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Juniperus virginiana encroachment into upland oak forests alters arbuscular mycorrhizal abundance and litter chemistry

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ABSTRACT

Upland oak forests in the ecotone between the eastern deciduous forest and the southern Great Plains are threatened by encroachment of eastern redcedar (*Juniperus virginiana*) due to fire suppression. The rapid rate of encroachment caused concern about concomitant alterations of site characteristics including nutrient cycling and the soil microbial communities (SMC) that could lead to positive feedbacks reinforcing eastern redcedar encroachment. We studied eight upland oak forests across central and western Oklahoma with stands representing three levels of encroachment: oak-dominated, eastern redcedar-dominated, and an intermediate mixture of both species. We analyzed litter chemistry (carbon, lignin, and nitrogen), soil chemistry (soil organic matter, NH_4N, NO_3-N, PO_4, K, and pH), and profiled soil microbial communities using phospholipid fatty acid analysis (PLFA). Eastern redcedar encroachment was accompanied by reduced litter carbon along with higher levels of arbuscular mycorrhizal (AM) fungi while litter N was lower in mixed stands. However, we detected no change in soil chemistry. Our results indicate eastern redcedar encroachment in these upland oak forests reduced litter quality and altered the SMC through increases in AM fungi, a symbiont associated with eastern redcedar. These alterations may create positive soil–microbial feedbacks by reducing the fitness of the dominant oak species and facilitating rapid increase in eastern redcedar in this threatened, oak-dominated ecosystem.

1. Introduction

Upland oak forests in North America's midcontinent ecotone between the eastern deciduous forest and the southern Great Plains are threatened by *Juniperus virginiana* L. (eastern redcedar) encroachment due to fire suppression over the past century (DeSantis et al., 2010). Eastern redcedar was nearly non-detectable in upland oak forests in the 1950s (Rice and Penfound, 1959) and increased to 13% of the basal area and 23% of the sapling density in the early 2000s, a trend recruitment patterns indicate is accelerating (DeSantis et al., 2011). Although there have been studies assessing eastern redcedar encroachment into grasslands, consequences of eastern redcedar encroachment into forests have not received much attention. Previous studies have reported invasion into grasslands by eastern redcedar resulted in a loss of native plant species richness (Gehring and Bragg, 1992; Briggs et al., 2002; Linneman and Palmer, 2006), and alterations in nutrient accumulation and cycling (Norris et al., 2001, 2007; McKinley and Blair, 2008; McKinley et al., 2008). Few studies have addressed how eastern redcedar encroachment affects belowground communities in either grassland or forest.

Eastern redcedar encroachment into upland oak forests gradually replaces dominant post oak (*Quercus stellata* Wangenh.) and blackjack oak (*Q. marilandica* Münchh.) increasing diversity of canopy trees. Increased forest canopy diversity has been found to increase spatial heterogeneity in soil physiochemical properties (Augusto et al., 2002; Kamei et al., 2009; Turk et al., 2008) and soil microbial community diversity (SMCs) (Grayston and Prescott, 2005; Bach et al., 2008; Mitchell et al., 2010). Soils under mixed forests of hardwood and coniferous species contrast pure conifer forests largely due to differences in litter chemistry (Saetre, 1999; Li and Han, 2008; Turk et al., 2008). Conifers generally have lower soil nutrient uptake rates than hardwoods and produce nutrient-poor litter (Fisher and Binkley, 2000; Rothe and Binkley, 2001; Augusto et al., 2002) with higher lignin and polyphenol concentrations (Perry et al., 1987); consequently, conifer litter often has lower decomposition and mineralization rates (Perry et al., 1987; Fisher and Binkley, 2000). The lower pH (Ovington, 1953; Alban, 1982; Hornung, 1985) and higher C/N of conifer litter favor greater fungal development over bacteria (Witkamp, 1963). The greater input and

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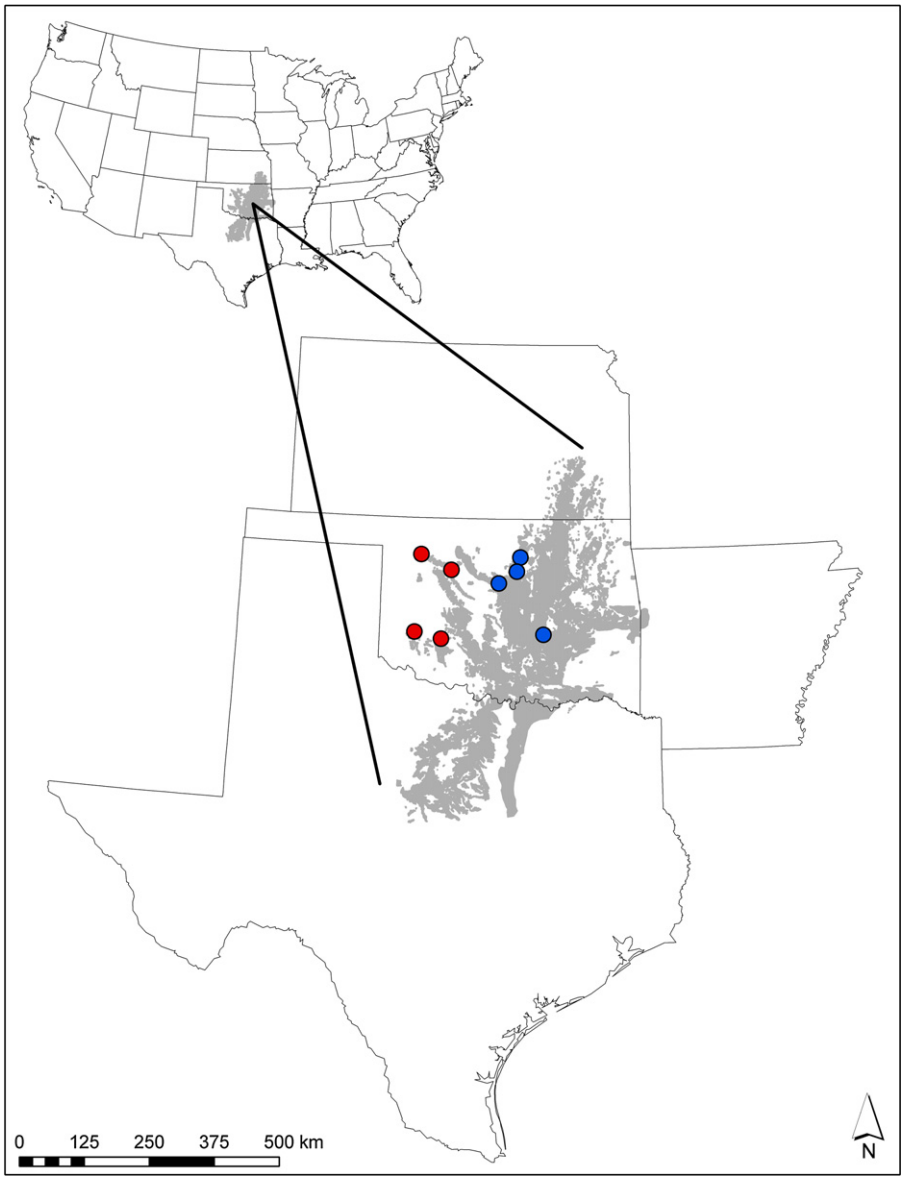

Fig. 1. Map of Cross Timbers forest and sites used in study. Red circles represent western sites and blue circles represent central sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more rapid decomposition of hardwood litter (Fried et al., 1990) may increase soil organic matter (SOM) thereby increasing porosity and reducing bulk density. On the other hand, increased litter accumulation under conifers may also promote SOM storage and decreased bulk density (Challinor, 1968). The complexity of alterations to soil ecosystems under hardwoods and conifers makes it difficult to predict changes due to eastern redcedar encroachment into post oak-blackjack oak forests.

Fungal symbionts are key players in ecosystem function and can shape both plant and soil microbial communities (Rillig, 2004; Cheng and Baumgartner, 2006; Rillig et al., 2006). Oak species like post oak typically form associations with ectomycorrhizal (EM) fungi and may depend on the symbiosis for survival and growth (Marks and Kozlowski, 1973; Cavender-Bares et al., 2009). EM fungi assist with nutrient uptake, pathogen resistance, and can play an important role in nutrient cycling (Read and Perez-Moreno, 2003; Karlinski et al., 2007; Smith and Read, 2008). *Juniperus* species, such as eastern redcedar and other members of the Cupressaceae, form a symbiotic relationship with arbuscular mycorrhizal (AM) fungi (Newman and Reddell, 1987). Competitive effects between EM and AM fungi can negatively affect host plant fitness and survival (Sylvia and Jarstfer, 1997; McHugh and Gehring, 2006). AM and EM fungi have differential effects on other components of SMCs, possibly interacting with nutrient cycling members of the community (Phillips and Fahey, 2006; Shah et al., 2009). Therefore, increases in AM fungi in a post oak-dominated soil due to eastern redcedar encroachment could contribute to changes in ecosystem functioning and reduce the fitness of the dominant post oak.

We conducted a study to determine the consequences of encroachment of eastern redcedar into upland oak forests for the soil ecosystem. The study was conducted at eight widely distributed forests where the degree of encroachment in individual stands was minor (stands with less than 30% eastern redcedar), intermediate (50% eastern redcedar) or severe (more than 70% replacement of oak trees by eastern redcedar). We measured litter chemistry, soil chemical and physical properties, and determined SMC composition based on PLFA. The ultimate goal of the study was to assess eastern redcedar encroachment on litter and soil quality with potentially long-term impacts on nutrient cycling and forest succession.

2. Materials and methods

2.1. Study area

Forests in our study were selected from 30 sites sampled in the 1950s by Rice and Penfound (1959) and resurveyed in 2007 (DeSantis et al., 2010). We selected eight forests ranging in size from 16 to 130 ha that were historically dominated by post oak and experienced substantial encroachment by eastern redcedar between the 1950s and 2000s (DeSantis et al., 2010). The location of forests used in our current study ranged from central to western Oklahoma near the panhandle (Fig. 1). These stands had a range of soil types from fine sands to sandy fine loams (Table 1). Mean annual precipitation ranges from 690 mm in the west to 1070 mm in the east, and rainfall is well distributed throughout the year. Mean annual temperature ranges from 14 °C in the north to 16 °C in the south (Oklahoma Mesonet, 2011). The area is prone to drought because evapotranspiration can often equal precipitation especially in the west (Pettyjohn et al., 1983). The elevation decreases from 500 m in the west to 300 m in the southeast.

2.2. Plot establishment

Each forest was surveyed to locate stands with different levels of eastern redcedar encroachment. Basal area was measured with a basal area factor 10 prism and qualifying stands were placed in one of the following three canopy types representing different levels of eastern redcedar encroachment: oak dominated (*Q. stellata* > 70% and eastern redcedar < 30%), mixed stands (60% > eastern redcedar > 40% and 60% > *Q. stellata* > 40%) and eastern redcedar dominated (eastern redcedar > 70% and *Q. stellata* < 30%). Two representative stands of each canopy type were sampled within each site (6 per site). The sampled stands were separated by at least 100 m. A 1 m^2 sample plot that fell under our canopy-type criteria was established in each of the representative stands for sampling as explained in the following sections. This single plot was used to minimize variance caused by soil heterogeneity within a small area under the same canopy type.

2.3. Understory vegetation and litter measurement

All understory vegetation < 1.4 m tall was identified to either species or genus level and percent cover was estimated using a customized Braun-Blanquet cover scale (Kent and Coker, 1992). Species that could not be identified in field were collected and identified in the laboratory. All litter was removed to mineral soil in the four corners and center of the quadrat with a total comprised area of 40 cm^2. Litter was stored in bags for transport, washed gently for 30 s to remove mineral soil, and dried at 70 °C for 48 h. Dried litter samples were finely ground and analyzed by the Soil, Water, and Forage Analytical Laboratory (SWFAL) at Oklahoma State University for total carbon (TC), total nitrogen (TN), and lignin content. Carbon and nitrogen were determined using a dry combustion nitrogen analyzer (LECO TruSpec® Micro Elemental Series CHN/CHNS/O; St. Joseph, MI) (Undersander et al., 1990; Bremner, 1996; Nelson and Sommers, 1996). Lignin was quantified by the method of determining acid detergent lignin in beakers (ANKOM Technology, 2011). First, samples were extracted by the acid detergent fiber method with an ANKOM 200 Fiber Analyzer (ANKOM Technology; Macedon, NY). Then they were extracted with a 72% sulfuric acid in a beaker that left only lignin and recalcitrant materials to be determined by weight.

2.4. Soil collection and measurement

After collecting all litter to mineral soil, we collected soil cores to a depth of 10 cm with an 8 cm diameter cylindrical core sampler directly beneath the location of the litter samples. The 5 cores collected from each plot were combined, homogenized in the field, and sub-sampled for chemical and soil microbial analyses. Two additional soil cores were collected within each quadrat to measure bulk density and soil moisture. Soil was sieved through 4 mm and 2 mm screens to remove root fragments and rocks. Samples were analyzed by the SWFAL at Oklahoma State University for pH, total nitrogen (TN), soil organic matter (SOM), total soil organic carbon (SOC), extractable inorganic N (NO_3-N, NH_4-N), plant-available phosphorous (H_2PO_4-P), and total soil potassium (K). Soil samples were dried at 60 °C over night and ground to pass a 2 mm sieve. Soil pH was measured by glass electrode in a 1:1 soil:water suspension (Sims, 1996; Sikora, 2006). TN, SOM and SOC were determined using a dry combustion nitrogen analyzer (LECO TruSpec® Micro Elemental Series CHN/CHNS/O; LECO Corporation, St. Joseph, MI) (Bremner, 1996; Nelson and Sommers, 1996). Soil NO_3-N and NH_4-N were extracted with 1 M KCl solution and quantified by a flow injection autoanalyzer (Lachat QuikChem® 8500 Series 2 Flow Injection Analysis System; Lachat Instruments, Hach Company, Loveland, CO). Plant available P and total K were extracted using Mehlich 3 solution (Mehlich, 1984) and quantified in the extract by an inductively coupled plasma spectrometer (SPECTRO CIROS CCD-ICP spectrometer; SPECTRO Analytical Instruments GmbH; Kleve, Germany) (Soltanpour et al., 1996). For bulk density and gravimetric soil moisture, soil cores were weighed before and after drying at 105 °C for 48 h.

2.5. Soil microbial community analysis

Soil was freeze-dried and finely ground with a mortar and pestle. Five grams of each sample were mixed with 4 ml phosphate buffer, 10 ml methanol followed by 5 ml chloroform for lipid extraction. The soil–solvent mixture was separated by centrifugation and then decanted with 1:2 mix of chloroform and methanol. Phosphate

Table 1
Soil series and taxonomic class (Soil Survey Staff, 1999) of soils in study.

Site	Soil series	Taxonomic class	Soil texture	Reference
Central				
1	Endsaw	Fine, mixed, active, thermic Oxyaquic Hapludalfs	Fine sandy loam	Long (1968)
1	Hector	Loamy, siliceous, subactive, thermic Lithic Dystrudepts		Long (1968)
2, 4	Stephenville	Fine-loamy, siliceous, active, thermic Ultic Haplustalfs	Fine sandy loam	Henley et al. (1987), Wilson et al. (2006)
3	Darsil	Thermic, shallow, coated Ustic Quartzipsamments	Fine sandy loam	Henley et al. (1987), Wilson et al. (2006)
3	Derby	Mixed, thermic Alfic Ustipsamments	Loamy fine sand	Henley et al. (1987)
4	Darnell	Loamy, siliceous, thermic, shallow Udic Ustochrepts	Fine sandy loam	Henley et al. (1987)
West				
5	Devol	Coarse-loamy, mixed, superactive, thermic Typic Haplustalfs	Fine sandy loam	Fisher (1968)
5	Nobscot	Loamy, mixed, superactive, thermic Arenic Paleustalfs	Fine sand	Fisher (1968)
6	Eda	Mixed, thermic Lamellic Ustipsamments	Fine sand	Nance et al. (1963)
7, 8	Brico	Clayey-skeletal, mixed, active, thermic Typic Argiustolls	Cobbly loam/Loam with rock fragments	Mobley and Brinlee (1967)

buffer was added and left for phase separation to occur overnight. After phase separation, the chloroform layer containing the lipids was recovered and reduced by nitrogen flow at 50 °C. The lipids were separated into neutral lipids, glycolipids, and phospholipids by solid phase extraction by eluting with chloroform, acetone, and methanol, respectively. Phospholipids were hydrolized and methylated. The methylated fatty acids were extracted with hexane and evaporated under nitrogen at 37 °C. Phospholipid fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector.

The nomenclature used to describe the identified fatty acid is as follows (Bossio et al., 1998): total number of C atoms:number of double bonds, *cis* or *trans* isomers identified by *c* or *t*. Prefixes of *a* or *i* indicate anteiso branching or iso branching, respectively. Following the total number of C atoms, the ω followed by a number represents the number of C atoms from the terminal methyl group (Ruess and Chamberlain, 2010). Other terms like "cy" represent cyclopropyl ring molecules and "3-OH" represent the position of hydroxyl groups (Ruess and Chamberlain, 2010). We selected the following biomarkers: 16:1ω5c for AM fungi (Olsson et al., 1995); 3-OH 14:0, 16:1ω7c, cy17:0, 2-OH 16:0, 18:1ω7c, cy19:0 for Gram-negative bacteria; i15:0, a15:0, i16:0, i17:0 for Gram-positive bacteria; 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 for common (non-specific) microbes; 18:2ω9,12c for saprotrophic fungi (McKinley et al., 2005) and 18:1ω9c for saprotrophic/ectomycorrhizal fungi (Ushio et al., 2008; Frostegård et al., 2011). It has been noted previously that the AM fungal marker 16:1ω5c can also be produced by Gram-negative bacteria (Joergensen and Wichern, 2008; Frostegård et al., 2011), however Joergensen and Wichern (2008) suggested analyzing cy17:0 and cy19:0 for possible interference between Gram-negative bacteria (GNB) and AM fungi. We analyzed bivariate relationships between individual or aggregated Gram-negative bacterial markers and 16:1ω5c to test if our assumption that 16:1ω5c was representative of AM fungi was reasonable. Common non-specific fatty acid biomarkers were only included in our analysis to express alterations in overall microbial biomass, although these cannot allow assessment of shifts of specific microbial community groups and were therefore not included in any other analyses. PLFA data is reported in percent of the total mole fraction, which can be interpreted as a relative abundance.

2.6. Data analysis

Lignin:N ratios were calculated by dividing percent lignin TN, creating an index of %lignin:%TN by mass of soil. Plant-available phosphorus, K, NH_4-N, and NO_3-N were log transformed prior to analysis (Palmer, 1993). Analysis of variance was used to test for significance of eastern redcedar encroachment on cover of understory plant functional groups, litter nutrients, soil chemical properties, and SMCs (SAS Institute, 2008). All variables were transformed when necessary to account for deviations from normality. We employed a split-plot design that averaged replicate plots ($n = 3$) within a site ($n = 8$) using a mixed-effects model with region (central or western) and canopy type as main effects with site and subsamples as random effects to test the differences between canopy types. The same model was used to determine the differences between the western and central regions of the state, independent of canopy type. Results of statistical tests were considered significant at $P \leq 0.05$.

When testing the differences between GNB and AM fungi markers, we used standard major axis regression (SMA) in the lmodel2 package in R v 2.11.1. In short, this method is a form of orthogonal regression where neither variable is held as independent. Bivariate tests were also performed using the linear model function in R, but differences in these methods did not affect our interpretation (data not shown). Principal component analysis (PCA) was conducted using the vegan package in R v 2.11.1 where replicate plots were averaged at each site by treatment (canopy type) in order to look at the relationship between treatments and microbial PLFA biomarker composition (ter Braak and Šmilauer, 2002). To test differences in SMC composition between canopy types we used a multiple response permutation procedure (MRPP) with a Sørensen dissimilarity index using the vegan package in R v 2.11. This procedure was run for both a full model and pairwise comparisons between canopy types. We used a Bonferroni correction to adjust *P*-values for multiple comparisons. In short, MRPP is a nonparametric method that can be used to compare dissimilarities between groups of multiple species, or in this case individual PLFA biomarkers (McCune et al., 2002).

3. Results

3.1. Understory vegetation, litter nutrients, and soil chemical properties

Eastern redcedar encroachment and region did not show an effect on understory community composition. Percent cover of the understory plants ranged from 44 to 51%. Understory cover was greatest for woody plants (38%) followed by graminoids (5%), forbs (3%), and legumes (2%). Woody plant cover was typically 5 to 10 times greater than forb, graminoid, and legume cover within the plots (data not shown).

Easter redcedar encroachment reduced litter TN for the mixed stands and not for the eastern redcedar stands (Table 2). Eastern redcedar encroachment reduced litter TC for both mixed and eastern redcedar stands and had no effect on litter C:N ratio, lignin content, or lignin:N ratio (Table 2). Region did not show an effect on litter nutrients.

Eastern redcedar encroachment and region did not show an effect on soil chemical properties (Table 3). In general, soil pH was slightly lower than neutral. Bulk density averaged 1.0 g cm^{-3}, and the percent moisture averaged approximately 9%. Soil organic matter averaged 4%, SOC averaged 2.44% and TN averaged 0.17%. Phosphorus averaged 4.19 g m^{-2}, and K averaged 25.54 g m^{-2} across all plots. Average NO_3-N values were typically lower than NH_4-N (1.06 and 2.19 g m^{-2}, respectively).

3.2. Soil microbial community analyses

We first determined if 16:1ω5c was indicative of AM fungi and not GNB using bivariate regression (Supplementary Fig. 1 and Supplementary Table 1). Despite a weak positive relationship between 3-OH 14:0 and 16:1ω5c, all GNB markers and their sum had either no relationship or a negative relationship with 16:1ω5c. Furthermore, markers suggested by Joergensen and Wichern (2008) (cy17:0 and cy19:0) as potential indicators of differences between GNB and AM fungi were negatively correlated. Therefore, we assumed that 16:1ω5c is only indicative of AM fungi in our use of PLFA.

Supplementary data related to this article found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2012.12.020

Of the 13 specific PLFA biomarkers assigned to microbial categories that we assessed in this study, 2 showed a significant effect of eastern redcedar encroachment (Table 4). The biomarker for AM fungi, 16:1ω5c, was 54% higher in soils dominated by eastern redcedar, as compared to stands dominated by oak. The GNB marker, cy17:0, was 16% lower in soils collected from eastern redcedar dominated stands when compared to those dominated by oak. Biomarkers for saprotrophic fungi (18:2ω9,12c) and

Table 2
Effect of eastern redcedar encroachment on litter in oak dominated forests.

Litter variables	Oak	SE	Mixed	SE	Redcedar	SE	*P*-value
%TN	1.18*a*	0.05	1.06*b*	0.04	1.12*ab*	0.03	0.046
%TC	36.03*a*	0.56	31.70*b*	0.26	33.08*b*	0.53	<0.001
C:N	31.49	1.76	30.55	1.25	30.01	1.08	NS
%Lignin	28.98	1.36	28.27	1.00	29.69	0.86	NS
Lignin:N	25.14	1.37	27.08	0.94	27.03	0.94	NS

Total nitrogen (TN), total carbon (TC), lignin content, C:N, and lignin:N ratios are represented in the above table. Letters indicate significant differences among levels of eastern redcedar encroachment at $P \leq 0.05$. NS represents $P > 0.1$. SE = standard error, $n = 8$.

Table 3
Effect of eastern redcedar encroachment on soils in oak dominated forests.

Soil variables	Oak	SE	Mixed	SE	Redcedar	SE	*P*-value
pH	6.19	0.20	6.53	0.32	6.94	0.13	0.066
%SOM	3.31	0.45	4.99	0.70	4.32	0.75	NS
%SOC	1.92	0.28	2.90	0.42	2.50	0.38	NS
%TN	0.14	0.03	0.18	0.03	0.18	0.03	NS
P	4.13	0.72	4.26	0.87	4.19	0.92	NS
K	23.96	4.13	25.78	4.87	26.89	3.98	NS
NH_4-N	2.09	0.47	2.17	0.36	2.30	0.60	NS
NO_3-N	1.01	0.31	0.98	0.25	1.19	0.27	NS
C:N	16.09	1.58	17.60	2.52	14.72	1.91	NS
% water	8.02	1.25	9.10	1.59	10.14	2.29	NS
Bulk density	1.04	0.03	0.97	0.04	1.01	0.05	NS

Soil pH, %SOM, %SOC, %TN, concentration of P, K, NH_4-N, and NO_3-N ($g\,m^{-2}$), C:N, % water and bulk density ($g\,cm^{-3}$) are represented in the above table. NS represents $P > 0.1$. SE = standard error, $n = 8$.

Table 4
Effect of eastern redcedar encroachment of oak dominated forests on PLFA biomarker values as percent of the total molar fraction (relative abundance).

Categories	Marker	Oak	SE	Mixed	SE	Redcedar	SE	*P*-value
GNB	3-OH14:0	0.64	0.21	0.95	0.23	0.98	0.15	NS
GNB	16:1ω7c	5.85	0.36	6.54	0.55	5.99	0.37	NS
GNB	cy17:0	3.19*a*	0.12	3.02*a*	0.11	2.67*b*	0.13	<0.001
GNB	2-OH16:0	2.24	0.37	2.28	0.27	2.30	0.23	NS
GNB	18:1w7c	1.42	0.10	1.60	0.11	1.45	0.22	NS
GNB	cy19:0	10.56	0.66	9.94	0.45	8.49	1.10	NS
GPB	i15:0	11.01	0.13	10.93	0.30	10.72	0.41	NS
GPB	a15:0	6.12	0.15	6.03	0.37	5.92	0.33	NS
GPB	i16:0	6.39	0.53	6.76	0.31	6.71	0.35	NS
GPB	i17:0	3.23	0.13	3.21	0.12	3.22	0.10	NS
AM Fungi	16:1ω5c	3.74*a*	0.27	4.19*a*	0.17	5.65*b*	0.66	0.017
SAP Fungi	18:2 ω9,12c	3.42	0.37	3.67	0.48	3.62	0.41	NS
SAP Fungi	18:1ω9c	11.89	0.66	11.15	0.56	10.61	0.45	NS

GNB, Gram-negative bacteria; GPB, Gram-positive bacteria; NS, non-specific microbes; AM fungi, arbuscular mycorrhizal fungi; SAP fungi, saprotrophic fungi. Letters indicated significant differences among levels of eastern redcedar encroachment, $P \leq 0.05$. NS represents $P > 0.1$. SE = standard error, $n = 8$.

saprotrophic/ectomycorrhizal fungi (18:1ω9c) did not show an effect of eastern redcedar encroachment. Relative abundance for functional groups other than arbuscular mycorrhizal fungi (GNB, Gram-positive bacteria, saprotrophic fungi, total fungal and bacterial biomarkers) and biomass measurements (bacterial, fungal, and total microbial) were not significantly affected by eastern redcedar encroachment (Table 5). Additionally, there were no significant differences among levels of eastern redcedar encroachment for total bacterial, total fungal, or total microbial biomass, as determined by PLFA analysis.

Table 5
Effect of eastern redcedar encroachment of oak dominated forests on PLFA biomarker values for composite values as percent of the total molar fraction and microbial biomass.

Categories	Oak	SE	Mixed	SE	Redcedar	SE	*P*-value
GNB (%)	23.9	0.8	24.34	0.69	21.86	1.41	NS
GPB (%)	26.74	0.63	26.94	0.86	26.57	0.93	NS
NSP (%)	30.3	0.72	29.71	0.83	31.69	0.97	NS
AM Fungi (%)	3.74*b*	0.27	4.19*b*	0.17	5.65*a*	0.66	0.017
SAP fungi (%)	15.31	0.76	14.82	0.73	14.23	0.52	NS
Bacterial (%)	80.95	0.77	80.99	0.83	80.12	1.13	NS
Fungal (%)	19.05	0.77	19.01	0.83	19.88	1.13	NS
Total bacterial PLFA mass	1979.47	272.39	3570.23	272.39	3050.68	272.39	NS
Total fungal PLFA mass	769.34	115.15	801.61	115.15	911.187	115.15	NS
Total microbial PLFA mass	2748.81	357.83	3089.60	357.83	2769.47	357.83	NS

Masses are reported in $ng\,g^{-1}$ soil and represent mass calculations without NS markers. GNB, Gram-negative bacteria; GPB, Gram-positive bacteria; NSP, non-specific microbes; AM fungi, arbuscular mycorrhizal fungi; SAP fungi, saprotrophic fungi. Letters indicate differences among levels of eastern redcedar encroachment, $P \leq 0.05$. NS represents $P > 0.1$. SE = standard error, $n = 8$.

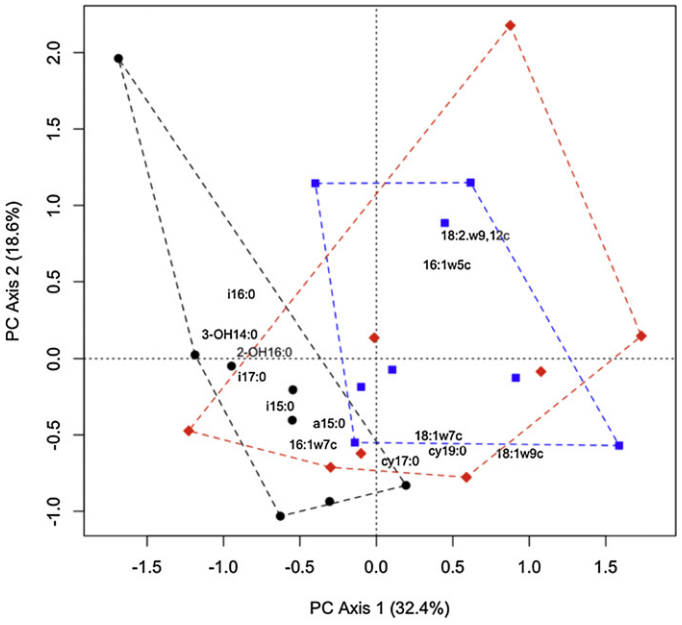

Fig. 2. Principal component analysis (PCA) displaying soil microbial community composition based on 13 PLFA biomarkers. Circles represent oak plots, squares represent mixed plots, and diamonds represent eastern redcedar plots. Black dashed lines outline oak plots while blue and red dashed lines represent mixed and eastern redcedar plots respectively. Species scores for each individual biomarker are represented by its label. The percentage of variation explained by axes is displayed by the respective axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Two PLFA biomarkers were significantly different between the central and western stands surveyed in this study. A GNB biomarker, 2-OH 16:0, was 35% greater in west ($2.75 + 0.24\ ng\ g^{-1}$), as compared to the central stands ($1.79 + 0.24\ ng\ g^{-1}$; $P = 0.03$). The saprotrophic biomarker (18:1ω9c), was 11% lower in the west ($10.61 + 0.37\ ng\ g^{-1}$), compared to the central region of the state ($11.83 + 0.37\ ng\ g^{-1}$; $P = 0.049$). The total saprotrophic fungal biomarkers (18:1ω9c and 18:2ω9,12c) were also 15% more abundant ($15.80 + 0.46\ ng\ g^{-1}$) in the central region than in the western forest stands ($13.77 + 0.46\ ng\ g^{-1}$; $P = 0.0204$). Total saprotrophic fungal biomass was positively correlated with annual precipitation data (cm) from the closest local Oklahoma Mesonet stations (2011) ($R^2 = 0.1645$, $P = 0.049$). There were no significant differences observed for total microbial, bacterial, or fungal biomass between central and western plots.

The PCA biplot (Fig. 2) suggested differentiation in SMC composition associated level of eastern redcedar encroachment (Fig. 2). The first axis represents a transition from oak dominated stands to eastern redcedar dominated stands. Though there appeared to be a large area of overlap and little change in the majority of PLFA biomarkers, there was some differentiation among levels of eastern redcedar encroachment. This may be largely driven by the biomarkers cy19:0 and 16:1ω5c which are representative of GNB and AM fungi respectively, suggesting a relationship between these two groups. This relationship between GNB and AM fungi was supported by a weak but significant negative correlation between the AM fungal biomarker and GNB biomarkers ($R^2 = 0.2336$, $P = 0.0167$).

Through the MRPP we observed significant differences between SMCs under oak and mixed canopy types, though we did not observe a significant difference between oak and eastern redcedar canopies (Table 6). The low A values observed within each group comparison shows high levels of homogeneity suggesting a high level of similarity between PLFA biomarker profiles under each canopy type despite significant *P*-values.

Table 6
Results of MRPP testing SMC composition among levels of eastern redcedar encroachment.

	δ	Expected δ	A	P-value
All canopy types	0.0980	0.1038	0.0559	0.010
Oak vs. mixed	0.0961	0.1031	0.0678	0.014
Oak vs. eastern redcedar	0.0982	0.1031	0.0477	0.086
Mixed vs. eastern redcedar	0.0997	0.1011	0.0140	NS

The observed and expected δ represent the weighted mean within group distance and the expected value based on the permutation. The chance corrected within group agreement (A) represents the within group homogeneity ranging between 0 and 1. Lower values represent greater homogeneity within the comparison between groups. NS represents $P > 0.1$.

4. Discussion

Significant changes in litter chemistry and soil microbial and mycorrhizal communities accompanied eastern redcedar encroachment into the upland oak forests. Previous studies likewise found evidence of alterations in litter chemistry (Sariyildiz and Anderson, 2003; Meier and Bowman, 2008; Talbot and Finzi, 2008), soil physiochemical properties (Augusto et al., 2002; Turk et al., 2008; Kamei et al., 2009), and SMCs due to different overstory tree species (Grayston and Prescott, 2005; Bach et al., 2008; Mitchell et al., 2010). Although mineral soil chemical properties did not change with the forest canopy species conversion from oak to eastern redcedar, changes in litter chemistry could possibly lead to future alterations in carbon and nitrogen cycling within these forest soils. Higher AM fungal abundance in eastern redcedar dominated forests could also generate a soil environment detrimental to the success of EM symbiotic species, including post oak.

4.1. Changes in litter and soil chemistry

We found eastern redcedar replacement of oak in the overstory was accompanied by decreases in C and N concentrations in litter with no measurable changes soil chemistry. Changes in litter N did not follow the same pattern seen in earlier experiments examining differences between soils under hardwoods and conifers, as we found that pure stands of oaks and eastern redcedar were not significantly different despite lower litter N in mixed stands (Perry et al., 1987; Fisher and Binkley, 2000). If lower levels of litter N persist and eventually alter C:N or lignin:N ratios, rates of soil processes, such as decomposition, may differ between stand types (Jacob et al., 2010). This transitional step between oak-dominance and eastern redcedar-dominance requires further investigation to quantify effects on lower litter N on N-transformations below in the mineral soil. Nitrogen leaching from litter to soils in forest areas that are an even mix of oaks and eastern redcedar may differ strongly from oak-dominated stands, requiring further investigation into N-cycling in forest stands suffering intermediate levels of invasion.

Although litter C decreased with the degree of eastern redcedar encroachment, SOC remained the same. This could be due to differences in litter mass and the conversion of litter carbon into SOC through decomposition or leaching. Similarity, SOC stocks among different levels of eastern redcedar encroachment may also be influenced by properties of the SMC. The greater AM fungal biomass from soils associated with eastern redcedar dominated stands suggests a possible source of SOC that may counter-balance a lack potential input from C-rich litter as observed under oaks (Miller and Jastrow, 1992; Zhu and Miller, 2003). Glomalin, a glycoprotein produced by AM fungal hyphae, also contributes to increased soil aggregation and SOC (Rillig et al., 2001; Wilson et al., 2009). These belowground contributions to SOC may balance the difference in potential carbon inputs from litter as seen between oak- and eastern redcedar-dominated stands.

The lower TN in litter from mixed stands compared to oak stands was consistent with earlier findings that N dynamics differed between mixed and single species litter (Chapman et al., 1988; Blair et al., 1990) and in hardwood-conifer comparisons (Perry et al., 1987; Fisher and Binkley, 2000). Litter mixtures may have fostered novel combinations of microarthropods and microbial communities whose processing of the litter increased leaching and reduced subsequent accumulation of high N litter (Blair et al., 1990). However, we observed no differences in soil N that may be expected with increased leaching from litter. Litter mixtures may have accelerated decomposition and N-mineralization, stimulating forest vegetation N uptake and growth (Chapman et al., 1988). The potential for increased growth from encroachment of eastern redcedar in oak forests due to changes in N mineralization from litter is an interesting possibility worthy of further study. It would suggest the transition from oak to eastern redcedar dominance could promote a period of mixed forests with higher rates of N-cycling but relatively stable N-pool sizes within the soil ecosystem.

4.2. Effect of precipitation gradient on SMCs

The 14% increase in saprotrophic fungi from west to east across the study area was associated with a gradient of increasing annual precipitation from 69 cm in the west to 107 cm in the east. Though it is known saprotrophic fungi can remain active in relatively dry seasonal conditions (Bell et al., 2009), the overall abundance may be related to annual precipitation. Increasing abundance in saprotrophic fungi can influence litter decomposition rates (Beare et al., 1992). Precipitation gradients along with inherent differences in the soil environment at the landscape scale could result in discontinuities in rates of nutrient cycling across the landscape and differential responses to increased eastern redcedar productivity throughout the upland oak forests of this ecotone.

4.3. Ramifications of high AM fungal abundance

Over a fifty year period, eastern redcedar increased from less than 0.5% to greater than 13% of the basal area and 23% of the sapling density of upland oak forests in the ecotone between the eastern deciduous forest and the southern Great Plains (DeSantis et al., 2010). Although our results did not find large changes in overall SMC composition, our study showed one potentially important consequence of redcedar encroachment is significant increases in AM fungal biomass. Increases in this microbial functional group may induce positive plant–microbial feedbacks facilitating redcedar encroachment (Shah et al., 2009). The increase in AM fungi could also result in loss of EM hyphal production, an additional plant–microbial feedback further facilitating redcedar encroachment, as oak species form mycorrhizal associations with EM fungi, as opposed to AM fungi (Marks and Kozlowski, 1973; McHugh and Gehring, 2006; Cavender-Bares et al., 2009). Since EM symbiosis increases plant fitness, decreasing EM abundance of post oak and blackjack oak may hinder disease resistance and reduce competitive ability with eastern redcedar for resources (Smith and Read, 2008). Due to common PLFA biomarkers in saprotrophic and EM fungi we did not assess EM fungi abundance. Future research should assay EM abundance directly by quantifying EM colonization of oaks to determine effects of eastern redcedar encroachment on fungal cohorts of post oak and blackjack oak (McHugh and Gehring, 2006).

Our results showed eastern redcedar encroachment was associated with important changes in the litter and SMC compared to upland oak forests. The change in overstory plant species composition may be detrimental to conserving an ecosystem that may be one of the largest areas of old-growth forest in the eastern United States (Therrell and Stahle, 1998). Significant increases in AM fungal biomass may enhance the ability for eastern redcedar and other AM fungal-associated plant species to compete in a relatively nutrient poor ecosystem while possibly impeding the long-term survival of the historically dominant post oak. Through changes occurring in the soil environment, these threatened forests may become increasingly difficult to maintain as oak-dominated ecosystems.

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