. The positions of the sample plots were established by GPS and mapped on a digital elevation map overlain with an IMI score map in an ArcView

environment to verify the IMI score for each sample plot.

In June 2005, samples of approximately 100 g of mineral soil were taken by removing the unconsolidated litter layer (O_i and O_e) and sampling the underlying (fragmentary) O_a and well-developed A horizon with a trowel. We took samples at each corner of a total of 24 of the established plots, one plot per IMI class ($N = 3$)-restoration treatment ($N = 4$)-study site ($N = 2$) combination. The total number of samples taken was 96. Geostatistical analysis of the spatial autocorrelation in soil properties in this site indicated that samples taken at such distances from each other (25–55 m) constitute spatially uncorrelated, statistically independent samples (Boerner and Brinkman, 2004, 2005). All samples were brought to the laboratory and stored in field moist condition at 4 °C for 3–4 weeks until analysis (Speir and Ross, 1975).

2.3. Laboratory methods

Soils were passed through a 2 mm sieve to remove stones and root fragments, and then analyzed for the activities of three enzymes: phosphomonoesterase (acid phosphatase), chitinase, and phenol oxidase. Acid phosphatase was chosen as an indicator of overall microbial activity as acid phosphatase activity is often strongly correlated with microbial biomass (Clarholm, 1993; Kandeler and Eder, 1993), fungal hyphal length (Häussling and Marschner, 1989) and N mineralization (Decker et al., 1999). Chitinase is a bacterial enzyme that catalyzes the breakdown of chitin, a by-product of both fungi and arthropods, into carbohydrates and inorganic N. As chitin is intermediate in its resistance to microbial metabolism, its synthesis is only induced when other, more labile C and N sources are absent (Hanzlikova and Jandera, 1993). Phenol oxidase is produced primarily by white rot fungi, and is specific for highly recalcitrant organic matter, such as lignin (Carlisle and Watkinson, 1994). Increases in phenol oxidase activity relative to other enzymes gives another indication of changes in the relative contribution of bacteria and fungi to microbial activity as well as an additional indication of the quality of the organic matter present. Thus, as a group these three enzymes supply insight into changes in the relative importance of bacteria and fungi, as well as the nature of the organic matter complex.

The enzyme activities were determined on field-moist soil using methods developed by Tabatabai (1982), as modified by Sinsabaugh (Sinsabaugh et al., 1993; Sinsabaugh and Findlay, 1995). Subsamples of

approximately 10 g of fresh soil were suspended in 120 ml of 50 mM NaOAc buffer (pH 5.0) and homogenized by rapid mechanical stirring for 90 s. To minimize sand sedimentation, stirring was continued while aliquots were withdrawn for analysis. Samples were incubated for 1 h (acid phosphatase, phenol oxidase) or 2 h (chitinase) at 20–22 °C with constant mixing. Following incubation, samples were centrifuged at $3000 \times g$ for 3 min to precipitate particulates.

Acid phosphatase (EC 3.1.3.2) and chitinase (EC 3.2.1.14) activities in soil suspensions were determined using *p*-nitrophenol (*p*NP) linked substrates: *p*NP-phosphate for acid phosphatase and *p*NP-glucosaminide for chitinase. An aliquot of 2.0 ml of the supernatant was transferred to a clean, sterile tube, and 0.1 ml of 1.0 M NaOH was added to halt enzymatic activity and facilitate color development. Prior to spectrophotometric analysis at 410 nm each sample of the supernatant was diluted with 8.0 ml of distilled, deionized water.

Phenol oxidase (EC 1.10.3.2) activity in soil suspensions was measured by oxidation of L-DOPA (L-3,4-dihydroxyphenylalanine). Following incubation, samples were centrifuged as above, and analyzed at 460 nm without dilution. Parallel oxidations using standard horseradish peroxidase (Sigma Chemical) were used to calculate the L-DOPA extinction coefficient. Time course analysis indicated that the L-DOPA concentration in both soil extracts and standards were stable for 2.5–3.0 h.

Subsamples of 7–10 g of each field moist soil sample were weighted, dried at 70 °C to constant weight (usually 48–72 h), and reweighed to determine initial moisture content. All enzyme activities were expressed per unit dry mass.

Fungal and bacterial functional diversities were analyzed by developing community-level physiological profiles using Biolog[®] assays as described in Classen et al. (2003). A 2 g of sieved soil were extracted in 50 mmol KH₂PO₄ sterile buffer, and inoculated into a sterile solution of 0.40% NaCl, 0.03% Pluronic F-56, and 0.01% Gellan Gum. The soil solutions were dispensed into Biolog[®] microtiter plates (ECO plates for bacteria and SF-N2 plates for fungi) and incubated at 20–22 °C (72 h for bacteria and 96 h for fungi). To avoid bacterial growth in SF-N2 plates, 10 µl of an antibiotic cocktail made up of 0.5 g each of streptomycin sulfate (Sigma S6501) and chlortetracycline (Sigma C4881) in 1 L of deionized water were applied when dispensing the soil solution.

Each ECO plate was read at 590 nm (for color development plus turbidity) and at 750 nm (for turbidity).

Each SF-N2 plate was read at 750 nm. Readings from the wells were first adjusted by subtracting the readings from the wells containing water. Then, to reduce the influence of differences in initial inoculum density, the responses were normalized by adding all the single substrate absorbances on a plate to give a plate total, and dividing each single substrate absorbance by the plate total. Total activity, an indicator for rates of substrate utilization for each community, was calculated as the sum of all absorbances >0.100. Substrate richness was calculated as the number of positive readings, i.e. readings >0.100.

Soil subsamples of ~15 g were extracted in 40 ml of 0.5 M K₂SO₄. Aliquots from that extracts were used for inorganic N analysis (Sims et al., 1995). After inorganic N determinations were done, 15 ml of the soil extracts were digested in 0.148 M K₂S₂O₈ for dissolved organic N determination (Amees et al., 1993). Microbial biomass N was determined by fumigating 15–20 g of fresh soil with CHCl₃ under vacuum for 24 h, followed by 0.5 M K₂SO₄ extraction and K₂S₂O₈ digestion (Tate et al., 1988). Total inorganic N was calculated as the sum of NO₃⁻ and NH₄⁺ from the initial soil samples. As K₂S₂O₈ digestions convert dissolved organic N into NO₃⁻, dissolved organic N was calculated as the difference between the NO₃⁻ content after the K₂S₂O₈ digestions and the NO₃⁻ from the initial soils. Microbial N biomass was calculated as the difference between the N content before and after CHCl₃ fumigation, using a *k*_{EN} of 0.18 (Tate et al., 1988).

2.4. Data analysis

Treatment effects were analyzed with a mixed-model analysis of covariance, with IMI as a covariate and $\alpha = 0.05$ as the significance level. The maximum likelihood method was utilized, and differences among treatments were determined by least squares analysis (SAS, 1995). In cases where there was a significant site-by-treatment interaction, we analyzed each site independently using a one-way analysis of covariance.

We then used non-parametric multidimensional scaling (NMS), a form of ordination, to help visualize the holistic responses of the microbial assemblages in the soils of these treatment units to the four alternative management strategies (McCune and Grace, 2002). The initial NMS run was unconstrained with respect to the number of axes and a full range of distance metrics was evaluated. Based on the resulting stress plots, the final ordination was done with three axes, Verimax rotation to maximize resolution, and Bray-Curtis (Sorenson) distance for distance estimation (McCune and Grace, 2002).

Table 1

Analysis of covariance of the effect of restoration treatments on selected soil and microbial parameters in two deciduous forests, using the integrated moisture index (IMI) landscape position metric as a covariate

Parameter	Treatment	Treatment-site interaction	IMI covariate
Acid phosphatase activity	$F = 4.18, p < 0.008$	$F = 1.61, p < 0.192$	$F = 0.01, p < 0.910$
Chitinase activity	$F = 1.49, p < 0.222$	$F = 1.48, p < 0.225$	$F = 0.08, p < 0.773$
Phenol oxidase activity	$F = 1.15, p < 0.332$	$F = 6.01, p < 0.001$	$F = 8.23, p < 0.005$
Summed bacterial absorbances	$F = 3.19, p < 0.029$	$F = 0.02, p < 0.995$	$F = 0.07, p < 0.794$
Summed fungal absorbances	$F = 0.77, p < 0.516$	$F = 1.62, p < 0.193$	$F = 0.88, p < 0.353$
Bacterial substrate richness	$F = 2.45, p < 0.072$	$F = 0.22, p < 0.886$	$F = 0.01, p < 0.961$
Fungal substrate richness	$F = 1.10, p < 0.354$	$F = 1.33, p < 0.272$	$F = 1.23, p < 0.273$
Soil organic C	$F = 0.18, p < 0.907$	$F = 0.08, p < 0.969$	$F = 0.42, p < 0.519$
Soil C:N ratio	$F = 3.38, p < 0.022$	$F = 0.10, p < 0.960$	$F = 0.14, p < 0.703$
Dissolved inorganic N	$F = 0.39, p < 0.763$	$F = 0.65, p < 0.585$	$F = 0.42, p < 0.517$
Dissolved organic N	$F = 0.47, p < 0.703$	$F = 0.71, p < 0.549$	$F = 0.50, p < 0.480$
Microbial biomass N	$F = 0.12, p < 0.947$	$F = 1.15, p < 0.332$	$F = 3.70, p < 0.058$

3. Results

3.1. Microbial activity and functional diversity

Acid phosphate activity varied significantly among treatments (Table 1), such that activity in the soils from the thin-only treatment exceeded those of the other three treatments (Fig. 1). In contrast, chitinase

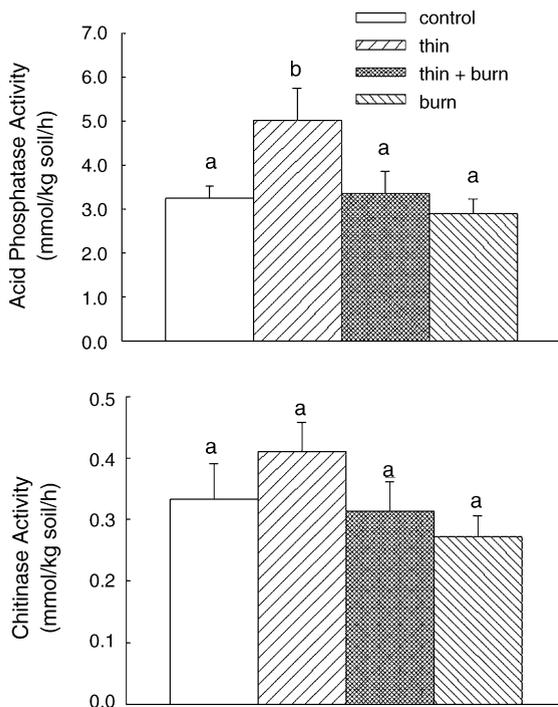


Fig. 1. Variations in acid phosphatase and chitinase activity (both in mmol/kg soil/h) in relation to four forest restoration treatments. Each histogram bar shows the mean of $N = 24$, with the standard error of the mean indicated. Histogram bars labeled with the same lower case letter were not significantly different at $p < 0.05$.

activity was not significantly affected by treatment (Table 1, Fig. 1).

Although there was no significant overall effect of treatment on phenol oxidase activity, there was a significant site-by-treatment interaction (Table 1). In Zaleski State Forest, soils from the thin-only and burn-only treatments had significantly greater phenol oxidase activity than did soils from the thin + burn treatment and controls; there was no significant effect of treatment on phenol oxidase activity in REMA (Fig. 2).

Bacterial activity was affected significantly by the restoration treatments, but fungal activity was not (Table 1). Bacterial activity was significantly greater in soil from the burn and thin + burn unit than in the control soil (Table 2). Substrate richness of bacteria and fungi were not affected significantly by treatment (Tables 1 and 2).

The NMS ordination of bacterial functional diversity resulted in a separation of the thin + burn treatment from the others along NMS axis 3, which accounted for 56.2% of the variance in substrate utilization (Fig. 3). This axis correlated negatively with carbohydrate utilization ($r = -0.937, p < 0.01$) and positively with amino acid utilization ($r = 0.776, p < 0.01$).

NMS ordination of fungal functional diversity arrayed the thin-only treatment at the lower end and the thin + burn treatment at the upper end of NMS axis 2, which accounted for 35.9% of the variance in substrate utilization. The burn treatment and control clustered around the middle of this axis (Fig. 3). Axis 2 correlated negatively with utilization of alcohols/esters ($r = -0.619, p < 0.01$), and polymers ($r = -0.775, p < 0.01$) and positively with the utilization of amines/amides ($r = 0.673, p < 0.01$) and phosphorylated C compounds ($r = 0.656, p < 0.01$).

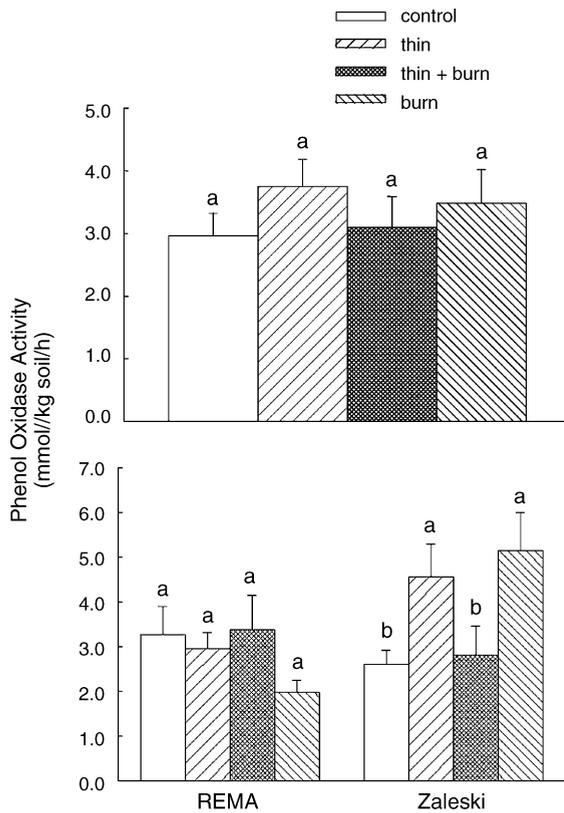


Fig. 2. Variations in phenol oxidase activity (in mmol/kg soil/h) in relation to four forest restoration treatments. The two study sites are pooled in the top panel and presented separately in the bottom panel. Each histogram bar shows the mean of $N = 24$ (top panel) and $N = 12$ (bottom panel), with the standard error of the mean indicated. Histogram bars labeled with the same lower case letter were not significantly different at $p < 0.05$.

3.2. Soil organic C and N

There were no significant differences among treatments in soil organic C quantity (Tables 1 and 2); however there were significant effects of the treatments

Table 2

Biological and chemical responses to four restoration treatments in southern Ohio forests

Parameter	Control	Thin-only	Thin + burn	Burn-only
Summed bacterial absorbances	11.57 b (1.31)	12.42 ab (1.13)	14.58 a (1.37)	14.58 a (1.36)
Summed fungal absorbances	23.79 a (2.26)	20.45 a (1.84)	24.74 a (2.00)	24.76 a (2.02)
Bacterial substrate richness	51.6 a (5.2)	51.6 a (1.8)	54.9 a (1.1)	49.8 a (3.4)
Fungal substrate richness	52.8 a (8.1)	53.2 a (4.9)	57.3 a (5.4)	48.3 a (9.7)
Soil organic C ($\mu\text{C kg}^{-1}$)	58.70 a (3.98)	58.21 a (6.47)	57.19 a (4.85)	54.13 a (4.11)
Soil C:N ratio	12.32 a (0.96)	23.10 b (5.36)	13.25 a (1.10)	12.90 a (1.40)
Dissolved inorganic N (mgN kg^{-1})	28.35 a (2.74)	24.39 a (2.89)	27.14 a (2.99)	28.05 a (2.96)
Dissolved organic N (mgN kg^{-1})	9.51 a (1.32)	8.57 a (1.64)	7.47 a (0.81)	8.47 a (1.38)
Microbial biomass N (mgN kg^{-1})	10.90 a (0.84)	10.62 a (0.92)	10.98 a (0.93)	11.46 a (1.05)

Means of $N = 24$ are given, with standard errors of the means in parentheses. Within a response parameter, means labeled with the same lower case letter were not significantly different at $p < 0.05$ following analysis of covariance.

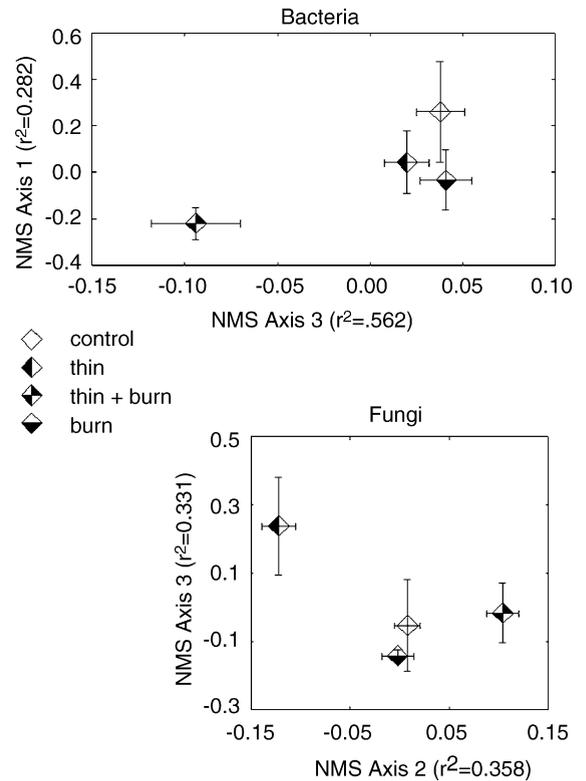


Fig. 3. NMS ordination of bacterial and fungal functional diversity, in relation to four forest restoration treatments. Standard errors of the means of $N = 24$ along each ordination axis are indicated.

on soil organic matter quality (Table 1). The C:N ratio of the soils in the thin-only treatment was significantly higher (by an average of 78%) than those of the soils given the other three treatments (Table 2).

There were no significant differences among treatments in dissolved inorganic N or dissolved organic N in the soil solution (Table 1). Overall there was approximately three times as much inorganic N as dissolved organic N in the soil solution in these sites

(Table 2). Similarly, treatments did not affect soil microbial biomass N (Tables 1 and 2), and microbial biomass N was similar in magnitude to dissolved organic N (Table 2).

4. Discussion

Our first objective was to determine whether the application of prescribed fire, thinning, and/or their combination affected soil microbial activity and microbial functional diversity in ways that would persist through the fourth growing season after the sites were thinned. This time lag was a critical aspect of our experimental design, as it permitted us to avoid the immediate and relatively short-term effects of these treatments on the forest floor and soil surface (Boerner and Brinkman, 2003, 2004), and focused the attention instead on effects which might be persistent enough to influence ecosystem function over a significant period of time. We approached this evaluation by both analyzing the activity of three enzymes and characterizing the utilization of different carbon substrates. Most studies (compare, for example Decker et al., 1999; Ratcliff et al., 2006) have employed one approach or the other, with the result that comparisons across studies become problematical.

We found that thinning of the tree canopy resulted in increased acid phosphatase activity relative to the untreated control, but neither prescribed fire nor the combination of fire and thinning had this effect, even though the second fire in these sites took place only 2–3 months prior to sampling. This result was consistent with those from a study of the same suite of restoration treatments applied to a loblolly pine-oak (*Pinus taeda*–*Quercus* spp.) ecosystem in South Carolina (Boerner et al., in press).

We observed no significant differences among restoration treatments in chitinase activity, and the effects of the treatments on phenol oxidase varied between sites. In the more fertile of the two study sites (Zaleski; data from Boerner and Brinkman, 2003) there was greater phenol oxidase activity in soils of the thin-only and burn-only treatments than in soils from the untreated control or the combined thin + burn treatment. In contrast, in the REMA site we observed no significant effect of the restoration treatments on phenol oxidase activity. As phenol oxidase is one of the enzymes involved in the degradation of lignin and other recalcitrant compounds, its increase is consistent with an increase in the utilization of low quality soil organic matter. In the thinning treatment, the deposition of a large amount of woody debris could account for this. In

the prescribed burn treatment the increase in the utilization of relatively poor organic matter could be the result of the direct combustion of the more labile organic matter fractions (e.g. leaf litter). The latter would be consistent with other studies of phenol oxidase activity and fire in this region (Boerner and Brinkman, 2003). In the units that were thinned and then burned, the fine woody debris added by the thinning treatment may have, to some extent, been consumed by the subsequent fire, with the result that the thinning and burning had offsetting effects on the organic matter complex. Based on soil enzyme assays, this suite of restoration treatments had only modest impact on microbial activity in these sites, at least during the fourth post-treatment growing season after thinning.

The second approach for evaluating effects on microbial activity was through the analysis of carbon substrate utilization using Biolog[®] plates. We found significant differences among restoration treatments in total substrate utilization by bacteria (but not fungi), and no differences in the diversity of substrates utilized by either fungi or bacteria. Total substrate utilization by bacteria was greater in those treatments where fire was applied, with or without following thinning.

The discrepancy between this result and the significant effect of the thinning treatment on microbial activity when measured as acid phosphatase activity is intriguing. It is possible that the breadth of substrates available for bacterial metabolism (but not fungal metabolism) in the Biolog[®] plates was better matched to those present in sites that had been burned than to those present in control or thinned sites. Similarly, the protocols by which the samples were prepared prior to Biolog[®] and enzyme activity assays would have minimized the impact of charcoal/black carbon in the former but not the latter. Black carbon can comprise as much as 40% of the soil organic matter in ecosystems exposed to frequent fire (Ponomarenko and Anderson, 2001), and this material may have sorptive properties that are important in regulating soil solution chemistry and biochemistry for some time after fire (Wardle et al., 1998). Finally, the difference between the Biolog[®] and enzyme assay estimates of microbial activity may have been caused by a failure of one or the other analysis to account for the activity of actinomycetes (Crawford and Sutherland, 1978; Isaac and Nair, 2005) or other groups of microbes not amenable to culturing. Although acid phosphatase is also a common constituent of plant roots and inclusion of plant root fragments in the enzyme assays might have resulted in increased activity of this enzyme, we do not think this is a viable explanation as (1) we carefully sieved all soil samples specifically to

ensure that roots were not carried over into the assays, and (2) soils from all treatments were treated identically, thus the greater activity of acid phosphatase in soils from the thinned areas should not have had greater content of fine root fragments than those from areas both thinned and burned.

The carbon substrate utilization approach also identified unique community physiological profiles in soils that received different restoration treatments. For the bacteria, samples from areas where the combination of thinning and burning were applied were clearly separated from the other three treatments. The bacteria from thin + burn areas exhibited considerably heavier carbohydrate utilization, whereas bacteria from the other treatments exhibited greater utilization of amino acids. To the degree that Biolog[®] utilization patterns represent what is happening in the real world, such a pattern might suggest that the combination of thinning and burning removed N as a limiting factor for bacterial productivity, resulting in greater use of C-rich, labile substrates in that site; in contrast, the still N-limited bacteria in the soils of the other three treatments remained dependent on amino acids.

The NMS ordinations of fungal functional diversity arrayed the treatments along a gradient that ranged from the thin-only treatment at one extreme (which correlated with heavy use of alcohols, esters and polymers) to the thin + burn treatment at the other extreme (which correlated with heavier utilization of amine/amide and phosphorylated compounds, all of which are relatively N-rich compared to alcohols and esters). With the understanding that Biolog[®] utilization patterns may not be true representations of field conditions, such a pattern might suggest relative limitation of fungal growth by C in the thin-only treatment and by N in the thin + burn treatment. The fungal functional diversities of the control and the burn-only treatment were clustered at the middle portion of the axis, suggesting a more balanced substrate utilization profile.

The second objective of our study was to evaluate whether any changes in either soil microbial activity or microbial functional diversity could be linked to changes in organic matter C quantity/quality or soil N status. We found no significant differences in soil organic C content among the four treatment alternatives, though we did observe a significant increase in C: N ratio (i.e. a decrease in soil organic matter quality) in the thin-only treatment. We consider this increase in C: N ratio to be related to accumulations on the forest floor of woody remains from the trees cut during the thinning treatment, and this result is consistent with other similar studies (Boerner et al., *in press*).

Soil N content, measured as dissolved organic N, dissolved inorganic N, and microbial biomass N did not vary significantly among treatments. These findings agree with studies done one month after the application of dormant season prescribed fire in sites similar to ours (Boerner et al., 2000b). In our study sites, dissolved inorganic N was considerably greater than was dissolved organic N in all treatments. Similar situations have been reported in highly productive systems, such as limed, fertilized agricultural grasslands (Bardgett et al., 2003), whereas the opposite condition (i.e. dissolved organic N > dissolved inorganic N) is common in less productive ecosystems, such as coastal and high elevation forests (Hannam and Prescott, 2003) and over-exploited grasslands (Bardgett et al., 2003). In those systems, organic N is recognized as an important source of N for direct plant uptake (Lipson and Nasholm, 2001). Even though these mixed-oak forests have been historically considered N-limited ecosystems, atmospheric N deposition over the last 50 years might be causing a shift towards N-saturated conditions (Boerner and Sutherland, 1997; Vitousek et al., 1997; Aber et al., 1998; Morris and Boerner, 1998; Peterjohn et al., 1999; Boerner et al., 2004).

In our study sites microbial N biomass was similar in magnitude to dissolved organic N. Consistent with what we noted in the previous paragraph, this is likely the result of stronger microbial N immobilization, either through microbial biomass production or mycorrhizal assimilation and exudation, or both (DeMars and Boerner, 1995; Aber et al., 1998; Knorr et al., 2003). Greater microbial N immobilization would be expected in N-limited ecosystems when N deposition increases, as has been demonstrated in conifer forests along a N deposition gradient in northwestern Europe (Gundersen et al., 1998).

To meet the restoration goals established at the beginning of the FFS study (Yaussy, 2001), a successful restoration treatment would result in soil biological and chemical conditions “adequate to sustain a mixed-oak ecosystem.” The criteria we have developed to determine if these treatments are accomplishing that goal are: no loss of microbial functional diversity, a reduction in overall microbial activity, and an increase in relatively recalcitrant soil organic matter. Four years after the application of three alternative, manipulative restoration treatments and several months after a second application of prescribed fire, the success at meeting these goals has been mixed. There has been no observable loss of microbial functional diversity, though treatment-specific shifts in microbial functional diversity have taken place. No significant change in soil

organic C content could be resolved and only one treatment (thin-only) resulted in a decrease in organic matter quality. Whether microbial activity, as a whole, increased or remained the same in a given treatment alternative depended on which method one relied upon; however, neither of the methods suggested an overall reduction in microbial activity.

Acknowledgments

This is publication 110 of the Fire and Fire Surrogates Network Project, funded by the Joint Fire Sciences Program. We thank Jennifer Brinkman, Jessica Miesel, Jianjun Huang, and Philip Ruse for assistance in the field and laboratory, and Mariano Iannuzzi for editorial assistance.

References

- Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. *Bioscience* 39, 378–386.
- Aber, J.D., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems. *Bioscience* 48, 921–934.
- Amees, J.J., Axler, R.P., Owen, C.J., 1993. Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters. *Am. Environ. Lab.* 10, 1–11.
- Bardgett, R.D., Streeter, T.C., Bol, R., 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology* 84, 1277–1287.
- Boerner, R.E.J., Sutherland, E.K., 1997. The chemical characteristics of soil in control and experimentally thinned plots in mesic oak forest along a historical desposition gradient. *Appl. Soil Ecol.* 7, 59–71.
- Boerner, R.E.J., Brinkman, J.A., 2003. Fire frequency and soil enzyme activity in southern Ohio oak-hickory forests. *Appl. Soil Ecol.* 23, 137–146.
- Boerner, R.E.J., Sutherland, E.K., 2003. Physiography, geology, and soil classification. In: Sutherland, E.K., Hutchinson, T.F. (Eds.), *Characteristics of Mixed-Oak Forest Ecosystems in Southern Ohio Prior to the Reintroduction of fire*. USDA General Technical Report NE-299, Newtown Square, PA, pp. 43–46.
- Boerner, R.E.J., Brinkman, J.A., 2004. Spatial, temporal and restoration treatments effects on soil resources in Ohio hardwood forests. In: Yaussy, D.A., Hix, D., Goebel, P.C., Long, R.B. (Eds.), *Proceedings of the 14th Central Harwood Forest Conference*. USDA General Technical Report NE-316, Newtown Square, PA, pp. 251–254.
- Boerner, R.E.J., Brinkman, J.A., 2005. Effects of prescribed fire at two frequencies on N dynamics at two spatial scales in mixed-oak forests. *Fire Ecol.* 1, 28–49.
- Boerner, R.E.J., Decker, K.L.M., Sutherland, E.K., 2000a. Prescribed burning effects on soil enzyme activity in a southern Ohio hardwood forest: a landscape-scale analysis. *Soil Biol. Biochem.* 32, 899–908.
- Boerner, R.E.J., Morris, S.J., Sutherland, E.K., Hutchinson, T.F., 2000b. Spatial variability in soil nitrogen dynamics after prescribed burning in Ohio mixed-oak forests. *Landsc. Ecol.* 15, 425–439.
- Boerner, R.E.J., Brinkman, J.A., Sutherland, E.K., 2004. Effect of fire at two frequencies on forest soils in a nitrogen-enriched landscape. *Can. J. For. Res.* 34, 609–618.
- Boerner, R.E.J., Waldrop, T.A., Shelburne, V.B., in press. Wildfire mitigation strategies affect microbial activity and soil organic matter in loblolly pine (*Pinus taeda* L.) forests. *Can. J. For. Res.*
- Carlisle, M.J., Watkinson, S.C., 1994. *The Fungi*. Academic Press, NY, 482 pp.
- Clarholm, M., 1993. Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizer. *Biol. Fertil. Soils* 8, 128–133.
- Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., Hart, S.C., 2003. Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. *FEMS Microbiol. Ecol.* 4, 319–328.
- Crawford, D.L., Sutherland, J.B., 1978. The role of actinomycetes in the decomposition of lignocellulose. In: Underkofler, L.A. (Ed.), *Proceedings of the 35th General Meeting of the Society for Industrial Microbiology*, Society for Industrial Microbiology, pp. 143–151.
- Decker, K.L.M., Boerner, R.E.J., Morris, S.J., 1999. Scale-dependent patterns of soil enzyme activity in a forested landscape. *Can. J. For. Res.* 29, 232–241.
- Delcourt, P.A., Delcourt, H.R., 1997. Pre-columbian native American use of fire on southern Appalachian landscapes. *Cons. Biol.* 11, 1010–1014.
- DeMars, B.G., Boerner, R.E.J., 1995. Mycorrhizal dynamics of three woodland herbs of contrasting phenology along topographic gradients. *Am. J. Bot.* 82, 1426–1431.
- Gundersen, P., Emmett, B.A., Kjonass, O.J., Koopmans, C.J., Tietema, A., 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *For. Ecol. Manage.* 101, 37–55.
- Hanzlikova, A., Jandera, A., 1993. Chitinase and changes of microbial community in soil. *Folia Microbiol.* 38, 159–160.
- Hannam, K.D., Prescott, C.E., 2003. Soluble organic nitrogen in forests and adjacent clearcuts in British Columbia, Canada. *Can. J. For. Res.* 33, 1709–1718.
- Harmon, M.E., 1982. Fire history of the westernmost portion of the Great Smoky Mountains National Park. *Bull. Torrey Bot. Club* 109, 74–79.
- Häussling, M., Marschner, H., 1989. Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce (*Picea abies* (L.) Karst.) trees. *Biol. Fertil. Soils* 8, 128–133.
- Hutchinson, T.F., Boerner, R.E.J., Sutherland, S., Sutherland, E.K., Ortt, M., Iverson, L.R., 2005a. Prescribed fire effects on the herbaceous layer of mixed-oak forests, Ohio, USA. *Can. J. For. Res.* 35, 877–890.
- Hutchinson, T.F., Sutherland, E.K., Yaussy, D.A., 2005b. Effects of repeated prescribed fires on the structure, composition, and regeneration of mixed-oak forests in Ohio. *For. Ecol. Manage.* 218, 210–228.
- Isaac, S.R., Nair, M.A., 2005. Biodegradation of leaf litter in the warm humid tropics of Kerala, India. *Soil Biol. Biochem.* 37, 1656–1664.
- Iverson, L.R., Dale, M.E., Scott, C.T., Prasad, A., 1997. A GIS-derived integrated moisture index to predict forest composition and productivity of Ohio forests (USA). *Landsc. Ecol.* 12, 331–348.
- Iverson, L.R., Yaussy, D.A., Rebeck, J., Hutchinson, T.F., Long, R.P., McCarthy, B.C., Riccardi, C.L., 2003. Spatial and temporal

- distribution of fire temperatures from prescribed fires in the mixed oak forests of southern Ohio. In: Van Sambeek, J.W., Dawson, J.O., Ponder, Jr., F., Loewenstein, E.F., Fralish, J.S. (Eds.), *Proceedings of the 13th Central Hardwood Forest Conference*, USDA General Technical Report NC-234, St. Paul, MN, pp. 293–294.
- Iverson, L.R., Yaussy, D.A., Rebbeck, J., Hutchinson, T.F., Long, R.P., Prasad, A., 2004. A comparison of thermocouples and temperature paints to monitor spatial and temporal characteristics of landscape-scale prescribed fires. *Int. J. Wildl. Fire* 13, 1–12.
- Kandeler, E., Eder, G., 1993. Effect of cattle slurry in grasslands on microbial biomass and on activities of various enzymes. *Biol. Fertil. Soils* 16, 249–254.
- Knorr, M.A., Boerner, R.E.J., Rillig, M., 2003. Glomalin content of forest soils in relation to fire frequency and landscape position. *Mycorrhiza* 13, 205–210.
- Lemaster, D.D., Gilmore, G., 1993. *The soils of Vinton County, OH*. Ohio Department of Natural Resources, Columbus, OH, 36 pp.
- Lipson, S., Nasholm, T., 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* 128, 305–316.
- McCune, B., Grace, J.B., 2002. *Analysis of Ecological Communities*. MJM Software Design, Gleneden Beach, OR, 300 pp.
- Morris, S.J., Boerner, R.E.J., 1998. Landscape patterns of nitrogen mineralization and nitrification in southern Ohio hardwood forests. *Landsc. Ecol.* 13, 215–224.
- Morris, S.J., Boerner, R.E.J., 1999. Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils. I. Scale dependency and landscape patterns. *Soil Biol. Biochem.* 31, 887–902.
- Peterjohn, W.T., Foster, C.J., Christ, M.J., Adams, M.B., 1999. Patterns of nitrogen availability within a forested watershed exhibiting symptoms of nitrogen saturation. *For. Ecol. Manage.* 119, 247–257.
- Ponomarenko, E.V., Anderson, D.W., 2001. Importance of charred organic matter in black Chernozem soils of Saskatchewan. *Can. J. Soil Sci.* 81, 285–297.
- Ratcliff, A.W., Busse, M.D., Shestak, C.J., 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. *Appl. Soil Ecol.* 34 (2–3), 114–124.
- SAS, 1995. *Statistical Analysis System, User's Guide, Version 9.1e*. Cary, NC (on-line documentation, <http://www.sas.com>).
- Sims, G.K., Ellsworth, T.R., Mulvaney, R.L., 1995. Microscale determination of inorganic N in water and soil extracts. *Commun. Soil Sci. Plant Anal.* 26, 303–316.
- Sinsabaugh, R.L., Findlay, S., 1995. Microbial production, enzyme activity and carbon turnover in surface sediments of the Hudson River estuary. *Microbiol. Ecol.* 30, 127–141.
- Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., McLaugherty, C.A., Rayburn, L., Repert, D., Weiland, T., 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology* 74, 1586–1593.
- Speir, T.W., Ross, D.J., 1975. Effects of storage on the activities of protease, urease, phosphatase, and sulphatase in three soils under pasture. *N. Z. J. Sci.* 18, 231–237.
- Sutherland, E.K., 1997. The history of fire in a southern Ohio second-growth mixed-oak forest. In: Pallardy, S.G., Cecich, R.A., Garret, H.E., Johnson, P.S. (Eds.), *Proceedings of the 11th Central Hardwood Forest Conference*. USDA General Technical Report NC-188, St. Paul, MN, pp. 172–183.
- Sutherland, E.K., Hutchinson, T.F., 2003. Characteristics of Mixed-Oak Forest Ecosystems in Southern Ohio Prior to the Reintroduction of Fire. USDA General Technical Report NE-299, Newtown Square, PA, 159 pp.
- Sutherland, E.K., Hutchinson, T.F., Yaussy, D.A., 2003. Introduction, study area description, and experimental design. In: Sutherland, E.K., Hutchinson, T.F. (Eds.), *Characteristics of Mixed-Oak Forest Ecosystems in Southern Ohio Prior to the Reintroduction of Fire*. USDA. General Technical Report NE-299, Newtown Square, PA, pp. 1–16.
- Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L. (Ed.), *Methods of Soil Analysis*. American Society for Agronomy, Madison, WI, pp. 903–948.
- Tate, K.R., Ross, D.J., Feltham, C.W., 1988. A direct method to estimate soil microbial C. effects of experimental variables and some different calibration procedures. *Soil Biol. Biochem.* 20, 329–335.
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.M., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7, 737–750.
- Vogt, K.A., Publicover, D.A., Vogt, D.J., 1991. A critique of the role of ectomycorrhizae in forest ecosystems. *Agric. Ecosys. Environ.* 35, 171–190.
- Wardle, D.A., Zackrisson, O., Nilsson, M.C., 1998. The charcoal effect in Boreal forests: mechanisms and ecological consequences. *Oecologia* 115, 419–426.
- Yaussy, D.A., 2001. Study plan and establishment report: consequences of fire and fire surrogates treatments: the Ohio Hills site. In: McIver, J. (Ed.), *FFS Study: A National Study on the Consequences of Fire and Fire Surrogate Treatments*. USDA Pacific Northwest Research Station, LaGrande, OR, pp. 450–500, 583 pp.
- Yaussy, D.A., Hutchinson, T.F., Sutherland, E.K., 2003. Structure, composition, and condition of overstory trees. In: Sutherland, E.K., Hutchinson, T.F. (Eds.), *Characteristics of Mixed-Oak Forest Ecosystems in Southern Ohio Prior to the Reintroduction of Fire*. USDA General Technical Report NE-299, Newtown Square, PA, pp. 99–112.