Essential oil, insect, and microbe relationships in *Juniperus osteosperma* (Cupressaceae) trees killed by wildfire

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ABSTRACT

Pinyon-juniper forests figure prominently in the Intermountain West (USA) with *Juniperus osteosperma* (Torr.) Little (Utah juniper) being the most common tree in the state of Utah. Wildfires frequently impact these woodlands. Dead Utah juniper trees (n = 16), which were killed by different wildfires over a range of 21 years, were harvested to research the relationship between abiotic and biotic influencers of decomposition. Specifically, dead juniper trunks were divided into four segments to research essential oil (volatile oil) content and composition, water content, boring insect populations, and fungal and bacterial colonization. Volatile oil samples, produced through steam distillation of trunk portions, were analyzed by GC-FID and GC-MS. Prominent compounds include α-cedrene (10.4%), cis-thujopsene (19.8%), widdrol (7.6%), cedrol (20.1%), and cedr-8-en-13-ol (5.7%). Based on the year that trees were burnt, older trees displayed a trend of containing a higher yield of volatile oil. Essential oil positively correlated with water content (Spearman's correlation test: $\rho = 0.519$, $p = 0.039$). Evidence of boring insects from the family Buprestidae were found in most trunk samples (75%). DNA sequencing identified 1258 bacterial and 326 fungal taxa, with samples dominated by fungal reads. Fungal communities were dominated by Ascomycota and Basidiomycota, and bacterial communities by Actinobacteria, Proteobacteria, and Bacteroidetes. The presence of two volatile compounds, α- and β-acorenol, was demonstrated to be a negative predictor of fungal diversity (GLM; $p = 0.017$). The content or composition of volatile oil demonstrated little-to-no impact on bacterial diversity or insect populations. This study establishes that volatile oil remains in the trunk wood of Utah juniper trees for at least 20 years, but has little predictive impact on biotic influencers of decomposition. *Published online* ***www.phytologia.org*** *Phytologia 103(4): 106-118 (December 22, 2021). ISSN 030319430.*

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Pinyon-juniper forests are estimated to cover over 14.5 million (Shaw et al. 2005) or 18 million hectares (Miller and Tausch 2001) of the Intermountain West. Three tree species that are common in the pinyon-juniper forest of Utah and produce quantifiable amounts of volatile oils include *Juniperus osteosperma* (Torr.) Little (Utah juniper), *Pinus edulis* Engelm. (pinyon pine), and at certain elevations and growing conditions, *Juniperus scopulorum* Sarg. (Rocky Mountain juniper) (Cronquist et al. 1972; Welsh

1993). Utah juniper is the most common tree in Utah (Cronquist et al. 1972). The trunk wood of all three species has been found to contain volatile oil (Poulson et al. 2020, 2021; Wilson et al. 2019).

The aromatic profile for Utah juniper trunk essential oil has been found to be prominent in α-pinene (59.4%), δ-3-carene (4.4%), cis-thujopsene (11.0%), and cedrol (3.0%) (Wilson et al. 2019). The aromatic profile for pinyon pine trunk essential oil has been found to be prominent in α-pinene (50.3%), δ-3-carene (7.3%), ethyl octanoate (2.9%), longifolene (6.7%), and germacrene D (5.8%) (Poulson et al. 2020). The aromatic profile for Rocky Mountain juniper trunk essential oil has been found to be prominent in α-pinene (20.5%), cis-thujopsene (34.2%), and cedrol (18.9%) (Poulson et al. 2021). A primary difference between Utah juniper and Rocky Mountain juniper is the latter's unique purple-red heartwood (Cronquist et al. 1972; Poulson et al. 2021; Welsh 1993).

Pinyon-juniper forests are often casualties of forest fires which result in the deaths of many trees (Gruell 1999). It has long been known that the decomposition of dead trees is an intricate process involving both abiotic and biotic factors (Shigo 1979). Examples of biotic decomposers include saprophytic fungi and bacteria (Janusz et al. 2017; Kubartová et al. 2009; Song et al. 2017) and insects through both indirect and direct means (Jacobsen et al. 2018; Krivosheina 2016; Ulyshen et al. 2016).

Previous work (Clark et al. 1990) has shown both antimicrobial and antifungal properties of volatile oil extracted from Utah juniper heartwood, sapwood, and bark. Additionally, Utah juniper heartwood demonstrated termiticidal activity (Adams et al. 1988). The durability and abundance of Utah juniper underscore its popular use as fence posts (Cronquist et al. 1972). Volatile compounds, which act as preserving agents, in the wood appear to contribute to the long-lasting nature of dead juniper wood (Adams 2014).

We hypothesized that dead Utah juniper trees retain essential oils, and that these volatile compounds help slow the process of decomposition. Utilizing wildfire data from 1998-2018, the present study aimed to investigate whether essential oils remain in dead Utah juniper trunks following wildfires. We also sought to characterize any changes in essential oil composition following tree death, and to examine the relationship between biotic decomposition influencers of dead juniper trees, namely insects, fungi, and bacteria.

MATERIALS AND METHODS

Burnt trunks of *J. osteosperma* were collected from sixteen locations, one tree per site, throughout the state of Utah (Table 1). Historical fire dates were obtained from the Bureau of Land Management (BLM) records (https://www.blm.gov/services/geospatial/GISData/utah). Selected burnt trunks of *J. osteosperma* were cut 0.25 m above ground and then cut to 1 m in length. Due to the lack of leaf and reproductive tissue, conventional voucher samples were not obtained. However, the identity of specimens was determined through means of volatile compound analysis (Poulson et al. 2020; Poulson et al. 2021; Wilson et al. 2019) and, in the case of Rocky Mountain juniper, the color of the heartwood (Cronquist et al. 1972; Poulson et al. 2021; Welsh 1993). For simplicity and consistency, samples are referred to by the year they burned by wildfire (Table 1).

Samples of burnt *J. osteosperma* trunk (n = 16) were collected and processed as follows for each group of researchers: 30-cm section for steam distillation and analytical analysis (Richard E. Carlson; D. Gary Young Research Institute); 30-cm section for entomology analysis (Ryan Davis; Utah State University); 30-cm section for fungal and bacterial analysis (Geoffrey Zahn; Utah Valley University); and 10-cm section for water content analysis (Michael T. Stevens; Utah Valley University) (Figure 1). All sections were stored in a sealed bag at ambient temperature and out of direct sunlight until analysis occurred.

Immediately prior to distillation, cross sections of the trunk were cut with a chainsaw to produce sawdust. Laboratory-scale distillation occurred as follows: 3 L of water added to the bottom of a 12-L distillation chamber (Albrigi Luigi S.R.L., Italy), plant material (sawdust) accurately weighed before being added to the distillation chamber, distillation for 4 hours by direct steam, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were filtered and stored in sealed amber glass bottles at 25°C until analysis.

Table 1. Sample details, including Bureau of Land Management (BLM) fire site name, wildfire year, and coordinates of sample collection.

BLM fire site name	year of wildfire	latitude	longitude
Minersville	1998	38.2399	-112.8383
Meadow Spring	1999	38.3633	-113.9149
Fisher	2000	38.6792	-109.2999
Substation	2001	39.3052	-112.0301
Vance	2004	38.4009	-113.8894
Neck	2005	38.0547	-113.1844
Monarch	2007	40.9520	-112.8348
Devil	2008	41.5442	-113.6921
Engleman	2009	39.9885	-113.1322
Nowhere	2011	39.9785	-113.1177
Shanty Canyon	2012	40.8316	-112.4833
Playground	2013	41.5335	-113.7096
Lion Peak	2014	39.9422	-112.7181
Hicks Creek	2016	37.5799	-113.0851
Knob	2017	40.9202	-113.0017
Ridge	2018	41.5719	-113.7488

Essential oils were analyzed, and volatile compounds identified by GC-MS using an Agilent 7890B GC/5977B MSD and J&W DB-5, 0.25 mm x 60 m, 0.25 μm film thickness, fused silica capillary column, 1.0 μL Agilent syringe. Operating conditions: 0.1 μL of neat sample, 150:1 split ratio, initial oven temperature of 40°C with an initial hold time of 5 minutes, oven ramp rate of 4.5°C per minute to 310 °C with a hold time of 5 minutes. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150°C. Volatile compounds were identified using the Adams volatile oil library (Adams 2007, pdf at www.juniperus.org) using Chemstation library search in conjunction with retention indices. Note that in some samples γ-eudesmol/α-acorenol and widdrol/cedrol elute as single peaks, but their amounts are determined by the ratio of masses 119 (α-acorenol), 189 (γ-eudesmol), 151 (widdrol), and 150 (cedrol). Volatile compounds were quantified and are reported as a relative area percent by GC-FID using an Agilent 7890B and J&W DB-5, 0.25 mm x 60 m, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of sample (5% soln. for essential oils, 1% for reference compounds in hexane), 25:1 split ratio, initial oven temperature at 40°C with an initial hold time of 2 minutes, oven ramp rate of 3.0°C per minute to 250°C with a hold time of 3 minutes. For quantification, compounds were identified using retention indices coupled with retention time data of reference compounds.

The essential oil percent yield was calculated as the ratio of mass of processed plant material immediately before distillation to the mass of essential oil produced, and then multiplied by 100. The corrected essential oil yield takes into consideration the percent of the trunk mass that is water weight. In regard to essential oil content and composition, Spearman's correlation test was used to evaluate the relationship between variables themselves over the 21-year range. To determine the water content of the sawdust generated from the 16 burnt trunk samples, we first separated out coarse wood fragments by passing each sample through a no. 10 mesh sieve (screen size = 2.0 mm). From each of the 16 screened samples, we took five replicates and placed them into 80 separate coin envelopes. The average fresh weight of the 80 resulting samples was 2.4 g. The samples were put into a drying oven set at 60°C and dried until preliminary weight assessments showed that they had reached a constant weight. After 13 days the samples were removed from their envelopes, and their final dry weight was determined. Percent water content was calculated using [(fresh weight-dry weight)/fresh weight].

Figure 1. From bottom to top: 30-cm section for steam distillation and analytical analysis (red), 30-cm section for entomology (orange), 30-cm section for fungal and bacterial analysis (pink), 10-cm section for water content analysis (white). Cuts were made in the field using a chainsaw and, as such, are approximations.

For each trunk sample selected for entomological analysis, height and circumference (of the top and bottom of each trunk section) were measured to determine outer trunk surface area. Trunk samples were destructively sampled for the presence of insects and insect activity, including number of adult exit holes, exit hole height, width, and length, larval gallery depth, number of live adults, number of live larvae, and larvae taxa identified visually to family. The number of successful attacks was estimated using the average number of emergence holes per cm^2 in the bark or outer xylem.

For microbial community measurements, three replicate spots on each trunk were selected and surface-sterilized with isopropyl alcohol. Using a sterilized drill bit, triplicate 10-cm holes were drilled into

sapwood. If bark material was present, it was removed prior to surface sterilization. Wood shavings and dust were collected in a sterile container and homogenized. Homogenized sawdust (0.25 g) from each sample along with negative controls were subjected to DNA extraction using Qiagen PowerSoil DNA kits according to manufacturer instructions.

Bacterial 16S DNA was amplified using 515f-806r primers. Fungal ITS2 DNA was amplified using ITS3-mix (5'-CANCGATGAAGAACGYRG-3') – ITS4 (5'-TCCTSCGCTTATTGATATGC-3') (Tedersoo et al. 2014).

All primers were modified with the addition of Illumina adaptors (Caporaso et al. 2011) and DNA was amplified using the following protocol: 98°C for 2 min; 22 cycles of: 98°C for 15 s, 52°C for 20 s, 72°C for 30 s; 72°C for 2 min. After 22 cycles, the PCR product was diluted 1:12 and 1 µL of this was used as a template for 8 more rounds of PCR with a 60°C annealing temperature in which bi-directional barcodes bound to reverse complimented Illumina adaptors acted as primers. Resulting barcoded libraries were cleaned, normalized, and sequenced with the Illumina MiSeq platform (V3 chemistry, 2 × 300 bp).

All bioinformatics steps and analyses have been documented via reproducible code at https://github.com/gzahn/Juniper_Microbes. Briefly, processing raw DNA reads included: 1) removal of primer sequences from all reads using cutadapt (Martin 2011); 2) extraction of the ITS2 region of fungal reads using itsxpress (Rivers et al. 2018); 3) Amplicon Sequence Variant (ASV) calling on fungal and bacterial reads using DADA2 (Callahan et al. 2016); 4) detection and removal of putative chimeras and contaminant sequences using decontam (Davis et al. 2018); and 5) assignment of taxonomy for fungi and bacteria using the UNITE + EUK database (Abarenkov et al. 2020) and the RDP 16S training set, respectively, with the RDP Naive Bayesian Classifier algorithm (Wang et al. 2007). All microbial community analyses were performed in R v 3.6.2 (R Core Team 2017) using the phyloseq, vegan, and corncob packages (Martin et al. 2020; McMurdie and Holmes 2013; Okansen et al. 2016).

We tested geographic location, essential oil composition, and time since burn as predictors of bacterial and fungal community composition. Geographic location was tested via a Mantel test with 9999 permutations. Years since burn and essential oil composition were tested with generalized linear models and a permutational ANOVA model using the vegan package.

RESULTS AND DISCUSSION

The overall health and condition of each tree, prior to being burnt, is unknown. Due to the geographical isolation of particular wildfires, it was only feasible to collect samples from 16 different years over a 21-year range.

The aromatic profiles of each trunk can be found in Table 2. Interestingly, while α-pinene and δ-3-carene were prominent compounds in Utah juniper trunk essential oil from living trees (Wilson et al. 2019), neither greatly contributed to the profile of burnt Utah juniper trees (α-pinene: from nd to 3.9%; δ-3-carene: nd). These lighter fractions were either readily lost to the atmosphere or were primarily contained in the outer layers of the trunk, which were completely burnt. Prominent compounds, which were defined as an average area % from all samples > 5, include α-cedrene (10.4%), cis-thujopsene (19.8%), widdrol (7.6%), cedrol (20.1%), and cedr-8-en-13-ol (5.7%). Based on the date when these trees were burnt, older trunks displayed a trend of a higher relative abundance of more volatile fractions (α-cedrene, cis-thujopsene, widdrol) (Figure 2) and lower relative abundance of less-volatile fractions (cedrol, cedr-8-en-13-ol) (Figure 3). However, a larger dataset is needed to determine statistical significance.

Table 2. Aromatic profile of *J. osteosperma* essential oil from burnt trunks (n = 16). Compounds not detected are denoted as not detected (nd) and values less than 0.1% as traces (t). Unidentified compounds less than 1.0% are not included. KI is the Kovat's Index using a linear calculation on DB-5 column (Adams 2007). Relative area percent is determined by GC-FID. Essential oil samples were analyzed in triplicate to ensure reproducibility (SD < 1 for all compounds). **KI** indicated in bold font was calculated using alkane standards.

	Year of Fire:	1998	1999	2000	2001	2004	2005	2007	2008	2009	2011	2012	2013	2014	2016	2017	2018
KI	**Compound**																
932	α-pinene	0.1	nd	nd	nd	nd	3.9	t	t	0.5	t	nd	nd	0.8	0.3	2.0	1.0
953	thuja-2,4(10)-diene	nd	nd	nd	nd	nd	0.8	nd	nd	t	nd	nd	nd	nd	t	0.2	nd
1020	p-cymene	nd	0.2	2.9	0.5	1.7	1.2	0.6	0.3	1.0	0.6	0.2	t	0.3	1.4	0.5	0.5
1186	α-terpineol	0.3	t	3.2	1.8	5.3	1.5	0.6	0.3	1.5	3.5	0.3	t	1.1	1.2	0.5	2.8
1194	myrtenol	t	t	t	0.1	0.7	0.4	t	0.1	0.3	0.1	0.1	t	2.7	0.1	0.2	0.7
1195	myrtenal	t	t	t	0.1	0.4	1.1	t	0.2	0.3	0.3	0.2	t	1.3	0.4	0.2	0.1
1225	Unknown 1	nd	nd	nd	nd	nd	t	t	nd	nd	nd	nd	nd	nd	2.0	nd	nd
1241	carvacrol methyl ether	0.2	t	0.9	0.4	0.3	0.7	t	0.3	0.1	0.3	0.2	nd	1.0	1.1	0.4	0.5
1249	piperitone	nd	nd	t	t	0.2	nd	nd	1.0	nd	nd	nd	nd	1.3	t	nd	t
1387	α-duprezianene	0.7	1.1	1.0	0.7	0.7	1.2	0.9	1.0	0.6	0.5	0.5	1.2	0.8	1.2	0.9	0.3
1410	α-cedrene	14.3	16.7	3.6	2.4	5.6	18.1	7.2	34.1	8.1	8.0	11.4	7.8	7.1	11.7	8.0	2.1
1419	β-cedrene	2.9	3.3	0.9	0.6	2.3	4.1	1.9	7.2	2.4	2.5	1.8	0.7	1.9	2.2	2.0	0.9
1421	β-duprezianene	0.4	0.8	0.8	0.6	0.3	0.8	0.8	0.7	0.4	0.4	0.4	2.1	0.6	0.8	0.8	0.4
1429	cis-thujopsene	8.7	16.1	30.7	25.8	26.4	15.2	30.2	15.8	13.9	27.2	12.0	13.8	22.4	12.0	20.7	26.0
1449	α-himachalene	t	0.6	0.4	0.4	0.4	0.4	0.4	0.3	nd	0.2	0.1	t	0.3	0.4	0.3	0.2
1469	β-acoradiene	0.4	0.7	0.8	0.5	0.6	0.9	0.7	0.7	0.1	0.3	0.5	0.6	0.5	0.9	0.4	0.3
1474	10-epi-β-acoradiene	0.3	0.7	0.6	0.4	0.9	0.9	0.8	0.7	0.1	0.4	0.4	0.8	0.5	0.6	0.6	0.3
1476	β-chamigrene	0.5	0.9	1.5	1.0	0.7	1.4	0.8	1.5	0.3	0.4	1.1	0.8	0.8	1.6	0.9	0.3
1498	pseudowiddrene	0.9	2.3	1.9	1.1	1.2	2.2	1.1	2.3	0.6	0.5	1.6	1.6	1.3	3.7	1.5	0.2
1504	cuparene	1.6	2.3	3.5	2.8	2.1	2.5	2.4	2.5	1.6	1.4	2.9	1.9	2.0	3.2	1.9	1.5
1541	8,14-cedranoxide	t	0.5	0.9	0.9	0.3	0.3	t	t	nd	nd	2.5	1.4	0.8	1.9	0.4	0.8
1589	allo-cedrol	0.3	0.4	0.4	0.5	0.4	0.2	0.6	t	0.6	0.5	t	0.7	0.5	t	0.3	1.2
1595	cis-dihydro-mayurone	0.5	1.4	0.6	0.6	0.4	0.6	0.4	t	0.4	t	0.5	1.9	0.3	0.4	0.2	0.3
1599	widdrol	7.5	7.1	10.1	10.3	6.0	7.9	7.0	5.5	4.3	2.6	10.0	15.5	7.4	11.8	6.4	1.4
1600	cedrol	37.3	17.8	5.8	7.4	15.1	14.6	18.4	15.9	36.6	38.1	15.2	20.5	20.6	8.6	15.0	35.2
1607	β-biotone	0.3	0.4	t	0.3	0.3	t	0.3	t	0.6	nd	0.3	0.6	0.5	0.3	0.3	0.2
1630	γ-eudesmol	0.5	1.1	2.0	3.4	0.4	0.4	0.3	0.2	1.2	0.2	0.7	0.8	0.2	2.8	2.4	nd
1632	α-acorenol	0.8	0.4	1.2	1.1	1.4	0.9	1.6	0.4	1.2	1.5	0.4	2.6	1.1	nd	0.7	3.1
1636	β-acorenol	0.4	0.5	0.5	0.7	0.6	0.4	0.7	t	0.3	0.5	0.3	0.9	0.6	0.3	0.5	0.8
1639	1,7-diepi-alpha-cedrenal	1.3	0.9	1.4	1.7	0.8	0.8	0.9	0.7	1.0	0.4	1.6	1.6	0.8	1.1	0.5	0.5
1649	β-eudesmol	1.0	1.1	4.2	6.2	0.9	1.0	1.9	1.0	3.9	1.0	0.9	3.2	0.7	1.9	3.1	1.5
1652	α-eudesmol	0.6	0.7	2.2	3.1	0.5	0.6	0.6	t	2.0	0.5	0.5	2.0	0.4	1.6	1.9	0.6
1681	Unknown 2	0.8	0.8	0.7	1.1	0.7	0.5	0.8	0.4	0.4	0.5	0.9	1.2	0.9	0.8	0.9	0.9
1688	cedr-8-en-13-ol	4.9	5.7	1.5	2.2	7.8	5.0	9.2	3.0	6.1	0.8	13.0	3.3	6.5	8.8	11.4	1.8
1714	cedr-8-en-15-ol	3.3	2.9	2.1	3.4	2.2	2.1	2.9	1.6	2.8	1.7	3.9	2.3	3.1	3.1	3.7	2.7
1709	mayurone	0.4	0.5	0.8	0.8	0.4	0.4	t	t	0.5	t	0.5	0.6	t	0.3	nd	0.1
1708	thujopsenal	0.7	0.5	0.6	0.9	0.5	0.4	0.4	0.4	t	0.7	0.5	0.5	0.5	0.7	0.5	0.5
1725	Unknown 3	1.2	1.5	0.7	1.4	2.1	0.9	2.4	0.5	1.4	1.0	1.1	1.0	2.0	0.9	2.4	1.8
1739	4a,8,8-Trimethylocta-hydrocyclopropa[d] naphthalen-2(3H)-one	1.1	2.6	1.6	1.4	0.9	1.3	0.9	0.7	0.5	0.7	1.6	2.2	0.7	1.3	0.5	0.2
1746	3,3,4-Trimethyl-4-(4-methylphenyl) cyclopentanol	0.4	0.3	0.3	0.9	0.4	0.2	0.4	t	t	t	0.9	nd	0.4	0.5	0.4	0.3
1788	8-cedren-13-ol acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	0.4	nd	nd	nd	nd	nd
1806	nootkatone	t	t	1.0	0.4	t	t	t	t	nd	nd	nd	t	nd	nd	nd	0.2
1889	8S,14-cedranediol	t	0.2	0.6	0.7	0.4	0.3	0.3	t	nd	nd	1.4	0.7	1.0	1.2	0.6	0.9
	column total:	94.6	93.0	91.9	88.6	92.3	96.1	98.4	99.3	95.6	97.7	90.8	94.8	95.7	93.1	94.1	93.1

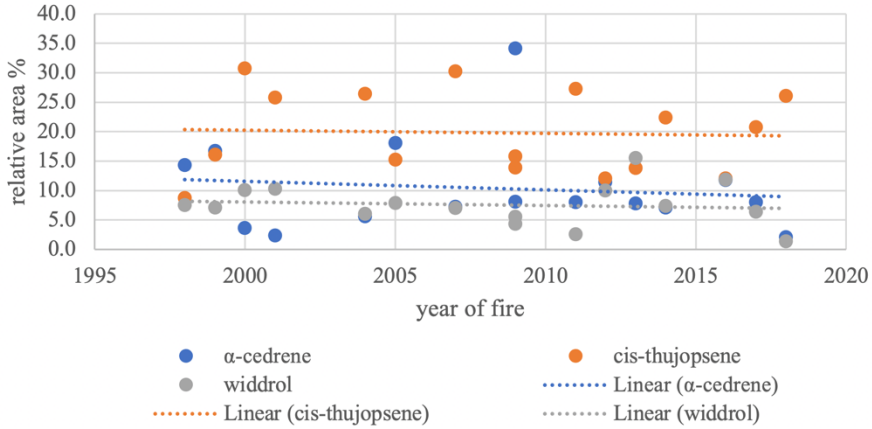

Figure 2. Scatter plot of α-cedrene, cis-thujopsene, and widdrol. Levels of these compounds tend to be lower in more recently burned trunks.

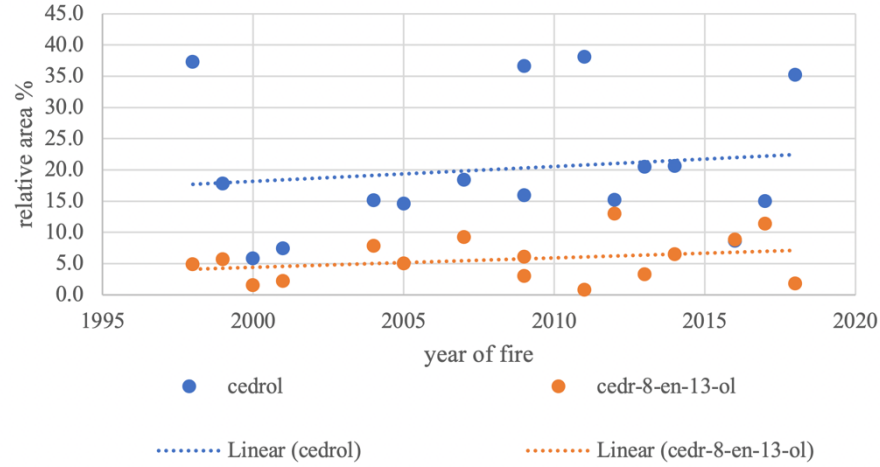

Figure 3. Scatter plot of cedrol and cedr-8-en-13-ol. Levels of these compounds tend to be higher in more recently burned trunks.

The raw material mass and essential oil yield are detailed in Table 3. Not surprisingly, older trees, defined by when the tree was burnt, tended to contain less water and, as a result, a higher yield (relative to the weight of the raw material) of essential oil (Figure 4). There was a positive and moderate correlation (Spearman's correlation test: $\rho = 0.519$, $p = 0.039$) between essential oil and water content in burnt trunks. The corrected essential oil content by year of wildfire is detailed in Figure 5.

In our examination of trunk samples, flatheaded borer exit holes were easily discernable. Insect exit holes from flatheaded/metallic woodboring beetles (Buprestidae) were present in 75% of trunk samples, ranging from one to thirty-five exit holes per sample. A total of five living flatheaded borer larvae were recovered from the trunk samples (2013 (Playground), 1 larva; 2014 (Lion Peak), 4 larvae). Previous observations by other authors from living *J. osteosperma* trunk wood include species of Buprestidae (*Chrysobothris texana*), Cerambycidae (*Semanotus ligneus*), and Curculionidae (*Phloeosinus* spp.) (Furniss and Carolin 1977; Kannenberg et. al 2021).

Insect activity can be found in Table 4. While the essential oil content and the number of insects exit holes (per cm^2) appear to decrease from 1998-2018 (Figure 6), the correlation between the two sets of data was not statistically significant (Spearman's correlation test: $\rho = -0.185$, $p > 0.05$). The woodboring beetle species listed above preferentially attack stressed living and recently dead *J. osteosperma* and are not considered a causal agent of mortality (Kannenberg et al. 2021). Therefore, it is likely that most of the

woodborer exit holes recorded in this study were created while the trees were living or within a few years after the trees were injured by fire or dead. In this case, woodborer colonization of stressed living, or recently burned or dead trees may be more likely driven by the essential oil composition of living *J. osteosperma* (Wilson et al. 2019) rather than older dead or older fire-injured or fire-killed trees.

Table 3. Mass distilled and essential oil (EO) yield from single *J. osteosperma* trees (the number was limited to one as per permit restrictions) collected from 16 different locations. Each tree was cut 0.25 m above ground; all measurements and calculations are reflective of above ground portions.

year of fire	mass distilled (g)	mass water content (%)	EO yield (g)	corrected EO yield (%)
1998	900.2	4.9	1.7	0.2
1999	813.5	5.4	3.8	0.5
2000	1050.5	4.6	4.4	0.4
2001	832.7	5.0	2.3	0.3
2004	936.1	5.1	7.6	0.9
2005	1416.7	4.7	5.2	0.4
2007	1205.7	5.2	5.6	0.5
2008	1426.1	5.5	11.0	0.8
2009	1030.7	5.5	4.4	0.5
2011	984.6	6.0	9.4	1.0
2012	844.1	5.7	4.5	0.6
2013	933.6	5.3	0.7	0.1
2014	1127.5	5.1	4.7	0.4
2016	1109.9	5.3	3.7	0.4
2017	1641.5	5.0	4.8	0.3
2018	1196.2	5.4	2.2	0.2

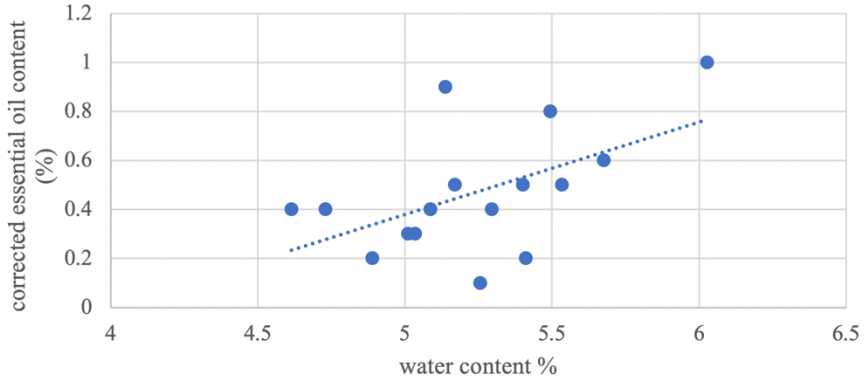

Figure 4. There is a positive and moderate correlation (Spearman's correlation test: $\rho = 0.519$, $p = 0.039$) between percent corrected essential oil content and percent water content (mean of 5 replicates) in each sample.

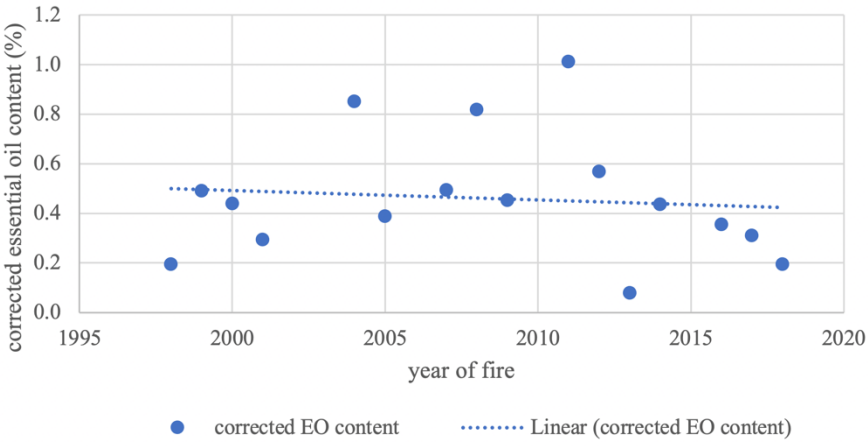

Figure 5. Corrected essential oil content percentages by year of fire. Water content ranges between 4.6% and 6.0% in all samples.

Table 4. Entomological data from the dead trunk sections (n = 16). All insect activity, and associated measurements, were determined to be from the family Buprestidae. There were a total of 5 living larvae counted (2013, 2014). The column denoting non-woodborer insect taxa present includes taxa that could not be identified with confidence or non-boring arthropods (Argasidae, Dermestidae, Polyxenidae).

year of fire	trunk surface area (cm^2)	insect exit hole (count)	insect exit hole (per cm^2)	average exit hole width (mm)	average exit hole height (mm)	average gallery depth (mm)	flatheaded borer larvae (count)	non-woodborer unidentifiable insect taxa present
1998	1988	1	0.0006	*	*	*	0	no
1999	2711	7	0.0034	3.9	2	8.9	0	no
2000	2355	0	0.0000	-	-	-	0	no
2001	1253	11	0.0104	4	2	9.2	0	no
2004	2046	8	0.0050	4	2	9.3	0	unidentifiable
2005	1718	35	0.0253	4.6	2.7	10.8	0	unidentifiable
2007	1949	10	0.0063	4	2	8.9	0	Argasidae
2008	2384	1	0.0006	4	2	6	0	no
2009	1356	7	0.0061	4.3	2	8	0	Dermestidae
2011	1921	0	0.0000	-	-	-	0	no
2012	1340	1	0.0009	4	2	4	0	no
2013	1801	8	0.0056	4	2	13.6	1	unidentifiable
2014	2959	11	0.0050	3.3	2.2	8.2	4	unidentifiable
2016	1979	0	0.0000	-	-	-	0	Polyxenidae
2017	1389	10	0.0085	3.6	1.9	9.7	0	no
2018	1770	0	0.0000	-	-	-	0	no

*Incomplete data collected due to branch union in trunk sample.

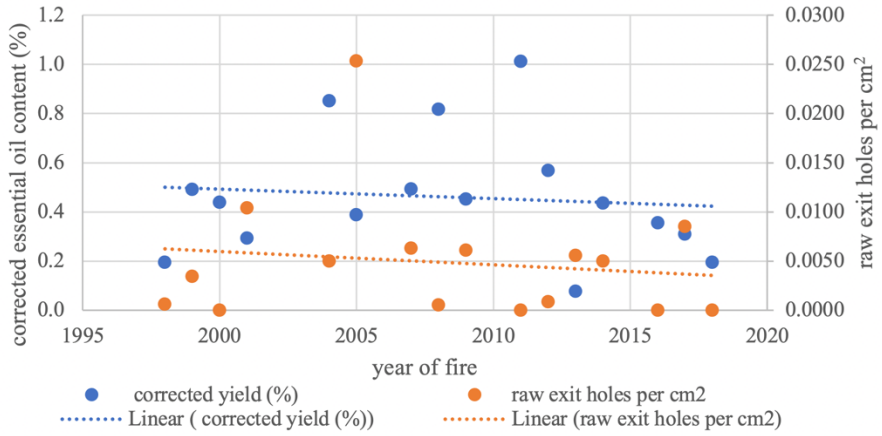

Figure 6. Corrected essential oil content (%) and insect exit holes (per cm^2) in relation to the year of wildfire. The correlation between essential oil content and insect exit holes is not statistically significant.

After quality filtration and processing, we identified 1258 bacterial and 326 fungal taxa. Samples were dominated by fungal reads, and some wood samples did not have detectable bacteria (Figure 7). Bacterial communities were dominated by Actinobacteria, Proteobacteria, and Bacteroidetes (Figure 8). In terms of fungi, Ascomycota were significantly more abundant than Basidiomycota except for in one case (2018).

A Mantel test did not show any community separation by distance for either bacterial or fungal communities ($p = 0.073$, $p = 0.605$, respectively). Therefore, geographic location was not a significant indicator of microbial composition. Linear regressions showed that two compounds (α-acorenol and β-acorenol) had significant impacts on fungal diversity, but no compounds were found to affect bacterial diversity.

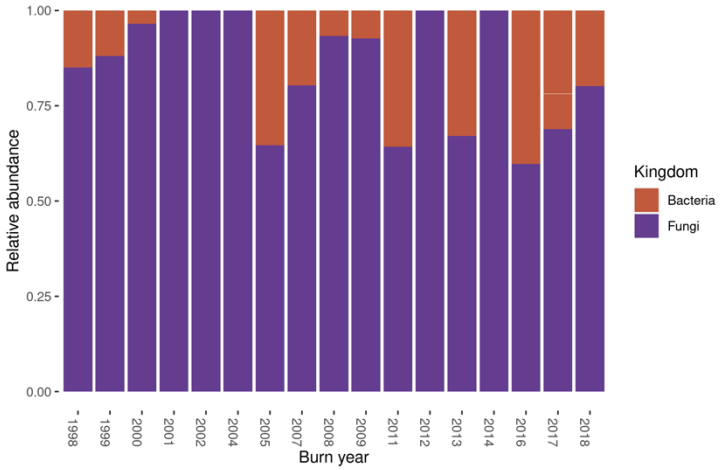

Figure 7. Relative abundance of bacterial and fungal taxa for each burn year.

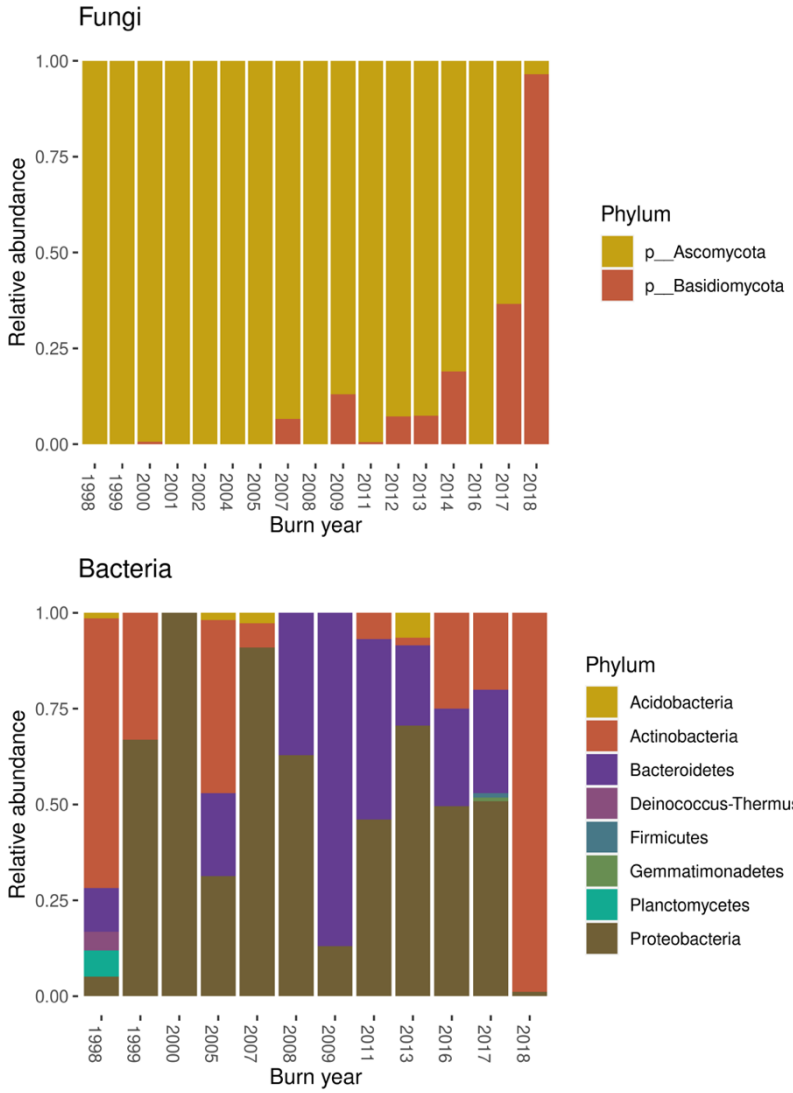

Figure 8. Relative abundance of observed fungal (top panel) and bacterial (bottom panel) phyla for each burn year.

We fitted a linear model (estimated using ML) to predict fungal Shannon diversity with β-acorenol, α-acorenol and years_since_burn. The explanatory power of the model was substantial (R^2 = 0.480) and showed that the combination of α- and β-acorenol was a statistically significant and negative predictor of fungal diversity (GLM; p = 0.017). Neither compound was significant on its own, suggesting synergy between them.

Years since burn did not have a detectable effect on fungal diversity or composition, but it did influence bacterial diversity (GLM; p = 0.005) and community composition (PermANOVA; p = 0.042). Overall, both bacterial and fungal communities in these dead trees appear to be stochastic in nature. Though years since burn affected bacterial diversity, and acorenol compounds affected fungal diversity, we did not find any predictable effect of these factors on discrete microbial taxa.

Sequence data has been deposited in the Sequence Read Archive under accession PRJNA730829. All microbial data and code can be found at https://github.com/gzahn/Juniper_Microbes.

CONCLUSIONS

This study establishes, for the first time, that wildfire-killed Utah juniper trees retain volatile oil for at least 20-years. We further demonstrated that volatile oil in dead Utah juniper trees differs from that in living conspecific trunks, and that α-cedrene (10.4%), cis-thujopsene (19.8%), widdrol (7.6%), cedrol (20.1%), and cedr-8-en-13-ol (5.7%) are prominent compounds in the dead trees. Based on the year the trees were burnt, older burnt trunks also showed an increase in relative abundance of more volatile aromatic compounds and a decrease in relative abundance of less volatile aromatic compounds, as well as having a lower water content and thus a higher yield of essential oil. Water content in the trunk and essential oil yield were positively correlated. While the presence of water in dead plant material is an important factor in decomposition, the presence of volatile oil had little predictive impact on the influence of decomposition.

When examined for insect activity, evidence of flatheaded borers (Buprestidae) predominated, and these insects likely attacked the sample trees prior to or recently after the trees' deaths. While there appeared to be a trend between corrected essential oil yield and number of exit holes, there was no statistical correlation between the two factors. Given the timing of attack of the woodborers commonly found in *J. osteosperma*, number of exit holes may be better tested against essential oil concentrations of living or recently dead trees. These findings suggest that flatheaded borers have a negligible direct impact on decomposition of dead Utah juniper trees.

Numerous bacterial (1258) and fungal (326) taxa were found in the burnt trunk samples. While the diversity of bacteria was much greater than the diversity of fungi, the relative abundance of fungi was much higher than that of bacteria. Actinobacteria, Proteobacteria, and Bacteroidetes were the most prominent bacterial communities. Of the fungi, Ascomycota tended to be more abundant than Basidiomycota. No predictive effects on discrete microbial taxa were found between location, essential oil composition, or year since burn, but there were still some interesting findings. Years since burn did not have a detectable effect on fungal diversity or composition, but it did influence bacterial diversity and community composition. Moreover, combination of α- and β-acorenol in the essential oil was a significant negative predictor of fungal diversity, which may be due to synergy between the two compounds. However, additional research is needed to address this and to better understand the potential impact on decomposition.

While this study establishes the foundation for better understanding abiotic and biotic factors that contribute to decomposition of dead Utah juniper, additional research is needed. To better understand the impact that essential oil compounds have on insect and microbial populations, a baseline is needed. Performing similar research on non-essential oil-bearing species in the same habitat could provide such

insight and lead to a better understanding of the interactions between the many species that make up pinyon-juniper communities.

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