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Microbial properties and litter and soil nutrients after two prescribed fires in developing savannas in an upland Missouri Ozark Forest

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ABSTRACT

On some landscapes periodic fire may be necessary to develop and maintain oak-dominated savannas. We studied the effects of two annual prescribed burns to determine their effect on microbial activity and soil and litter nutrients 1 year after the last burn. Surface litter and soil from the upper 0–5 cm soil layer in three developing savannas (oak-hickory, *Quercus-Carya*), oak-hickory-pine (*Quercus-Carya-Pinus*), and pine (*Pinus*) were collected one year after the second of two annual prescribed burns. Surface litter was analyzed for nutrients and soil was analyzed for phospholipid fatty acids (PLFAs) and nutrients. Surface litter chemistry differed across the three savannas for potassium (K) and boron (B), being significantly ($P < 0.05$) higher for unburned forest than for burned forest. Among savannas, only sulfur (S) was higher for the pine savanna and B for the oak-hickory savanna, both were higher for unburned forest than for burned forest. For soil, calcium (Ca) and B differed across savannas, being higher for burned forest than for unburned forest. Among savannas, soil pH, Ca, and B concentrations were higher in soil from burned forest than from unburned forest. Total PLFA differed among savannas, but was not affected by burning treatments. However, the amounts of biomarkers for Gram-positive and Gram-negative bacteria were higher while the amount of biomarker for fungal PLFA was lower for burned forest than for unburned forest. Our results indicate that the two annual prescribed burns moderately affected PLFA microbial community structure and litter and soil nutrient concentrations. However, the long-term effects of fire on these study sites are not known and merit further study.

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1. Introduction

The use of fire for developing and maintaining tree savanna structure in temperate forest ecosystems has drawn considerable interest (White, 1986; Nuzzo, 1986; Blake and Schuette, 2000). Savannas declined by more than 99.9% in the Midwestern United States during the last 100 years primarily because of fire suppression and land use changes (Nuzzo, 1986). Disturbances caused by fire can limit successful tree seedling germination, establishment, and growth to mature size classes (Hochberg et al., 1994; Higgins et al., 2000). Perhaps the term "disequilibrium" best describes savannas in the Midwest because the long-term coexistence of grasses and trees is not possible and the system would converge to a wooded or grassy state following the cessation of fires and/or herbivory (Sankaran et al., 2004). Hence, disturbances are essential for the persistence of trees and grasses in this region.

While fire-associated changes in vegetation are within the rim of predictability, fire-induced changes in soil nutrients are sporadic, possibly due to the natural variability of fire. Such changes may be negligible (Knighton, 1977; Eivazi and Bryan, 1996), detrimental but eventually replaced (McKee and Lewis, 1983), or positive by increasing available nutrients (Vance and Henderson, 1984). Some authors (Dhillion et al., 1988; Dobrowolski et al., 1992) suggest that the major effects of fire may be due to litter removal and associated changes in the microclimate rather than to direct soil heating or fire-induced nutrient changes. The changes exerted by heat on chemical properties of humus have also been shown to cause changes in microbial composition (Pietikäinen et al., 2000). Microorganisms are the nutrient cyclers of forest ecosystems, decomposing all forms of detritus and releasing vital nutrients back into the system (Alexander, 1991; Atlas and Bartha, 1993; Lloyd, 1993). Thus, microbial activity is essential to the biological determinant of forest site quality. Wells et al. (1979) argued that reduced competition between plants and

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microorganisms following fire may be a better explanation for increases in plant biomass than the incorporation of ash into the soil. Different types of fire effects on the soil system have been examined in a wide range of previous studies and it has become clear that many variables need to be considered in order to understand fire's impact on the physical, chemical, and biological soil properties (Úbeda et al., 2005). Further, considering all the variables in natural systems, Neary et al. (1999) stated earlier that the impacts of fire on belowground systems can be highly variable and may not be predictable. However, in order to manage and preserve landscapes requiring fire effectively, it is important to challenge this concept in attempting to provide a better understanding of fire impacts on soil properties. Also, because of the low nutrient concentration in most of the upland forest soils in the Ozarks (King, 1997; Kaczmarek et al., 1995), fire could contribute to losses of nutrients from this ecosystem, significantly affecting the nutrient cycling process and soil microbial composition and activity.

Prescribed fires normally burn at low intensity and if the organic layer is moist should ensure that the temperature a few centimeters below the soil surface is kept low, however some changes in the soil system may be unavoidable (Úbeda et al., 2005). The objective of this study was to determine the effects of two annual prescribed burns on microbial activity and soil and litter nutrients 1 year after the last burn and, therefore, to provide insight as to whether this practice can be used sustainably as a management tool without striking effects on belowground systems in this Ozark forest.

2. Methods and materials

2.1. Site description and prescribed fire

We studied an oak-hickory (*Quercus-Carya*), oak-hickory-pine (*Quercus-Carya-Pinus*), and pine (*Pinus*) developing savanna on the Sinkin Experimental Forest in south central Missouri in the Ozark Highlands (lat. 37.5°N, long. 91.2°W). The soil is the Clarksville series, a Typic Paleudult developed from Ordovician and Cambrian dolomites, cherty dolomites, and some sandstones of the Gasconade formation (Gott, 1975). The oak-hickory savanna had an average tree age of 95 year; it consisted primarily of black oak (*Quercus velutina* Lam.), with average tree height of 18.2–19.8 m at age of 50 years for black oak. The 1.1-ha area was on the upper slope with a western aspect. The pretreatment overstory was composed primarily of black oak, white oak (*Q. alba* L.), post oak (*Q. stellata* Wagenh. var.), and hickory (*Carya* spp.) with midstory and understory composed primarily of black gum (*Nyssa sylvatica* var. Marsh.), dogwood (*Cornus racemosa* Lam.), and sassafras (*Sassafras albidum* (Nutt.) Nees). Stand density was approximately 24.8 m^2 ha^{-1} basal area.

The oak-hickory-pine savanna was 1.6 ha in size and had an average tree age of 85 years. It consisted primarily of scarlet oak (*Q. coccinea* Muenchh.), black oak, white oak, hickory, and shortleaf pine (*Pinus echinata* Mill.), with average tree height of 18.2 m at age of 50 years for black oak. Understory trees were principally hickory, black gum, and dogwood. Stand density was approximately 18.4 m^2 ha^{-1} basal area. Because of the relative low density of this stand, there was a well-developed understory and midstory of oak advance reproduction and woody shrubs.

The pine savanna was 1.2 ha in size and had an average tree age of 80 years. It had an overstory of principally shortleaf pine, and an average tree height of 19.8 m for black oak at age of 50 years. Trees in the understory included white oak, post oak, black oak, scarlet oak, hickory, black gum, and dogwood. There were few understory trees in this stand compared to the other two, probably due to the relatively high density (basal area of 35.1 m^2 ha^{-1}).

The prescription for the savannas was for the overstory structure to have approximately 8 m^2 ha^{-2} of large, well-spaced trees. This density would equate to approximately 50 percent canopy closure if the trees had been open grown (Law et al., 1994). However, since the initial stands were fairly high density, closed canopy stands, the crowns of the residual stems were less developed than open grown trees and the resulting canopy closure was reduced. As the residual overstory trees expand their crowns, adjustments to stand density will be made in future years to ensure that canopy closure remains within the 10–50% range cited as typical for Missouri Ozark savanna systems (Nuzzo, 1986).

Following pretreatment inventory, the three study sites were thinned to 9.2 m^2 ha^{-1} of basal area in all stems $\geq$16 cm in diameter at 1.4 m above the soil surface. Leave trees were selected based on canopy dominance, species, vigor, and spacing. Thinning was done by horse logging, which removed trees with almost no residual stand damage; the main skid trail resembled a back-country hiking trail.

Prescribed burns were conducted in April for two consecutive years. However, there was difficulty in getting the fire to carry across the stands during the second-year burn because of low fuel loads and discontinuous fuels.

2.2. Sample collection, preparation, and analyses

Soil and litter samples were collected 1 year after the second burn in early summer for chemical and phospholipid fatty acid analyses. Because we did not collect pretreatment samples, we compared each savanna to an adjacent part of the savanna that was not burned (control). From each savanna, a total of 36 samples (18 per burn treatment) were collected at equally spaced locations in each treatment plot (burned) and adjacent untreated plot (unburned) along a 75-m transect that bisected each burned plot and extended an equal distance into the adjacent unburned plot. Samples were collected 2 m apart along transect lines having a north–south orientation. For litter, a .09-m^2 metal frame was placed on the ground at each sampling point, and all litter within the frame was collected down to the mineral soil, put in plastic bags, and stored in an ice chest. Soil was carefully collected at the same locations where litter was collected from the surface to a depth of 5 cm using a garden spatula to remove an approximate area of mineral soil 13 cm × 13 cm. Both soil and litter samples from each of three locations along that portion of a transect line for a burn treatment were composited after collecting three consecutive samples, mixed thoroughly, and a sub-sample withdrawn, placed in plastic bags, stored in an ice chest before being transported to the lab, yielding a total of 12 sub-samples for each savanna. Samples were prepared and analyzed for chemical properties. Litter samples were ovendried at 65 °C for 72 h, ground in a Wiley mill to pass through a 2-mm mesh sieve and analyzed by the Agriculture Diagnostic Laboratory at the University of Arkansas. Total nitrogen (N) in the litter was determined by dry combustion (Pella, 1990). For other nutrients in the litter, the Mehlich III extraction (Mehlich, 1984) was analyzed with an inductively coupled plasma emission spectroscopy (Perkin-Elmer Corp, 1983).

Soil samples were immediately transferred to a refrigerator in the laboratory and within 24 h sieved using a 2-mm sieve, and separated into two lots (one for lipid and PLFA analyses and the other for nutrient analyses). Soil samples for nutrient analyses were ovendried at 65 °C for 72 h, sieved again using a 2-mm mesh sieve, and analyzed by the Agriculture Diagnostic Laboratory at the University of Arkansas. Carbon (C) was by combustion (Nelson and Sommers, 1982), pH and electrical conductivity (EC) in 1:2 (soil to water, w/v) suspension after 1 h by electrode, and other nutrients

by Melich III extract using an inductively coupled plasma emission spectroscopy (Perkin-Elmer Corp, 1983).

Fresh, not dried, soil samples used for lipid and PLFA determinations were shipped overnight-delivery to Microbial Insights, Inc. (Rockford, TN) for analysis. Lipids were analyzed as described by Bligh and Dyer (1959) and White et al. (1979). Extractions were performed using one-phase chloroform-methanol-buffer extractant. Lipids were recovered, dissolved in chloroform, and fractionated in disposable silicic acid columns into neutral-, glyco-, and polar-lipid fractions. The polar-lipid fraction was transesterified with mild alkali to recover the PLFA as methyl esters in hexane. PLFA were analyzed by gas chromatography with peak conformation performed by electron impact mass spectrometry (GC/MS) (Guckert et al., 1985). The lipid-extracted residue was subjected to strong acid hydrolysis, and the hydroxy fatty acids of the lipopolysaccharide (LPS) were analyzed by GC/MS. PLFA nomenclature follows the pattern of A:BωC. The "A" position identifies the total number of C atoms in the fatty acid. Position B is the number of double bonds from the aliphatic (ω) end of the molecule. Position "C" designates the C atom from the aliphatic end before the double bond. This is followed by a "c" for *cis* or a "t" for *trans* configuration. The prefixes i, a, or br indicate *iso*, *anteiso*, or *branched* (position undetermined). Mid-chain branching is noted by "me," and cyclopropyl fatty acids are indicated with the prefix "cy" with the ring position indicated from the aliphatic end of the molecule (Ringelberg et al., 1989). For the purpose of community structure analyses, PLFAs were divided into biomarkers for six different groups: terminally branched saturates (i14:0, i15:0, α15:0, i16:0, i17:0, α17:0) (Kaneda, 1991; O'Leary and Wilkinson, 1988), monounsaturates (15:1ω6c, 16:1ω9c, 16:1ω7c, 16:1ω7t, 16:1ω5c, 17:1ω6c, cy17:0, 17:1α, 18:1ω7c, 18:1ω6c, 18:1ω5c, 19:1ω6c, cy19:0), (Parke and Taylor, 1983; Wilkinson, 1988), branched monounsaturates (br15:1α, i16:1α, i16:1b, br16:1α, br16:1b, i17:1ω7c, br19:1α, br19:1b) (Boon et al., 1977; Edlund et al., 1985), mid-chain-branched saturates (br15:0α, br15:0b, br16:0, 10me16:0, 11me16:0, 12me16:0, br17:0α, 10me17:0, 11me17:0, 12me17:0/18:2, 10me18:0, 12me18:0) (Dowling et al., 1986, 1988), normal saturates (14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0) (Pinkart et al., 2000), and eukaryotes or fungal (18:2α, 18:2ω6, 18:3ω3, 18:1ω9c, 20:4ω6, 20:5ω3, 20:1ω11c, 20:1ω9c, 20:1ω7c, 22:5ω6, 22:6ω3, 22:1ω9c) (Erwin, 1973). The relative amount of each group of the total PLFA pool was determined from the sum of the amount of all the PLFA markers for a particular group divided by the total amount of PLFA in the sample. The metabolic status ratios, consisting of the starvation index, was determined from the sum of the ratios of cy17:0 to 16:1ω7c and cy19:0 to 18:1ω7c (Kief et al., 1994) and the membrane stress index, was calculated from the ratio of 16:1ω7t to 16:1ω7c. A factor of 20,000 cells $pmol^{-1}$ of PLFA, developed by Microbial Insight, was used to calculate the number of viable organisms.

2.3. Statistical analyses

The three savannas were physically separated by intervening forest stands with replicated sampling within savannas for two treatments (burned and unburned). There was no true replication of savannas neither was there pretreatment data collected. Thus, the use of inferential statistics to compare savannas and treatments are subject to the error of pseudo-replication (Hurlbert, 1984).

Litter and soil nutrient values for the three savannas that were treated (burned) and not treated (unburned) were compared statistically by analysis of variance (ANOVA) using PROC GLM procedure in SAS Version 8.2 (SAS Institute, Cary, NC). The PLFA compositions and their structure group abundances were also subjected to an ANOVA. Tukey's test for mean separation was used where significant differences ($P < 0.05$) were indicated. Data were not transformed.

3. Results

3.1. Litter and soil nutrients

Surface litter nutrient concentrations differed across the three savannas for potassium (K) and boron (B) (Table 1), being significantly ($P < 0.05$) higher for unburned forest than for burned forest. Among savannas, the only litter nutrient other than K or B that was significantly higher in unburned forest than in burned forest was sulfur (S) in the pine savanna. With few exceptions, the concentration of surface litter nutrients tended to be higher for unburned forests than for burned forest for individual savannas. The concentration of no litter nutrients were significantly higher for burned forest than for unburned forest.

For soil, only Ca and B differed significantly across savannas, being higher in burned forest than in unburned forest (Table 2). Among savannas, soil pH, Ca, and B were higher for the burned forest than for the unburned forest. Iron, however, which was different between fire treatments for the pine savanna, was higher in the unburned forest than in the burned forest, while soil P was significantly higher in soil from burned forest than in soil from unburned forest for the oak-hickory savanna.

3.2. Total PLFA, community structural groups, and individual PLFAs

Analyzing the microbial community by quantifying fatty acids present in phospholipids is useful to estimate soil microbial biomass and to gain insight into the diversity of microbial community structure in response to site manipulations (Guckert et al., 1985; Zelles, 1997) such as fire (Blankenship and Arthur, 1999). In our study, total PLFA biomass differed significantly ($P < 0.05$) among savannas; being highest in the oak-hickory savanna than in the pine savanna suggesting that conditions in the pine savanna were less favorable for microbial growth (Tables 3 and 4). However, total PLFA was not affected by burning treatments in any of the savannas (Tables 3 and 4). Among community structural groups, only the mean percent of terminally branched saturates, which are general indicators of gram-positive or anaerobic gram-negative bacteria, differed among savannas; being lowest in the pine savanna (Tables 3 and 4). Terminally branched saturates, monounsaturates, which are general indicators of gram-negative sulfate-reducing bacteria, and branched saturates, anaerobic metal reducing bacteria, were greater in the burned forest than in the unburned forest (Table 4). Neither mid-chain-branched saturates and polyunsaturates, indicators of sulfate-reducing bacteria decreased for burned forest nor normal saturates, which are common to a wide range of microorganisms, were significantly affected by burning. But burned forest had significantly lower eukaryotes or fungal biomarkers, indicators of fungal and higher organism biomass than unburned forest.

The concentration of individual PLFA biomarkers making up terminally branched saturates was highest in the oak-hickory savanna followed by oak-hickory-pine and pine savannas (Table 5). In general, these biomarkers were consistently higher in burned forest soil than in unburned forest soil for all savannas. However, only in the pine savanna was all PLFA biomarkers in this group higher in burned forest soil than in unburned forest soil. Individual PLFA biomarkers i17:0 and a17:0 were higher for the burned forest soil for all savannas, but i14:0 and i15:0 were higher in burned than in unburned forest soil for the oak-hickory-pine and pine savannas but not for the oak-hickory savanna.

Table 1
Litter nutrients in oak-hickory, oak-hickory-shortleaf pine, and pine savannas for burned and unburned control treatments ($n = 6$).

Savanna	Fire	Litter nutrient (mg/kg)											
		N	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
Oak-hickory	Burned	1.1 (0.2)[1]	565.7 (99)	812.7**b**[2] (120)	12860 (3276)	1051.3 (197)	907.3 (210)	53.0 (12)	3670 (669)	3647 (1105)	48.4 (9)	15.1 (2)	10.6**b** (1)
	Unburned	1.0 (0.3)	700.7 (171)	1014.5**a** (127)	13068 (8747)	1353.8 (321)	1009.7 (271)	60.7 (15)	4013 (2186)	2784 (1070)	40.8 (9)	14.8 (2)	13.3**a** (0.7)
Oak-hickory-pine	Burned	1.1 (0.2)	579.6 (59)	830.4 (64)	14044 (2527)	975.6 (166)	1006.4 (240)	63.8 (16)	2919 (691)	2287 (750)	54.3 (18)	16.5 (3)	10.1 (1)
	Unburned	1.2 (0.3)	611.3 (131)	908.8 (167)	9565 (4086)	1094.3 (664)	1111.2 (246)	54.2 (13)	3360 (3036)	1970 (1333)	41.7 (22)	13.6 (2)	11.3 (4)
Pine	Burned	0.8 (0.2)	485.8 (108)	686.3**b** (58)	9075 (2158)	829.8 (141)	717.7**b** (152)	57.0 (14)	3698 (1176)	1742 (890)	47.7 (17)	13.6 (3)	9.1 (1)
	Unburned	1.0 (0.1)	525.4 (55)	793.0**a** (88)	9522 (3796)	827.2 (199)	919.0**a** (128)	52.0 (10)	3558 (1305)	1635 (681)	35.4 (9)	11.9 (1)	11.0 (3)
Across savannas	Burned	1.0	541.6	773.3**a**	11872	950.9	869.5	57.6	3459	2575	49.9	14.9	9.9**a**
	Unburned	1.1	617.6	912.1**b**	10789	1107.4	1018.8	55.8	3649	2159	39.5	13.5	11.9**b**

[1] Values in parentheses are mean ± standard deviations.
[2] Values for a nutrient within a savanna followed by a different letter are significant at the 0.05 level.

Table 2
Soil nutrients in oak-hickory, oak-hickory-shortleaf pine, and pine savannas for burned and unburned treatments ($n = 6$).

Savanna	Fire	Soil nutrient mg/kg													
		pH	EC	C%	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
Oak-hickory	Burned	5.5**a**[1] (0.4)[2]	76.0 (21)	3.8 (1.7)	66.1**a** (14)	250.7 (133)	2198.6**a** (1471)	194.7 (81)	20.1 (5)	41.1 (15)	211.1 (51)	481.6 (191)	9.8 (5)	3.7 (1)	0.7**a** (0.3)
	Unburned	4.9**b** (0.2)	85.0 (19)	4.7 (1)	36.2**b** (21)	191.5 (62)	1435.3**b** (798)	269.2 (110)	22.8 (4)	37.0 (11)	238.2 (60)	393.9 (111)	11.5 (3)	3.3 (1)	0.4**b** (0.1)
Oak-hickory-pine	Burned	5.6**a** (1)	94.7 (44)	5.9 (2)	42.2 (23)	227.7 (99)	4876.5**a** (5411)	242.7 (101)	25.9 (8)	55.1 (39)	270.5 (113)	189.3 (160)	9.0 (4)	3.3 (4)	0.9**a** (0.5)
	Unburned	4.4**b** (1)	89.3 (26)	4.6 (2)	21.5 (10)	174.7 (48)	943.0 **b** (697)	183.7 (138)	25.0 (3)	35.1 (14)	322.4 (125)	216.4 (129)	7.3 (2)	2.4 (1)	0.4**b** (0.1)
Pine	Burned	5.5**a** (0.3)	75.0 (16)	4.3 (2)	27.2 (10)	151.2 (42)	2610.2**a** (1257)	179.6 (71)	20.3 (4)	39.2 (10)	222.5**b** (53)	272.5 (72)	9.1 (4)	2.8 (36)	0.9 (0.1)
	Unburned	4.9**b** (0.4)	99.3 (30)	3.6 (1)	20.0 (5)	175.1 (49)	1269.3**b** (841)	152.7 (64)	25.9 (6)	33.0 (11)	303.0**a** (53)	308.4 (109)	8.9 (3)	2.8 (29)	0.8 (0.1)
Across savannas	Burned	5.5	81.9	4.7	31.4	187.4	2882.4**a**	183.6	19.6	40.3	209.6	288.8	8.3	2.9	0.8**a**
	Unburned	4.7	91.2	4.3	32.0	161.1	1085.6**b**	180.2	21.9	31.3	257.0	273.4	8.3	2.6	0.5**b**

[1] values for a nutrient within a savanna followed by a different letter are significant at the 0.05 level.
[2] Values in parentheses are mean ± standard deviations.

Table 3
Analysis of variance (*P*-values) for phospholipid fatty acid (PLFA) and relative percentages of PLFA in community structural groups in oak-hickory, oak-hickory-shortleaf pine, and pine savannas for burned and unburned treatments.

Parameter	Community structure[1] (% of total PLFA)						
	TPLFA	TBSAT	MOSAT	BSAT	MB SAT	NSAT	EUKAR
Savanna	**0.0009**[2]	**0.0146**	0.3922	0.7163	0.3761	0.9617	0.6086
Treatment	0.4997	**0.0001**	**0.0363**	**0.0001**	0.1718	0.1460	**0.0001**

[1] Structural groups are assigned according to PLFA chemical structure, which is related to fatty acid (f. a.) biosynthesis including terminally branched saturates (TBSAT) f. a., monounsaturates (MOSAT) f. a., branched saturates (BSAT) f. a., mid-chain-branched (MBSAT) f. a., normal saturate (NSAT), f. a., and eukaryote (EUKAR) f. a.
[2] The *P*-values less than 0.05 are in bold in the table.

Table 4
Total phospholipid fatty acid (PLFA) and relative percentages of PLFA in community structural groups in oak-hickory, oak-hickory-shortleaf pine, and pine savannas for burned and unburned treatments ($n = 6$).

Parameter	Savanna							
	Oak-hickory		Oak-hickory-pine		Pine		Across savannas	
	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned
PLFA								
Viable biomass (Total PLFA in nmol g^{-1} of soil)	990867	979601	752268	795813	703437	518386	815524	764600
Community structure (% of total PLFA)								
Terminally branched saturates	15.47*[1]	13.15	14.37*	11.25	13.70*	9.67	14.51*	11.44
Monounsaturates	35.58	34.57	38.10	36.48	39.88	33.93	37.86*	34.99
Branched saturates	4.62*	3.77	4.42*	3.40	4.85*	3.32	4.63*	3.49
Mid-chain-branched saturates	8.92	8.50	8.70	8.32	8.50	7.08	8.72	7.97
Normal saturates	18.17	18.70	18.47	18.13	16.95	19.48	17.86	18.77
Eukaryotes	17.25*	21.35	15.96*	22.23	16.10*	26.52	16.44*	23.37

[1] *Indicates that PLFA value is significantly different between treatments foa a savanna at the 0.05 level according to Tukey's test. Values for a parameter in a row not followed by a symbol are not significant.

Table 5
Percent of individual phospholipid fatty acids (PLFAs) in microbial communities in soil from three forested areas (savannas) composed primarily either of oak-hickory, oak-hickory-pine (shortleaf pine) or pine that was burned compared to an unburned control ($n = 6$).

PLFA	Percent PLFA in community group							
	Oak-hickory		Oak-hickory-pine		Pine		Across savannas	
	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned
Terminally branched saturates								
il4:0	0.28	0.20	0.28*	0.18	0.23*	0.08	0.27*	0.16
il5:0	5.27	4.98	5.10*	3.90	4.25*	3.45	4.87*	4.11
al5:0	3.30	2.50	2.73	2.20	3.03*	1.68	3.02*	2.14
il6:0	2.87	2.65	2.60	2.35	2.47*	1.83	2.64*	2.28
il7:0	1.73*[1]	1.27	1.72*	1.25	1.72*	1.20	1.72*	1.24
al7:0	2.05*	1.55	1.97*	1.65	2.05*	1.47	2.02*	1.56
Monounsaturates								
15:1co6c	0.08	0.10	0.10	0.10	0.08	0.10	0.09	0.10
16:1co9c	1.08	1.06	1.15	1.03	1.13	0.93	1.12*	1.01
16:1co7c	4.30*	3.75	4.80*	3.58	4.15	3.25	4.42*	3.53
16:1co7t	0.33*	0.57	0.37	0.47	0.33*	0.48	0.32*	0.51
16:1co5c	3.17*	2.50	3.95*	2.55	3.23*	1.93	3.45*	2.33
17:1co6c	0.15	0.10	0.23*	0.10	0.20	0.12	0.19*	0.11
cyl7:0	1.88	2.11	2.47	2.13	2.33	2.43	2.23	2.23
17:1a	0.22*	0.35	0.23*	0.30	0.20*	0.30	0.22*	0.32
18:1co7c	9.98	8.60	11.37*	8.86	13.20*	9.20	11.52*	8.89
18:1co6c	0.40	0.25	0.32	0.47	0.62	0.35	0.44	0.36
18:1co5c	1.57	1.50	1.55	1.83	1.45	1.42	1.52	1.58
19:1co6c	0.15	0.15	0.22	0.17	0.15	0.23	0.17	0.18
cy19:0	12.38	13.52	11.43*	14.93	12.90	13.23	12.24*	13.89
Branched saturates								
brl5:1a	0.23	0.27	0.13	0.22	0.22	0.13	0.19	0.21
il6:1a	0.45	0.37	0.45	0.32	0.38*	0.27	0.43*	0.32
il6:1b	0.10	0.10	0.10	0.10	0.12	0.08	0.11	0.09
brl6:1a	0.08	0.10	0.07	0.07	0.05	0.07	0.07	0.08
brl6:1b	0.05	0.05	0.07	0.08	0.08	0.13	0.07	0.09
il7:1co7c	1.88*	1.30	1.90*	1.07	1.88*	1.12	1.89*	1.16
brl9:1a	1.58	1.27	1.57	1.48	1.80*	1.45	1.65*	1.40
brl9:1b	0.27	0.38	0.20	0.10	0.28	0.15	0.25	0.03
brl5:0b	0.15	0.10	0.07	0.08	0.07	0.05	0.09	0.07

Table 5 (*Continued*)

PLFA	Percent PLFA in community group							
	Oak-hickory		Oak-hickory-pine		Pine		Across savannas	
	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned
brl6:0	0.32	0.28	0.35*	0.17	0.47*	0.23	0.38*	0.22
10mel6:0	3.35	3.65	3.43	3.02	3.33	2.73	3.36	3.13
llmel6:0	0.22	0.15	0.23	0.22	0.18	0.13	0.21	0.17
12mel6:0	1.18	1.37	1.22	1.98	1.05	1.15	1.15*	1.50
brl7:0a	0.62*	0.33	0.43*	0.25	0.52	0.35	0.52*	0.31
10mel7:0	0.62	0.55	0.50	0.55	0.45	0.50	0.52	0.53
11me17:0	0.28*	0.15	0.33*	0.12	0.20	0.18	0.27*	0.15
12mel7:0/18:2	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00
Mid-chain-branched saturates								
10mel8:0	1.72	1.88	0.82*	1.77	1.78	1.60	1.44*	1.75
12mel8:0	0.47*	0.02	1.82	0.18	0.22	0.27	0.62*	0.16
Normal saturates								
14:0	0.60	0.62	0.63	0.57	0.58	0.43	0.61	0.54
15:0	0.55	0.70	0.62*	0.78	0.58	0.97	0.58*	0.82
16:0	13.53	13.68	13.77	13.37	12.53	14.28	13.28	13.78
17:0	0.55	0.68	0.58	0.52	0.53	0.90	0.55*	0.70
18:0	3.00	3.03	2.88	2.90	2.73	2.95	2.87	2.96
20:0	0.38	0.55	0.47	0.47	0.53	0.57	0.46	0.52
22:0	0.50	0.83	0.65	0.68	0.73	0.97	0.64	0.83
Eukaryotes								
18:2a	0.22	0.25	0.42	0.25	0.23	0.25	0.29	0.25
18:2co6	4.23	5.68	3.35*	7.57	3.50*	12.03	3.64*	8.34
18:3co3	0.32	0.23	0.32	0.22	0.35	0.45	0.33	0.30
18:lco9c	9.83	11.83	8.83*	11.38	9.28	10.90	9.32*	11.32
2O:4co6	0.58	0.48	0.80*	0.48	0.58	0.48	0.66*	0.48
2O:5co3	0.20	0.13	0.35*	0.13	0.22	0.13	0.26	0.13
2O:lcollc	0.13	0.10	0.13	0.10	0.10	0.12	0.12	0.11
2O:lco9c	0.17	0.12	0.13	0.12	0.18	0.10	0.16*	0.11
2O:lco7c	0.20*	0.10	0.25*	0.10	0.23	0.15	0.23*	0.12
22:5co6	0.08	0.05	0.12	0.08	0.07	0.03	0.08	0.05
22:6co3	0.13	0.17	0.15	0.18	0.16	0.15	0.15	0.17
22:lco9c	0.30*	0.83	0.27*	0.52	0.15	0.25	0.23*	0.53
Metabolic status (ratio) Sum cy 17:0/16: 1ω7c and cy 19:0/18: 1Ω7c								
Cyc/mono[2]	1.68*	2.13	1.52*	2.28	1.54*	2.19	1.57*	2.21
16:1ω7t/16:1ω7c								
Trans/cis[3]	0.08*	0.15	0.08	0.13	0.08*	0.15	0.08*	0.14

[1] *Indicates that PLFA value is significantly different from the control at the 0.05 level according to Tukey's test. Values for a parameter in a row not followed by a symbol are not significant.

[2] *cyc/mono,* ratio of cyclopropane to monoenic precursor fatty acids.

[3] *trans/cis*, ratio of monounsaturated *trans*- to *cis*-isomers.

Among the monounsaturate PLFA biomarker concentrations, burning differentially affected individual PLFAs in this group (Table 5). For biomarkers 16:1ω7c, 16:1ω5c, and 18:1ω7c, when differences occurred between burned forest and unburned forest soil, they were always higher in soil for the burned forest, while PLFA biomarkers such as 16:1ω7t, 17:1a, and cy19:0 were lower in burned forest soil than in unburned forest soil. For PLFA biomarkers in the branched saturates group, only i16:1a, i17:1ω7c, and br19:1a were higher in soils in burned forest than in unburned forest.

With few exceptions, individual PLFAs in the mid-chain-branched saturates group, whose amounts were different, were usually highest in soil for burned forest, including biomarkers br15:0a, br16:0, br17:0a, 11me17:0, and 12me18:0. Only the amount of 12me16:0 and 10me 18:0 was higher in unburned forest than in burned forest. Among the biomarkers for normal saturates, only 15:0 and 17:0 were lower in soil from burned forest than from unburned forest (Table 5).

Eukaryote or fungal PLFA biomarkers occurring in significantly ($P < 0.05$) different amounts were more common among treatments in the oak-hickory-pine savanna than in either the oak-hickory or pine savannas (Table 5). Among eukaryote biomarkers occurring in the largest amounts, 18:2ω6 and 18:1ω9c, their percentages of individual eukaryote PLFA was often higher in soil from the unburned forest than in soil from the burned forest. Also, there were several markers, 20:4ω6, 20:1ω9c, and 20:1ω7c, whose concentrations were higher in soil from burned forest than in unburned forest. Although, each biomarker contributed only a small amount to the total community group, some eukaryote biomarkers in soil from unburned forest were more than double the amount in soil from the burned forest.

The ratios of cyclopropyl fatty acids to their monoenoic precursors (i.e., cy17:0/16:1ω7c and cy19:0/18:1ω7c) and the ratio of *trans*- to *cis*-monoenoic (i.e., 16:1ω7t/16:1ω7c) were greater on the average in the unburned soil than in the burned soil (Table 5), suggesting that microbial growth or metabolic status of organisms in the unburned soil was substrate limited (Michalsen et al., 2007). Gram-negative bacteria also generate *trans*-fatty acids to minimize the permeability of their cellular membranes in order to adapt to more hostile environments (Guckert et al., 1986). The larger ratio of 16:1ω7t (*trans*-unsaturated fatty acid) to16:1ω7c (*trans*-fatty acids) for these organisms in soil from unburned forest indicates a decrease in membrane permeability (Table 5).

4. Discussion

4.1. Litter and soil nutrients

Conditions created by fire such as soil heating and ash deposition, either singularly or in combination, can alter physical, chemical, and biological soil properties (DeBano et al., 1976; Neary et al., 1999; Neary et al., 2005; Giardina et al., 2000; Rhoades et al., 2004; Doerr and Cerdá, 2005). However, the magnitude of these effects is influenced considerably by fire severity (Wells et al., 1979; Pietikäinen et al., 2000; Guerrero et al., 2005). Also, the impacts of fire on belowground effects can be highly variable due to a variety of factors including the distribution of the burning fuels and the insulation of soils from heating by differences in soil moisture (Neary et al., 1999). Although we did not measure soil surface temperatures, the blackened soil surface after the fire indicated that the heat generated during the burn was insufficient to completely consume the litter and other organic material at the soil surface, suggesting that both fires were likely of low severity with some areas of moderate severity.

Some apparent differences among savannas in litter and soil nutrient contents and PLFAs can likely be attributed to differences in dominant tree types on the savannas. Overall, litter from the pine savanna was lowest in nutrients while the oak-hickory and oak-hickory-pine savannas were either first or second highest. Except for litter K and soil pH and Ca, the content of no other litter or soil nutrient measurement behaved similar in response to fire treatments for all three savannas. There were, however, other litter and soil nutrients that were either higher or in response to fire treatments, but differences were often not significant. Covington et al. (1991) and Bauhus et al. (1993) agreed that it is not unusual for soil pH and cation nutrients to be higher following forest burning and for the effect to last for one to several years. During this time, equilibrium between litter production and its decomposition/nutrient release occurs. Rhoades et al. (2004) reported that soil pH, NO_3N, P, K, and Ca were all higher in log burnouts (herbaceous vegetation-free areas for more than 3 years after combustion of downed logs) compared to adjacent soil.

On the nutrient-poor sites used in the present study (Ponder et al., 1999), nutrient released from litter would be expected to be immediately taken up by plants or immobilized by microbes (Blankenship and Arthur, 1999). However, higher soil nutrient concentrations attributed to burned forest usually did not lead to corresponding higher litter nutrient concentrations in the savannas, and for some litter nutrients, their concentrations for burned and unburned forest were the reverse of soil nutrients for these treatments (Tables 1 and 2). This suggests that nutrient uptake might have been limited by nutrient availability on these savannas causing vegetation to retain a greater portion of the nutrients taken up rather than loose them at leaf fall.

4.2. Total PLFA, community structural groups, and individual PLFAs

Nutrient values for litter and soil and bacterial PLFA were generally lower for the pine and the oak-hickory-pine savanna than for the oak-hickory savanna (Tables 1, 2 and 4). However, soil microbes may decrease or be eliminated (depending on fire temperatures) from the litter and upper soil layers, but will reestablish during incubation following the burn (Dunn et al., 1979; De Marco et al., 2005). The soil microbial community in all three savannas appears to have more bacterial organisms than fungal organisms (Table 4). Differences in soil microbial community structure of bacterial origin due to burning were indicated by more gram-positive and gram-negative bacteria. This response may have been associated with the higher pH due to the burning. The combustion of organic matter during the fires caused the subsequent release and deposition of basic cations such as Ca on the soil surface, hence yielding a rise in pH. In the present study bacterial biomarkers were higher in soil from burned forest which had a higher pH. Bååth and Arnebrant (1994) found that culturable bacteria were up to 5.1 times higher in the high pH, limed, and ash amended soils compared to the untreated control. For our study, there were fewer biomarkers for terminally branched saturated bacteria, which are gram-positive bacteria, than biomarkers for monounsaturate bacteria, indicators of facultative and anaerobic bacteria. While the overall response to burning for both groups was higher compared to the amount in soil from unburned forest, monounsaturates did not differ between burning treatments (Table 4). It should be noted, however, that while differences in PLFA community structure have been attributed to burned and unburned forest, an individual bacteria species can have numerous fatty acids, and the same fatty acids may occur in bacteria, fungi, algae, and higher plants causing an overestimation of the species abundance of an organism (Lechevalier, 1977).

Overall, fungal PLFA was lower in burned forest than in unburned forest for all savannas (Tables 4 and 5). The fungal PLFA biomarkers 18:2ω6 and 18:ω9c made up the largest portion of the eukaryotic community for all savannas. Soil pH was negatively correlated with fungal PLFA while S and Fe were positively correlated with fungal PLFA (Data not shown). Blankenship and Arthur (1999) found no consistent effect of two annual prescribed fires on fungal PLFA. Further, in another study, mycorrhizal biomass in mineral soil layers was not significantly reduced by fire (Stendell et al., 1999). Changes in or reduced available substrate could affect fungal organism populations due to environmental effects of pH on plant growth and root exudation (Bolton et al., 1993). Also, fire-induced changes in the soil environment may favor one soil organism to the detriment of another. Reaves et al. (1990) reported that the growth of *Trichoderma*, a soil fungus, was encouraged in soils sampled from a ponderosa pine (*Pinus ponderosa*) site that had been burned by prescribed fire. Although these species of *Trichoderma* inhibited the growth of *Armillaria ostoyae*, one of several species of *Armillaria* responsible for serious root diseases in both artificially and naturally regenerated coniferous forests, we have no evidence of fungal growth inhibition in our study.

Among the tools used to determine the physiological or metabolic state of nutritionally challenged microorganisms is the analysis of PLFA profile. Bacteria are known to alter their membrane fatty acid components in response to environmental stress, thereby generating characteristic PLFA stress signatures (Kief et al., 1994). Higher ratios of both cyclopropyl fatty acids to their monoenoic precursors and rations of monounsaturated *trans*- to *cis*-isomers have been linked with starvation, stationary growth phase, and nutrient deprivation (Knivett and Cullen, 1965; Guckert et al., 1986; Kief et al., 1994; Thomas and Batt, 1969). The higher PLFA ratio for burned forest compared to the lower ratio for unburned forest is consistent with evidence reported for starved bacteria under physiological stress conditions (Kief et al., 1994). While we have primarily attributed differences in PLFA profiles between treatments to nutrient deprivation, there may be other reasons since our data is from plots in the uncontrolled environment of forest conditions. In laboratory conditions, increases in cyclopropyl PLFA have also been shown to be associated with cell age and anaerobiosis (Guckert et al., 1985, 1986).

5. Conclusion

We found that the burning effect across savannas on litter nutrients was minimal with only K and B being higher in unburned

than in burned treatments. But for these two nutrients in the soil, only B was affected, being higher in burned forest than in unburned forest across savannas. The increases in soil pH, Ca, and B among savannas were likely due to increase in ash deposited on the soil surface during the fire and will likely be of short duration (Marion et al., 1991). Further, these overall moderate changes in litter soil nutrient contents attributed to fire indicate that the two annual fires did not strongly promote fire cycling of nutrients.

Burning had little effect on total PLFA, but did affect community structure as noted by more biomarkers for terminally branched saturate (Gram-positive), monounsaturate (Gram-negative, and branched saturate (anaerobic metal reducing) bacteria and less of those for fungal PLFA. Effects on microbial community related to burning likely resulted from variation in nutrient and substrate availability associated with differences in nutrient concentrations caused by burning and further evidenced by differences in the ratio of the *trans*- to *cis*-monoenoic fatty acids and in ratio of cyclopropyl fatty acids to their monoenoic precursors. Our results suggest that annual low intensity prescribed fires can cause some moderate changes in nutrient contents and microbial community. Further research will need to be done to determine the long-term consequences of repeated burning as a management tool to develop savannas, protect forest resources, and on the sustainability of soil nutrients and microbes in this nutrient-poor upland oak-hickory ecosystem. On sites such as these, the practice of annual burning may not allow sufficient time for soils to fully recover between burns (Úbeda et al., 2005).

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